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Evolution of the centrosome, from the periphery to the center

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Abstract

Centrosomes are central organelles that organize microtubules (MTs) in animals, fungi and several other eukaryotic lineages. Despite an important diversity of structure, the centrosomes of different lineages share the same functions and part of their molecular components. To uncover how divergent centrosomes are related to each other, we need to trace the evolutionary history of MT organization. Careful assessment of cytoskeletal architecture in extant eukaryotic species can help us infer the ancestral state and identify the subsequent changes that took place during evolution. This led to the finding that the last common ancestor of all eukaryotes was very likely a biflagellate cell with a surprisingly complex cytoskeletal organization. Centrosomes likely derived from the basal bodies of such flagellate, but when and how many times this happened remains unclear. Here, we discuss different hypotheses for how centrosomes evolved in a eukaryotic lineage called Amorphea, to which animals, fungi and amoebozoans belong.

Introduction

Centrosomes are single-copy organelles that organize dynamic arrays of microtubules (MTs). Other basic properties of centrosomes include duplication once per cell cycle, a physical link to the nucleus, and the ability to position themselves at the cell center due to their MT organizing capacity [1]. The centrosome of animal cells is formed by two centrioles surrounded by a pericentriolar material (PCM) from which MTs are nucleated [2]. Other well characterized examples include the acentriolar centrosomes of yeasts and the slime mold *Dictyostelium discoideum*. The budding yeast centrosome, called spindle pole body (SPB), is a multi-layered organelle embedded within the nuclear envelope. The outer layer organizes cytoplasmic MTs and the inner layer organizes spindle MTs during mitosis. The fission yeast SPB has no obvious layer structure and is inserted into the nuclear envelope only during mitosis but otherwise shares many features with its budding yeast counterpart [3]. In *Dictyostelium*, the nucleus-associated body (NAB) consists of a layered core surrounded by a corona of more amorphous material from which the MTs are organized [4]. Despite this great diversity of shape, key functional and molecular aspects are shared between the centrosomes of animals, yeasts and *Dictyostelium*, suggesting a common evolutionary origin [3,4].

The older centriole within the animal centrosome, called mother centriole, can also migrate to the cell periphery and nucleate the assembly of a cilium [5]. In this context, the mother centriole is functionally similar to the basal bodies (BBs) that nucleate cilia or flagella in a range of unicellular eukaryotes. This suggests that centrioles and BBs are related structures, which is strongly supported by both ultrastructural and molecular comparisons [6–9]. This conservation and the presence of centrioles/BBs in all branches of the eukaryotic tree of life implies that the centriolar structure evolved only once and was already present in the last eukaryotic common ancestor (LECA), which is thought to have lived 1.1-1.7 billion years ago [1,6,10,11]. Furthermore, centrioles/BBs are only found in species that form cilia during at least part of their life cycle, which supports that centriole function is tightly and ancestrally linked to ciliary assembly [8,12,13]. Thus, centrioles and cilia were already present in the LECA, but is this also true of centrosomes? To answer this question, we need to figure out what this ancestor was like.

The LECA was a flagellate with a complex cytoskeleton

Observing living species can help us identify ancestral traits and thus infer the cellular organization of the LECA. Determining the ancestral state and subsequent evolution of a given trait requires knowledge of the phylogenetic relationships between species, especially the structure of deep-branching groups and the position of the root. Important progress has been made in this respect through sequencing of species at key positions in the phylogenetic tree, and development of phylogenomics. The position of the root is still unclear, but consensus is emerging on the constitution

