THE KARYOMASTIGONT AS AN EVOLUTIONARY SEME

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In Memory of Lynn Margulis (1938–2011)

Lynn Margulis was an American evolutionary biologist, one of the founders and perhaps the foremost exponent of modern Serial Endosymbiotic Theory (SET). SET asserts that eukaryotic cells evolved not only by classical Darwinian selection on individual genes, but also by symbiotic mergers involving at least three prokaryotic organisms: a host cell (now largely accepted as being of archaean ancestry) and its two acquired eubacterial symbionts, an α-proteobacterium and a cyanobacterium, ancestors respectively of mitochondria and chloroplasts. The host cell acquired not only metabolic faculties but the entire genomes of the symbionts, which thus became heritable organelles. In contrast to Darwinian gradualism, symbiogenesis is a saltatory mode of evolution whereby new species can arise in a single generation. Against considerable resistance, Lynn tirelessly promoted her ideas until, by the 1980s, they were accepted as orthodoxy. The impact of her contribution to the life sciences cannot be overstated. Not many of us can claim to have changed the way our colleagues view even our own narrow fields. Yet, Lynn's insight and perseverance caused the whole world to think differently about living things and how they evolve.

Lynn was a forceful advocate of the karyomastigont's importance in eukaryotic evolution. She knew that the authors saw a great deal of merit in her model, but also that this article represented something of a "reset" to (hopefully) a point just before all the disagreement begins. In her last private meeting with Chapman, the day before she fell ill, she eagerly asked where things stood with this manuscript. Had it already been submitted? Was it ready to go out the door? She was her ever-enthusiastic self, eager to pursue the debate, certain that she had the truth in her sights, if not all the details.

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The world knows the Lynn Margulis who had boundless energy, a remarkable store of knowledge, and no fear of controversy. However, fewer knew the complete Lynn. She was a kind and generous person who took people into her home and graciously offered financial assistance, encouragement, and confidence. She relished cooking for friends and family and even little-known guests. Lynn was someone special who made others feel special. Out in the world she was a giant. Among friends and family, she was a kind woman with a warm maternal streak. Lynn Margulis has made a place for herself in history, and in the hearts of her friends and colleagues. She will be sorely missed.

ABSTRACT

The problem of eukaryogenesis—the evolutionary mechanism whereby eukaryotic cells evolved from prokaryotes—remains one of the great unsolved mysteries of cell biology, possibly due to the reductionist tendency of most scientists to work only within their subdisciplines. Communication between biologists who conduct research on the nucleus and those working on the cytoskeleton or endomembrane system are sometimes wanting, and yet, all of these quintessentially eukaryotic elements of the cell are interdependent, and are physically associated in many protists as the karyomastigont organellar system: nucleus, one or more basal bodies and flagella, nuclear connector, and Golgi apparatus. Here we suggest a more holistic view of the karyomastigont as not simply an organellar system, but an evolutionary seme, the archaic state of the eukaryotic cell. We also present a scheme whereby the karyomastigont may have dissociated, giving rise in more derived cells to one or more free nuclei and discrete flagellar apparati (akaryomastigonts).

INTRODUCTION

PRODUCTIVE debate on the nature of the last eukaryotic common ancestor (LECA) has been hampered by artificial borders between biological disciplines. Cell biologists specializing in the cytoskeleton, nucleus, membranes, etc. have been unable to produce a unified theory of eukaryogenesis to date, and the subject is even more clouded among the broader disciplines of cell biology, protistology, and bacteriology. There has always been some amount of crosstalk between disciplines, of course, and the borders separating disciplines may fade with time (see Kutschera 2009, 2011). Still, separate histories and perspectives on eukaryogenesis can conflict with one another in detail, thus obscuring points of agreement and hindering advancement in the characterization of LECA.

In some cases, the problem may arise from simple differences in terminology. For example, the basal body of cell biology literature has long been known as the kinetosome to protozoologists. A flagellum to the former may be called an undulipodium by the latter. Indeed, the single term flagellum represents two vastly different organelles in bacteria and eukaryotic cells, their only common characteristics being that they are elongate and motile.

