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Mitosis, Microtubule Dynamics and the Evolution of Kinesins

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All living organisms must transmit genetic information through successive generations, and all six eukaryotic supergroups utilize a mitotic spindle to accomplish this task. The mitotic spindle is the cellular machinery responsible for chromosome segregation during mitosis, and it is comprised of hundreds of proteins. During evolution, eukaryotic cells have developed different mechanisms and different kinds of mitotic spindles to segregate the chromosomes (Drechsler and McAinsh, 2012). Regardless of the differences, the common characteristic of all these different mitotic spindles is the utilization of microtubules and kinesins, as all known eukaryotes studied to this date possess kinesins (Wickstead *et al.*, 2010).

The mitotic spindle is a bipolar array of microtubules of varied lengths that continuously grow and shrink. These highly dynamic microtubules are nucleated by centrosomes and contact the chromosomes in the centromeric region to facilitate chromosome attachment and segregation. Although chromosome movement is powered in part by changes in microtubule assembly (Shelden and Wadsworth, 1992), kinesins associated with microtubules and other spindle structures refine the movement of chromosomes in the spindle. Kinesins participate in chromosome attachment, influence microtubule dynamics and contribute to anaphase spindle elongation (reviewed in (Cross and McAinsh, 2014)). Thus, kinesins, in conjunction with dynamic microtubules, ensure the proper distribution of genetic material between the two daughter cells to avoid aneuploidy.

Kinesins are a class of molecular motors that use the energy from hydrolysis of ATP to translocate along the microtubule or control microtubule end dynamics (Vale *et al.*, 1985, 1996; Desai *et al.*, 1999). They have been identified in members of all six eukaryotic supergroups including extremely deep-rooted members (Fig. 1). Processive kinesins are able to perform successive work-producing cycles of ATP hydrolysis without detaching from the

substrate microtubule whereas non-processive kinesins readily detach from microtubules but can be quite effective working in ensembles. There are 14 families of kinesins, and most members possess two distinct functional domains: an ATP-hydrolyzing motor domain and a tail domain that can associate with cellular structures or cargo (Lawrence *et al.*, 2004; Miki *et al.*, 2005). The motor domain is very well conserved among the different kinesins families while the tail domains are more divergent. Most kinesins translocate to the plus ends of the microtubule and possess an N-terminal motor domain. There are kinesins with the motor at the C-terminus that translocate to the minus end of the microtubule. Besides this hand over hand "walking" activity (Yildiz *et al.*, 2004), there are some kinesins that are able to control microtubule dynamics through promoting polymerization, promoting depolymerization or pausing polymerization activity (Desai *et al.*, 1999; Bringmann *et al.*, 2004; Cui *et al.*, 2005).

In Opisthokont metazoans there appear to be Kinesins involved in every step of mitosis, belonging to the families 4, 5, 6, 7, 8, 10, 12, 13, 14 (reviewed in (Cross and McAinsh, 2014)). Here we are going to review the kinesins involved in each phase of mitosis with an emphasis on the stages of mammalian cell division. Additionally, we will consider the role of kinesins in deep-rooted eukaryotes.

Kinesins in Mitosis

Prophase: Centrosome separation

During prophase, the two centrosomes close to the nuclear envelope separate and travel to opposites sides of the cell to form a bipolar spindle. At the same time the chromatin begins to condense to form chromosomes. The most important kinesin family involved in the formation of the bipolar spindle is the kinesin-5 family (Kashina *et al.*, 1997). Kif11 (also known as Eg5) is the Kinesin-5 family member involved in the bipolar spindle formation in humans (Slangy *et al.*, 1995). Kif11 acts as a tetramer with two kinesin heads contacting one microtubule and the other pair of heads contacting a parallel or antiparallel microtubule. Thus, Kif11 acts as a microtubule crosslinker that is able to force two microtubules to glide with respect to each other, and uses this activity to separate the centrosomes during the beginning of mitosis (Kapitein *et al.*, 2005). A representative Kif11 member is present in many deep-rooted eukaryotes and lacking in others, even across those (such as *T. brucei* vs. *S. cerevisiae*) with closed mitosis (Fig. 2).