of deep-branching “supergroups” (Figure 1) [14,15]. Once phylogenetic relationships are established, the cytoskeletal features are analyzed across the supergroups to infer the ancestral traits. Despite the apparent variability of cytoskeletal organization in eukaryotes, careful comparison of individual components of the cytoskeleton allows us to identify many conserved, and thus likely ancestral features. The LECA was probably a biflagellate cell with a complex cytoskeleton resembling extant protists referred to as typical excavates (Figure 1) [16,17]. Typical excavates are heterotrophic cells with a ventral feeding groove and two flagella with distinct functions: one for motility (usually gliding motility) and the other for feeding. In most flagellates, BBs are associated with microtubular and fibrous roots of fixed morphology and position within a given species (Figure 2) (16). These roots connect the BBs to each other and organize the whole cellular architecture (17). In typical excavates, the oldest BB (referred to as BB1) at the base of the posterior flagellum is associated with microtubular roots (called R1, R2 and S – R stands for root and S for singlet as the S root is formed by a single MT) that delineate the ventral groove in which the posterior flagellum is encased. The youngest BB (BB2), at the base of the anterior flagellum, is associated with two microtubular roots (R3 and R4), one of which (R3) organizes an array of superficial MTs supporting the dorsal part of the cell (the dorsal fan). After cell division, the anterior centriole moves to the posterior position and acquires the corresponding roots, a process called flagellar transformation [16,17]. The conservation of this architecture across all supergroups (Figure 1, 2) [17], together with the finding that the eukaryotic tree is possibly rooted within excavates [14], thus supports that the LECA had an exquisitely complex excavate-like cytoskeletal architecture.

Was the mitotic apparatus ancestrally associated to the BBs in the LECA?

A conserved centrosome function is to facilitate the assembly of the mitotic spindle and control its position within the cell [2]. In animal cells, the centrosome forms around centrioles that also drive ciliary assembly, but does this reflect the ancestral state? In various extant species, spindle MTs are organized by nuclear MT-organizing centers (MTOCs) that are separate from the BB complex. Nuclear MTOCs and BBs could thus be derived from ancestrally distinct MTOCs [18]. Different hypotheses have indeed been proposed to account for centriole/BB formation during early eukaryotic evolution [11,18,19]. What happened prior to the LECA is likely to remain a mystery, however, since there are no extant lineages descending from earlier eukaryotic ancestors, and no fossil record. All we can say is that key centriole/BB and ciliary components originated in eukaryotes, supporting the idea that BBs and cilia formed endogenously at some point between the first eukaryotic common ancestor (FECA) and the LECA [6,11,19]. Observation of extant species can nevertheless help us infer the configuration of mitosis in the excavate-like LECA. In some of the few typical excavates and other deep-branching protists for which mitosis has been observed, the spindle appears to be organized in close vicinity of the BB complex [20–22]. In *Chlamydomonas*, BBs are attached to the spindle poles

and perturbing BB numbers alters spindle morphology - similar to the effects induced by abnormal numbers of centrioles in animal cells [23]. In addition, ciliated/flagellated eukaryotes in which the spindle is organized from MTOCs independent of the BB complex, such as *Trypanosoma*, *Plasmodium* or ciliates, are highly derived with respect to the ancestral cytoskeleton [17,24]. It is thus conceivable that the mitotic spindle was already linked to the BB complex in the LECA. Future characterization of additional eukaryotic species, in particular excavate-like flagellates, will help addressing this question.

When and how many times did the centrosome evolve?

Deciphering centrosome evolution is not a trivial task, in part because some key centrosome features are shared with the BB complex of flagellates. Furthermore, many eukaryotes can take different forms during their life cycle or in response to a change in their environment. In some species, the BB complex is internalized and acts as a centrosome only transiently. For instance, flagellated fungi possess an encysted stage called zoosporangium, in which MT arrays are organized by a centriolar centrosome initially derived from the BB complex [25]. The slime mold *Physarum polycephalum* produces swarm cells that interconvert between flagellates and amoebae depending on the environment [26]. In amoebae, the BBs adopt a central position, the microtubular roots are reduced and a MTOC anchored at the anterior BB nucleates interphase and mitotic MTs [27,28]. The LECA was possibly also capable of switching to amoeboid motility, as this mode of actin-based motility and key associated actin regulators are found across the tree of eukaryotes [29]. Delineating the evolutionary history of the centrosome thus not only involves understanding the evolution of individual cellular architectures but also of complex life cycles.