Integral to the debate on eukaryogenesis is the origin of the microtubule-based cytoskeleton. Prokaryotes possess proteins with structures and properties similar to tubulin, but we understand comparatively little of their function and origin. Moreover, there are but few reports of microtubule-like structures in bacterial cells (Bermudes et al. 1994). So how did the transition occur between the bacterial state and eukaryotes that, so far as we know, universally possess microtubules composed of tubulin proteins? Our purpose here is not to champion a particular theory, nor is this a treatise on the semantics of different biological disciplines. Rather, our aim is to acquaint—or reacquaint—investigators with two important terms that have been in use for some time, albeit not broadly across disciplines: *seme* and *karyomastigont*. A new conceptual perspective, driven by recent observations and relevant to the debate

on eukaryogenesis, is growing up around these terms. What is a mastigont (much less a karyomastigont)? What is a seme? In short, they are basic functional units, the former structural, the latter evolutionary. We aim to show how the perspective of the *karyomastigont seme* suggests a highly pragmatic way of thinking about the structural and evolutionary relationships of cell motility organelles.

THE SEME

"Seme" is a term introduced by Hanson (1976) to identify a coherent phylogenetic unit. He defines a seme as "an information-containing entity in an interbreeding population of organisms . . . [that] will be used in reference to a structural or functional part of an organism" (Hanson 1977:89). A seme is a functional unit upon which natural selection may act, or which may confer some evolutionary advantage. Examples of semes include body parts (e.g., pectoral fins), organs or tissues (such as liver), cellular organelles or organelle systems, or macromolecular complexes (such as ribosomes). Although defining the phylogenetic history of individual molecules may generate useful data, it can also distract us from functional units (semes), especially units that are linked only discretely. This also holds true at the organelle level. Additionally, the more we have come to understand the roles of symbiogenesis and lateral gene transfer as evolutionary forces shaping cells and their genomes, the clearer the necessity to define units, as best we can, with a common functional purpose and evolutionary origin. Thus, Hanson's seme.

Yet, "seme" is a term too seldom used. Although the cell biology literature is more and more replete with discussion of the evolutionary history and pressures that shaped the cell and its components, the term "seme" is still not well known. The seme concept is, nonetheless, an intuitive part of all evolutionary discourse. When we discuss the origin and evolutionary history of mitochondria, their ultrastructure, biochemistry, or genetics, we are discussing a seme. Likewise, when we discuss the origin and evolution of endomembranes, we are discussing another seme.

Recognizing where one seme ends and another begins can be a difficult problem, since semes can merge into a functional continuum over evolutionary time, as may be the case for the endomembrane system. Thus, the components of a seme may have different origins, but become blended as a functional evolutionary unit. Or, components that originated together (by symbiogenesis, for example) may become disengaged according to new needs and constraints within which the seme operates. It is just such a debate that pervades our understanding of the microtubule-based motility system. In animal cells, this seme consists of microtubules, the centrosome (microtubule organizing center, MTOC), centrioles, and several microtubule assemblages that vary depending on cell type and physiological activity. These include cilia and flagella, the basal bodies from which they arise (themselves derived from centrioles), and the mitotic spindle. There is evidence to suggest that certain intranuclear structures may be considered part of this system as well (Allen 1951, 1953; Tanaka 1973; Laane and Haugli 1974; Alliegro et al. 2010, 2012).

Shared composition, concerted function, and physical linkage join these microtubule-based motility components into a system. Yet they are not all directly connected in the cell nor even present at the same time. The mitotic spindle is present for only a short period during the cell cycle, and only rarely concurrent with a cilium or flagellum. Cilia and flagella may themselves come and go during the life cycle of a cell. They may be at some distance in the cytoplasm from the centriole, but their basal body, in which all cilia and flagella are rooted, is structurally almost identical to the centriole. Once again, shared composition, concerted function, and physical linkage (although sometimes transient), join these structures into a seme.

