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Centrosome: The Cell Spherical Reference System Organizer

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ABSTRACT

The orthogonal arrangement of the centrioles in the centrosome suggests that this organelle is the "spherical reference system" organizer in metazoan cells: based on two orthogonal protractors, one to manage geometry in the "x y" plane, the second in an orthogonal plane containing the "z" axis, the centrosome, through its aster of microtubules, subdivides the cell cortex in a map of distinct, wired, "labeled", discrete compartments, generating a fine-tuned polarity, indispensable to organize and manage cell geometry. Moreover, counterclockwise circumferential polarity of the mother centriole organizes the molecular basis of Metazoan bilateral symmetry.

KEYWORDS Centrosome, Bilateral Symmetry, Cell Geometry, Morphogenesis

Introduction

Centrosomes are made up of two orthogonal centrioles (centrioles and basal-bodies at the base of cilia are the same organelles) composed of nine-fold orderly symmetric equi-spaced blades of three parallel microtubules, "triplets", linked to a central hub by nine radial spokes: an axial Centriole, named "Mother" (MC) and an eccentric orthogonal "Daughter" Centriole (DC) embedded in a protein matrix called PeriCentriolar Material (PCM), made of coaxial layers and subdivided into 9 sectors/wedges corresponding to the 9 MC blades (Sonnen et al., 2012, Mennella et al., 2012; Gopalakrishnan et al., 2011): the PCM is responsible for the nucleation and anchoring of microtubules (MTs): an "aster" of non-intersecting robust MTs irradiates radially and centrifugally from the centrosome to the cell cortex. MTs are nucleated by γ-TuRCs (γ-Tubulin Ring complexes), displaced on the PCM surface and supported by a protein scaffold: the direction of each MT is the consequence of the orientation and inclination of its own γ-TuRC.

The centrosome shows unique characteristics: like the nucleus and the primary cilium (its basal body is the MC of the centrosome) it is the only organelle that exists in a single copy per cell (so ts duplication is unique and peculiar Regolini, 2014); it is also the only organelle that does not have a membrane; it is in contact through its MTs with each other organelle and each cytoplasmic and cortical location.

The centrosome plays a role in mitosis in metazoan cells, but it is not indispensable: rather, its peculiar geometric structure suggests its geometric role as the organizer of the spherical reference system of the cell.

A spherical reference system is well shown by a globe: longitude, latitude and distance from the center are the coordinates of each point.

Centrosome loss in Planaria

Planarians (flat worms) have centrioles (basal bodies of cilia) but do not build centrosomes, (two orthogonal centrioles embedded in a structured PCM): they cannot perform the characteristic spiral cleavage of their ancestors equipped with centrosomes.

"When centrioles are experimentally ablated, spindles drift within the cell" (Azimzadeh and Marshall, 2010): cells cannot control the orientation of their division planes.

"The embryogenesis of freshwater planarians is equally intriguing: cleavage of the fertilized egg was described as 'anarchic'. No overt gastrulation or epiboly has been described." (Sànchez Alvarado).

Without centrosomes, division plane orientation and convergent extension (two highly geometric processes, indispensable in cleavage and gastrulation) become respectively runaway and impossible; the geometric role played by the centrosome is undeniable: planarian cells are no longer capable of changing the geometric disposal of their (cell-cell and cell-extracellular matrix) adhesion junctions to invaginate inside the embryo. Without centrosomes, cells lose the capability of managing the geometry of extra-cellular fibers. Gastrulation, the "most" animal characteristic, fails.

So, Planarians, centrosome-free like plants, are capable of performing only simple structures, with cylindrical or laminar arrangement.