Prometaphase and Metaphase: Organization of a bipolar spindle and chromosome congression

During prometaphase the centrosomes localize to opposites sides of the cell to form a bipolar spindle. There is evidence that Kif15 (a Kinesin-12 family member) functionally overlaps Kif11 in the separation of the centrosomes and in the formation of a bipolar spindle (Tanenbaum *et al.*, 2009; Sturgill and Ohi, 2013). KifC1 (a kinesin-14 motor) is a minus-end directed motor that generates an inward force during the formation of the spindle. The outward force created by Kif11 and Kif15 compensates this inward force to help maintain the spindle length (Mountain *et al.*, 1999). Interestingly, some organisms that lack Kif11, such as *P. tetraurelia* and *T. brucei*, do possess Kif15 (Wickstead *et al.*, 2010).

During this phase, microtubules contact the chromosomes in the kinetochores and move them to the middle of the spindle forming the metaphase plate. Several kinesin families are involved in the capture and congression of the chromosomes: Kinesin-4 (Kif4), Kinesin-7 (Kif10), Kinesin-8 (Kif18A), Kinesin-10 (Kif22), Kinesin-13 (Kif2B, Kif2C) and Kinesin-14 (KifC1).

The first contact is usually a “lateral connection”, where the kinetochores contact the microtubule via the lattice rather than the microtubule tip. In these cases, the molecular motors Dynein and Kif10 (also known as CenpE, a Kinesin-7 member) are the players involved in the transport of the chromosomes to the plus-end tip of the microtubule to establish a stronger connection kinetochore-microtubule (Schaar *et al.*, 1997; Wood *et al.*, 1997; Kapoor *et al.*, 2006; Cai *et al.*, 2009). Kinesin-7 is widely represented in deep-rooted organisms.

Members of the Kinesin-8 family are important for the correct chromosome alignment in metaphase. For example, the deletion of Kinesin-8 members Klp5 and Klp6 in *S. pombe* and Kip3 in *S. cerevisiae*, alters the alignment of chromosomes in metaphase (Garcia *et al.*, 2002; West *et al.*, 2002; Wargacki *et al.*, 2010). In human cells the depletion of Kif18A generates a congression defect with chromosomes dispersed through all the spindle (Mayr *et al.*, 2007; Stumpff *et al.*, 2008, 2012). Kinesin-13 member's role in chromosome alignment at the metaphase plate is not as well understood as Kinesin-8, but it is known that the lack of Kif2C (MCAK) affects attachment (Domnitz *et al.*, 2012) and congression in human cells (Zhu *et al.*, 2005) possibly by limiting microtubule length within the spindle.

Another activity influenced by kinesins is chromosome oscillation around the metaphase plate. Once two sister chromatids are attached to microtubule tips emerging from respective opposite poles, the chromosome is bi-oriented. Stable, bi-oriented attachment of the chromosomes to the mitotic spindle is a requirement to turn off the mitotic checkpoint. The checkpoint assures that both daughter cells receive the same amount of genetic material by inhibiting the initiation of anaphase chromosome segregation in the presence of improperly attached chromosomes. Bi-oriented chromosomes establish a meta-stable position at the metaphase plate by oscillating back and forth across the spindle midpoint. It is hypothesized that in addition to properly positioning chromosomes for anaphase, oscillations may facilitate the shedding of improper microtubule attachments during prometaphase and metaphase (Holt *et al.*, 2005; Wordeman *et al.*, 2007). These oscillations are possible thanks to forces derived from changes in microtubule polymerization rates that are controlled in part by kinesins. There are several kinesins implicated as participating in the oscillation movements: Kif2B, Kif2C, Kif4, Kif10, Kif18A and Kif22.

Polar ejection forces help to push chromosomes away from the spindle poles and relocate them to the metaphase plate if they have moved far away from it (Rieder *et al.*, 1986). This is achieved thanks to a group of kinesins that interact with chromosomes called chromokinesins. Kif22 (also known as Kid, member of the Kinesin-10 family) is a chromokinesin that uses solely plus-end directed motility to facilitate chromosome congression (Stumpff *et al.*, 2012). Kif4 (Kinesin-4 family) is a plus-end directed chromokinesin that can also regulate microtubule dynamics and microtubule length to

influence congression (Oh *et al.*, 2000; Samejima *et al.*, 2012; Stumpff *et al.*, 2012; Wandke *et al.*, 2012). This unusual kinesin family member can be detected in a number of deep-rooted eukaryotes (Fig. 2).