MASTIGONT AND KARYOMASTIGONT

We may call this seme the microtubule-based motility seme, or coin another term for it. Or we may choose to use the name that it was given over 80 years ago and is still in active use in some biological disciplines, such as protistology: the mastigont. In parabasalids and other protists, as well as mammalian sperm cells, the nucleus is at-
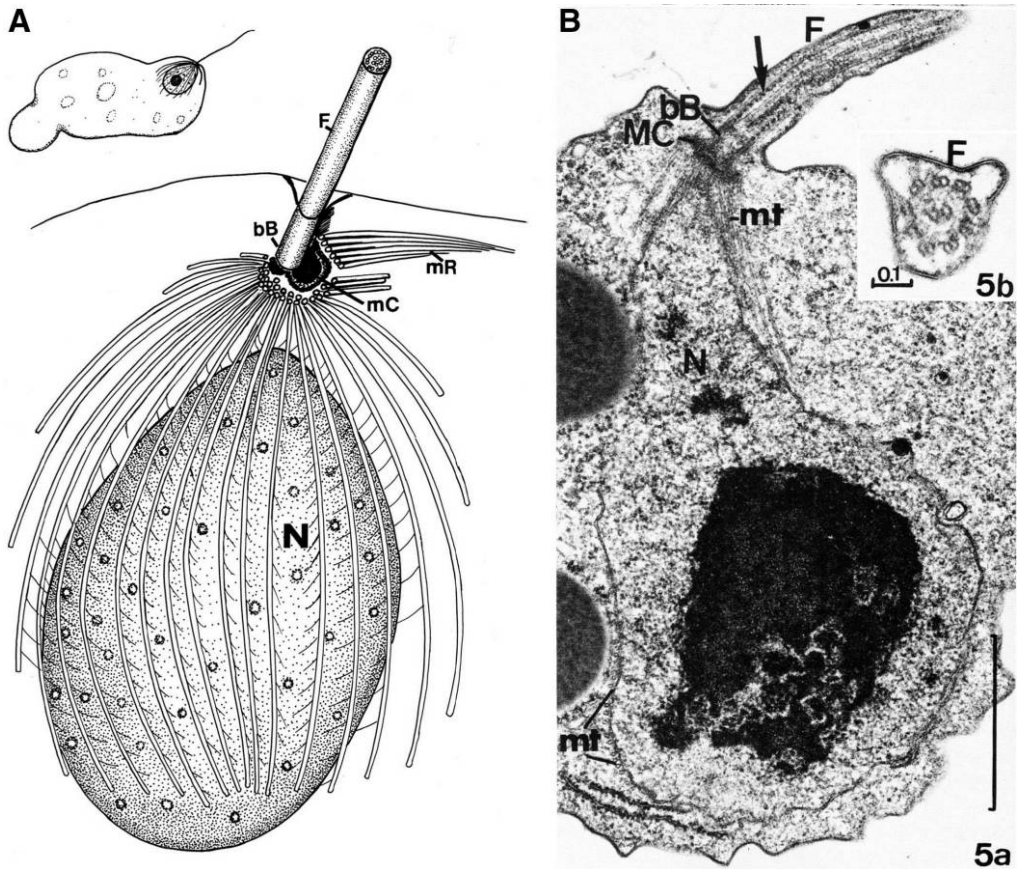
FIGURE 1. KARYOMASTIGONT STRUCTURE

A) Diagram of the flagellar/cytoskeletal system forming one karyomastigont in a mastigameboid type: *Mastigina*. From a microtubule organizaing centre (*mC*) associated with the basal body (*bB*) of the unique flagellum (*F*) arises a cone of microtubules that caps and attaches to the nucleus (*N*). A surface root (*mR*) is also differentiated. B) Electron micrograph of the *Mastigamoeba* karyomastogont. The cone of microtubules (*mt*) arises from a center (*MC*) at the base of a short basal body (*bB*) and is associated with the nuclear envelope. The large endosomal structure is remarkable in the pear-shape nucleus (*N*). The flagellar axoneme (*F*) appears normal (*b*), but a helix-like structure is apparent in the basal region (arrow). (Figures and captions from Brugerolle 1991, Figures 1 and 5a, respectively; reproduced with permission from Springer-Verlag Wien).

tached to the basal body (or bodies) via a nuclear connector made of centrin protein. With the nucleus added, this *kar*yomastigont (Janicki 1915) often also includes Golgi elements that function in sorting and targeting proteins to the flagellar compartment. Regardless of terminology, the karyomastigont is a ubiquitous signature system and seme of eukaryotic cells well known in some fields (Figure 1).