Results

Many studies on Protists have demonstrated that the nine triplets of centrioles are different (not-equivalent) and arranged in an ordered sequence: the circumferential polarity in the arrangement of triplets is accorded to the disposition of the cytoskeleton (Beisson and Jerka-Dziasdosz, 1999): "Among flagellates, the centriolar appendages are also varied, biochemically and morphologically; even microtubule appendages may have highly complex shapes as, for example, in Physarum or in Ochromonas…It seems that in Protists and in Metazoa the triplets of basal bodies are not-equivalent" (Beisson and Jerka-Dziasdosz, 1999). Different (non-equivalent) shapes imply different molecular composition (quaternary structure): each triplet possesses its own molecular distinct identifying mark (an "address" receptor).

Metazoan development occurs through highly directional processes: division plane orientations, cell migration, differential adhesions, morphogen gradients, extracellular fibers orientation; Metazoa, which possess centrosome, have cells (cytology), tissues (histology) and organs (anatomy) showing sophisticated morphology, not comparable to histology of plants, which do not have centrosomes (Regolini, 2013): how do metazoan cells organize their intrinsic geometry?

"The spatial resolution of gradients based on diffusible molecules has fundamental limitations set by the ability of receptors to discriminate small differences in ligand concentration, such that sensitivity to concentration changes in one part of the gradient comes at the cost of saturation in the rest of the gradient. Such considerations lead to the idea that global positional information might provide at most a low-resolution

map of position within the cell that must then be refined by local determination of structure and organization" (Marshall, 2011).

The centrosome, made of two orthogonal centrioles showing 9-fold symmetry and circumferential polarity (non-equivalence of triplets), indeed is a geometric organelle with the proper structure of a tool really capable of realizing the "high-resolution map of position within the cell" hypothesized by Marshall: it possesses all the structural properties to be a molecular interface that recognizes targeted complexes and delivers each of them into its expected location: by its aster of MTs and correlated cargo-motor-proteins (kinesins and dyneins), it connects, like a wiring device, each targeted complex with its final location through the corresponding correctly-oriented microtubule.

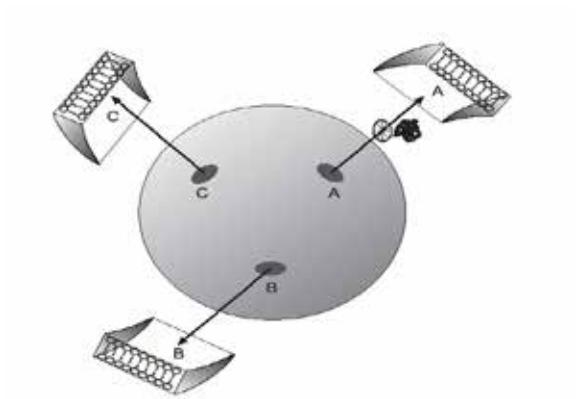


Fig. 1 Centrosome theoretical geometrical model: functioning.

Small ellipses represent γ-TuRCs on the centrosome (large sphere): each one is identified by its own private receptor (capital letters: A, B, C) which recognizes only the corresponding targeting sequence. Each γ-TuRC has the orientation of the plane which, in that point, is tangent to the centrosome "spherical" surface. MTs (arrows) are nucleated with directions imposed by the orientation of the corresponding γ-TuRC: like orientation, like direction (one "discrete" orientation, one "discrete" direction). So, a molecular complex (twisted tube) through its "geometric" targeting sequence ("A"), recognizes and links exclusively the receptor (A or B or C) that marks that γ-TuRC which has the correct orientation to nucleate a microtubule directed to the desired (corresponding: A or B, or C) destination, reached through a kinesin carrier (wheel): one targeting sequence, one γ-TuRC receptor, one cortical compartment: one-to-one univocal correspondence.

"The genetic instructions often include information pertinent to the localization of the product. Targeting sequences direct proteins to the plasma membrane, nucleus, mitochondria, or lysosomes. Certain proteins and mRNAs are transported individually to particular locations in cell space, and this localization depends on having an appropriate sequence. Transport vesicles recognize specific target membranes, such as the Golgi, vacuole, or plasma membrane, with the aid of SNARE proteins. But there is much more to growth and division than manufacturing the parts. A rod-shaped cell must also elongate with constant diameter, construct an efficient apparatus to partition its chromosomes, locate its midpoint, lay down a septum, and undergo fission. In eukaryotic cells, targeted vesicle fusion requires, in addition to the SNAREs, both a delivery system and a secretory apparatus" (Harold, 2005).

