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SERIAL ENDOSYMBIOTIC THEORY (SET): UNDULIPODIA, MITOSIS AND THEIR MICROTUBULE SYSTEMS PRECEDED MITOCHONDRIA

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Key Words: *Caedibacter*, cell evolution, cell symbiosis, mitochondria, microtubules, undulipodia, protist phylogeny, protoctista, tubulin, xenosomes.

Summary: Arguments are presented for the symbiotic acquisition of microtubule systems prior to the acquisition of mitochondria in the evolution of eukaryotic cells. These arguments are based on distribution of organellar systems (microtubules more widely distributed than mitochondria, mitochondria more widely distributed than plastids) and on polyphyly (multiple origins of mitochondria, plastids, motility symbionts, extracellular bacteria and other xenosomes are easily demonstrable). All mitotic and meiotic eukaryotes are purported to have evolved from associations of *Thermoplasma*-like archaeobacteria with *Spirochaeta*-like symbiotrophs. The former bacteria are thought to have been ancestral to the protonucleocytoplasm whereas the latter supplied eukaryotes with their microtubule-based internal motility systems.

INTRODUCTION

The SERIAL ENDOSYMBIOSIS THEORY (SET) of the origins of eukaryotic cells has assumed that mitochondria preceded undulipodia in the origin of cell organelles (1, 21). The endomembrane system (including the nuclear membrane) was purported to have evolved as a consequence of protomitochondrial symbiosis (21, 24). Reevaluation of recent information has led to a revision of that view. This paper supports and extends the concept of Schwemmler (29, 30) that microtubule systems, including those of the undulipodia, preceded the symbiotic acquisition of

mitochondria by the ancestors to nucleocytoplasm (31). Mitochondria probably originated by transformed necrotrophic associations of predaceous oxygen-respiring bacteria, e.g., *Daptobacter*, *Bdellovibrio* (14, 15) with *Thermoplasma*-like archaeobacteria as hosts (32). Reasons to suspect that microtubule systems of symbiotic origin preceded mitochondria acquisition are presented here.

II. ORIGIN OF UNDULIPODIA AND OTHER EUKARYOTIC MICROTUBULE SYSTEMS VIA MOTILITY SYMBIOSES

The thesis that undulipodia (cilia, eukaryotic flagella and other [9(2)+2] microtubule organelles of animal, plant and protoctist cells) originated from symbiotic spirochetes (bacteria) in motility associations has not been proven. (For discussion of terms for motility organelles see 1, 22, 24 and Table 1.) The hypothesis posits that microtubules (composed of tubulin proteins) evolved in spirochetes. Some of these spirochetes entered motility symbioses, e.g., they subsequently were acquired as surface symbionts and merged with their bacterial hosts to form the ancestors to eukaryotes. Various kinds of evidence support this hypothesis.

1. Microtubules, from 20-24 nm in diameter, have been observed by electron microscopy in thin transverse sections of large spirochetes (5). Two proteins, purified by warm-cold cycling from *Spirochaeta bajacaliforniensis*, show self-assembly, immunological and other properties similar to those of purified tubulin of eukaryotic microtubules (3).

Table 1

Motility organelle terminology

AXONEME. The [9(2)+2] microtubular shaft of an undulipodium (excluding the membrane).

AXOPOD. Long, thin cell structure of protoctists (most conspicuous in the phylum Actinopoda, such as heliozoans) used for locomotion or feeding; the centers of axopods are made of e.g., spirally arranged microtubular shafts.

AXOSOME. Fuzzy body where the central pair of microtubules of the axoneme contacts the kinetosome.

CENTRIOLE. Kinetosome lacking a [9(2)+0] axoneme; a structure that forms at each pole in the mitotic spindle during division in most animal cells.

CILIUM (*pl.* cilia). Undulipodium; generally short [9(2)+2] wavy cell protrusion found on eukaryotic cells and used for moving cells or extracellular fluids.

DYNEIN. The microtubule-associated protein with ATPase activity comprising the "arms" on the outer doublet tubules of undulipodia.

KINETID. Elementary repeating unit in all undulipodiated cells that consists of a kinetosome (or kinetosomes) and associated organelles, such as undulipodia, striated fibers and sheets of microtubules.

KINETOSOME. Organelle at the base of all undulipodia composed of microtubules arranged in a [9(2)+0] array; centriole from which axoneme emerges; confusingly called a "basal body".

MEIOTIC SEX. The life-cycle combination of meiosis and fertilization that characterizes sexual eukaryotes.

MICROTUBULES. Hollow, cylindrical, intracellular structures the walls of which are composed of the tubulin protein heterodimers (α tubulin, β tubulin) arranged as 13 protofilaments and associated with other proteins (MAPs); each microtubule is about 25 nanometers in diameter but of extremely variable length.

MICROTUBULE-ASSOCIATED PROTEINS (MAPs). A class of proteins, including dynein, which copurify with tubulins; several are high molecular weight relative to tubulin.

Table 1 (cont.)

MICROTUBULE ORGANIZING CENTER (MTOC). Site in a cell regularly associated with the appearance and organization of microtubules, such as in the development of the axoneme from the kinetosome in an undulipodium; the smallest are occasionally seen under the electron microscope as fuzzy spots, or their existence may be inferred from appearance of microtubule-based organelles; among the largest are heliozoan MTOCs.

MICROTUBULE PROTEIN. Tubulin; any of a class of proteins comprising microtubules and having molecular weights of about 50,000 daltons. The assembly of tubulin into microtubules is inhibited, in many organisms, by colchicine, vinblastine and other microtubule-protein polymerization-inhibiting drugs.

