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Centriole asymmetry determines algal cell geometry

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Abstract

The mechanisms that determine the shape and organization of cells remain largely unknown. Green algae such as Chlamydomonas provide excellent model systems for studying cell geometry due to their highly reproducible cell organization. Structural and genetic studies suggest that asymmetry of the centriole (basal body) plays a critical determining role in organizing the internal organization of algal cells, through the attachment of microtubule rootlets and other large fiber systems to specific sets of microtubule triplets on the centriole. Thus to understand cell organization, it will be critical to understand how the different triplets of the centriole come to have distinct molecular identities.

Introduction: why study cell geometry in green algae

An important unsolved question in cell biology is what mechanisms determine the shape and internal organization of cells. One reason this question has been so hard to answer is that the transformed cell lines so popular in cell biological research appear to be quite amorphous, making it difficult to compare one cell to the next. In this regard, algal cells stand out as excellent systems for exploring cell geometry, because of their highly reproducible shapes and well-defined geometries. Consider for example the *Chlamydomonas reinhardtii* cell. Every cell shares the same egg-like shape, with exactly two flagella located at the apical pole and pointing in opposite directions. The chloroplast always forms a cup abutting the opposite pole from the flagella, and the nucleus sits in a depression in the cup. This much is just cell polarity. But *Chlamydomonas* cells also have a defined chirality [1]. An eyespot is always located at a longitudinal angle of 45 degrees clockwise relative to the plane of the two flagella, nearer to the daughter basal body. Another important organelle, the contractile vacuole, also occupies a defined position. Each cell has two contractile vacuoles, and these are located near the apical pole, with the pair of contractile vacuoles running perpendicular to the flagellar plane. Thus most, and possibly all, components of the Chlamydomonas cell are located in predictable positions that do not vary from one cell to another.

Where does all this organization come from? Here we hypothesize that self assembly of centrioles into chiral structures ultimately determines the global organization of algal cells. There is substantial evidence in favor of this view, and at least it provides a falsifiable model for how the geometry of the cell is built.

The flagellar apparatus patterns the whole algal cell

The dominant structural feature of unicellular green alga is the “flagellar basal body apparatus”, which consists of a pair of centrioles called basal bodies, each of which nucleates a flagellum. Centrioles or basal bodies are cylinders composed of nine microtubule triplet blades, arranged in a turbine-like chiral arrangement (Figure 1A). New centrioles usually arise adjacent to pre-existing centrioles, although centrioles can form de novo in algal cells if existing centrioles are removed [2]. When the newly formed centriole is first forming it is called a pro-centriole or pro-basal body, and when it reaches its final size it is termed a daughter centriole or daughter basal body. The daughter basal body remains linked to the pre-existing centrioles that it formed next to, and this centriole is then named a mother centriole. In *Chlamydomonas*, the mother and daughter centrioles are joined by two fibers, a distal striated fiber and a proximal connecting fiber (Figure 1B). The microtubule triplets of the centriole extend into the microtubule doublets that form the axoneme, which is the core structural framework of the flagellum.

After the basal bodies themselves, the next most visible structure is the nucleus-basal body connector or NBBC (Figure 1C), a pair of fibrous bundles composed of centrin which run from the basal bodies down to the nucleus [3]. Two newly-formed pro-basal bodies (procentrioles) can often be seen on either side of the mature centriole pair (see for example [4]). Four microtubule bundles, called rootlets, extend out from the basal bodies [1]. Two of these rootlets contain four microtubules, and two contain two microtubules and together the four rootlets make a cross-shaped pattern that joins in the middle of the two centrioles. The rootlets extend out from the basal body region, remaining associated with the surface of the cell, down towards the posterior of the cell. The components of the flagellar basal body apparatus are physically attached to each other and can be purified as a stably associated complex [3].