A "biological reference system organizer" made up of two orthogonal protractors

One 360°-protractor (a complete ring) is responsible for longitude, the other, orthogonal to the first, is responsible for latitude (it is composed of two symmetric 180° half-protractors facing each other to compose a complete ring; a globe is normally equipped with only one 180° half-protractor): together, they divide the space into nine meridian wedges and five parallel sectors (two polar caps and three parallel disks). In the centrosome the MC is responsible for longitude (9 meridian wedges), the DC for latitude (5 parallel rings).

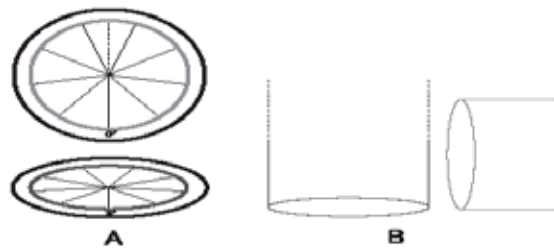


Fig. 2 Centrosome theoretical geometrical model: a spherical reference system composed of two orthogonal protractors/goniometers.

A: frontal view of two orthogonal goniometers, subdivided into nine sectors, which represents the two orthogonal centrioles.

B: schematic lateral view of the proximal end of both centrioles (during S, G2) to show the respective position of the above two sections.

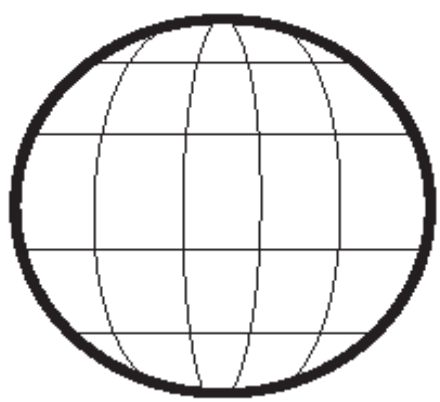


FIG. 3

Nine meridians and four parallels subdivide the centrosome surface into 45 small areas (scaffolds for γ-TuRCs, each oriented in correspondence to its position: their inclination is the result of the addition of two inclinations, one imposed by the MC (longitude) and the other by the DC (latitude). As on a globe, longitude covers the entire circumference (2π; 9 different meridians or 9 different meridian 40° wedges) while latitude covers (symmetrically) only half circumference (π; 2 caps and 3 parallel discs).

This "two-protractors instrument" is sufficient to subdivide the space into 45 pyramidal frustums with the little base at the center, each one identifiable by its own longitude and latitude: the large base (subtending a vertex solid angle of 4π /45 steradians) has an extension of $4\pi r^2/45$, thus, in a cell with a diameter of 10 μm (radius: 5 μm; surface: $4\pi r^2$ approximately 314 μm^2) it corresponds to a cell cortex extension of about 7 μm^2 (a circle with a diameter of 3 μm, or a square with a side of 2.6 μm). These dimensions together with the physical properties of the MTs (bending-resistance and rigidity) give an idea about the interesting order of magnitude of the noise-resistance of this system and of its fine-tuned precision, much better than that of chemical gradients: 45 cell cortex compartments (or rather poles) are much more than six poles (anterior, posterior, dorsal, ventral, left and right). Let's re-read

Marshall's words to better appreciate the previous geometrical model: "Such considerations lead to the idea that global positional information might provide at most a low-resolution map of position within the cell that must then be refined by local determination of structure and organization" (2011)".