The Kinesin-8 family member, Kif18A, is able to control microtubule dynamics (Roostalu and Surrey, 2013; Su *et al.*, 2013) and chromosome oscillations probably by pausing or reducing the polymerization rates of microtubules ends close to the kinetochores (Du *et al.*, 2010; Stumpff *et al.*, 2012). Studies in human cells have shown that Kif18A localizes in the spindle with a higher concentration at the tip of the plus-end microtubule close to kinetochores (Mayr *et al.*, 2007; Stumpff *et al.*, 2008). Kinetochores closer to the metaphase plate have longer k-fibers, and therefore accumulate more Kif18A molecules. Growth suppression of these longer k-fibers would expected to be enhanced, preventing the chromosomes from moving farther from the metaphase plate. In contrast, the loss of Kif18A allows chromosomes to stray farther from the metaphase plate during each oscillation. This would explain why there are chromosomes dispersed through all the spindle in the Kif18A knockdown (Mayr *et al.*, 2007; Stumpff *et al.*, 2008, 2011, 2012). There are very few eukaryotes that do not possess either a kinesin-13 member, a kinesin-8 member or both, which suggests microtubule length modulation by kinesins is an essential activity. Curiously, one primitive eukaryote, *Cyanidioschyzon merolae*, that lacks kinesin-8 and -13 family members accomplishes much of its G1 activities without utilizing assembled microtubules (Imoto *et al.*, 2011). In this organism MTs are only present in the mitotic spindle, and the authors suggest the idea that MTs first evolved associated to mitosis, and that the cytoskeleton and transport functions evolved later.

Besides chromosome congression and positioning, another process regulated by kinesins is the turnover of the kinetochore microtubules. The kinesin-13 family members (Kif2A, Kif2B and Kif2C) are involved in this process likely because they are capable of using the energy of ATP to directly disassemble microtubules (Desai *et al.*, 1999; Hunter *et al.*, 2003; Cooper *et al.*, 2010). During the first contacts of the microtubule with the kinetochores, erroneous connections are common (Cimini *et al.*, 2003). Kinetochore-associated Kif2C is implicated in the correction of MT-KT attachments (Kline-Smith *et al.*, 2004; Wordeman *et al.*, 2007). Kif2C is found both on kinetochores and also as a complex with EB1 on microtubule plus-ends. It facilitates end-on attachment of kinetochores to microtubule tips by suppressing plus-end microtubule length within the spindle (Domnitz *et al.*, 2012). Kif2A also controls spindle microtubule length but its centrosomal position suggests control of microtubule minus ends so its overarching role may be to set overall spindle length (Wilbur and Heald, 2013).

Anaphase, Telophase and Cytokinesis

During anaphase the separated sister chromosomes move from the center of the spindle in the metaphase plate toward opposite poles of the cell (anaphase A) and the mitotic spindle is elongated (anaphase B). Microtubule depolymerization is the principle driving force for anaphase chromosome segregation (Gorbsky *et al.*, 1987; Shelden and Wadsworth, 1992). In *Drosophila* the Kinesin-13 members KLP59C and KLP10A may influence microtubule depolymerization to facilitate chromosome segregation as well (Rogers *et al.*, 2004). Spindle

elongation is controlled by kinesin-5 members in flies and yeast (Straight *et al.*, 1998; Brust-Mascher *et al.*, 2009). Interestingly, it is not well-understood what controls anaphase B spindle elongation in mammals.

The central spindle is a structure of antiparallel microtubules formed between the two sets of segregating chromosomes important for the regulation cytokinesis. Kif2A and Kif4 control the size of the central spindle through the control of microtubule dynamics (Bieling *et al.*, 2010; Hu *et al.*, 2011; Uehara *et al.*, 2013). There are other kinesins important for cytokinesis like Kif10, Kif14, Kif20A, Kif20B and Kif23 (reviewed in (Lee *et al.*, 2012) and chapter xxx in this same number).

Kinesins and the control of Microtubule dynamics

The classical or conventional role of kinesins is the transport of cargo from one place in the cell to another, e.g. the transport of cargo down the axon of a neuron. But as described above, some kinesins are able to influence MT dynamics. Microtubules are dynamic biological polymers that constantly grow and shrink in an assembly/disassembly cycle known as dynamic instability (Mitchison and Kirschner, 1984).