NAOs. Nucleus-associated organelle; a type of MTOC (q.v.); also called "extranuclear division center".

[NINE PLUS TWO, [9(2)+2]. Axoneme structure; description of the arrangement of nine doublets [9(2)] of microtubules surrounding a central pair [(2)] of singlets; shaft of undulipodium.

PERICENTRIOLAR DENSE BODY. Tiny amorphous structure seen with the electron microscope to be associated with [9(2)+0] centrioles.

PROTIST. Protoctist composed of a single or a few cells.

PROTOCTIST. A member of the kingdom Protoctista, unicellular (protists) and multicellular eukaryotic microorganisms and their descendants excluded from animals (K. Animalia) because they lack blastular embryos, from plants (K. Plantae) because they lack plant embryos enclosed in internal tissue and from fungi (K. Fungi) because they do not develop from zygo-, asco- or basidiospores.

SPIROCHETE. Corkscrew-shaped heterotrophic bacterium similar in morphology to spirilla except that the flagella of spirochetes are inside the outer membrane of the gram-negative cell wall; many spirochetes are extremely rapid swimmers; and they proliferate especially in anaerobic environments, such as muds and the guts of animals.

SPIROCHETE HYPOTHESIS. The hypothesis that undulipodia originated from motile anaerobic spirochetes which entered, reproduced in and became symbiotic with protoeukaryotic cells. These spirochetes, in the course of co-evolution of the merged symbionts, lost the ability to reproduce outside

Table 1 (cont.)

their hosts' cytoplasm; they differentiated into functioning structures at the basis of eukaryotic cell motility, such as MTOCs.

UNDULIPODIUM. [9(2)+2] eukaryotic organelle (confusingly referred to as a "eukaryotic flagellum" when long and few per cell and as a "cilium" when short and many per cell); all undulipodia from sperm tails to protist cilia, because of their [9(2)+2] ultrastructure, are thought to have a common origin (*see* SPIROCHETE HYPOTHESIS).

XENOSOME. Membrane-bounded intracellular structure (organism/organelle?) of presumed original foreign origin.

The search for other centriolar-kinetosomal-axonemal proteins (besides tubulin) in free-living spirochetes is underway.

2. Preliminary data, derived from *Spirochaeta bajacaliforniensis* DNA transcribed in an expression vector on a sequence of forty-five bases, indicate detectable homology between these tubulin-like proteins of *Spirochaeta* and all sequenced tubulins to date, e.g., mammalian brain, *Chlamydomonas*, *Saccharomyces*, *Arabidopsis* and *Stylonychia* (19, 36).

3. Various spirochetes have longitudinally-aligned microtubules, i,e., hollow proteinaceous structures 24nm in diameter that resemble eukaryotic microtubules (5, 11, 16).

4. Spirochetes easily enter motility symbioses and have been observed to orient themselves, adhere, attack and penetrate eukaryotic hosts, remaining motile throughout the association process (21, 25).

5. Some spirochetes have formed permanent hereditary symbioses with some protists, e.g., *Mixotricha* (13, 21).

6. Cortical inheritance in ciliates, variation in protist mitosis and meiosis as well as other "oddities and peculiarities" are best understood as evolutionary legacies, i.e., by the application of Darwin's "Panda Principle" to the origin of centrioles, kinetosomes and their undulipodia via spirochete associations. This principle claims that peculiarities and oddities in extant morphology, physiology and behavior are only explicable in the context of their evolutionary histories. (For details of this point see 2, 24.)

7. In protists and tissue preparations cilia have been mistaken for spirochetes and spirochetes for cilia in the cell biological literature (13, 26). A resemblance of the mitotic apparatus of the protist *Pansporella* to spirochetes was even noted (8).

Possible modes of transition from active, free-living spirochetes to a permanently-attached and functional undulipodium underlain by a kinetosome were detailed by Szathmary (35), who cogently argues a multiple origin (polyphyly) of eukaryotic microtubule systems. Because the primary sequence of the amino acid residues in tubulin proteins (from all sources mentioned in #2 above) is clearly homologous and the structure of undulipodia ubiquitous in all eukaryotes studied, no one doubts the ultimate homology of microtubule systems. The undulipodial system of

eukaryotes, on the other hand, displays no homology to prokaryotic flagella and hence the use of the term flagella for eukaryotes must be abandoned (22).

This paper argues that the events of spirochete acquisition occurred before eukaryotic ancestors became fully aerobic (via symbiotic acquisition of the respiring bacteria that evolved into mitochondria). The two lines of evidence that undulipodial-microtubule systems preceded mitochondria are (a) the distribution argument and (b) the polyphyly argument.

III. THE DISTRIBUTION ARGUMENT

When all members of a taxon above the level of genes show a hereditary organelle or symbiont, that organelle or symbiont must have been acquired prior to or during the evolution of that taxon (6, 20). Examples of this include plastids of all red algae, phycobionts of all lichens and mitochondria of all leguminous plants. On the other hand, when hereditary organelles or symbionts are not universally distributed in a higher taxon (above genus), they must have been acquired after the evolution of the taxon itself (6). For this reason red algae parasitic on other red algae (12), hypersymbionts such as actinobacteria of lichens (41) and *Rhizobium* of nitrogen-fixing root nodules of leguminous plants are all believed to have evolved after, respectively, the rhodophytes, lichens and legumes themselves evolved.

All species in the following higher taxa of protists always lack mitochondria: Parabasalia, Retortamonads and

Table 2

Protist taxa in which members lack mitochondria and have microtubules of undulipodia in all stages of life cycle*.