The karyomastigont insures physical association of the nucleus with the basal bodies, which can perform double duty as part of the mitotic MTOC, enabling simultaneous flagellar replication and mitosis. In basal eukaryotes, which have not yet evolved a diverse suite of targeting and recognition proteins, the karyomastigont would have been not merely a valuable seme, but an essential one. The nucleus is dependent on the microtubule-based motility system that forms the mitotic spindle. Likewise, the microtubule-based motility system depends on expression of nuclear genes that encode its more than 360 known proteins.

The mastigont and its components—the basal body plus associated structures such as the cilium or flagellum (undulipodium) and, in some cases, the parabasal body (Golgi)—is well-studied under this name in many protist groups, including ciliates (*Tetrahymena, Paramecium*), dinoflagellates (*Gonyaulax, Gymnodinium*), and trichomonads (*Trichomonas*). By definition, the mastigont is present in all ciliated or flagellated eukaryotes. However, visualization of the mastigont as a unit can be complicated by changing morphologies during the life cycle of a single organism so that sometimes its full presence is obvious, and at other times parts may be translocated or temporarily disassembled or incorporated into other modules. An example of such dynamics in a well-known model organism is the unicellular alga, *Chlamydomonas* (Johnson and Porter 1968). For most of the *Chlamydomonas* life cycle two roughly equivalent mastigonts are present (thus the designation as isokont). During cell division, however, the flagella are disassembled, and the basal bodies (kinetosomes) replicate, then move to the anterior end of the dividing cell to lie next to the cleavage furrow. As originally theorized by Henneguy (1898) and Lenhossék (1898), in many cells, mitotic centrioles replicate and move to the plasma membrane to function as basal bodies (i.e., centrioles and basal bodies are not only structurally equivalent, but in some cells are virtually the same organelle).

Chlamydomonas mastigonts, because they are linked to the nucleus, also serve to illustrate the next level of organization in this system—the karyomastigont. The complexity of the karyomastigont system is multiplied by several permutations that may exist, in that the ratio of mastigonts to nuclei can vary depending upon both the number of mastigonts and the number of nuclei in a given cell. In *Chlamydomonas*, there are two mastigonts associated with a single nucleus. In mammalian sperm, the ratio is 1:1. Other cells such as *Metacoronympha* may have multiple mastigonts, some of which are associated with a nucleus, and some of which are not (akaryomastigonts; see Figure 2C).

As the karyomastigont may change during the life cycle of a single organism, so it varies through evolutionary descent. Harold Kirby,

in establishing the taxonomy of the termite-symbiotic calonymphid protists (for example, *Calonympha, Coronympha, Metacoronympha,* and *Stephanonympha*; Figure 2) noted that karyomastigonts increase in number with increasing cell size (Kirby and Margulis 1994). Under selection for larger cell size and/or faster swimming, cells such as *Calonympha* (Figure 2A) evolved multiple karyomastigonts per cell (e.g., *Coronympha*, Figure 2B). Large numbers of intact karyomastigonts, however, create difficulties with mitosis, so in giant cells such as *Stephanonympha* (Figure 2D), which may be hundreds of microns in length, the hundreds of nuclei become detached from the basal bodies creating akaryomastigonts. An intermediate stage in this evolutionary process is represented by *Metacoronympha* (Figure 2C) some of whose nuclei are detached and others attached—components of intact karyomastigonts (Kirby and Margulis 1994).

Organelle multiplicity is of basic importance in evolution. This holds true for the karyomastigont. Kirby introduced a new taxonomic perspective based upon mastigont multiplicity in his classification of the calonymphids. Prior to his treatise, calonymphid taxa were organized into classes according to number of mastigonts. Kirby proposed that relationships defining descent within a group should be based on mastigont composition and morphology rather than mastigont number. That is, protists bearing different numbers of mastigonts, but with similar karyomastigont morphology, were related by descent. Kirby utilized the entire unit, the seme, to more accurately describe evolutionary taxonomic relationships in the Calonymphidae and other classes of protists. Use of the seme for taxonomic analysis incorporated more information than simple mastigont counts, which could vary according to nutritional states and other factors.

