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Centrosome: is it a geometric, noise resistant, 3D interface that translates morphogenetic signals into precise locations in the cell?

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Summary

The requirements of a spherical reference system based on two orthogonal goniometers show a surprising correspondence with the evidence emerging from numerous experimental studies on centrioles and centrosomes: the centrosome, because of the 9-fold symmetry of its centrioles, their orthogonal arrangement and their circumferential polarity, may play the role of an interface, composed by two orthogonal goniometers, that recognizes and decodes morphogenetic instructions, or, more generally, geometric molecular signals and translates them into their expected real locations in the cell. The purpose of this study is to outline a theoretical model of the centrosome and address the question on "how" the centrosome works, rather than investigate "what" centrioles might be or "what" might be their task, as many in-depth previous studies have discussed; the present analysis looks for the correspondence between structure and function in the centrosome, delineates a link between morphogenetic (DNA) instructions and their translation into actual locations into cells, tissues and organs, and finally analyzes centrosome behavior in many developmental processes: polarization, planar polarity, apical constriction, migration, morphogens transport, convergent extension, left-right bilateral symmetry and asymmetry establishment.

Abbreviations and common short forms: MT: MicroTubules; MTOC: MicroTubules Organizing Center; γ-TuRC: γ-Tubulin Ring Complex; PCM: PeriCentriolar Material; AJ: Adherens Junction; AP: Anterior-Posterior; DV: Dorsal-Ventral; PD: Proximal-Distal; LR: Left-Right; 2/3D: two/three dimensions.

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"La filosofia naturale è scritta in questo grandissimo libro che continuamente ci sta aperto innanzi agli occhi, io dico l'universo, ma non si può intendere se prima non s'impara a intender la lingua e conoscer i caratteri nei quali è scritto. Egli è scritto in lingua matematica, e i caratteri son triangoli, cerchi ed altre figure geometriche, senza i quali mezzi è impossibile a intenderne umanamente parola; senza questi è un aggirarsi vanamente per un oscuro labirinto."
(Galileo Galilei, 1564-1642)

[Natural philosophy is written in this great book which is continually open in front of our eyes, the Universe I say, but it cannot be understood if we, earlier, do not learn to speak the language and know the characters which it is written by. It is written in mathematical language, and its letters are triangles, circles and other geometric figures, without which it is impossible that we humans understand its words: without them it is like to vainly mist over in a dark labyrinth.]

Introduction

Two actors in morphogenesis

The shape of organs and organisms is the consequence of the spatial disposition of cells and extra cellular matrix fibers: our hands are different from our feet (even though they contain very similar anatomical and histological structures) because of the different spatial disposition of cells (osteocytes above all) and the different geometry of the extra cellular fibers; even more evident is, in our skin, the different cells arrangement between sebaceous and sweat glands: each type of gland possesses its own particular disposition of cells; there are a lot of these glands, often very close, but always and everywhere each type of gland is built in accord with its own characteristic cell arrangement, suggesting that an intrinsic mechanism guides cells. Anatomy and histology show that each organ (and each microscopic tissue structure like the neprhon or the cochlear Corti organ) has characteristic shape and dimensions as in different complex polyhedrons each face is arranged in a fixed position and has its own particular shape, orientation and extension; how do organs and organisms achieve their "geometrical" shape? There are two hot topics in morphogenesis: growth anisotropy and heredity of forms.

Growth anisotropy: when growth is rigorously isotropic (equal in every direction) spherical shapes are formed; directional, but unpredictable, differences of growth (random anisotropy) generate amorphous structures; to realize particular forms, anisotropy of growth must follow projected and forecast directions; so growth is orderly anisotropic from the earliest stages of zygote cleavage and becomes highly anisotropic during organogenesis, resulting in structures that develop according to predetermined directions; cells populations are correctly guided into fixed forecast 3D positions, following programs characteristic of each species: cleavage is radial in Amphibians, spiral in Molluscans, bilateral in Ascidians, syncytial in flies, discoidal in birds, rotational in Mammals; in mice, first embryonic divisions - described as irregularly rotational and asynchronous - occur along unpredictable cleavage planes; in this stage, the geometry of the arrangement of cells is of little use; inner mass cells com-