Another obvious approach is to trace the evolution of centrosome components, which is complicated by the fact that most components of the animal centrosome are required for centriole assembly [9]. These include ancestral proteins, but also proteins that appeared later in evolution to control centriole assembly or ciliogenesis – for instance, the kinase Plk4 and the centriole component CP110 are not ancestral [6]. Core components of the yeast SPBs and *Dictyostelium* NAB have also been identified, but they are overall not conserved outside their respective lineages [3,30]. The large scaffolding proteins that compose the PCM are thus probably the most relevant to decipher centrosome evolution. In animal cells, PCM components are recruited around the centrioles in a hierarchical manner and serve as docking sites for the γ -tubulin complexes that nucleate MTs [31]. These include CEP152 (Centrosomal Protein of 152 kDa; called asterless/asl in *Drosophila*) and CEP192 (Centrosomal Protein of 192 kDa; SPD-2 in *C. elegans* and *Drosophila*), which are also required for centriole assembly, as well as pericentrin (PCNT; D-PLP in *Drosophila*) and CDK5RAP2/CEP215 (Cyclin-Dependent Kinase 5 Regulatory subunit-Associated Protein 2/Centrosomal Protein of 192 kDa;

centrosomin/CNN in *Drosophila*) [31]. Outside of animals, orthologs of PCM proteins have been characterized in yeasts and *Dictyostelium*. In yeasts, CDK5RAP2 orthologs (Spc72 in *S. cerevisiae* and Pcp1 in *S. pombe*) dock γ -tubulin complexes at the outer layer of the SPB, whereas PCNT orthologs (Spc110 in *S. cerevisiae* and Mto1 in *S. pombe*) dock γ -tubulin complexes at the inner layer [3]. CEP192 and CDK5RAP2 orthologs in *Dictyostelium* are NAB components, and CDK5RAP2 is required for organizing the PCM equivalent - the corona - and for MT organization [30,32]. Interestingly, CDK5RAP2 cooperates with another NAB component called CP148, which shares common features with both PCNT and CEP152 [32], although *bona fide* orthologs of CEP152 are not identified outside animals [33,34].

Thus, centrosomes in *Dictyostelium*, yeasts and animals are functionally and molecularly related, but do they derive from a unique ancestral centrosome? These species all belong to the supergroup Amorphea, which also encompasses several lineages of protists (Figure 1). Analysis of extant species from early-branching amorphean lineages such as Amoebozoa (to which *Dictyostelium* and *Physarum* belong), and the protist lineages Apusomonada and Breviata support that the ancestor of all Amorphea was still an excavate-like flagellate (Figure 2, 3) [17,35]. In Amoebozoa, the excavate-like cytoskeleton was then simplified (*e.g.* in *Physarum*) or lost (*e.g.* in *Dictyostelium*) several times independently [35,36]. The actomyosin-based pseudopodial motility was also likely ancestral in Amoebozoa [36], with the ancestor possibly interconverting between flagellates and amoebae like in *Physarum*. It is tempting to speculate that the ancestor of all Amorphea had also an amoeboid stage, possibly with the internalized BB complex acting as a centrosome, which would thus constitute the ancestral centrosome (Figure 3). The finding that orthologs of PCM proteins are only found in Amorphea [6,33] suggests a possible link with such an evolution. Of note, the excavate *Naegleria gruberi* uses amoeboid motility with actin-based mechanisms very similar to those employed by amoebozoan and animal cells, but it does so in the complete absence of MTs or BBs [37]. Even though amoeboid motility is possibly ancestral, the BB complex might thus have acquired a new role in the amorphean lineage in organizing dynamic arrays of MTs (via PCM components) during amoeboid phases. Yet some amorphean lineages that diverged later than Amoebozoa, such as Apusomonada and Breviata, are excavate-like flagellates with no known amoeboid stage, although they form pseudopods for feeding [35,38,39]. The apusomonad *Thecamonas trahens* encodes putative orthologs of CEP192 and PCNT in its genome, which possibly contribute to organizing MTs from the BB complex. Interphase *Thecamonas* cells do not differ much from the typical excavate pattern (Figure 2), but PCM components could be involved in organizing mitotic MT arrays. Likewise, in animal cells, CEP192, PCNT and CDK5RAP2 are present at interphase centrosomes but recruited in much larger amounts during mitosis [31]. Even in the hypothesis that spindle poles were ancestrally connected to the BB complex, the composition of the connector might have changed during evolution of Amorphea. This could have happened concomitantly to a modification of the link between the BB complex and the

interphase nucleus. In Amorphea, centrosomes are tethered to the nucleus via the LINC (LInker of Nucleoskeleton and Cytoskeleton) complex, but this complex is only partially found in other supergroups [40], suggesting that the ancestral link was somehow distinct. In *Chlamydomonas* for instance, centrin-containing fibers connect BBs to both the interphase nucleus and to the spindle poles [23]. The possibility that PCM proteins evolved to perform functions independent of amoeboid motility, such as spindle organization, and the persistence of flagellates in the amorphean tree after the divergence of Amoebozoa would favor the idea that the centrosomes of Amoebozoa and other centrosomes derived independently from distinct flagellate ancestors (18, 24, 25). Alternatively, extant apusomonads and breviate might have derived from amoeboflagellate ancestors by losing amoeboid motility, in which case the hypothesis of a common origin of centrosomes would still stand.