I. Contain temporary mitotic microtubules

Phylum	Class	Genus
Zoomastigina	Amoebomastigota	Mastigina Mastigella
	Diplomonads	Diplomonas Giardia
	Retortamonads	Retortamonas
	Pyrsonymphida	Notila Saccinobaculus Pyrsonympha
	Parabasalia	Trichomonas Trichonympha

II. Permanently lack mitotic microtubules and have immotile undulipodia at some stages*

Zoomastigina	Karyoblastea	Pelomyxa

*For details see references listed in (40).

Diplomonads. These protists live in anoxic or reduced-oxygen conditions. They contain undulipodia at all stages in their life cycle (Table 2). Furthermore, other protist taxa have been described in which members with undulipodia have been detected but lack mitochondria at all stages in their development (Table 2). For example, non-motile standard [9(2)+2] undulipodia on the surfaces of the giant amoeba *Pelomyxa* have been reported [Griffin in (40)] yet these multinucleate amoebae always lack mitochondria. These amoebae contain at least three other distinct types of endosymbiotic bacteria including two kinds of methanogens (37), implying they evolved independently of other lineages of free-living amoebae. The existence of *Pelomyxa* in which undulipodia are present but all vestiges of microtubules used for the mitotic spindle are absent strongly argues that undulipodia preceded the evolution of mitosis in anoxic or low-oxygen environments. Finally, protists with mitotic spindle microtubules that lack both undulipodia and mitochondria have also been reported, e.g., microsporidians (Table 3). Since, where known, the protists listed in Table 2 use the motility of microtubules in the formation and elongation of the mitotic apparatus (in addition to their use in undulipodia), both the origin of undulipodia and mitotic motility probably preceded the symbiotic acquisition of mitochondria. The evolution of mastigote mitosis of the sort found in those groups, e.g., *Trichomonas* (Table 2) most likely occurred in environments containing reduced oxygen;

Table 3

Protist taxa in which members have mitotic spindle microtubules but lack microtubules of undulipodia and lack mitochondria at all stages of their life cycle*.

Protoctist Taxon	Examples of Genera
PHYLUM	GENUS
Rhizopoda	Entamoeba
Microspora	Metchnikovella Chytridiopsis Glugea Vavraia Unikaryon

*For details see references listed in (40).

these organisms are intolerant to atmospheric oxygen to which they probably never have been exposed.

Since all cells that have mitochondria also have microtubules (either microtubules of mitosis or those of undulipodia or both types), but many cells that have microtubules permanently lack mitochondria, microtubules preceded mitochondria in the development of eukaryotes. The secondary genetic loss of mitochondria might be expected to have occurred sporadically in a given species reoccupying anaerobic niches. Although this possibility cannot be

rigorously excluded, the absence of secondary mitochondrial loss in animals, plants and fungi occupying low-oxygen habitats renders secondary loss unlikely.

IV. THE POLYPHYLY ARGUMENT

Spirochetes (bacteria with periplasmic flagella) as well as other flagellated bacteria enter motility symbioses. The polyphyly of motility symbioses is obvious from the comparison of the details of a small sample of protist and bacterial associates [(13) and Table 4 lower portion]. Attachments of bacteria to mastigotes is common, especially in anaerobic habitats (Figs. 1, 2 and Table 4, upper portion). In hindguts of a single subterranean or drywood termite more than one kind of bacterial-host attachment can be observed (Figs. 2, 3; for electron microscopic preparation techniques and further examples see (25). From the ease with which extant spirochetes enter both necrotrophic and biotrophic associations (which may or may not become motility symbioses) we infer that various types of spirochete/host associations have been prevalent in low-oxygen environments. Such associations, so conspicuous in ultrastructural observations of live material, probably were already established prior to the appearance of eukaryotes. The earliest acritarchs, microfossils interpreted to be the first protoctists, are present in the Proterozoic Eon beginning about 1200 million years ago (38). Thus extant spirochete associations may be viewed as preadaptations for the origin and evolution of undulipodia. The abundance,

Table 4

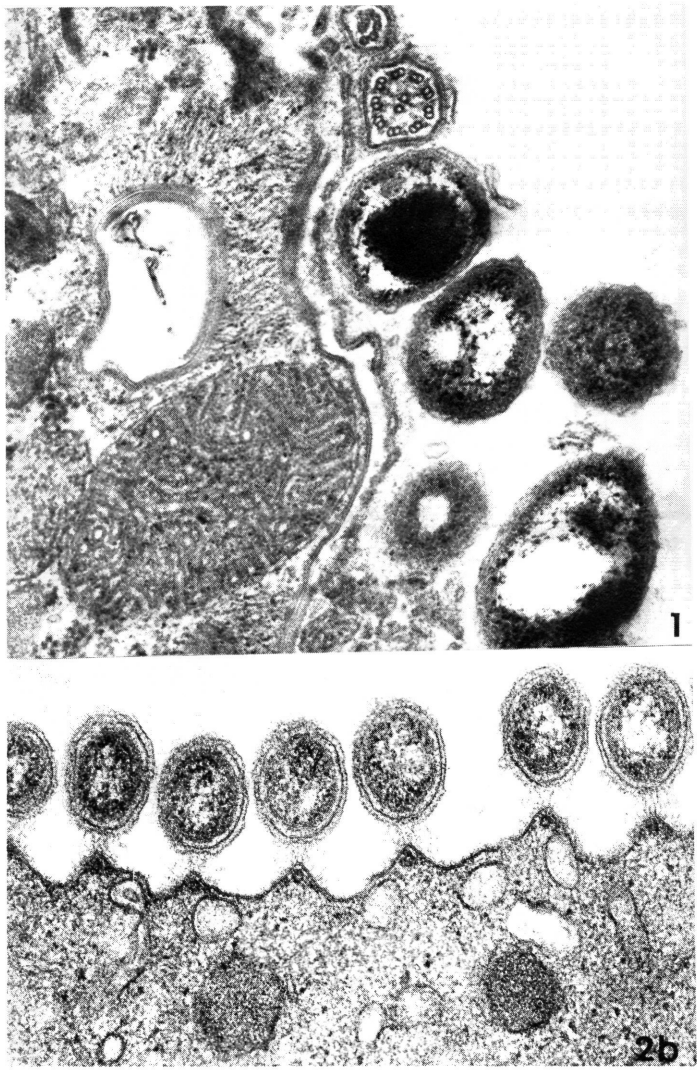
Polyphyly of symbioses: protist and bacterial associates. Selected examples