pactation does not follow any architectural design and blastomeres lack centrioles, that will appear only at the blastocyst stage (Gueth-Hallonet et al., 1993); gastrulation and neurulation show programmed movements of groups of cells characteristic of each species. The biological mechanisms involved in tissues and organs development are highly directional: orientation of division planes, cell movements and convergent extension take place according to precise directions (Baena-Lopez et al., 2005); internal forces (osmotic pressure) and external (tension of extra-cellular fibres) lead to stretching and bending of cylindrical structures according to the angle between cell axes and the direction of the extra-cellular fibres (Keller, 2006); adhesions between cells are not random but accurately localized and continuously remodeled (differential adhesion: Cagan, 2009); gradients of morphogens are formed using directionally selective transport (Ibañes and Izpisúa-Belmonte, 2008). Cells must be orientated and "informed" about the physical real directions of their movements, adhesions, cleavage orientations, disposal of the extra cellular fibers that they are producing; programmed cell migration is induced by chemo-attractants and implies the previous correct spatial location of the source of attracting molecules. How can a not-polarized zygote (*C. elegans*, for example) or first blastomeres know "where" the real physical location of the "anterior" or "dorsal" or "ventral" pole is? How are they able to coordinate their own polarity with that of the neighbouring cells? "Centriole duplication is part of the mechanism by which the cytoskeleton of the daughter cell is patterned upon that of the mother" (Harold, 2005). How can cells build and orientate the orthogonality of their three axes, AP, DV, and LR? Cells must have a biological tool, conceivable as roughly resembling and comparable to a 3D-compass or 3D-GPS navigator (obviously intrinsic and with no connection to magnetism) that is able to grasp coded spatial indications and translate them to find and reach the intended directions and locations.

"Heredity of forms" means that the zygote's genome contains and transmits to the offspring all the necessary programs that control growth, capable of precisely indicating the geometry (accurately forecast, memorized and stored in DNA) which growth must respect: in fact fertilized eggs only generate organisms that acquire the characteristic shape of the species (in the few crosses that occur successfully, morphological characteristics are blended). The famous nucleus transfer from a mammary gland cell of a Finn Dorset sheep into the cytoplasm of an enucleated oocyte of a Scottish Blakface sheep produced Dolly, which showed the morphological features of a Finn Dorset sheep. The genome, the first actor in morphogenesis, controls the growth and development of the organism: it provides "directional signals" that guide cells to reach and occupy the forecast positions: thus DNA strictly rules the orientation of cell movements, cell-cell adhesions and division planes, in addition to master the directional organization of the extra cellular matrix. Comparative anatomy and molecular phylogenetics (Strachan and Read, 2010) indicate that the homology of structures, organs and tissues is founded on the homology of genomes: comparisons of whole genomes show high degrees of similarity (sequences homology) that fit anatomical and morphological features; morphological taxonomy corresponds to sequences homology. Therefore DNA contains heritable morphogenetic (coded) guidelines that, by means of molecular targeting signals, drive and orientate populations of cells, suggesting the correct direction that cells must follow to reach their proper locations and to correctly assemble the extra cellular matrix. This is astonishingly evident in the

organization of *D. melanogaster* imaginal discs and in their process of extroflection. "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). For reaching precise locations in the cell cortex (apical, basal, anterior, posterior, dorsal, ventral, proximal, distal), firstly the cortex must be "mapped" and the cell must have at its disposal all the instruments necessary to manage this "map": a tool is necessary that can "understand" geometric and directional coded signals and translates them finding and physically reaching the intended cortical position. Whatever the signals (polynucleotides or polypeptides), how are they decoded and translated, identifying with precision the desired directions, locations and orientations?