Left-Right

This model opens a very intriguing new point of view abut Left-Right patterning and bilateral symmetry. By reverting (clockwise > counterclockwise) the sequence of its marks, a protractor becomes symmetric in respect to the original one: a centrosome equipped with a "reversed" MC individuates a sector of the plane which is symmetric, compared to that found by a non-reverse MC: it "translates" the same coded signal into a symmetric location. A simple reversion of the mark sequence of the protractor responsible for longitude realizes bilateral symmetry: changing an enormous number of topogenic instructions is not necessary to build bilateral symmetric organs. In Left or Right cells, the same topogenic sequence matches the same γ- TuRC receptor which is in a point of the PCM bilateral symmetric (Left/Right) wired to a symmetric cortical location. one simple change in place of thousands of changes is much more likely in evolution.

Bilateral symmetry is characteristic of Metazoa, whereas in plants it is only apparent: the curvature of the leaf edges (particularly near the petiole), the edge indentation, the position of petiole and apex, the arrangement of the veins that start alternately from the central vein and, above all, meristem histology and developmental biology exclude the existence of a true bilateral symmetry. Also the flowers of zygomorphous plants (orchids for example), described as roughly bilaterally symmetric, after a thorough morphological and developmental analysis, lead to the same conclusions: bilateral symmetry of paired sepals and petals, as symmetry of the two halves of unpaired structures (especially for the arrangement of veins and pigments) is only apparent, not supported by anatomical, histological and developmental foundations. Regolini, 2013).

DNA, through the centrosome, generates a 3D oriented map of distinguishable discrete compartments in a previously homogenous cell cortex

In a cell, a "two-protractors" tool is a biological geometric interface (a biological "switching wire-hub" or a "mail-sorting-machine") that receives (molecular) coded signals (input: targeting or topogenic sequences of polypeptides or polyribonucleotides), each one intended for a particular cell compartment, recognizes them through their tertiary structure, matches each one with the corresponding γ-TuRC mark (ligand-receptor interaction) and indicates the spatial position (output) of the desired locations: any location can be easily reached through an oriented ray (an oriented MT) arising from the selected mark (oriented and "labeled" γ-TuRC).

"Cortical compartmentalization of proteins into discrete clustered domains may be a general feature exploited by cells to control spatiotemporal signalling events with high fidelity. As we observe similar polarity factor clusters in Saccharomyces cerevisiae and Caenorhabditis elegans cells, the clustered organization of polarity factors within cortical subdomains and regulated cluster segregation may be key regulatory features across many organisms. In the case of cell polarity, cortical nodes and their spatial segregation could contribute to robust 'on' or 'off' switching of polarity at the right place and time in cells, following intra- or extracellular signals…This suggested that localized microtubule delivery of polarity factor could suffice for the formation and maintenance of cortical nodes … A quantitation of node showed that a typical cell consistently organizes 20–30 nodes of each protein dispersed along the cell cortex" (Dodgson et al., 2013).

DNA, through the centrosome, generates ex novo a 3D labeled environment, a grid line(or a graticule) in a previously homogenous cell, transforming and converting it in a discrete, mapped, wired, "webbed" and polarized-in-detail cell: a "useless" homogenous cell cortex becomes a "useful and usable" compartmentalized space. Through the centrosome, a "blind" DNA becomes a "sighted" DNA: by the centrosome, DNA, so to say, turns on the light in the cell.

Discussion

Centrosome geometry and architecture necessarily imply its function: as a technical designer first squares a sheet, similarly, during the last period of each cell division, through the centrosome geometry and its "wiring" MT aster, DNA maps and wires the non-polarized and homogeneous cortex of the new arising cell: so, DNA builds the intrinsic 3D map of the cell, transforming a "virtual" (DNA-coded) grid line into a real "actual" map (cortical topology) of distinguishable compartments (the "cell wide web": a "graticule" of cortical distinct compartments) with intrinsic points of reference to correctly dictate and orientate the position of membrane polarity factors. By the peculiar process of centriole and centrosome duplication (Regolini, 2013), DNA uses the centrosome to polarize (in coordination and agreement with the parental cell and, consequently with the whole tissue) the cellular cortex and membrane in order to assume the spatial control and the mastery of the cellular and extracellular (orientation of fibers) environment.