Protist phylum	bacterial type	environment	reference
CILIOPHORA (*Kentrophorus*, *Coleps*)	spirochetes, rods	marine sands	Raikov[1]
		fresh water sulfurous lake	J. Mir, J. Gazol and I. Esteve (unpublished, Fig. 1 this paper)
ZOOMASTIGINA			
pyrsonymphids	spirochetes	termites, wood-eating cockroaches	Buhse and Smith[1]
devescovinids	gram-negative peritrichs	termite hindgut: *Cryptotermes cavifrons*	Tamm[1]
Staurojoenina assimilis	unidentified cocci	termite hindgut	David Chase (unpublished, Fig. 2 this paper)

[1] See reference (21) for discussion and complete references. See (13) for *Mixotricha* spirochete-protist motility symbiosis and (18) for phototrophic-motile bacterial consortia. Classification scheme of reference (40) is used in tables 1-4.

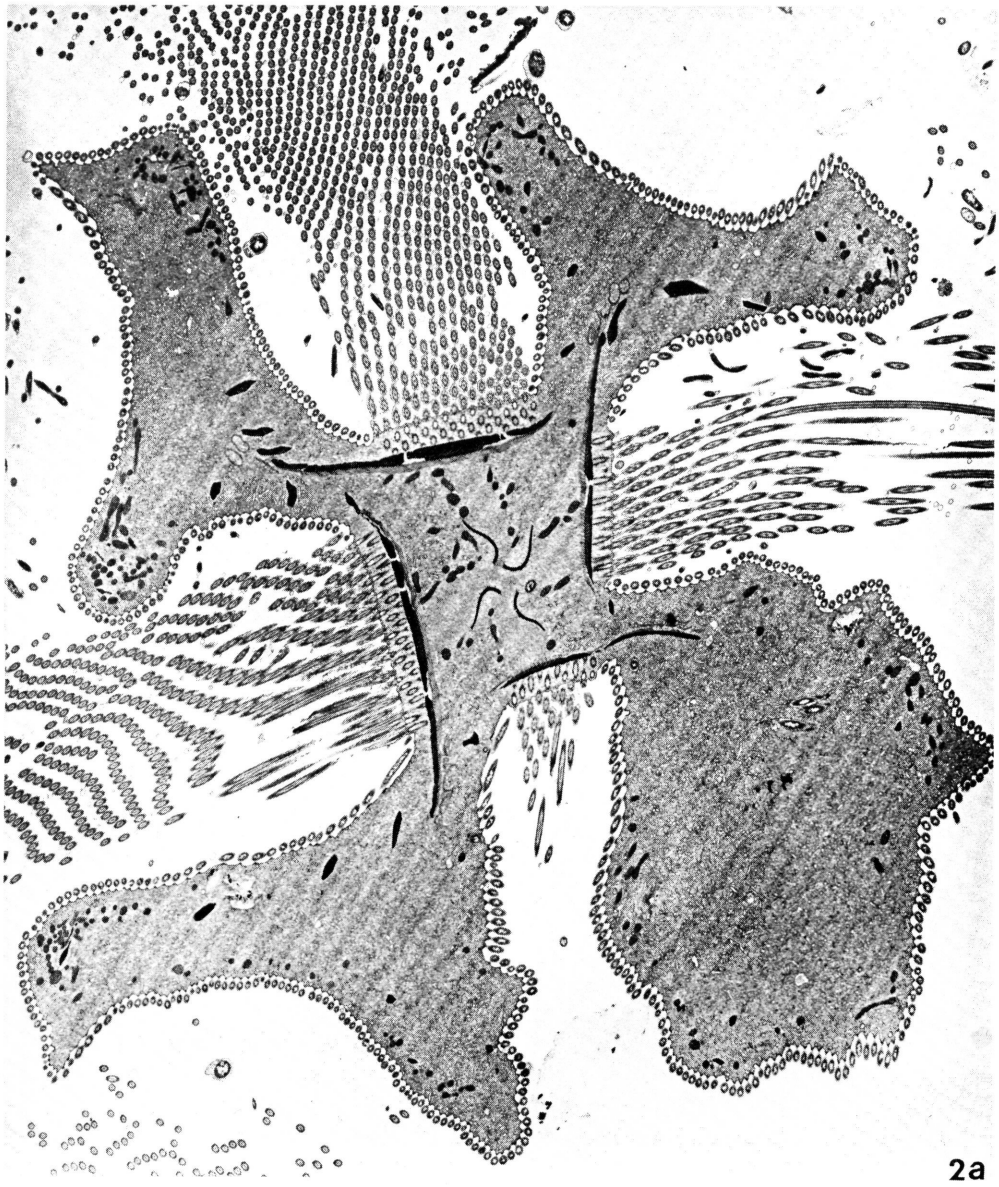
Figure 1. Three bacteria attached to amorphous material on the surface of the ciliate *Coleps*, Lake Ciso, northeast Spain. Note transverse section of ciliary axoneme and ciliary tip at left, courtesy of J. Mir, J. Gazol and I. Esteve (x60,000)

Figure 2. Bacterial attachments to protists in hindgut communities of the drywood termite *Incisitermes minor*.