The rootlets and fibers that project from the flagellar apparatus, in turn, appear to pattern the rest of the cell (for illustration of Chlamydomonas cell architecture, see Figure 2). The NBBC fixes the nucleus in position beneath the basal body pair, as evidenced by the fact that the nucleus position is randomized in vfl2 mutants in which the NBBC is missing [5]. The position of the contractile vacuoles is determined by the position of the basal bodies such that when basal bodies are mis-positioned the contractile vacuole shows a correspondingly defective position [5]. The position of the eyespot is determined apparently by the four-membered rootlet arising from the daughter basal body [1]. Not only does the longitudinal position of the eyespot correlate with the position of this rootlet, the latitude of the eyespot correlates with the length of the rootlet, and in a mutant with shorter rootlets, the eyespot shifts to a more apical position [6].

In addition to determining the position of specific sub-cellular structures, the flagellar apparatus likely influences global cell architecture. For example in the alga *Ochromonas*, microtubules arising from the flagellar apparatus extend in a rotationally asymmetric arrangement that appears to sculpt the shape of the cell [7]. In *Chlamydomonas*, the position of the microtubule rootlets appears to determine the cell division axis [8] and disruption of centriole positioning in ASQ2 mutants causes randomization of mitotic spindle orientation [9]. When algal cells fuse during mating, they are drawn together by their flagella and actin-rich mating structures into a defined relative orientation. The position of the mating structure in mt+ cells is a mirror image of its position in mt- cells, so that when the cells fuse, their flagellar basal body apparatus end up in a parallel direction so that both eyespots of the fusion product are pointing the same way [1]. Thus, at least in algae, the flagellar basal body apparatus plays a determining role in organizing the geometry of the entire cell. We thus

reduce the question of the origin of cell geometry to a simpler question - the origin of flagellar apparatus geometry.

Asymmetry of flagellar apparatus arises from asymmetry of the centriole

Since centrioles anchor the fibers of the flagellar apparatus, and mutants that displace centrioles produce corresponding displacements of flagellar apparatus structures [5], we will consider the centriole as determining a geometrical reference frame by which we can measure the position of other structures. For this purpose, the nine individual microtubule triplets of each basal body can be given numbers to distinguish them. The original basis for such numbering was the position of the central pair microtubules in the axoneme of animal sperm [10], but since the central pair rotates in *Chlamydomonas* flagella, an alternative numbering method was put forth by Hoops and Witman based on the direction of the flagellar bending [11]. In this scheme, triplets 5 and 6 face in the direction of flagellar bending during the power stroke, and triplet 1 faces away from the bend. Since the two basal bodies are oriented 180° opposite to each other, with their number 1 triplets facing each other, the two flagella beat away from each other in a breast stroke motion [11].

In reference to this numbering scheme, we find that the different fiber systems of the flagellar apparatus are attached to distinct sub-sets of triplets. The distal connecting fiber, which joins the mother and daughter centriole at their distal end, attaches to triplets 1, 2, and 9 on each of the mature basal bodies [12]. The VFL1 protein that mediates mother-daughter joining is found near triplets 1 and 2 [13]. Interestingly, this protein is also required for centriole pairs to have proper 180 degree rotational orientations. Another protein required for proper connection between the mother and daughter centrioles, ASQ2, does not localize in this region, however, but rather to a zone between the two NBBC fibers [9], so clearly there are multiple components necessary to properly connect and orient the two centrioles in a pair.

The distal striated fiber is not the only fiber with a defined attachment point. At the proximal end of the mother-daughter pair, the proximal connecting fiber joints triplets 2 and 3 on each centriole to triplets 8 and 9 on the other [12]. The so-called sinister fiber attaches triplets 2,3, and 4 to the 4-member rootlet, while the dextral fiber attaches triplets 8 and 9 to the 2 membered root, while the NBBC attaches to triplets 7 and 8 [12].

The asymmetry of the triplets is propagated to asymmetry of the axonemal microtubule doublets. For example, beak-like projections are visible on axonemal doublets 1, 5, and 6, while outer dynein arms are specifically missing from doublet 1 [11]. These structural asymmetries are presumably important for determining the direction of the flagellar beating movement, and indeed the activation of dynein arms is also asymmetric within the flagellum, with dyneins on doublets 2 and 3 being the most active [14].

