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REVIEW

Perspectives on the origin of microfilaments, microtubules, the relevant chaperonin system and cytoskeletal motors— a commentary on the spirochaete origin of flagella

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ABSTRACT

The origin of cytoskeleton and the origin of relevant intracellular transportation system are big problems for understanding the emergence of eukaryotic cells. The present article summarized relevant information of evidences and molecular traces on the origin of actin, tubulin, the chaperonin system for folding them, myosins, kinesins, axonemal dyneins and cytoplasmic dyneins. On this basis the authors proposed a series of works, which should be done in the future, and indicated the ways for reaching the targets. These targets are mainly: 1) the reconstruction of evolutionary path from MreB protein of archaeal ancestor of eukaryotic cells to typical actin; 2) the finding of the MreB or MreB-related proteins in crenarchaea and using them to examine J. A. Lake's hypothesis on the origin of eukaryote from "eocytes" (crenarchaea); 3) the examinations of the existence and distribution of cytoskeleton made of MreB-related protein within coccoid archaea, especially in amoeboid archaeon *Thermoplasm acidophilum*; 4) using *Thermoplasma* as a model of archaeal ancestor of eukaryotic cells; 5) the searching for the homolog of ancestral dynein in present-day living archaea. During the writing of this article, Margulis' famous spirochaete hypothesis on the origin of flagella and cilia was unexpectedly involved and analyzed from aspects of tubulins, dyneins and spirochaetes. Actually, spirochaete cannot be reasonably assumed as the ectosymbiotic ancestor of eukaryotic flagella and cilia, since their swing depends upon large amount of bacterial flagella beneath the flexible outer wall, but not depends upon their intracellular tubules and the assumed dyneins. In this case, if they had "evolved" into cilia and lost their bacterial flagella, they would immediately become immobile! In fact, tubulin and dynein-like proteins have not been found in any spirochaete.

Key words: *origin, actin, tubulin, motor proteins, flagella and cilia, spirochaete hypothesis.*

INTRODUCTION

The original and fundamental causative factor that eventually led to the emergence of eukaryotic cells was the continuous increase of the cell-size of archaeal ancestor of eukaryotic cells[1].

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#According to the data in GenBank, there is only one short hypothetical protein composed of 117 amino acids (GI: 21959823) from the genome of bacterium *Yersinia pestis* (not spirocheote!) containing a 57 amino acid sequence that has 34.84% identical to a 66 amino acid sequence in human axonemal dynein type II (4523 amino acids long, GI: 15395290). Although it is interesting, the corresponding sequences are too short at all.

##There are two copies of a gene "dhc" in the genome of archaeon *Methanosarsina acetivorans*. The gene encodes a "dynein heavy chain" composed of 159 amino acids (GI: 19913760 and 20088916). It is too short to be a homolog of dynein heavy chain in archea. Where did this gene come from? It might be a fragment of the real dhc gene transferred from an eukaryote to the archaeaon. But there are two copies of this gene, perhaps they have a certain cell physiological significance for this archaeon.

The enlargement of body size is one of the possible evolutionary directions for any species. The enlargement of the size would give the organism many benefits in natural selection, but at the same time would also bring on various new challenges. If these challenges could be met, then the species could evolve further on the new basis. In the earliest archaeal ancestor of eukaryotic cells the enlargement of the size decreased the ratio of plasma membrane area per unit of protoplasma. This challenge was met by the invagination of plasma membrane and the formation of endomembranous system. This let them evolve further along this direction until they hit a new severe challenge: the transportation of macromolecules and their complexes by simple diffusion became increasingly difficult within the protoplasma, which had already become enriched in membranous components. This severe challenge was met by the emergence of intracellular transportation system which probably appeared in the middle or late ancestors of eukaryotic cells. All eukaryotic cellular motions, such as amoeboid movement, cell crawling movement, even ciliary movement, are based upon this system or developed on the basis of this system. Without the formation of this system the eventual emergence of the real eukaryotic cell would be essentially impossible.