2 a. *Staurojoenina assimilis* (x6000) (see page 146)
Four sets of undulipodia (u) emerge from the rostral area of this protist; the rest of the surface is covered with a regular investment of coccoids attached by fuzz. Note the raised portion of the host protist bears a single microtubule at the point of the attached bacteria visible in b.

2 b. Bacteria lining the surface of *Staurojoenina assimilis* (x80,000), enlargement of 2a.

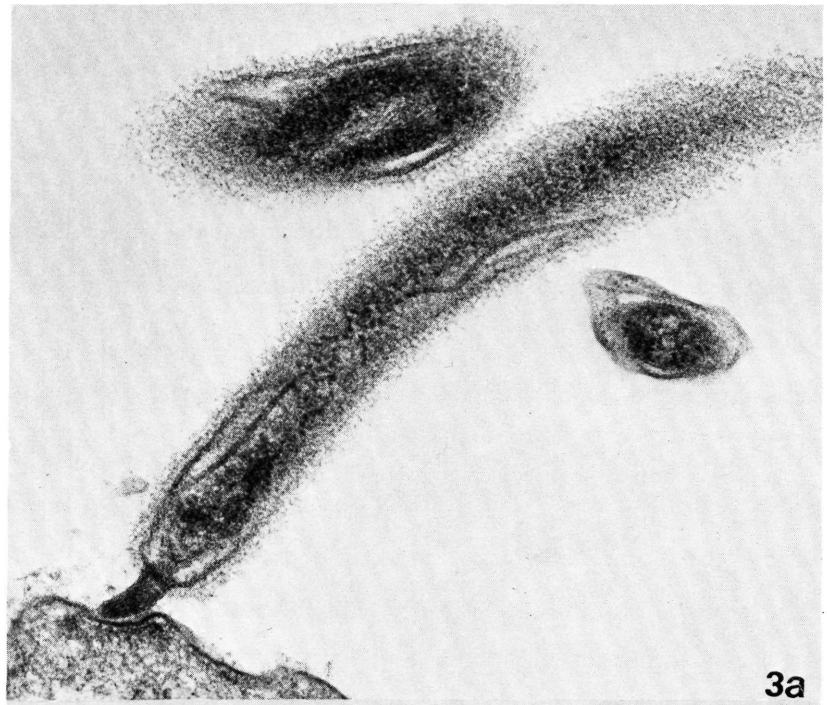


2a

diversity and ubiquity of polyphyletic bacterial-protist attachments alone suggests polyphyly of spirochetes that became undulipodia. Furthermore, the polyphyly of microtubule systems has been argued by analogy: nearly all biologists agree that plastids, mitochondria and many other microbial symbionts that became organelles were acquired polyphyletically (6, 20).

Mitochondria are probably of polyphyletic origin among eubacteria (10, 34, 39). Direct comparison of various mitochondrial 5S and 16S RNA sequences as well as the morphology of the mitochondrial cristae, e.g., tubular, vesicular or flattened (10, 34), have aided in the resolution of this issue.