Microtubule dynamics varies depending on the cell cycle, cell events and cellular structures encountered by the microtubule end. During interphase the half-life of MT is around 5 min, and in the mitotic spindle it is of around 5 seconds (Saxton *et al.*, 1984). But even inside of the spindle there are differences between microtubule subpopulations. The connection of MT with the KT (K-fibers) is very, stable, resulting in higher half-life than in the rest of the mitotic spindle (around 5 minutes) (Gorbsky and Borisy, 1989; Zhai *et al.*, 1995). The turnover of microtubules in the kinetochore fiber is an important contributor to error correction during cell division (Bakhoum *et al.*, 2009; Ertych *et al.*, 2014).

Microtubule dynamics is highly regulated by a complex of proteins like MAPs (microtubule associated proteins) and kinesins (Vaart *et al.*, 2009). Based on existing studies, we can differentiate three classes of kinesins regulating MT dynamics:

1. Kinesins that promote or enhance the addition of subunit to the MT: kinesin-7 (CenpE), Kinesin-10 (Nod)
2. Kinesins that enhance or promotes tubulin subunit loss: kinesin-8 (Kip3, Klp5/6, Kif19), Kinesin-13 (Kif2A, 2B and 2C/MCAK), Kinesin-14 (Kar3)
3. Kinesins that suppress the dynamics at the MT ends: Kinesin-4 (Kif4/Xklp-1), Kinesin-8 (Kif18A).

Kinesins proteins are the only cytoskeletal motors (kinesins, dyneins and myosins) that have been found in all eukaryotes studied to date (Richards and Cavalier-Smith, 2005; Wickstead and Gull, 2006, 2007; Wickstead *et al.*, 2010). This would put the apparition of the first kinesins between 1.6 and 2.2 billion years ago, with the apparition of the first eukaryotic cells. But, when did kinesins acquire the ability to modulate microtubule dynamics? Is that function previous or subsequent to translocation along the microtubule lattice? It is possible that regulation of microtubule assembly is an ancient and perhaps the original function of the earliest kinesins, with the transport function evolving later. With this in mind, what

would be the set of kinesins possessed by the FECA (First Eukaryotic Common Ancestor) and the LECA (Last Eukaryotic Common Ancestor)? In a 2010 paper Wickstead et al. studied kinesin diversity through a wide range of eukaryotes organisms concluding that is probably the LECA had a whole set of kinesins comprising members of families 1, 2, 3, 4, 5, 8, 9, 10, 13, 14, and 17 (the Kinesin-17 family is a new family only found in bikonts (Wickstead and Gull, 2006)). In addition, it appears that most eukaryotic organisms have kinesins controlling MT dynamics and most of them have some kind of MT-depolymerizing kinesin from families Kinesin-8, Kinesin-13 or Kinesin-14 (Wickstead and Gull, 2006; Wickstead *et al.*, 2010). Even the primitive red alga *C. merolae*, which lacks kinesin-8 and -13 family members, possesses a kinesin-14. We have performed a simple manual BLAST analysis of kinesins in eukaryote protists from the groups Excavata, Alveolates, Opisthokonts and Amoebozoa (Fig. 1). Some of these organisms are pathogens, and some of them are known as deep-rooted eukaryotes because they are usually positioned close to the root of the eukaryotic tree of life. We have not found kinesins from families 6 and 11, and our results fit with the results from previous papers.

Polymerizing kinesins—There are several kinesins in different systems that promote MT polymerization or MT nucleation. Kif10 (also known as Cenp-E) is a plus-end directed kinesin from the Kinesin-7 family (Wood *et al.*, 1997). It localizes to MT ends, is able to stabilize GTP-microtubules and promote the elongation of the stabilized MTs (Sardar *et al.*, 2010). The *Drosophila* Nod (Kinesin-10 family) is a non-motile kinesin that plays an important role in chromosome segregation during meiosis. It localizes to MT ends and promotes MT polymerization (Cui *et al.*, 2005). Additionally, there is the special case of the *S. pombe* KLP5/6 (Kinesin-8) motors that promote both MT nucleation and catastrophe (Cui *et al.*, 2005). In our analysis we have found proteins similar to Kif10 (CenpE, kinesin-7) in most of the eukaryotes analyzed (Fig. 2).