Mutations in genes coding for centriolar proteins cause morphogenetic disorders, from Protists to humans: *Chlamydomonas bld* mutant does not have flagella, *uni* has only one flagellum, *vfl* shows multiple ectopic flagella (Pearson and Winey, 1993): they all present abnormalities in the cytoskeleton, in the location of the nucleus and in the formation of the mitotic furrow; in humans, ciliopathies are associated with many morphological disorders: renal and hepatic malformations, polydactyly, Meckel-Gruber and Bardet-Biedel syndrome, etc. (Brugmann et al., 2010). Cilia are assembled by basal bodies/centrioles: what is the role of centrioles in development? What is the link between the genes involved in morphogenesis and the architectural organization of cells? The last step of each morphogenetic process is necessarily geometric and directional, in order to drive cells towards the position they must occupy. Morphogenetic signals are molecules (targeting sequences of nucleotides or amino-acids) that impose directions: then, there must be a noise-resistant structure, capable of precisely recognizing and identifying geometric signals, decoding, interpreting and translating them to find and reach the desired locations. This implies the existence of a three-dimensional reference system, the second actor in morphogenesis, made up of real cellular structures; evidently, the classical Cartesian reference system with three axes crossing the cytoplasm, does not exist; on the contrary, however, a spherical reference system does exist, which requires a structure, as small as is desired, composed of two "goniometers" orthogonal to each other and capable of generating oriented rays: this is the centrosome, with its two orthogonal centrioles, built with a 9-fold symmetry and capable of assembling robust microtubules (MTs) in oriented directions. "Some characteristics of mouse early development could be linked to the absence of centrioles: (1) the absence of regular cleavage planes during early development; (2) the absence of any detectable axis of polarity within the blastomeres before the 8-cell stage; (3) the random position of the spindle relative to the axis of polarity within the blastomere during the fourth cleavage. Centrioles may act to keep Peri-Centriolar Material components in a precise position throughout the cell cycle and so

be useful in the control of the position of the axis of polarity and division. This may become more important in differentiated cells, such as those found in the outer layer of the blastocyst" (Gueth-Hallonet et al., 1993).

Plants and animal anatomy

Fixed in the ground, plants control their anisotropic growth by an extrinsic reference system (gravity and light); animals, on the other hand, need an intrinsic reference system to manage their geometry: plants do not have orthogonal centrioles (Katsaros et al., 2006; Bornens, 2012), animals do. Is the centrosome the intrinsic reference system of animals?

Plants have developed simple anatomical and histological structures, with cylindrical or laminar arrangement: beautiful but anatomically simple, repeated a large number of times. In contrast, in animals, anatomical forms are particularly varied and complex (3D rather than 2D laminar arrangements: Gibson et al., 2011): e.g. the peacock's livery, the shells of crustaceans; they show also a high architectural accuracy and precision at the tissue level: e.g. kidney cortex and medulla or spongy bone osteons and trabeculae; the same holds true for organs: e.g. skeleton and heart. The shape of structures that perform complex functions is astonishing: in Vertebrates, the curvature of cornea, lens and retina strictly meets the need for projecting and focusing images; the inner ear - labyrinth and cochlea - in Mammals and Birds has a shape perfectly suited to measure the different vector-components of acceleration and analyze the frequency of acoustical signals (the basilar membrane performs a "biological Fast Fourier Transform"). Are complex 3D shapes correlated with orthogonal centrioles? In-depth previous studies have investigated "what" centrioles might be and "what" might be their task: Albrecht-Buehler (1981), Kirschner and Mitchison (1986), Beisson (1999), Harold (2005), Feldman (2007), Schatten (2008), Bornens (2012). Now the question is: "How does the centrosome work?"

Centrioles and centrosome

"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 and basal bodies (at the base of cilia and flagella) (found only in Eukaryotes, but not in plants) are the same organelle: the centriole of the spermatocyte becomes the basal body of the sperm, which, after fertilization, is again a centriole in the zygote; they are cylinders (or, rather, prisms) which, depending on the species, have a height of 150 to 500 nm and a base diameter of 100-200 nm; their lateral wall is made up of nine longitudinal bundles, each consisting of three parallel MTs (named A, B and C) that form "triplets"; inside, the structure of the proximal portion of a new centriole has the appearance of a cartwheel, with a central axis and nine spokes, each perpendicularly directed towards a triplet (Cottee et al., 2011). The centrosome, found only in Metazoa and in multicellular algae but not in higher plants and most fungi (Bornens, 2012), is a sphere of electron-dense material inside