Animals and fungi belong to a group called Opisthokonta, in which flagellated cells have a single posterior flagellum. Overall, the flagellar apparatus of opisthokonts is highly simplified compared to typical excavates [17]. In the zoosporangium of early-branching flagellated Fungi such as chytrids, MTs are organized from a centriolar centrosome similar to the centrosome of animal cells [3,25]. During the evolution of higher Fungi, centrioles and flagella were subsequently lost and replaced by SPBs, which anchor MTs via PCNT and CDK5RAP2-orthologs [3]. One interesting model to explain centrosome evolution in Fungi is that a SPB precursor first associated with the centriolar centrosome of the fungal ancestor, then interacted with PCM components and the nuclear envelope, eventually replacing the centrioles [3]. In line with this hypothesis, the SPB of the zygomycete *Coemansia reversa* contains a cylindrical structure formed by nine MTs reminiscent of a centriole [41], even though centriole assembly factors are missing from its genome [3]. If the fungal ancestor had a centriolar centrosome, is this also true for the last common ancestor of opisthokonts? Several very diverse but essentially unicellular lineages diverged from the animal ancestor later than Fungi. Among them, some predominantly flagellated species have actually very complex life cycles that include cysts and even syncytium-like stages [42], suggesting that the BB complex might be converted into a centrosome during specific stages like in chytrids. The best-studied opisthokont flagellates are choanoflagellates, the sister group to animals. In choanoflagellates, the older BB anchors a crown of stable cortical MTs that symmetrically surrounds the cell body. During mitosis, the flagella are retracted and the BBs organize the mitotic spindle [43]. It was recently discovered that choanoflagellates can switch to amoeboid motility when submitted to spatial confinement, a switch that involves remodeling the MT cytoskeleton [44]. Gaining knowledge of more diverse lineages will be key in the future to determining whether centrosome evolution was linked to the evolution of life styles in Amorphea, and how many times it derived from the BB complex.

Centrosome evolution in Metazoa

Centrosome evolution within animals is not well understood either. In particular, little is known about centrosome structure in early-branching lineages such as sponges and ctenophores, the two possible earliest-branching animal lineages [45]. Sponge feeding cells, called choanocytes, have received some attention because of their striking resemblance with choanoflagellates, which led to the hypothesis that metazoans derived from a choanoflagellate-like ancestor [45,46]. In choanocytes, the BB has a basal foot and a ciliary rootlet like in multiciliated cells of diverse bilaterian animals [47]. The basal foot is an appendage that anchors MTs and is required for polarizing the BBs at the apical surface of multiciliated cells. In mouse and planarian flatworms, basal foot assembly requires a conserved protein called ODF2 (Outer Dense Fiber 2)/cenexin [48,49], which is also present in the genome of early-branching animals. In addition, ODF2 is required for the assembly of appendages that decorate the mother centriole in a nine-fold symmetrical fashion in vertebrate cells [50]. Subdistal appendages share additional components with basal feet and also serve as anchoring sites for MTs [47,48,51,52]. Observations in choanocytes suggest that the presence of a single MT-anchoring appendage is the ancestral state for animal monociliated cells. In most opisthokont flagellates, the mature BB anchors a single microtubular root [42,53,54], the symmetrical MT array of choanoflagellates being likely a derived character [55]. Thus, the basal foot and its associated MTs possibly derived from the microtubular root of earlier-branching opisthokonts, which itself might be homologous to one of the posterior roots of the excavate-like cytoskeleton. Interestingly, conserved proteins of the VFL1 (Variable FLagella number 1) and VFL3 families are required for centriole polarization and proper assembly of microtubular roots in *Chlamydomonas* and *Paramecium* [56–58], basal foot assembly in planarian multiciliated cells [49], and subdistal appendage function at the mammalian centrosome [59]. This suggests that some molecular aspects underlying MT anchorage at the centrioles/BBs are ancient and conserved between the microtubular roots of flagellates and centriole appendages of vertebrate cells.