SELECTIVE ADVANTAGES OF THE KARYOMASTIGONT

Attempts to model the principal events of eukaryogenesis have historically suffered from the reductionist tendency in science to focus on individual parts of the system, rather than the whole. Focusing on two components of, say, nucleolar physiology separately, can yield
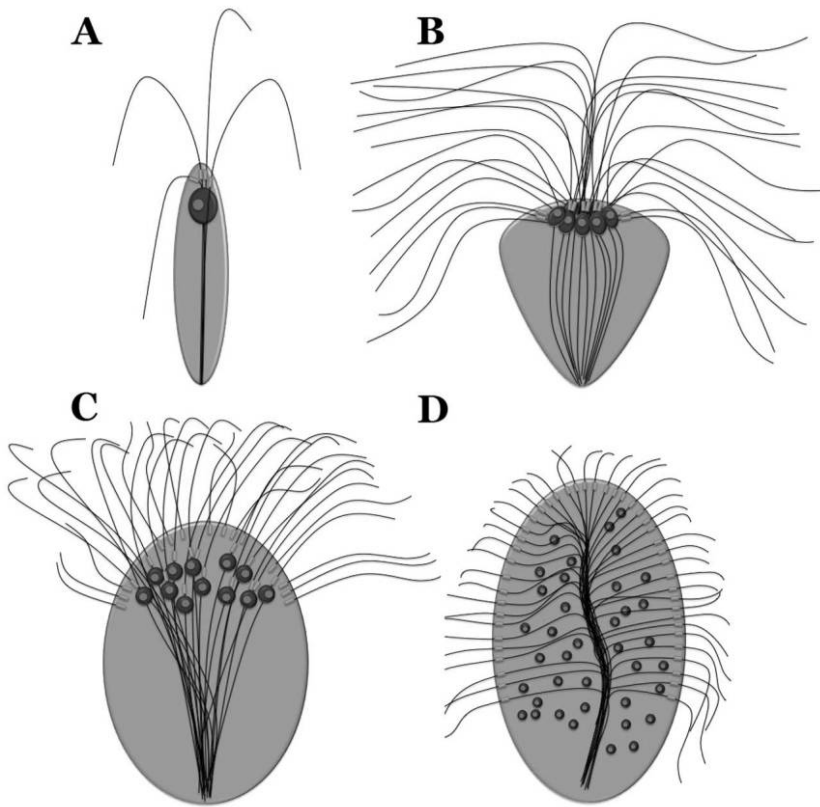
FIGURE 2. STAGES OF MASTIGONT MULTIPLICITY LEADING TO EVOLUTION OF DETACHED NUCLEUS
A) Generic trichomonad (this order of anaerobic protists includes the genera *Trichomonas*, *Mixotricha*, and *Histomonas*, among others) with one quadriflagellate karyomastigont; B) *Coronympha octonaria* with ring of eight karyomastigonts, each with four flagella; C) *Calonympha* with karyomastigonts and akaryomastigonts; and D) *Snyderella* with akaryomastigonts and free nuclei.

two very different models of evolutionary origin (Jékely 2008; Ohyanagi et al. 2008). Indeed, two nearly opposite theories of nucleolar origins have been drawn from the analysis of the very same protein set (Moreira et al. 2004; Staub et al. 2004). Although gene- or protein-based molecular clocks are often powerful tools for phylogenetic analysis, an overreliance on such criteria at the expense of classical evolutionary focus on shared characters can be dangerous. Molecular clocks derived from foraminiferan cytochrome c, for example, place the origin of that well-studied group 300–500 million years prior to the earliest known fossil evidence of life on Earth (S. Bowser, personal communication).

In terms of function then, a seme must be considered *in toto*. In this respect, all functional attributes of the karyomastigont—genetic reorganization from genophore to chromosomes, the origin of introns, microtubule-based cell motility, and membranous fusion both between cells (as in fertilization) and within cells (as in vesicular transport)—must be considered together in order to reach a coherent evolutionary hypothesis. In terms of genetic structure, the most striking eukaryotic innovation over prokaryotes is reorganization of the genome into multiple discrete, yet interdependent, units (chromosomes) as opposed to a unitary genophore, sometimes accompanied by plasmids. Proper distribution of a compound, multiple chromosome-based genome is highly dependent upon spindle-based mito-

sis. Flagellar motility, cell fusion, and vesicular transport are equally dependent on microtubules and motor proteins.