The position of flagellar apparatus structures within the overall cell is also asymmetric, for example in Chlamydomonas the flagellar pair is always located at the apical end of the cell, opposite from the chloroplast [1]. Since pre-existing mother centrioles are located at the apical end, the process of centriole duplication ensures that daughter centrioles will also form at the apical end, thus the mother-daughter interactions of centriole duplication may play an important role in propagating the asymmetric position of the flagellar apparatus.

Symmetry breaking mechanisms

It thus appears that the chiral organization of the algal cell ultimately arises from the radial asymmetry of the basal bodies, as manifest by attachment of distinct fiber systems to

reproducibly different sub-sets of triplets. What is the source of radial asymmetry in the centrioles?

Centriole duplication could explain centriole asymmetry. Since a new centriole typically forms at a defined site on a pre-existing mother centriole, the triplet that forms closest to the nucleation site on the mother could be differentially marked, and then propagate that asymmetry to the other triplets.

Since centrioles are usually found in mother-daughter pairs, one interesting idea is induced asymmetry, whereby the presence of a nearby centriole influences the molecular fate of triplets on a neighboring centriole, for example by increasing the local concentration of a centriole-associated protein. This type of mechanism is commonplace in physics, for example if two uniformly charged conducting spheres are brought close together, mutual repulsion of like charges will induce asymmetry in the charge distribution in each sphere.

Induced or propagating asymmetry models require the asymmetry-inducing influence to come from outside the centriole itself. The other possibility is that the centriole/basal body structure is able to break symmetry within itself during assembly. The cartwheel, a radial array of nine spokes whose formation is thought to be the initiating event in centriole assembly, appears to be radially symmetric consistent with the ability of the core cartwheel protein SAS-6 to assemble into structures with nine-fold rotational symmetry [15], but radial averaging was used to obtained high resolution images in that report, hence the radial symmetry could be a self-fulfilling prophecy.

It is however possible that the initial assembly is symmetric but then some symmetry breaking step happens during later assembly stages. An asymmetrical structure called the “acorn” can be seen in the lumen of the Chlamydomonas centriole [12], and the same workers found that the EF hand protein centrin, in addition to being part of the NBBC and distal striated fibers, also forms a V-shaped fiber within the distal lumen of the centriole, attached to triplets 4 and 5 [16]. The VFL1 protein also has an asymmetric localization within the centriole as mentioned above. The acorn, the centrin V-fiber, and VFL1 protein are present early in centriole assembly, prior to attachment of the other fiber systems, and so the incorporation of one or more of these asymmetric structures during centriole assembly may constitute early symmetry breaking events [16]. Specifically, it is possible that the 9 triplets are initially identical, and then the V-fiber forms as a stochastic event on one randomly selected pair of adjacent triplets. Once the triplets are thus marked, interactions with the V-fiber could then determine the position of attachment of other structures, further elaborating the initial symmetry breaking event into a complete marking of all 9 triplets. Centrioles that form de novo [2] could be used to test intrinsic symmetry breaking, since such centrioles arise separately from any pre-existing centrioles, hence if they are still asymmetric, it would argue that the symmetry is inherent in the self-assembly of the centriole structure itself.

In addition to the question of the initial origin of asymmetry, a related question is how are the specific triplet identities determined. For instance, does triplet 8 take on the triplet 8 fate because it is adjacent to triplets 7 and/or 9, or because it is opposite triplets 4 and 3? Mutants such as the bld12 mutants of Chlamydomonas, which form centrioles with abnormal numbers of triplets ranging from 7 to 11 [17], should provide information on this process by asking how altering triplet number affects the relative position of different attached fibers. At a more molecular level, the analysis of the VFL1 protein localization to a defined set of triplets by Silflow [13], discussed above, illustrates the power of using classical genetics in green algal cells with stereotyped geometries. The availability of a proteome for the Chlamydomonas centriole [18] should provide a basis for systematic localization studies to

detect asymmetrically localized proteins. For instance, the basal body proteome protein POC1 was found to be distributed over the centriole surface in a highly non-uniform pattern, with specific accumulation at the sites where flagellar apparatus fibers join the centriole triplets [19].