A geometrical model of centrosome functioning

"Several principles of construction of a microscopically small device for locating the directions of signal sources in microscopic dimensions: it appears that the simplest and smallest device that is compatible with the scrambling influence of thermal fluctuations as are demonstrated by Brownian motion is a pair of cylinders oriented at right angles to each other." (Albrecht-Buehler, 1981).

Centrioles are the only cell organelles which show a real geometrical organization, an intriguing 9-fold symmetric structure very resembling to a protractor: effectively, a pair of 9-fold symmetric cylinders, oriented at right angles to each other and capable of irradiating an aster of radially directed MTs, combined to the molecular non-equivalence of triplets (molecular "labels" or "address receptors") constitutes a spherical reference system organizer.

- Centrioles have a circumferential ordered and discrete asymmetry because of macromolecular differences of the quaternary structure of their triplets (molecular identifiers).
- Centrioles are platforms for a set of regulatory molecules that assist and facilitate the semi-self-assembly of the PCM.
- The MC transmits its 9-fold symmetry (longitude-dependent inclination: φ coordinate of a spherical reference system) to PCM γ-TuRC scaffolds /docking platforms, subdividing the PCM and its γ-TuRCs into 9 meridian wedges in each of which γ-TuRC longitude-inclination is the same.
- The DC transmits its 9-fold symmetry (latitude-dependent inclination: θ coordinate of a spherical reference system) to PCM γ-TuRC scaffolds /docking platforms subdividing the PCM and its γ-TuRCs into 5 parallel rings in each of which γ-TuRC latitude-inclination is the same.
- Each γ-TuRC on a centrosomal scaffold/docking platform is oriented in accord with the tilt of the local tangent plane: scaffold's (and γ -TuRC's) inclination is the addition of the latitudinal (DC) to the longitudinal (MC) tilt.
- The nine meridian wedges are subdivided by the five parallel rings: the PCM is then organized in 45 different compartments, in each of which γ-TuRC inclination is the same.
- Both centrioles transmit to the PCM wedges and ring their circumferential asymmetry and impress their molecular non-equivalence (longitude and latitude molecular identifying receptors).
- Each γ-TuRC (or its centrosomal scaffold / docking platform) is "labeled" by the "address" receptors, (longitude and latitude) corresponding to its own position and orientation, organized by both centrioles.
- Each γ-TuRC (or its centrosomal docking platform) displays its receptors (longitude and latitude) to recognize the "address" signal (a molecular ligand with a particular targeting

sequence) corresponding to its own orientation and to the intended location in the cell cortex.

- The direction of centrosomal MTs depends on the orientation (tilt) of the γ-TuRCs by which they are nucleated.
- Each signal (ligand) has a 3D shape that recognizes only the receptors of that γ-TuRC which is oriented in order to nucleate an MT with the desired direction: there is a precise and invariable one-to-one correspondence between geometric signals and γ-TuRC receptors.
- The correspondence between centrosomal and cortical compartments is univocal (one-to-one) but time-variable, since it depends on the position and orientation of the centrosome: a same γ-TuRC can be connected (under the strict control of DNA), at different times, to diverse cortical compartments. This is the consequence of the different position of the centrosome: in dividing cells, during G1 (interphase) the centrosome is near the nucleus, at the center of the cytoplasm, whereas in non dividing cells it moves to the cell cortex where the MC is the basal body of the primary non-motile cilium.
- The one-to-one correspondence between targeting signals and γ-TuRC receptors is constant and invariable, whereas the one-to-one correspondence between targeting signals (and related γ-TuRC receptors) and cortical compartments is time-variable.
- The centrosome is a geometric discrete interface that receives geometric coded signals (input), matches each one with the corresponding γ-TuRC receptor (decoding: ligand-receptor interaction) and nucleates oriented MTs (translation) to reach the required locations (output).
- Reverse or opposite rotational polarity of the MC is the base of Left-Right patterning and bilateral symmetry.

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