However, the exploration of the evolutionary origin of intracellular transportation system has been being very difficult. In the monograph "The Primitive Nucleus Model and the Origin of the Cell Nucleus" (Li, 1999)[1], the author investigated various aspects of the evolutionary origin of the cell nucleus and cytoplasmic structures and systems to the furthest possible extent (such as the eukaryotic protein synthesis machinery, the eukaryotic proteasome, etc.) based on the perspectives of the latest discoveries and information from the achievements of our laboratory in Kunming Institute of Zoology, Chinese Academy of Sciences. However, the origin of the intracellular transportation system was still not sufficiently explored. The main reason was that when the monograph was written, the trace on the origin of microfilaments and the prokaryotic homolog of actin had still been unknown, despite the fact that the relationship between prokaryotic FtsZ protein and tubulin had already been discovered. In that monograph the author could only introduce the initial and important efforts of Prof. D. G. Searcy on the origin of microfilaments[2, 3]. At present, although the situation has already progressed, there are still many problems waiting to be investigated. In this article we want to summarize the information on the origin of intracellular transportation system and propose several perspectives. The famous spirochaete hypothesis (Margulis, 1970) on the origin of eukaryotic flagella and cilia is unexpectedly involved and doubted.

The evolutionary origin of microfilaments

One of the main structural components of eukaryotic cytoskeleton is microfilaments composed of actin. Actin is a most abundant protein in many eukaryotic cells. Monomeric actin molecules can polymerize into polymeric protofilaments, and two protofilaments twist around each other forming an actin filament, and bundles of actin filaments form microfilaments in cytoskeleton.

Actin is highly conserved, for example, human and chicken actins are entirely identical in sequence. The three-dimensional structural resemblance among ATPase domains of actins, heat shock protein HSP70s, hexosugar kinases and bacterial cell division protein FtsAs showed that all these proteins are distantly, but significantly related to each other[2] (for review see[1]). All these functionally very diverse proteins belong to the same actin superfamily of proteins.

The most important advance in understanding the origin of actin was the discovery of its homologs, MreB proteins, in various prokaryotes. MreB protein and its gene were first discovered in *Escherichia coli*, and the protein was shown to play a role in the determination of cell shape[3]. Later, MreB proteins were found in various species of bacteria and several euryarchaea with rod, curve or spiral shape, but they have not been found in coccoid species (for review see[4]). For many years the relationship between MreB protein and actin was unknown since the sequence similarity between them is not significant. In the first year of this century two important papers were published. Ent et al. (2001)[5] indicated that MreB molecules could assemble into filaments with a subunit repeat arrangement similar to that of actin subunits in actin protofilaments, and found that the three-dimensional structure of MreB subunits in fil-

aments is remarkably similar to that of actin subunits in protofilament[5]. These facts strongly suggested the homology between these two proteins. At the same time the immuno-fluorescent microscopical investigations on *Bacillus subtilis* with antibodies against MreB protein and antibodies against the MreB-related protein Mbl demonstrated that these two species of proteins form two independent long, spiral and thick bundles of filaments of bacterial cytoskeleton beneath the plasma membrane[4]. These two excellent works provided compelling evidences for the existence of the MreB cytoskeleton in prokaryotes. These works also mainly agreed with the previous immuno-electron microscopical studies on various bacteria and archaea using antibodies against actin[6]. Because a series of reviews have already been published[7-10], here we will only discuss some special points that are very interesting in the evolution, and propose several studies, which should be done in the future.

1) Actins are highly conserved and bacterial MreB proteins are also fairly conservative. The MreB proteins of euryarchaeon *Methanothermobacter thermoautotrophicus* (GI: 15679042) and the most primitive living euryarchaeon *Methanopyrus kandleri*[11, 12] (GI: 20093608) have very similar sequence to those of bacteria. It suggests that the sequence of MreB protein of the earliest archaeal ancestor of eukaryotic cells must be quite similar to that of the MreB of *Methanopyrus*. So, we can imagine that the sequence of ancestral actin in the middle or late ancestor of eukaryotic cells should be intermediate between those of the actin of the present-day lowest protozoa (free-living diplomonads) and the MreB protein of *Methanopyrus*. During evolution the sequence of MreB protein of the earliest ancestor of eukaryotic cells must have changed to modify the three-dimensional structure to make two polymeric filaments able to twist around each other forming a two-stranded filament just as an actin filament composed of two protofilaments. This change might be important for the filament to carry out an additional new function in the transportation within cell. The whole process might be simulated using computer in the near future.