The multiple origin of mitochondrial and undulipodiated ancestors is made plausible by analogy with xenosomes in general. Polyphyly of xenosomes, intracellular particles of symbiotic origin (7), has been revealed by direct study of "Greek letter" particles, bacteria symbiotic in the cytoplasm of *Paramecium*. Some of these have been assigned to the genus *Caedibacter* and others to *Cytophaga* (18). Polyphyly of plastids of algae has long been accepted. Polyphyletic origin of the phycobionts of lichens is obvious, especially when the phycobionts are both of cyanobacterial and chlorophytic origin. By analogy with the general prevalence of polyphyly of microbial symbionts, xenosomes and hereditary organelles and the obvious polyphyly of extant spirochete attachment associations, the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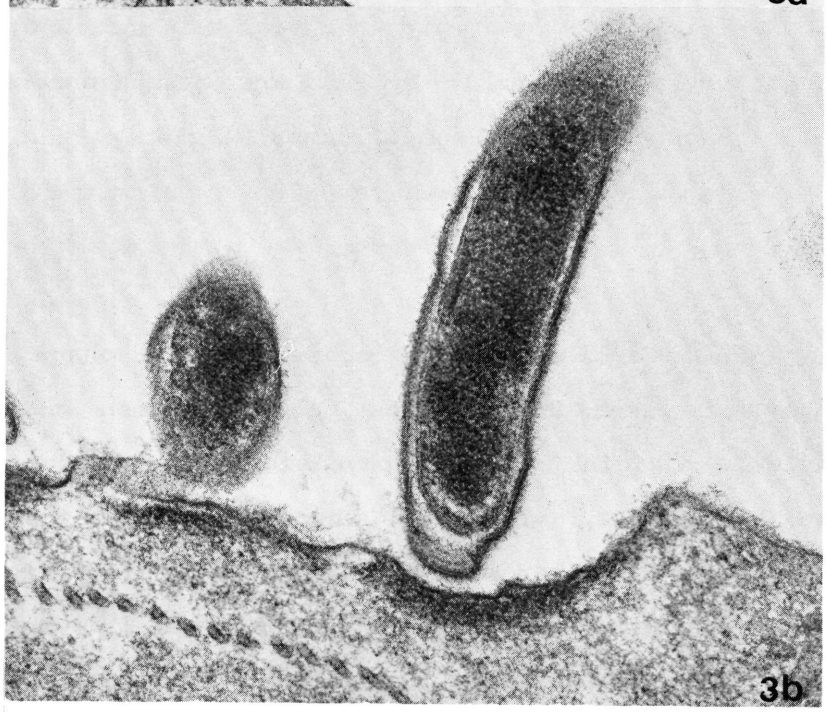
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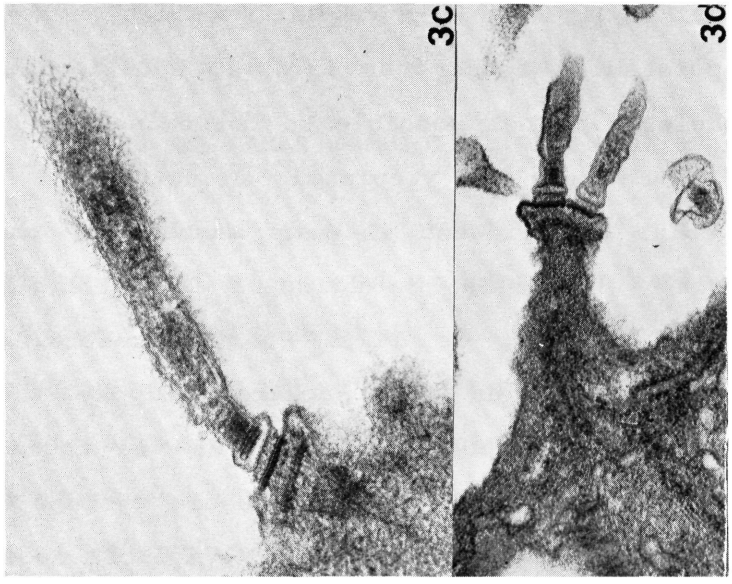

Figure 3. Internal modifications of bacteria for attachment to unidentified zoomastiginid protists from termites.

a. Curved densities. The attached bacteria, probably a spirochete, shows polarity: one terminus, curved and dense, is connected at a depressed portion of the protist host. *Incisitermes minor* hindgut. (x75,000)

b. Blunt rounded end. The attached bacterium, a spirochete judging from the conspicuous periplasm, is attached by fine fibrils to a dense portion of the protist host. *I. minor* hindgut. (x75,000)

c. and d. Pyrsonymphid-spirochete attachment structure showing "root-fiber"-type modification of the host, dense fibrils attaching the spirochete. Up to 3 spirochetes in proximity at same attachment site may be seen (two shown in 3d). Note deep penetration of "root" fibrils into protist host (left)(x75,000). Thickening of protist membranes at point of attachment especially prominent (center) (x75,000). Note attachment site continuous where two spirochetes appose protist membrane (right)(x50,000). All insect hosts are *Reticulitermes hesperus*.

general polyphyly of spirochete-host motility symbioses can be extrapolated. Whether the [9(2)+2] undulipodium itself is polyphyletic is not possible to determine at this time. However, since tubular cytoplasmic structures or tubulin-like proteins are present in many members of the genera *Spirochaeta*, *Borrelia*, *Pillotina*, *Hollandina* and *Clevelandina* (5), it is likely that ancestral tubulin-microtubules themselves were acquired polyphyletically as single or at most doublet microtubules via these various symbionts. Furthermore, the microtubule systems of early eukaryotes were probably acquired by symbiotic associations via different original spirochete-host partnerships. The different spirochete parts were deployed in different ways inside various types of hosts that formed motility symbioses; this seems consistent with the evidence of distribution of the complex mitotic and other microtubule apparatus of protists. The analogy with plastid origin is clear. Even though oxygenic bacterial photosynthesis may have ultimate common ancestry, the acquisition of plastids clearly represents separate evolutionary events (i.e., the polyphyletic acquisition of plastids). Let us note, for example, that comparisons of organelles with their free-living counterparts established the polyphyly of plastids, e.g., chloroplasts with *Prochloron*, and rhodoplasts with cyanobacteria such as *Synechococcus*. Independent acquisition of closely-related symbionts by closely-related

hosts is a well-known phenomenon that may apply to microtubule systems as well.

V. IMPLICATIONS OF EARLY SYMBIOTIC ORIGIN OF UNDULIPODIA FOR PHYLOGENY OF PROTOCTISTS

If certain amoebae, mitotic mastigotes, amoebomastigotes and other protists listed in Tables 2 and 3 evolved prior to mitochondrial acquisition, and since mitochondria were acquired polyphyletically during and after the origin of mitosis, no necessary correlation of protoctist type and mitochondrial type is required for the construction of protoctist phylogenetic trees. Furthermore, the great groups of organisms lacking undulipodia at all stages of their development (e.g., rhodophytes, fungi, conjugating green algae) as well as some minor protist groups (rhizopod amoebae, cellular slime molds, paramyxeans, myxozoans) probably acquired symbiotic spirochetes that evolved into mitotic motility systems but not into undulipodia (35). The innards of the former spirochetes on this view were deployed in the evolution of internal motility systems, including mitotic ones by an evolutionary process involving loss of spirochetal integrity. These permanently amastigote protoctists derived microtubules and eventually mitotic systems from adhering spirochetes prior to and during the evolution of other lineages of undulipodiated protoctists. This view, the convergent evolution of many mitotic and meiotic systems, reinforces the concept of polyphyly (23).