The MTOC, in whatever form it takes in a given cell, therefore, is the central component or linchpin of the karyomastigont, as essential to the eukaryotic condition as the nucleus itself. As shown by Henneguy-Lenhossék theory (Chapman 1998), cells produce a spindle only after retraction of their axonemes. The physical connection between nucleus and kinetosomes (basal bodies) is most apparent in anaerobic protists (parabasalids, oxymonads, pyrsonymphids), but also persists in derived protists (green algae, chrysophytes). Plants and animals have retained the karyomastigont in their flagellated sperm cells. Even the fungi, which like amoebae and foraminiferans have discarded their flagella in favor of cell (hyphal) elongation over the course of evolution, retain a physical connection between nucleus and MTOC in the form of their nuclear membrane-associated spindle pole bodies.

Taken together, the suite of shared characters involving the karyomastigont suggests that the physical connection between nucleus and MTOC was a crucial selective innovation that conferred an advantage over symbiotic associations such as the spirochete-*Thermoplasma* bacterial symbiosis, *Thiodendron* (Surkov et al. 2001). As shown by stratigraphic correlation between acritarchs (early eukaryotic fossils) and stromatolites (cyanobacterial fossils), the predominant environmental stress on early eukaryotes was increasing oxygen in the atmosphere due to cyanobacterial photosynthesis (Margulis 1993). A permanent physical connection between the nucleus and motility organelle, incorporating the motility system within the cell membrane, would have conferred the advantage of better motility through high-oxygen or low-sulfur environmental zones and consequent improved chances of reaching greener pastures. The simple *Thiodendron* association, by contrast, with its oxygen-sensitive spirochete partner and sulfide-requiring archaean partner, would tend to break down in stress environments. Moreover, through incorporation of the motility system within the cell membrane, the karyomastigont system conferred intra- as well as extracellular motility,

leading to mitosis, meiosis, and sexual fusion. The foregoing explanation of the selective advantage of the karyomastigont does not constitute an argument for the spirochete model of eukaryogenesis (Margulis 1993), nor for a symbiotic origin of the seme, although the selective advantages would be the same. Indeed, almost diametrically opposed theories of eukaryogenesis may still begin with the critical karyomastigont (e.g., Bornens and Asimzadeh 2007; Margulis et al. 2007).

THE KARYOMASTIGONT PERSPECTIVE

Reluctance to use the term karyomastigont may be due in part to its reputation in some circles as outdated. Perhaps a more descriptive name can be devised. Regardless of nomenclature, there are advantages to considering the karyomastigont as a unitary organelle rather than as separate entities associated by physical proximity and broad functional overlap. Also, the tangible connections between these seemingly distinct organelles may not yet be discovered, or at least obvious, but may nevertheless exist. The karyomastigont perspective seamlessly incorporates a number of observations, including some very recent and surprising findings. The data may only be correlative at present, but when viewed from the karyomastigont perspective, they are no longer surprising. It was quite unexpected, for example, to find that the Golgi can function as a MTOC (Efimov et al. 2007). Yet it is not so surprising when, as pointed out earlier in this discussion, the Golgi (parabasal body) has long been considered a karyomastigont component. From this perspective, the observation that a component of a unitary microtubule-based motility organelle could nucleate microtubules is no revelation. The karyomastigont perspective also addresses reports of MTOC components in the nucleus of some cells (Tanaka 1973; Laane and Haugli 1974; Alliegro et al. 2010, 2012). Viewed in light of the unitary karyomastigont, the relationship between the microtubule-based motility system and the nucleus is not simply one devised to segregate genetic material, but a much more integrated, reciprocal relationship with shared and exchanged elements.