According to a similarity tree (provided by COGs, www.ncbi.nlm.nih.gov?/COG/palox?COG1077) including twenty five bacterial MreB proteins, four MreB and MreB-related proteins of archaea, yeast actin and seven yeast actin-related proteins, yeast actin and actin-related proteins construct a separated big branch. In this branch five actin-related proteins branch earlier than actin, seemingly representing the last three steps during the origin of yeast actin. If the actins and actin-related proteins of protozoa, especially those of the present-day living lowest protozoa (free-living diplomonads) are used to establish the phylogenetic tree, we would be able to obtain more information about the eventual steps in the origin of actin. Thus, we would get a whole hypothetical evolutionary process from MreB protein of the archaeal ancestor of eukaryotic cells (represented by the MreB protein of *Methanopyrus*) through the assumed intermediate stage and the last steps, eventually arrive in the typical actin. Along this hypothetical process we can do effort to observe the continuous changes in the three-dimensional structure of the protein and modify the hypothetical process to make it more reliable.

2) Up to now MreB protein or MreB-related protein has not been found in any crenarchaeon. Lake and his colleagues assumed that eukaryotic cells originated from one of crenarchaea (termed as "eocytes" by him)[13]. If MreB or MtrB-related proteins of crenarchaea are found in the future, we can use them to examine Lake's hypothesis to see if they are surely more similar to actin than the MreB protein of *Methanopyrus*.

3) Although it has been concluded that MreB proteins are only existing in non-coccoid bacteria and archaea and not in coccoid species[4], genes encoding MreB-related proteins have already been found in genomes of three coccoid archaea: *Archaeoglobus fulgidus* (gene AF2021, GI: 2648511), *Thermoplasma acidophilum* (gene Ta0583, GI: 16081686), *Thermoplasma volcanium* (gene TVN0641, GI: 13541472). On the other hand, the immuno- electron microscopical studies with antibodies against actin[6] showed the label not only distributed at the periphery of rod-shaped bacteria indicating the cytoskeleton beneath the plasma membrane, but also distributed at the periphery of the coccoid archaeon *Methanococcus jannaschii*. This fact perhaps means an unknown MreB-related protein distributed there. Interestingly, the label is also distributed over the entire cytoplasm of rod-

shaped archaeon *Methanobacterium theroautotroplicun* and the coccoid *Methanococcus voltae*. This might mean the existence of a certain structural component made of MreB or MreB-related protein distributed within the cytoplasm of these archaea. This possibility is quite worthy to examine.

4) The facts discussed in 3) strongly remind us the previous discoveries of D.G. Searcy in the wall-less euryarchaeon *Thermoplasma acidophilum*[14,15]. This mini organism usually crawls on the surface of sulphur grains as an amoeba. In liquid it shows an irregular form (Fig 1) under suitable temperature (50-60 ^{o}C), but at room temperature becomes a sphere. These facts strongly suggest that under suitable environment they produce a certain structural component within the cells, which allow cell to produce an irregular shape. The later studies of Searcy and his colleague on cell physiology conformed this explanation[15]. Unfortunately, he had not enough funds to deepen his very interesting and important discovery. Now, MreB-related protein of this organism has already been found. So, the antibody against it would be made and used as a probe to do *in situ* detection and other examinations. Although this MreB-related protein is not closer to actin than common MreB proteins suggesting that *Thermoplasma* is not the direct ancestor of eukaryotic cells. The ability of *Thermoplasma* to produce flexible amoeba-like irregular shapes allow it to be used as a good model system for studying the ancestor of eukaryotic cell, since the ability to produce amoeba-like movement is essential for the archaeal ancestor of eukaryotic cells. By the way, a gene encoding myosin heavy chain-related protein has already been found in its genome (see section V (A) of this article). It is possible that the myosin-related protein mediates the amoeba-like movement.

The evolutionary origin of microtubules

Another cytoskeletal component, microtubule is also important for the intracellular transport. Microtubules are dynamic structures made of 12-15 protofilaments; each protofilament is a dynamics string of heterodimers of alpha- and beta-tubulin subunit. The two ends of a microtubule polymerize at different rates. The fast-growing end is called the plus end and the slow-growing end is called the minus end. The end of microtubule directed toward MTOC is always the minus end.