Depolymerizing Kinesins—The most studied group of kinesins controlling MT dynamics is the depolymerases, especially the Kinesin-8 and Kinesin-13 families.

Kinesin-13 family—Kinesins-13 members have the catalytic domain in the center of the protein, and do not walk over the MT, but use their ATPase activity to remove tubulin subunits from both ends of the MT (Desai *et al.*, 1999; Walczak, 2003), arriving to the ends mainly by diffusion (Helenius *et al.*, 2006). The first kinesins with depolymerization activity identified in mammals were Kif2A and Kif2C (MCAK) from the Kinesin-13 family (Noda *et al.*, 1995; Wordeman and Mitchison, 1995). Kif2A and 2C have been studied in a lot of detail, but the first studies showing the depolymerization activity of MCAK came studying the orthologous in *Xenopus:* XKCM1. The depletion of XKCM1 in *Xenopus* egg extracts causes an excessive growth of the MT (spindle and astral MT) and the prevention of mitotic spindle formation (Walczak *et al.*, 1996; Kline-Smith and Walczak, 2002). Cells overexpressing XKCM1 do not form a bipolar spindle because the MTs are too small and not able to grow (Ohi *et al.*, 2007). In human cells overexpression of MCAK cause similar defects, which MCAK loss promoted kinetochore attachment errors and spindle positioning defects (Maney *et al.*, 1998; Kline-Smith and Walczak, 2002; Wordeman *et al.*, 2007; Rankin and Wordeman, 2010; Domnitz *et al.*, 2012). *S. cerevisiae* doesn't possess members

of the Kinesin-13 family (Wickstead *et al.*, 2010). However, both Kar3 (a Kinesin-14) and Kip3 (a kinesin-8) have the ability to destabilize MT ends (Sproul *et al.*, 2005), so it is possible that they may have acquired the depolymerization activity to compensate for the lack of Kinesin-13. Alternatively, the kinesin-14, Kar3, may supply depolymerizing activity to functionally subsidize activities controlled by kinesin-13 in other organisms (Saunders *et al.*, 1997).

Kinesin-13 members are present in many deep-rooted eukaryotes. In *Giardia intestinalis* Kinesin-13 localizes to the median body and flagellum, affecting flagellum length, median body behavior and mitotic MT dynamics (Dawson *et al.*, 2007). Kinesin-13 in *Leishmania major* is involved in flagellar length control (Blaineau *et al.*, 2007). *Trypanosoma brucei* Kinesin-13 localizes to the flagellar tip but it seems that its role in regulating flagellar length is very modest (Chan and Ersfeld, 2010). And in the single cell green alga *Chlamydomonas reinhardtii* Kinesin-13 is involved in flagellum assembly/disassembly cycle (Piao *et al.*, 2009; Wang *et al.*, 2013).

Kinesin-8 family—Kinesin-8 proteins possess an N-terminal motor domain and use highly processive motility to reach the plus-end of the MT where they can influence MT dynamics (Gupta *et al.*, 2006; Varga *et al.*, 2006; Su *et al.*, 2012). Kinesin-8 has two MT interacting sites, one of them in the C-terminal of the protein, that allows the kinesin to keep attached to the MT and exhibit high processivity (Mayr *et al.*, 2011; Stumpff *et al.*, 2011; Su *et al.*, 2011; Weaver *et al.*, 2011). Thanks to this high processivity Kinesin-8 motors can accumulate at the end of the plus-side of the long MT creating a gradient of kinesin motors along the length of the MT that is more pronounced in longer MT fibers. In other words, greater numbers of motors can accumulate at the ends of longer MTs imparting more kinesin-8 activity at the ends of longer microtubules (Varga *et al.*, 2006, 2009).