Conclusion

The physical linkage between parts of the karyomastigont in basal organisms considered together with the interdependence and exchangeability of these components in so many derived taxa lacking an intact karyomastigont is thought-provoking. So, too, is the observation that karyomatigont components are all individually considered eukaryotic signature structures: the Golgi (Dacks et al. 2003; Mironov 2007), flagellum, and centriole/basal body/centrosome (Satir et al. 2007; Marshall 2009) are found across all taxonomic groups universally, or nearly so. In cases where they are absent, it is considered due to a secondary loss (Dacks et al. 2003; Mironov et al. 2007; Marshall 2009). The nucleus, of course, is presently considered definitional for eukaryotes by itself. We can therefore state with some confidence that, if not in its composite form, the building blocks of the karyomastigont are as close to the irreducible minimum for eukaryotic life as has yet been deliberated.

Constructing a testable hypothesis of origin for the karyomastigont is difficult, given the lack of data prerequisite for a reasonable model. Perhaps the only applicable test to falsify any immediate hypothesis would be documentation of a derived organism with an intact karyomastigont, and of its ancestor or ancestors lacking karyomastigonts. Still, we can speculate. To do so, we begin by allowing that the nucleus was derived symbiogenetically, as most modern theories of eukaryogenesis posit. If the unitary karyomastigont is truly the archaic form, it can only follow that the entire complex—nucleus, connector, MTOC (centriole/basal body/centrosome), and Golgi—was derived symbiogenetically. The moment of its incorporation into a host cell represents creation of the eukaryotic lineage and the elements of the karyomastigont are thenceforth conserved across virtually all eukaryotic taxa. We will not go so far as to speculate on whether the karyomastigont was, prior to this point, derived from a single organism or was itself a composite. However, from this point forward, we can borrow on Kirby's model for the transition from karyomastigont to the akaryomastigont of derived taxa. The interdependence of parts perhaps necessitated physical linkage in the early, simplified versions of the karyomatigont; i.e., the nucleus maintained a physical association with the basal body because that organelle became a spindle pole during mitosis; and similarly, maintained an axostyle of nonephemeral microtubules running caudally down the length of the cell. In its ancestral state, these two bundles of microtubules—the flagellar and axostylar—conferred intrinsic motility during interphase on the flagellum and cell body, respectively, then formed the two lobes of the spindle during mitosis. The basal body replicated to generate spindle poles during mitosis, then resumed its role in nucleation of the new flagellum in interphase in the offspring cell. The ancestral Golgi, probably a modified series of cisternae derived from the endoplasmic reticulum (Staehelin and Kang 2008), was closely apposed to the nucleus and nuclear connector because of its function. Highly complex in structure and subject to intense physical stress in its function, the flagellum needs concerted effort by the cell for its assembly and maintenance. This is the province of the IFT (intraflagellar transport) proteins, which shuttle components of the axoneme and flagellar membrane from the base to the tip and back again. Because one would expect ancestral IFT proteins to have been far fewer in number and less specialized than the 18 that are known today, close physical proximity might have been necessary between the ancestral Golgi and the basal body. The same is true of the centrin nuclear connector, whose component proteins had not yet diversified into the centrin family of proteins we know today. Perhaps in order to regulate the cell cycle, this ancestral centrin needed physical contact with both the nucleus and one of the spindle poles.

Meanwhile, it is tempting to choose individual molecules, or small groups of molecules that one or another investigator considers "core" to any given structure, and construct an evolutionary narrative for the seme or entire organism based on limited molecular phylogeny. This is perhaps a more likely trap when the chimeric eukaryotic cell, with its admixture of archaean and eubacterial proteins and its composite, yet unitary karyomastigont, is over-

looked and analyses are performed on a selected set from the basal body, flagellum, centrosome, or nucleus, each alone. Decisions on which members comprise the set of molecules chosen for analysis are always based on operational (functional/physiological) considerations, yet operational genes can be and are exchanged between organelles and organisms. The targets for analysis may therefore be rationally chosen, but are still arbitrary. Rather, we propose that the proteomes and transcriptomes of karyomastigont components should be assembled in their entirety and analyzed using a shotgun approach. This will let the core components and their origins reveal themselves to us, without bias.

In conclusion, given that all of these structures—nucleus, basal body, flagellum, and Golgi—have shared components, physical linkage, and concerted function in basal protists, it would be improvident to overlook the strong possibility of a shared evolutionary history. Viewed as a seme, the karyomastigont offers fresh evolutionary insights on all components of the system, and may ultimately shed light on the origin of eukaryotic cells.

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