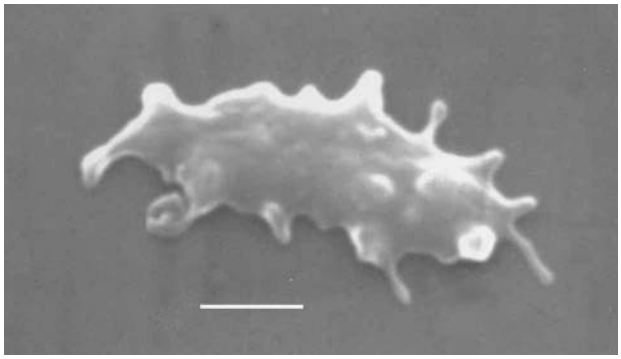

Fig 1. The scanning-electron microscopical photograph made by Dr. William Hixon, showing the irregular shape of the archaeon *Thermoplasma acidophilum* in liquid environment at suitable temperature. The bar is one micrometer. A gift from Dr. Dennis G. Searcy.

Alpha- and beta-tubulins are closely related and obviously derived from a common ancestral tubulin. Each tubulin has a specific binding site for GTP. Both tubulins bind GTP, but only the GTP bound by beta-tubulin in the heterodimer can be hydrolyzed or exchanged.

It is known that FtsZ proteins in prokaryotes are the homologs of tubulin. FtsZ proteins are present in all bacteria and archaea, and play a very important role in the prokaryotic cell division by assembling a ring with the leading edge of the invaginating plasma membrane until the separation is completed[16]. Like tubulin, FtsZ protein is also GTP-binding protein. The sequence motif essential for GTP-binding found in tubulin is highly similar to the motif for GTP-binding in FtsZ protein[17]. In addition, several short sequences outside the GTP-binding motif have been found similar between tubulin and FtsZ proteins[17]. The most exciting data indicating the similarity between FtsZ protein and tubulin are the findings that FtsZ protein can assemble into protofilaments and even into tubule structure. For detail information, see the monograph[1]. The similarity between the three-dimensional structure of tubulin dimer[18] and that of archaeal FtsZ protein[19] strongly suggests that tubulin and FtsZ proteins are homologs. Phylogenetic analysis showed that prokaryotic FtsZ proteins are distantly related to eukaryotic tubulins and archaeal FtsZ proteins are slightly closer to tubulin than bacterial FtsZ pro-

teins[20].

Margulis (1970) proposed her famous hypothesis that eukaryotic flagella and cilia originated from the ectosymbiotic spirochaete attached to the surface of ancestral eukaryotic cells. In the second edition of her book "Symbiosis in Cell Evolution" (1993) Margulis still hypothesized that all microtubules of eukaryotic cell originated from the tubules within the spirochaete as the ancestor of eukaryotic flagella[21]. The laboratory of Margulis had put great efforts for many years to search for tubulin in spirochaetes, but so far they have not succeeded. In 1987 they thought that they found a tubulin-like protein in *Spirochaeta bajacalforniensis*[22]. In 1991 they found a tektin-like protein in *Spirochaeta halophila*[23]. Although Margulis believed that all microtubules originated from the tubules of the spirochaete and this meant that all eukaryotic tubulin derived from the "spirochaete tubulin"[21], her laboratory found that the "tubulin-like protein" in *S. bajacalifoniensis* was actually just a heat-shock HSP65 protein[24]. In a review published in 1994 Margulis eventually admitted that there was no tubulin in spirochaetes and that FtsZ protein was a possible ancestor of tubulin[25]. In fact, up to now, we cannot find any tubulin or tubulin-closely-related protein in any spirochaete, neither in the complete genome of the spirochaete *Borrelia burgdorferi* (GI: 15594346).

Margulis has not abandoned her spirochaete hypothesis on the origin of flagella and cilia, although the fact that there is no tubulin in spirochaetes makes her hypothesis unrealistic. In this situation there is no reason to assume that all eukaryotic microtubules originated from spirochaete. She cannot use spirochaete FtsZ protein to replace the hypothesized "spirochaete tubulin", because FtsZ proteins have been found not only in spirochaetes, but also in all bacteria and archaea tested. According to the theory that eukaryotic cells originated from a very ancient archaeon (for review see [1]), tubulin and microtubules in eukaryotic cell proper should evolved from the FtsZ protein of the archaeal ancestor of eukaryotic cells. So, if Margulis still believes in the spirochaete hypothesis on the origin of flagella and cilia, she can only postulate that only the microtubules and their tubulin in flagella and cilia derived from spirochaete FtsZ protein. Then, there would be two distantly related groups of tubulins in the same cell. However this is not the case, she would have to throw away this hypothesis. Otherwise it has to be assumed that one group of tubulins has already been totally replaced by the other group during the evolution. We will discuss this problem further in section V (B) of this article. Recently, bacterial alpha- and beta-tubulins were reported found in a very less investigated new division of bacteria. We will discuss this report in section V (B) also along with the problem on the origin of flagella and cilia.