Beyond algae: centriole asymmetry in eukaryotic cell geometry

Although we have focused on Chlamydomonas in this review because the most information is available in this genetically tractable system, similarly elaborate arrangement of roots and fibers is a general feature of algal cells (see for example [20,21]). But what about non-algal species?

The basic structure of the centriole is highly conserved in all species, both in terms of ultrastructure and molecular composition. In addition, the tendency for centrioles to be equipped with associated fibrous structures that reach out into the cell is also fairly universal, although the size and extent of these fiber systems varies greatly from cell type to cell type. For example in humans, the basal bodies of ciliated epithelia in the airway, ependyma, and oviduct, extend two large appendages, the basal foot and striated rootlet. As in algae, the attachments of particular fibers or structures generally involve more than one adjacent triplet in vertebrates, for example the basal foot is anchored across three adjacent triplets on basal bodies of multiciliated epithelia [22]. We therefore expect that the fundamental architectural principle of having a rotationally asymmetric centriole that extends distinct fibers or projections from defined sub-sets of triplets is going to be a fairly universal feature of eukaryotic cell organization.

The cortex of ciliates such as Tetrahymena consists of parallel rows of basal bodies, which can either be single basal bodies or associated pairs. These basal body units are attached, via striated fibers, to microtubule bundles which resemble rootlet microtubules of algae. Other cellular structures have defined positions relative to these units, for example mitochondria are docked on the cortex to one side of the basal body units, and mutations which alter the arrangement of basal body units produce a corresponding rearrangement in the position of the mitochondria [23]. The ciliate cortex can thus be viewed as an array of repeating subunits, consisting of one or two basal bodies, associated fibers and microtubule bundles that extend in defined orientations, which in turn organize associated organelles into corresponding positions, thus resembling an array of algal flagellar apparatuses.

The dynamic cell polarity systems that determine cell directionality and shape in vertebrate cells seem inconsistent with the static pre-patterning imposed in algal cells by the highly stable flagellar basal body apparatus. However, cilia in mammalian cells do tend to occupy reproducible positions and orientations relative to the axis of cell polarity, for example pointing forward in migrating cells [24]. The mammalian ortholog of the *Chlamydomonas* ASQ2 involved in centriole positioning has been shown to be required not just for proper centriole positioning in migrating vertebrate cells, but also for proper positioning of the Golgi apparatus [25]. In multiciliated epithelia, the orientation of individual ciliary units is first established by the planar cell polarity (PCP) pathway, a long-range signal that spans across entire epithelial sheets and interacts with the basal bodies via unknown mechanisms to orient them relative to the overall orientation of the tissue and body axes [26]. However, once the basal bodies have oriented in response to the PCP cue, they become locked into place [27] and at this point, the ciliary units behave very much like the flagellar apparatus of algae, with the basal bodies locked into position via interaction with associated fiber systems. Overall, it seems likely that just as in algae, centriole associated structures might play a more extensive role in cellular architecture in mammals that is generally appreciated.

Acknowledgments

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Highlights

- algal cells are excellent systems for studying cell geometry because they have highly stereotyped structures
- the organization of the algal cell is determined by the flagellar basal body apparatus
- the flagellar basal body apparatus is built according to asymmetry of the centriole