which there are two centrioles orthogonal to each other. The centrosome possesses many peculiar and unique characteristics: it is the only organelle, together the nucleus, that exists in a single copy per cell; it is the only organelle that does not have a membrane: the material from which it is made up (PCM: PeriCentriolar Material), seemingly amorphous, is strongly organized because all of its components do not diffuse into the cytoplasm, although they show a remarkable turnover and a high spatiotemporal variability (Jakobsen et al., 2011); it is in contact (through its MTs) with each other organelle and each cytoplasmic location. Up now, more than 200 different molecular complexes, highly conserved from Protists to Mammals, have been identified in centrioles and centrosomes. When a cell enters the S-phase, the two centrioles separate and, orthogonally to each of them, a new one is assembled; the two new centrosomes, each containing an old centriole, named "mother", and a younger, newly assembled, centriole, the "daughter", participate (without being strictly indispensable) in mitosis and form the mitotic spindle, of which they constitute the poles; each centrosome will be inherited by one of the two daughters (sisters) cells. This unique semi-conservative duplication is reminiscent of something similar to DNA, but it has been shown that centrioles duplication does not occur by copying a template: there are two different pathways, one requiring a preexisting mother centriole that works like a platform to control the new daughter centriole assembly, and a *de novo* assembly pathway that is turned off when a mother centriole is present (with few exceptions, like in multiciliated epithelial cells: Loncarek and Khodjakov, 2009). Such duplication modality (in which the self-assembly capability of certain macromolecular centriolar complexes is particularly important: Song et al., 2008) allows the centrioles of daughters (sisters) cells to acquire the same circumferential polarity and orientation of their mother: so, from the zygote on, every cell has its global polarity coordinated with the architectural project of the whole organism.

The centrioles inside the centrosome are quite different (Vorobjev and Chentsov 1982): the older one, the "mother", has nine external radial distal ("distal" is toward the centrosome surface, "proximal" toward the centrosome center) and nine sub distal appendages, while the younger one (the "daughter") has nine small distal different ribs (structural difference); only the mother centriole can form a cilium (functional difference); the orthogonality between mother and daughter is asymmetric: the daughter longitudinal axis, if prolonged, crosses the other, so that the base of the daughter faces the lateral surface of the mother, but not vice-versa (geometric difference). After mitosis, the mother centriole remains fixed, anchored to the cell membrane by numerous MTs and, often, even to the nucleus, while the daughter may be more free in the cytoplasm (Piel et al., 2000). The centrosome is the cytoskeletal organizing center: from its PCM numerous MTs emerge, assembled by rings containing γ-tubulin (γ-TuRC: γ-Tubulin Ring Complex, composed of γ-TuSCs: γ-Tubulin Small Complexes) anchored by the participation of several proteins (ninein, centrin, pericentrin, etc.) which form a docking platform. MTs are also associated with a lot of proteins: kinesins and dyneins are two families of motor proteins that utilize MTs as rail tracks to carry organelles and macromolecules up and down; MAPs (Microtubules Associated Proteins) are a family of tissue-specific proteins with different activity: (de)stabilization, linkage to the cytoskeleton and association to the cell membrane (CLAPs). MTs are long polymers of α and β tubulin monomers, arranged in 13 polarized longitudinal filaments, with a "minus" end fixed on one γ-TuRC and a growing "plus" end centrifugally directed.

The centrosome is polarized: the "L" shape of the two centrioles (Fig. 4) allows a proximal-distal (central-peripheral) axis to be identified (mother centriole axis), with a distal pole characterized by the described appendages, and a second orthogonal axis along the daughter centriole. During duplication, one spoke of the "cartwheel" of the daughter centriole is parallel to the mother centriole axis (probasalbodies formation in *Chlamydomonas reinhardtii*: Geimer and Melkonian, 2004).

Proposal of a model

An input-output spherical reference system based on two orthogonal goniometers

"A new centriole normally arises in a definite spatial relationship to an existing one and at right angles to it" (Harold, 2005).

"Basal bodies and centrioles display structural and functional polarities that play an important role in the spatial arrangement of the cytoskeleton and hence the polarity of the cell " (Geimer and Melkonian, 2004).

Characteristic of the goniometers

The difference between a simple ring and a goniometer is the strictly ordered circumferential polarity and asymmetry of the goniometer: just as a ruler has two different poles, the beginning and the end, and is subdivided into an ordered row of equidistant diverse marks, a goniometer has different marks –asymmetry- sequenced in an ordered way at the same distance, beginning from a start pole and finishing at a stop pole –polarity- (in the cellular system, goniometers have 9 marks). One mark is considered the beginning ("0°" mark), fundamental to build and neatly assemble the ordered sequence of the other marks, and essential to orient the goniometer. So, the plane can be divided into 9 sectors. These goniometers (Fig. 1a), not used to measure, are translators of address-signals into their corresponding locations in the space; they are "geometric interfaces" that receive coded signals, each one intended for a particular sector (input), recognize them, match each one with the correspondent mark and return (or indicate) the spatial position of the desired locations (output): any location can be easily reached through an oriented ray arising from the selected mark.