The evolutionary origin of the chaperonin system for folding actin and tubulin

The nascant polypeptide chains of the great majority of proteins are folded by type I chaperon (HSP70) in eukaryotic cytoplasm. However, the polypeptide chains of actin and tubulin are folded by chaperonin CCT (HSP 60). Chaperonin CCT is the main folding machinary in archaea. The chaperonin complex has been found in all archaeal species investigated so far, but has not been found in any eubacterium[26]. This fact strongly suggests that the eukaryotic chaperonin complex must have originated from the chaperonin complex of the archaeal ancestor of eukaryotic cells (for review, see[1]). Type I chaperon seems to be obtained by the ancestor of eukaryotic cells from bacteria at a much later time, probably from the endosymbiontic bacterium which was the ancestor of mitochondria. Later, it became the most important folding machinery in eukaryotic cells, only nascent actin, tubulin and a few other proteins are still folded by the ancient archeal chaperonin.

The discovery of prefoldin in eukayotes[27, 28] and archaea[29, 30] further strengthened the theory that the eukaryotic chaperonin system originated from the archaeal ancestor of eukaryotic cells. Archaeal prefoldin is a hexomeric complex, built from two related classes of subunits, two alpha-subunits and four beta-subunits. Six subunits construct a jellyfish-like structure consisting of a double barrel with six long tentacle-like coiled-coils[30]. Eukaryotic prefoldin is also such a complex composed of six subunits[31]. The distal regions of the tentacles capture nascent actin, tubulin, archaeal MreB protein or archaeal FtsZ protein, and then transfer the nascent chain into the cavity of chaperonin complex. Pre-

foldin has not been found in any bacterium. This indicates that eukaryotic prefoldin complex could only originate from the prefoldin complex of the archaeal ancestor of eukaryotic cells.

The evolutionary origin of cytoskeletal motor proteins and the problem of the origin of flagella and cilia

In all eukaryotic cells motor proteins bind to polarized cytoskeletal filaments and use the energy derived from repeated cycles of ATP hydrolysis to move along them. There are many species of motor proteins existing in the same eukaryotic cells. They differ from each other in the type of cytoskeletal filaments they bind to, the direction they move along the polarized filaments, and in the cargos they carry. Motor proteins are divided into three major groups: myosins, kinesins and dyneins. Myosins move along microfilaments, kinesins and dyneins move along microtubules. The members of kinesin group usually move toward the fast growing plus end of microtubule, while dynein moves toward the opposite end, the minus end of microtubule. Myosin superfamily contains at least 18 types of myosins, including the muscle myosin (type II). Kinesin superfamily includes kinesins and kinesin-related proteins, such as KIF1B, KIF2, KIF2C.

The knowledge on the evolutionary origin of eukaryotic motor proteins is very important for understanding how the internal motion system of eukaryotic cells originated and evolved during the emergence of eukaryotic cells. However, up to date we only found a few interesting traces from genomes of archaea and bacteria. A lot of work has to be done in search for prokaryotic homologues of eukaryotic motor proteins.

(A) Evolutionary evidence of the origin of myosin and kinesin

Although myosin and kinesin move along different cytoskeletal filaments and are quite different in many properties, all the members of both superfamilies are composed of two structural components, the heavy chain and the light chain. Each of the heavy chain contains a globular motor domain, the head, usually at the N-terminal part of the chain (only the heavy chains of a few kinesin-related proteins are exceptional). The N-terminal extensions and the C-terminal tails of these heavy chains, even in the same superfamily, are diverse. The head domains of the heavy chains within each superfamily are highly conserved.

Myosin II (muscle myosin) is one of the earliest branches in myosin evolution history[32]. Both myosin II and kinesin are composed of two heavy chains and several light chains. Myosin head domains have nearly double the mass of the kinesin head domains and the two superfamilies show little sequence similarity. Nevertheless, the three-dimensional structure analyses demonstrated that their heads have essentially the same basic core structure[33]. The major differences between the two heads occur in the stretches that loop out from the cores. The greater size of myosin head domain is due to the much larger loops. Within these loops, the elements responsible for binding to actin filaments or microtubules appear to be homologues[34].