Members of this family can regulate MT dynamics by removing tubulin subunits or by blocking the addition of new tubulin. The mammalian Kinesin-8 Kif18A appears to suppress microtubule assembly at microtubule plus ends (Du *et al.*, 2010; Stumpff *et al.*, 2011, 2012). A pause in tubulin addition could allow GTP hydrolysis to reach the microtubule plus-end and increase catastrophes, but this mechanism has yet to be proven. Thus, these motors could induce MT depolymerization without actually removing tubulin subunits. *S. cerevisiae* Kip3p, in contrast, promotes the loss of tubulin subunits from the microtubule plus end (Varga *et al.*, 2006; Su *et al.*, 2011). To remove tubulin subunits, Kip3p accumulates on the plus-side of the MT and when new Kip3p arrives to the plus-end it pushes the previous molecules removing the motor and tubulin from the MT fiber (Varga *et al.*, 2009). In keeping with the controversial nature of kinesin-8 activity, it is not clear if Klp5/6 from *S. pombe* has depolymerizing activity or not, as there are studies showing depolymerizing activity (Erent *et al.*, 2012), and others showing the opposite (Grissom *et al.*, 2009). While yeast do not have a Kinesin-13 member, *Giardia* and some other deep eukaryotes have Kinesin-13, so it is possible that yeast has lost Kinesin-13 during evolution. However they have members of the Kinesin-8 family, so they still have kinesins with depolymerizing function. It is possible that organisms with simpler genomes and fewer kinesins have combined the depolymerizing functions in just one family.

Evolution of Kinesins

From our analysis we have reached similar conclusions to those in Wickstead. et al. There are organisms like *Crithidia*, *Eimeria*, *Leishmania*, *Neospora*, *Trypanosoma* or *Naegleria* that possess kinesins from 10 different families (Fig. 2). This would support the idea that the LECA was fully equipped with a complete set of kinesins for different cellular functions. However there are some other organisms like *Babesia* (Kinesin-8 and 13), *Enterocytozoon* (Kinesin-8 and 14) and *Theileria* (Kinesin-8 and 13) that have just two kinesins in their genomes (also previously reported by (Wickstead and Gull, 2006)). *Plasmodium* has just three kinesins (Kinesin-5, 8 and 13). And there are several other organisms with just three or four kinesins (Fig. 2). Importantly, in all the cases of organisms with just two, three or four kinesins, all of them possess representatives from kinesin families implicated in controlling MT dynamics, i.e. Kinesin-4, 7, 8, 13 and 14. So it is possible that the earliest eukaryotic cells (not the LECA) were equipped principally with a set of kinesins in charge of controlling MT dynamics and that kinesins specific for transport functions did not appear until later in evolution through gene duplication and specialization. It is possible that the organisms with only 2-3 kinesins have lost some kinesins members during evolution and would, therefore, not exemplify the most ancient eukaryotes. However, it is also possible that organisms with more than four kinesins have gained kinesins during evolution. Most of these deep-rooted eukaryotes possessing a broad range of kinesins are pathogens, so it is possible that horizontal gene transfer occurred between the host and the pathogen, or even between different pathogens. There are some reported cases of gene transfer between eukaryotic pathogens, and between the pathogen and a host (Andersson, 2005, 2009; Keeling and Palmer, 2008; Alsmark *et al.*, 2009; Bar, 2011; Selman *et al.*, 2011). For these reasons, the protist that epitomizes this putative ancient eukaryote remains to be unambiguously identified.

One characteristic common to most of these deep-rooted eukaryotes is the existence of flagella, and also a cytoskeleton composed of unusually complex MT structures (e.g. *Giardia*, *Trypanosoma*, *Plasmodium*, etc) (Wickstead and Gull, 2011; Dawson and Paredez, 2013). In some cases their life cycle depends strongly on the proper assembly and function of these flagella, for which they need to tightly control MT length and dynamics. It is difficult to separate the existence of a flagellum from transport. The IFT (IntraFlagellar Transport) is a system to build and maintain the eukaryotic flagellum that relies on the kinesins' transport activity. As the LECA is likely to have possessed a full 9+2 flagellar apparatus, which would presumably require transport activity, it is probable that it had several kinesins in charge of the transport of cargo from the cytoplasm to the end of the flagellum (Yubuki and Leander, 2013). The origin of the flagellum is not yet clear (Carvalho-Santos *et al.*, 2011; Yubuki and Leander, 2013), but one hypothesis proposes an autogenous origin of the flagellum from an MTOC organizing the mitotic spindle (Pickett-Heaps, 1974). *C. merolae* presents assembled MTs only during the formation of a mitotic spindle, suggesting that MTs first evolved to facilitate mitosis (Imoto *et al.*, 2011). If the first eukaryotic cells with a flagellum did organize this structure from a mitotic spindle, it is possible that they were able to do it without the transport-kinesins required for IFT. For example, the pathogen *Plasmodium* doesn't have an IFT system and it is able to build a

flagellum (Briggs *et al.*, 2004). Moreover, the flagellar machinery is often co-opted to build the mitotic spindle during cell division in some of these deep-rooted eukaryotes.