Concluding remarks

While much remains to be discovered about the evolution of the cytoskeleton and cellular architecture in general, important progress has been made in the past few years to pinpoint key ancestral features. This has been achieved by carefully examining the ultrastructure and life cycle of extant species at key positions within the tree, and decoding their genomes. Pursuing these efforts to describe a larger part of eukaryotic diversity will undoubtedly bring interesting new discoveries in the future. Better understanding centrosome evolution will also require gathering molecular data in additional amorphean lineages. Molecular tools are now available for working with chytrids and choanoflagellates, for instance [60–62]. Study of the function of orthologs of PCM components in these species will be of tremendous interest to decipher centrosome evolutionary history. Hopefully, such tools will be made available not only for additional flagellates but also for species that developed

divergent centrosomes [63]. This will be key to better understanding cytoskeleton evolution and how it was linked to changes in cell shape, division, and motility.

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Glossary

BB: basal body.

Deep-branching groups: groups that emerge at the base of a phylogenetic tree and are the closest from the root. Also called basal or early-branching groups.

First eukaryotic common ancestor (FECA): the first eukaryotic descendant of the last common ancestor of eukaryotes and their closest archeal relatives. The FECA is thus the oldest ancestor of eukaryotes that is not shared by any other extant lineages.

Last eukaryotic common ancestor (LECA): the last common ancestor of all extant eukaryotes.

MT: microtubule.

Nucleus Associated Body (NAB): the acentriolar centrosome of *Dictyostelium discoideum* and other Dictyosteliids.

Phylogenomics: field that aims at reconstructing the evolutionary relationships among species by comparing entire genomes, or large portions of genomes. By contrast, traditional phylogenetic analyses typically used single genes or a small number of genes.

Root (cytoskeleton): in addition to the BBs and flagella, the flagellar apparatus of protists comprises cytoskeletal elements called roots that have definite morphologies and positions in the cell. This includes microtubular roots, composed of stable MTs in specific numbers, and fibrous roots. The microtubular roots R1-R4 and S are found in extant members of all major groups of eukaryotes and were thus likely already present in the LECA.

Root (phylogeny): the root of a phylogenetic tree represents the ancestral lineage. The root of the eukaryotic tree is the LECA.

Spindle Pole Body (SPB): the acentriolar centrosome of higher fungi.

Supergroup: an informal term to design the most inclusive ensemble of species for which there is reasonable evidence that they form a monophyletic group. Most of the currently known eukaryotic diversity can be assigned to one of 7 supergroups, which all emerged relatively rapidly (about 300 million years) after the LECA (Figure 1) [14].

Figure legends

Figure 1. Presence of a centrosome and distribution of modes of motility in Amorphea.

A) Current view of the eukaryotic tree of life. The Last Eukaryotic Common Ancestor (LECA) was a biflagellate with a cytoskeleton similar to a subgroup of excavates referred to as ‘typical excavates’. This pattern is still present in extant species belonging to all supergroups (capitalized terms). Note that the supergroup Excavates is possibly not monophyletic (indicated by an asterisk). Adapted from [14,15]. **B)** Distribution of cell morphology and centrosome-like organelles in Amorphea. CC: centriolar centrosome; NAB: nucleus associated body; SPB: spindle pole body. Adapted from [44].

Figure 2. Organization of the MT cytoskeleton in the apusomonad *Thecamonas trahens*. The typical excavate cytoskeleton is to a large extent conserved in this amorphean flagellate. Among the conserved microtubular roots (R1-4, S), only R4 is missing. Unlike typical excavates, apusomonads use their posterior flagellum for gliding locomotion. During cell division, the anterior BB (BB2) matures into a posterior BB (BB1), and roots R3 and R4 (when present) are replaced by R1 and R2, respectively. Adapted from [38].