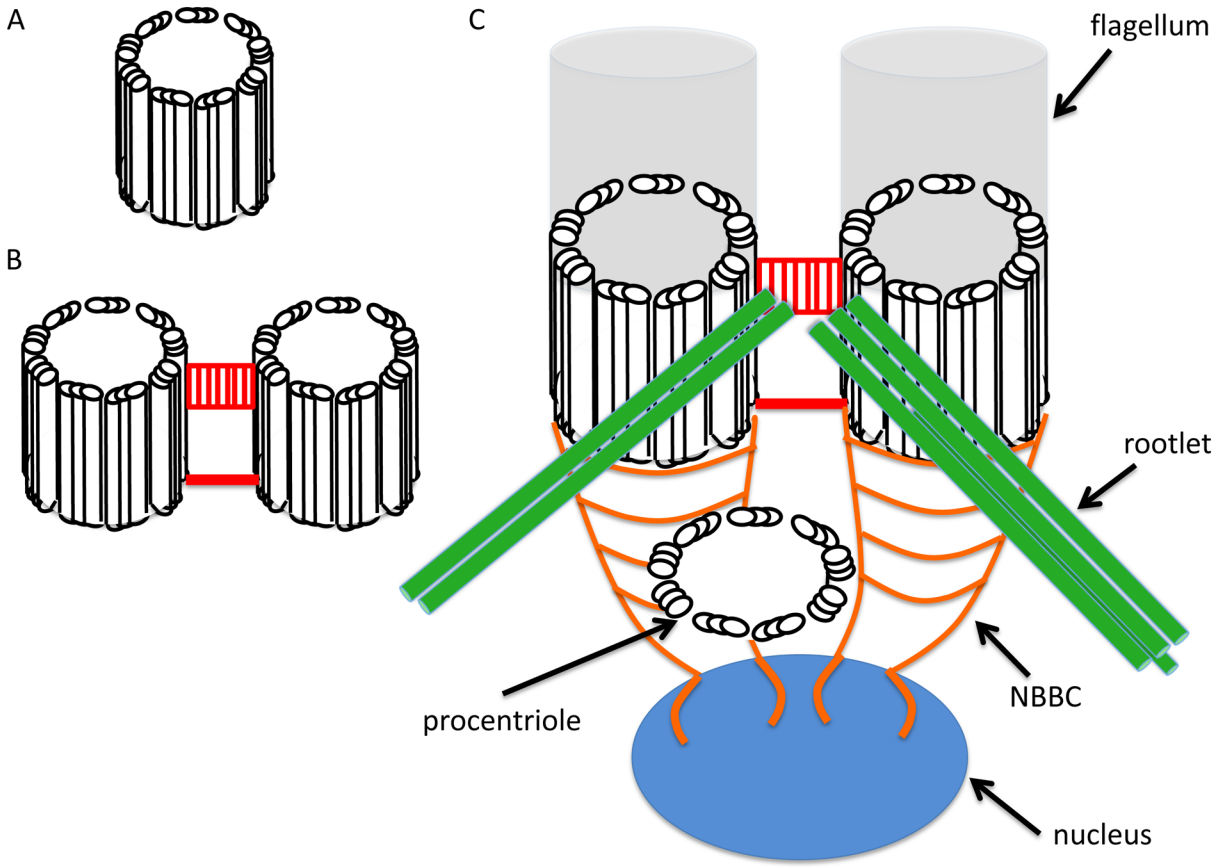

Figure 1.
Components of the flagellar basal body apparatus in Chlamydomonas. (A) Centrioles (also known as basal bodies) are cylinders composed of nine microtubule triplets. (B) Mother and daughter centrioles associate into a pair, joined by distal and proximal connecting fibers shown in red. The distal fiber is clearly striated and contains the protein centrin. It point of attachment to the centriole wall corresponds to the position of the VFL1 protein. (C) The centriole/basal body microtubule extend microtubule doublets to form flagella, microtubule-based projections from the cell surface that play motile and sensory roles. Additional components of the flagellar basal body apparatus are indicated. The rootlets are two and four-membered microtubule bundles that are attached to specific triplets of the centriole wall as described in the text. The procentriole is a newly forming basal body/centriole assembling around the cartwheel composed of SAS-6 protein.