Proof of the symbiotic origin of microtubule systems from spirochetes requires demonstration of homology between the proteins of the microtubule systems of eukaryotes and the morphogenetic motility proteins of spirochetes. Certain spirochetes and specific eukaryotes might display more homology than do certain other spirochetes and eukaryotes (for example, members of genus *Spirochaeta* could have given rise to the microtubules of fungal spindles whereas members of the genus *Borrelia* may have been ancestral to the microtubules of Zoomastigina). Such demonstrations require more detailed biochemistry, molecular biology and cytochemistry of both spirochete and protist motility systems than is currently available.

VI. IMPLICATIONS OF EARLY SYMBIOTIC ORIGINS OF UNDULIPODIA FOR THE INTERPRETATION OF SOME GENETIC DATA

If undulipodia were acquired symbiotically in a single step early in the history of protoctist cells, all genes determining its proteins were once contained within its spirochete nucleoid. Furthermore, morphogenetic assembly processes assuring the long motile cylindrical form of what became the axoneme were initially determined by genes inside the nucleoid and protoplasmic cylinder of the spirochetes that became organelles.

An unusual linkage group in *Chlamydomonas reinhardtii*, containing at least 19 markers affecting kinetosome assembly, has been identified (9, 27, 28). The transition zone between the kinetosome and axoneme is attenuated or

absent in many of these mutants. Furthermore, many are temperature-sensitive, unable to assemble kinetosomes at 32°C.

This "UNI" linkage group is unique. All of the mutants show phenotypes affecting kinetosome structure or function and when mapped these markers are linked to form a circle. The clustering of markers affecting the same organelle and circular linkage groups are properties heretofore reported only for plastids and mitochondria, the other inherited eukaryotic cell organelles. The marker order shows peculiar sensitivity to elevated temperatures: the linkage relationships change but only if the cells are exposed during a one-to-two-hour period five days before meiosis (28). No standard Mendelian nuclear markers (and over 200 are known) have been mapped to this UNI linkage group.

We interpret these findings as follows: 1) UNI, circular because of its bacterial ancestry, is the legacy of the symbiotic spirochete genome. The nucleic acid, which is the structural basis of UNI, will show more direct homology to the DNA of those free-living spirochetes containing tubulin-like proteins than it will to any other DNA from prokaryotes. 2) The peculiar temperature sensitivity of the relations between the markers of the UNI linkage group could be explained by the unique timing (five days before meiosis) of its nucleic acid synthesis/recombination. By hypothesis a kinetosomal nucleic acid exists that recombines from both parental sources during a one-to-two-hour period five days

before the onset of meiosis and zygote formation. Apparently the UNI genetic entity is synthesized asynchronously relative to the rest of the cellular DNA. Only UNI linkage relationships suffer from precisely timed heat sensitivity: standard nuclear, plastid and mitochondrial genes are unaffected. These observations argue for an exogenous--or at least different--origin of the UNI genetic determinant relative to the genetic material of the rest of the cell. Asynchrony of cell division by fission (or of organellar DNA and RNA replication) is well known in organelles and endosymbionts. Xenosomes generally reproduce out-of-phase relative to their hosts.

The phenotype of many of the markers of the UNI linkage group is temperature-sensitive undulipodial assembly. Mutant cells fail to construct competent undulipodia at 32°C, a lower-than-lethal temperature for *Chlamydomonas*. If undulipodia originate from spirochetes the temperature limitations may reflect ancestry: i.e., the immobility and inability of some members of the genus *Spirochaeta* (e.g., *S. aurantia* var. *stricta)* to grow above 30°C (18). In some free-living spirochetes, some of which may be co-descendant with undulipodia, morphogenetic processes are definitively inhibited above 30°C.

We know that in the close relatives of *Chlamydomonas* (i.e., *Chlorogonium*) kinetosomes disassociate from their axonemes during mitosis and may become centrioles for the mitotic division (17). By hypothesis, the UNI linkage group

should be physically associated with the migration of the centriole-kinetosome system (and the chromosomal centromeres or both). [Reasons for hypothesizing the spirochete ancestry of centriole-kinetosomes and centromeres are presented in (24).] The observations of the *Chlamydomonas* geneticists require that allelic markers of UNI segregate with the centriole-kinetosomes (centromeres or both) primarily during first division of meiosis whereas offspring copies of these organelles segregate in the second meiotic division. That UNI is the manifestation of centriole-kinetosomes, organelles interpreted to be spirochete remnants, has not been predicted by any other theory. Indeed, the existence of UNI is totally unexpected if kinetosomes and their undulipodia originated by direct differentiation from nuclear genes and their protein products.

VII. IMPLICATIONS FOR FURTHER RESEARCH

The hypothesis predicts that the physical basis for UNI, whether it is RNA, DNA, centriolar-kinetosomal and/or centromeric, will show direct nucleotide sequence homology with DNA from selected free-living spirochetes. Complementary (cDNA) made to the genes determining more than 200 axonemal and kinetosomal proteins must hybridize more efficiently with DNA from spirochetes, i.e., those containing microtubule-like protein, than it does with DNA from *Thermoplasma* or any other arbitrarily chosen bacterial DNA.

Antibodies made against centriolar-kinetosomal or axonemal proteins (e.g., antitubulins, antinexins, anti-axonemal calmodulin and anti-radial spoke proteins, etc.) should show cross-reactivity to certain spirochete proteins comparable to the cross-reaction of antitubulin antibodies made to brain tubulin with the 65 kilodalton protein (S1) from spirochetes (3).