Figure 3. Possible scenarios for the evolutionary origin of centrosomes. The last common ancestor of Amorphea, which is estimated to have lived ~300 million years after the LECA, was still sharing most traits of the excavate-like cytoskeleton present in the LECA. Key centrosome matrix proteins can be traced back to the origin of Amorphea (CEP192/SPD2, CDK5RAP2/CNN), or at least to the last common ancestor of apusomonads and opisthokonts (PCNT/D-PLP). Emergence of PCM components might have been linked to the evolution of an amoeboid form in which the BB complex was internalized to form a centrosome. Amoeboid stages would have then been lost secondarily in apusomonads and breviate. Alternatively, the centrosome possibly derived more than once from the BB complex. PCM components might have first evolved for other functions, like organizing mitotic MTs, even though the BBs were possibly also connected to the spindle poles in the LECA.

Figure 1

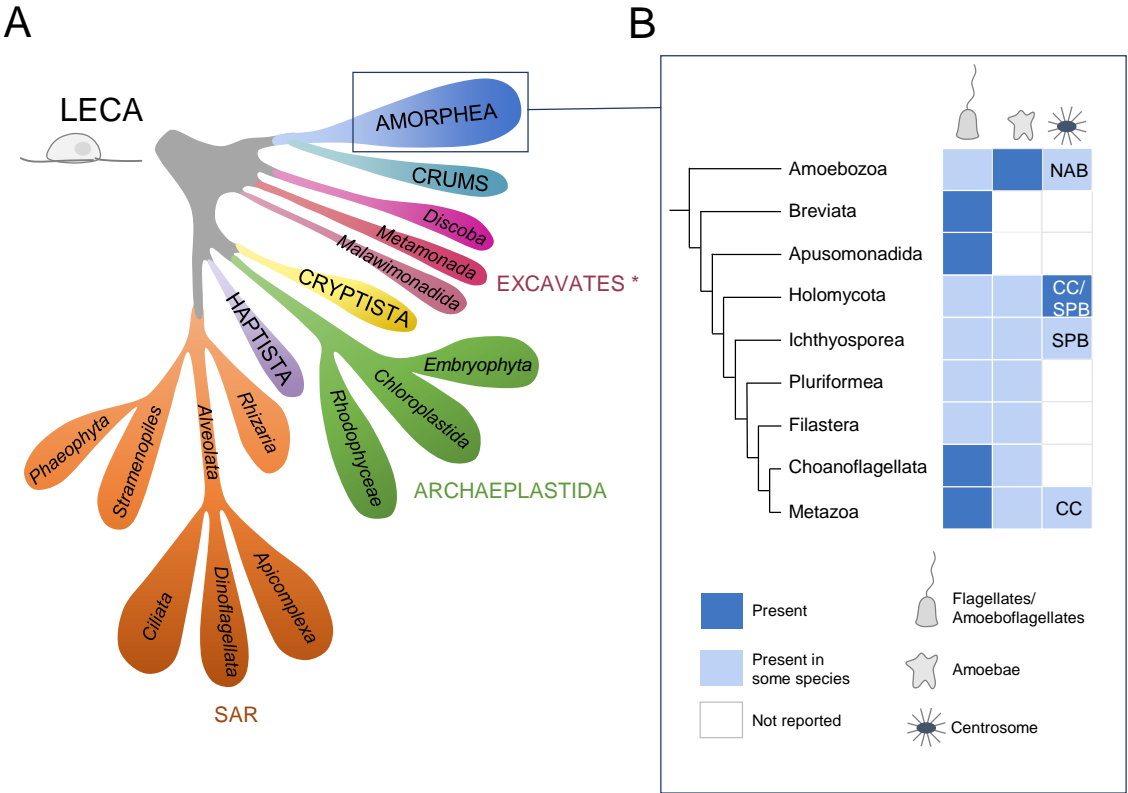


Figure 2

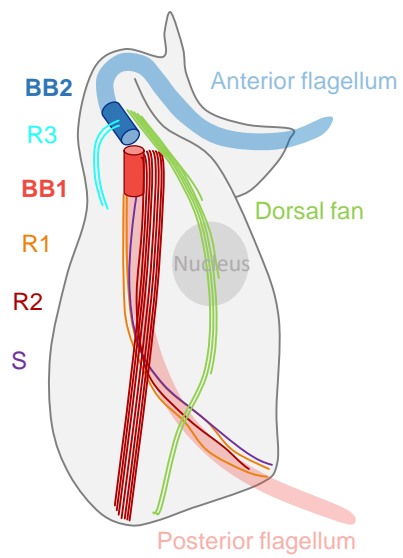


Figure 3