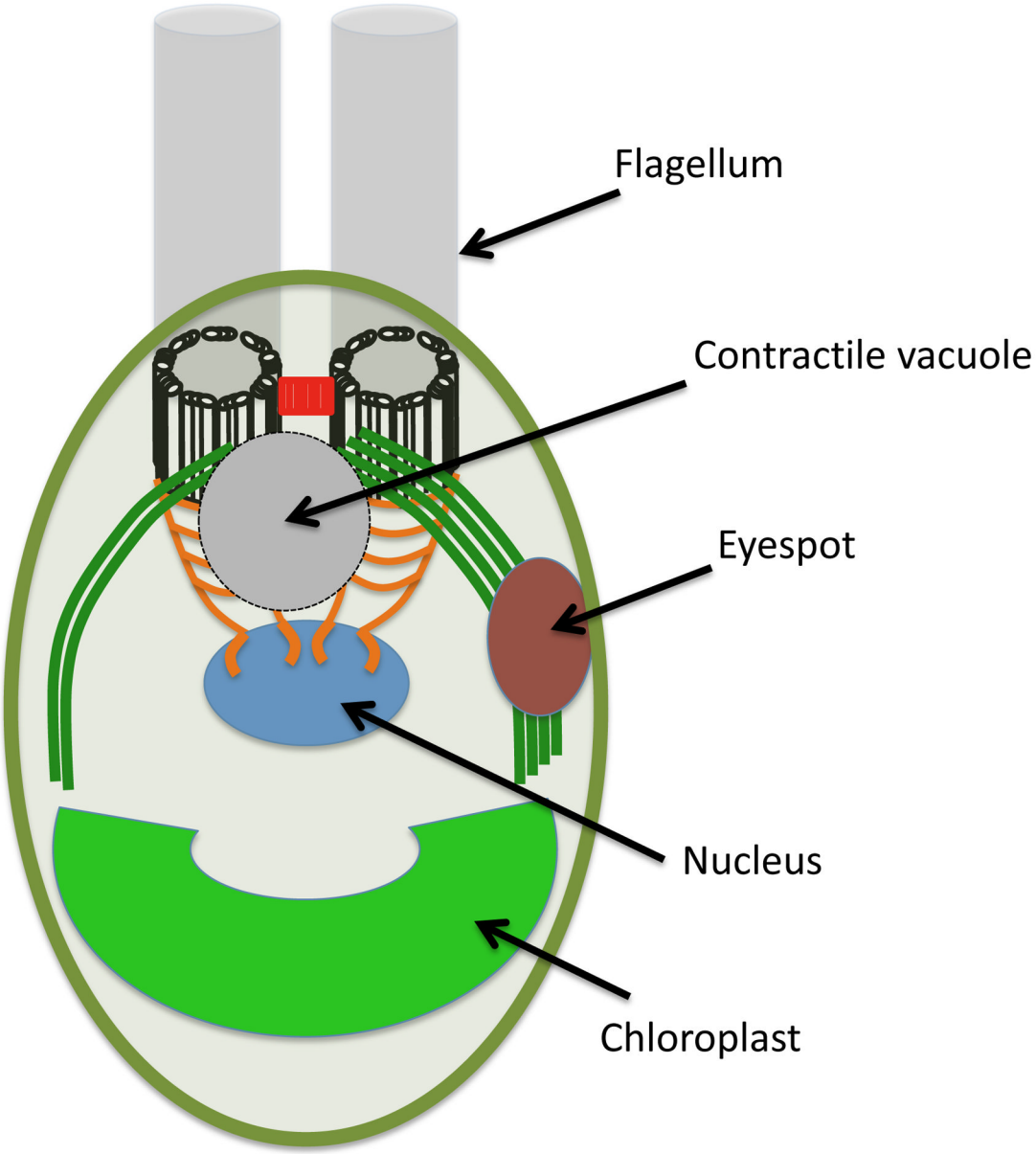

Figure 2.
Architecture of the Chlamydomonas cell, a typical green alga, showing position of the flagellar basal body apparatus from Figure 1 relative to key cellular structures as indicated.