It is possible that the very earliest eukaryotic cells, not the LECA, had just a minimum number of two or three kinesins in charge of MT dynamics to control mitotic spindle, cytoskeleton and an ancient flagellum. Other kinesins dedicated to transport may have appeared later in evolution as cells became larger and more specialized. More evolutionary cell biology research and more studies centering on these deep-rooted eukaryotes will be necessary to understand the nature and evolution of the kinesins controlling MT dynamics.

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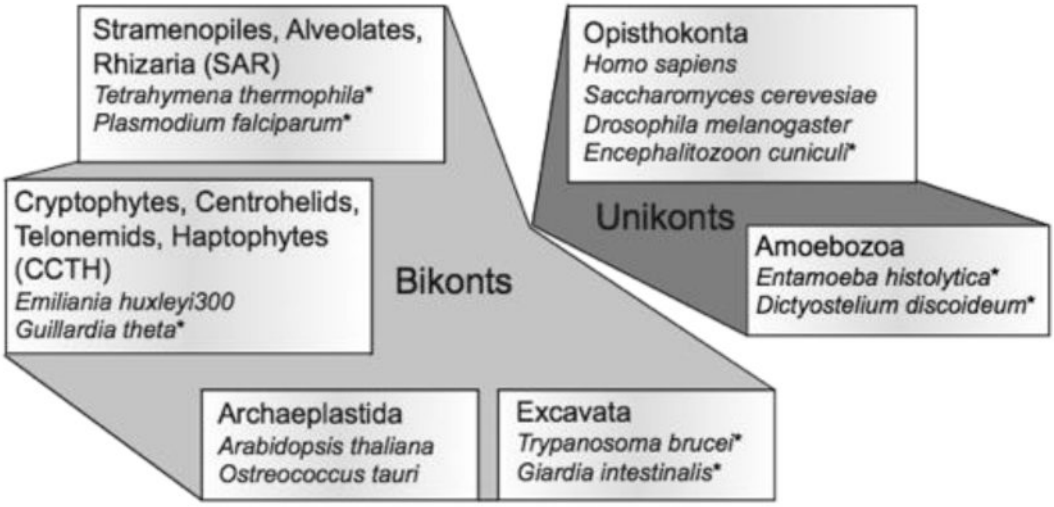

Figure 1.

Six eukaryotic supergroups including, in each case, a few members in which kinesins have been unambiguously identified. Organisms thought to be more deeply rooted are indicated (*).