All theses facts indicated that myosin and kinesin must have originated from a common ancestor composed of heavy and light chains. Therefore, it seems very interesting that there is a gene encoding myosin heavy chain-related protein in the genome of the archaeon *Thermoplasma acidophilum*. Two copies of Ta0157 gene encode myosin heavy chain (mhc A) related protein composed of 896 amino acids (GI: 10639302, 16081317). This protein is similar to the myosin heavy chain (mhcA) of protozoan *Entamoeba histolytica*, composed of 2139 amino acids (GI: 1850913). In the genome of archaeon *Sulfolobus solfataricus*, there are also two copies of gene sso2766 encoding a conserved protein composed of 452 amino acids (GI: 13816101, 15899482), which is described as "similar to myosin domain".

On the other hand, two copies of the gene MA2255 encoding "kinesin light chain" composed of 466 amino acids (GI: 19916191, 26091093) were found in the genome of archaeon *Methanosarcina acetivorans*. We postulate that in the archaeal ancestor of eukaryotic cells there was a common ancestor of myosin and kinesin, which is also composed of heavy chain and light chain, and the mhcA-related protein of *Thermoplasma* is the homolog of the heavy chain of this postulated ancestral protein and that the "kinesin light chain" of *Methanosarcina* is the homolog of its light chain. Investigating the

properties of these protein chains in living archaea would perhaps be able to give us useful information about the original functions of these ancient proteins before they combined into the common ancestor of myosin and kinesin. This common ancestor might perhaps already have the ability to move along filamentous structure within the middle or late ancestor of eukaryotic cells and then diversed into myosin and kinesin after both of the microfilaments and microtubules appeared.

(B) The evolutionary origin of dynein and the spirochaete hypothesis on the origin of flagella and cilia

All the members of dynein family are minus end-directed microtubule-based large motor proteins composed of one to three heavy chains and several light chains. Each dynein heavy chain has a very large head, the motor domain. The head is composed of seven to eight globules or lobes around a large central cavity. The very large size and its special structure make dynein head domain distinct from motor head domains of all members of myosin superfamily and kinesin superfamily. However, the microtubule-binding sites of dynein are similar to those of kinesins. The original function of dyneins is to participate intracellular transportation.

The members of dynein family can be divided into three subfamilies: cytoplasmic dyneins, IFT dyneins and axonemal dyneins. Cytoplasmic dyneins are homodimers having two heads and they carry various cargos moving along microtubules towards minus end. However, in metozoa, they take many new functions and became very diversed.

Axonemal dyneins are heterotrimers with three heads, composed of three different heavy chains and several light chains. They are very large proteins. In one axoneme dyneins have already differentiated into a lot of species constructing different axonemal structures. They are specifically responsible for driving the movement of flagella and cilia.

IFT dyneins are responsible for the intraflagellar transportation (IFT) in the direction from tip towards basal body, i.e. the retrograde intraflagellar transportation, while kinesins II are responsible for the anterograde transportation from basal body towards the the tip of the flagellum or cilium. Although ITF dyneins are located within flagella and cilia, they are quite different from axonemal dyneins. According to their amino acid sequences they derived from cytoplasmic dyneins.

The evolutionary relationship between cytoplasmic dyneins and axonemal dyneins involves the big problem of the evolutionary origin of flagella and cilia. As described in section III there are two different hypotheses. One hypothesis postulated that flagellum originated from the ancestor of the eukaryotic cell proper. Lynn Margulis postulated another hypothesis. According to Margulis' hypothesis, the original flagellum originated from the ectosymbiotic spirochaete attached to the surface of the ancestral eukaryotic cell. Contrary to endosymbiotic hypotheses on the origin of chloroplasts and of mitochondria, which have been proved by huge amount of molecular biological evidences from different aspects, the ectosymbiotic spirochaete hypothesis on the origin of flagella has not been supported by any molecular data.

The discussion in section III indicated that the studies of tubulin could not support the spirochaete hypothesis. Because tubulin has not been found in spirochaetes, in order to keep the spirochaete hypothesis one has to revise it and to assume that only axonemal tubulins and microtubules within flagella and cilia originated from spirochaete FtsZ protein, and the cytoplasmic tubulin and microtubules evolved from the FtsZ protein of the archaeal ancestor of eukaryotic cell proper. However, such two distantly related types of tubulins have not been found in any living eukaryote. So, if one wants to keep that hypothesis, one has to assume that one type of tubulins has already been totally replaced by another type of tubulin during the evolution. Although this possibility can not be completely excluded, it is something too far to be fetched. On the other hand, dyneins give us a much better opportunity to solve this problem, bacause there are two different types of dyneins in axoneme and in cytoplasm.