A spirochete protein with amino acid residue sequence homologous to that of the calmodulin of axonemes should be found as well.

Characterization and identification of the physical basis of the unique UNI linkage group will provide the impetus to understanding the genetic basis of meiotic and undulipodial microtubular systems. The significance of documenting the nucleic acid of the UNI linkage group can be compared to the status of the chromosomal theory of heredity following the discovery of DNA of chromatin or the resolution of the controversies concerning the origins of plastids and mitochondria from bacteria following the detection of DNA and protein synthetic systems in these organelles. The discovery of a nucleic acid basis for centriole-kinetosomes will have profound implications for the biology of all eukaryotes.

The report of the unique UNI linkage group by the *Chlamydomonas* investigators (David Luck and Susan Dutcher) has provided the means to a definitive, conclusive and unambiguous proof of the serial endosymbiotic theory of the

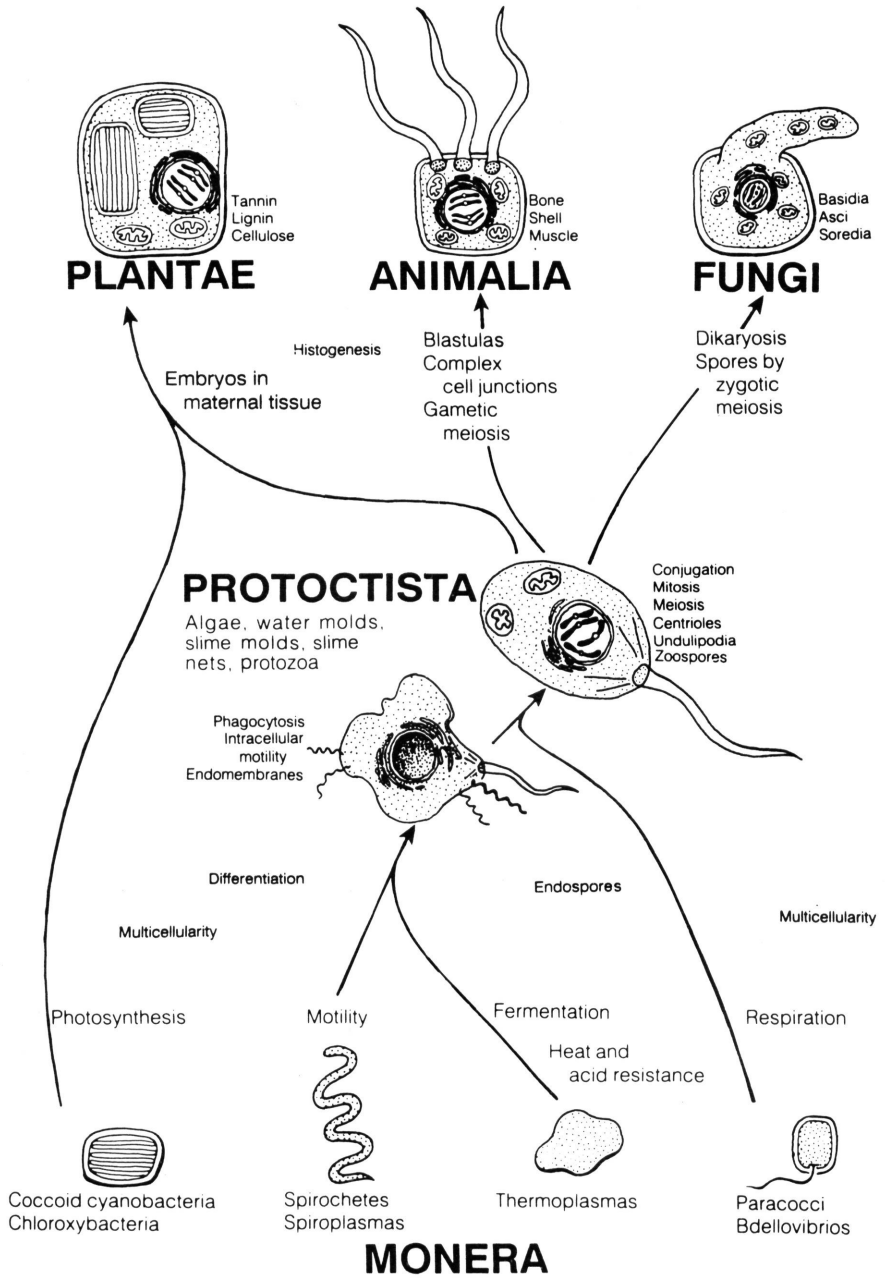

Figure 4. Serial endosymbiotic theory of the origin of eukaryotic cells. [Drawing by Rae Wallhausser modified from reference (21) p. 4].

origin of eukaryotic cells. The revised version of the theory based on the arguments presented here is diagrammed in Fig. 4.

We have argued that proliferation of membrane accompanies the development of intracellular associations (21, 24). The evolution of the membrane-bounded nucleus then becomes a specific example of the sort of proliferation of membrane that led to the origin of xenosome and organellar outer membranes, endoplasmic reticulum and Golgi apparatus. This argument is still valid, but, if Fig. 4 is an appropriate reconstruction of eukaryosis, then the origin of nuclear membranes occurred as part of the spirochetal-undulipodial transition and thus preceded the symbiotic acquisition of mitochondria. The study of the hypertrophy of lipoprotein membrane as a function of intracellular symbiosis acquisition is amenable to experimental investigation.

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