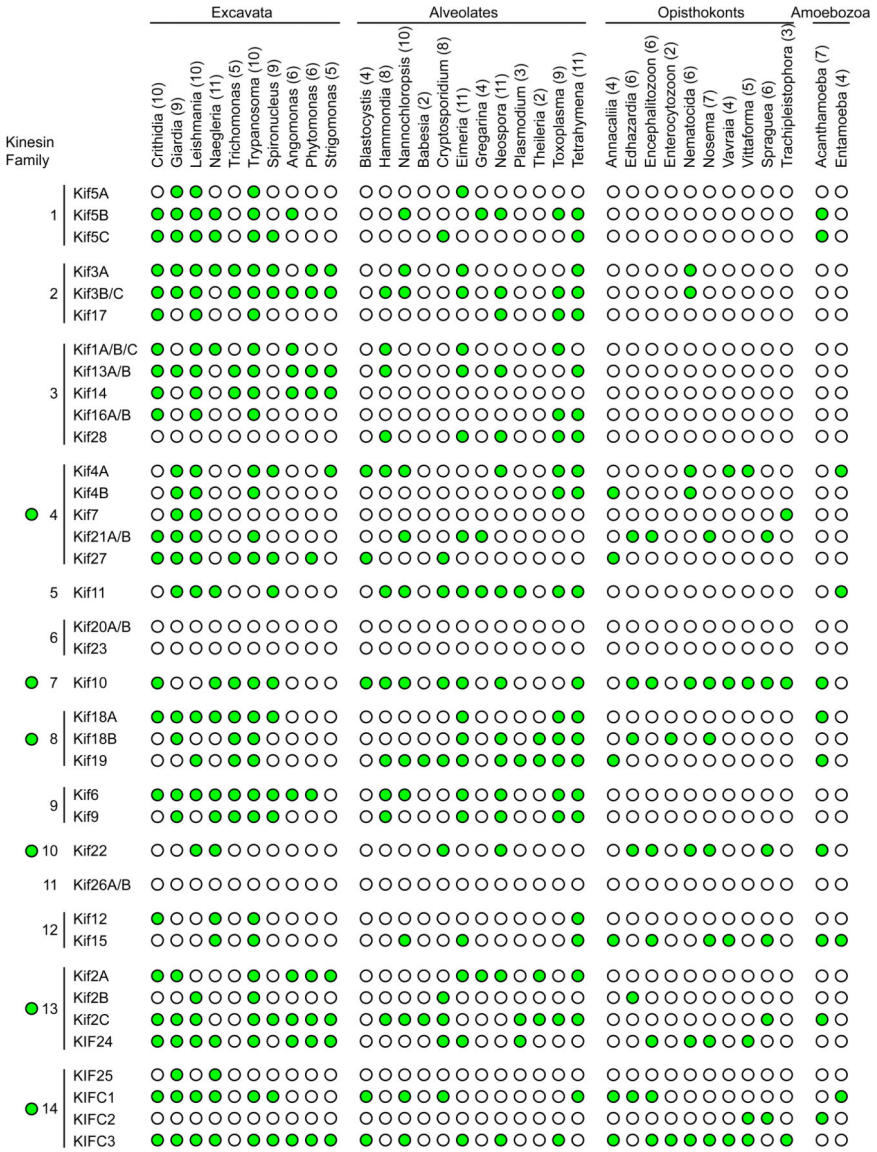

Figure 2.

Distribution of kinesins in deep-rooted eukaryotes. We manually analyzed the genome of several protists from the groups Excavata, Alveolates, Opisthokonts and Amoebozoa searching for kinesins. Presence of a kinesin member in the genome is indicated with a green filled circle. The number in parenthesis after the name indicates the number of kinesin families for each organism.

We have checked the genomes of the next eukaryotes: **Acanthamoeba** (*A castellanii*), **Angomonas** (*A deanei*), **Anncaliia** (*A algerae*), **Babesia** (*B. bovis, B. microti*), **Blastocystis** (*B. hominis*), **Crithidia** (*C. fasciculate*), **Cryptosporidium** (*C. hominis, C. muris, C. parvum*), **Edhazardia** (*E. aedis*), **Eimeria** (*E. acervulina, E. brunetti, E. falciformis, E. maxima, E. mitis, E. necatrix, E. praecox, E. tenella*), **Encephalitozoon** (*E. cuniculi, E. hellem, E. intestinalis, E. romaleae*), **Entamoeba** (*E. dispar, E. histolytica, E. invadens, E. moshkovskii, E. nuttalli*), **Enterocytozoon** (*E. bieneusi*), **Giardia** (*G. intestinalis*),

Gregarina (*G. niphandrodes*), **Hammondia** (*H. hammondi*), **Leishmania** (*L braziliensis, L donovani, L infantum, L major, L mexicana, L tarentolae*), **Naegleria** (*N. gruberi*), **Nannochloropsis** (*N. gaditana*), **Nematocida** (*N. parisii*), **Neospora** (*N. caninum*), **Nosema** (*N. bombycis, N. ceranae*), **Phytomonas spp** (different isolates), **Plasmodium** (*P. berghei, P. chabaudi, P. cynomolgi, P. falciparum, P. gallinaceum, P. knowlesi, P. reichenowi, P. vivax, P. yoelii*), **Spironucleus** (*S. salmonicida*), **Spraguea** (*S. lophii*), **Strigomonas** (*S. culicis*), **Tetrahymena** (*T. thermophila*), **Theileria** (*T. annulata, T. equi, T. orientalis, T. parva*), **Toxoplasma** (*T. gondii*), **Trachipleistophora** (*T. hominis*), **Trichomonas** (*T. vaginalis*), **Trypanosoma** (*T. brucei, T. congolense, T. cruzi, T. evansi, T. grayi, T. rangeli, T. vivax*), **Vavraia** (*V. culicis*), and **Vittaforma** (*V. corneae*).