According to the hypothesis of endogenous origin of flagella and cilia, cytoplasmic dyneins and axonemal dyneins all originated from their common ancestral protein within the ancestor of eukaryotic cells. All the differences between them are regarded mainly as functional differentiation. But according to the spirochaete hypothesis, there are two different possibilties. According to Margulis[21], all mi-

crotubules in eukaryotic cell originated only from the spirochaete. So, along with her hypothesis all dyneins should also come from the spirochaete, and the differences between two types are also functional ones. Another possibility is that only axonemal dyneins derived from the spirochaete and cytoplasmic dyneins originated from the ancestor of eukaryotic cell proper. So, the differences between two types should be very large, not only due to functional differentiation.

There are surely many differences between cytoplasmic dynein and axonemal dyneins. Not only cytoplasmic dyneins are homodimers and axonemal are heterotrimers, their heavy chains also have many differences. Comparsion of the heavy chains ammong cytoplasmic dyneins and axonemal dyneins from different species showed that in the C-termminal two third of the heavy chain, there are several highly conserved regions between cytoplasmic dyneins and axonemal dyneins in addition to the conserved regions within different cytoplasmic dyneins and the conserved regions within different axonemal dyneins[35, 36]. The N-terminal one-third of dynein haeavy chanins are variable and seem functionally specific.

According to these data, the two types of dyneins must have originated from a common ancestor and later diverged with their functions during evolution. So, the second possibility described above is incorrect. So far the molecular microbiological data do not favour the hypothesis of Margulis. In spirocheates any dynein or dynein-like protein has not been found[#]. The complete genome of the spirocheate *Borrelia* burgdoferi has already been sequenced. From the whole genome (GI: 15594346), no gene encoding dynein-like protein has been found, nor gene encoding kinesin-like protein.

Although spirochaetes have several long tubules within their body, they do not have dynein-like or kinesin-like proteins. It means that their tubules do not have any ability to participate in the cell movement or any intracellular transport of macromolecules. It seems very possible that these tubules only carry out a supporting role in keeping the spiral cell-shape during strenuous swinging movement.

When one hypothesizes that an ectosymbiotic bacterium would become an eukaryotic flagellum, one thinks that the bacterium could swing by using its intracellular tubules and the associated dynein-like proteins just as swinging of eukaryotic flagellum using its axoneme. If the swinging movement of spirochaetes were depending on their tubules and the associated proteins, the hypothesis would be quite reasonable. However, spirochaetes swing is depending upon large amount of bacterial flagella beneath the flexible outer wall. Bacterial flagella are present in all known spirochaetes. So, the hypthesis is against its principle. If such a spirochaete would "evolve" into a flagellum and lose its bacterial flagella, it would immediately become immobile.

Recently, two bacterial tubulins, BtubA and BtubB, were found in species of *Prosthecobacter* which belong to verrucomicrobia, a newly discovered division of bacteria[37]. Although the homologies between bacterial tubulins (BtubA and Btub B) and the eukaryotic tubulins (alpha- and beta- tubulins) are quite high (31-37% of identity), *Prosthecobacter* can not be used as a replacement of spirochaetes to support Margulis' ectosymbiotic hypothesis, even if the ancestor of eukaryotic cell obtained tubulin genes from them. According to "Bergey's Manual of Determinative Bacteriology" (the ninth edition, 1994), the species of verrucomicrobia are nonmotile.

At present we, the authors, have not found any believable homolog of the original dynein heavy chain in prokaryote[##], although we believe that the ancestral protein of this heavy chain existed in the archaeal ancestor of eukaryotic cells. In order to find the real homolog of dynein heavy chain in prokaryote, one should use the most highly conserved sequence regions in all dynein heavy chain genes as probes to search in all sequenced genomes of archaea. A more reliable way is to search among prokaryotic proteins for the three-dimensional structure similar to that of protozoan cytoplasmic dynein heavy chain. In our opinion, the comparison of three-dimensional structures of proteins is the most dependable way for finding the distantly related proteins.

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