Orientation of the first goniometer

Goniometers must be oriented: in a globe, one goniometer is "horizontal" (equatorial) the other, "vertical", passes through the North and South poles. The first goniometer, arranged on the equatorial plane, lies on the "x y" plane of the spherical reference system and its axis coincides with the "z" axis of the system: it is responsible to indicate the longitude (ϕ coordinate). The "0°" mark is used to orient the goniometer: on the globe it coincides with the meridian passing through Greenwich; on the "x y" plane it coincides with the "x" axis. Its nine marks indicate nine meridian (or vertical) spherical wedges.

Orientation of the second goniometer

The second goniometer, responsible for the latitude (θ coordinate) is vertical, orthogonal to the first (Fig. 1); it is possible to define its "top" and its "bottom", like in a clock on a tower the mark "12" is on the vertical axis and always at the top; the
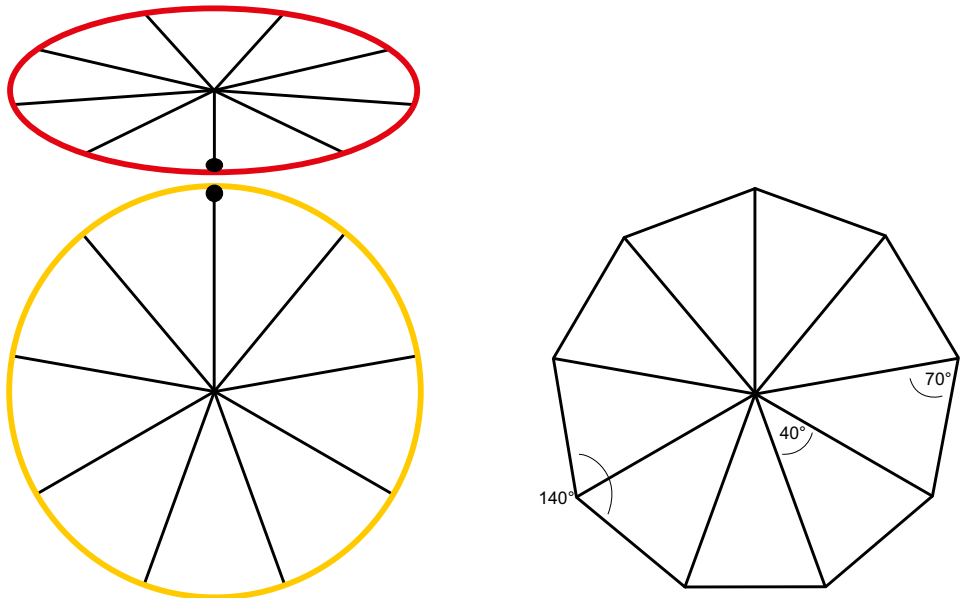
Figure 1 – left) Orientation of the goniometers, (frontal view). Red circle: first "horizontal" goniometer on the equatorial "x y" plane; yellow circle: second "vertical" goniometer (parallel to "z" axis). Both "0°" marks (black dot) correspond. right) Regular nonagon or enneagon.

diameter crossing its "0°" mark is vertical, parallel to the "z" axis (the axis of the first goniometer); the "0°" mark is positioned at the top and aligned with the "0°" mark of the first goniometer. In a classic spherical reference system, θ takes values from 0° to 180°, then it is convenient to consider the second goniometer divided, by the "vertical" diameter crossing its "0°" mark, into two halves (two facing symmetric hemi-goniometers) the "right" one showing on its round external border four marks (+40°; +80°; +120°; +160°) clockwise ordered starting from the "0°" mark, the "left" one showing the same four marks, but counterclockwise ordered (-40°; -80°; -120°; -160°). So, these eight marks (four "right" and four corresponding "left") are symmetrically positioned relatively to the "0°": they divide the space into five parallel "horizontal" sectors (two polar caps and three rings): each sector is subdivided in nine parts by the first goniometer. It is not necessary that goniometer centres coincide.

This "two-goniometers-instrument" is sufficient to subdivide the space into 45 pyramids (or cones) (Figs. 2, 3) with the apex at the centre, each one "labeled" by its own longitude and latitude (φ and θ coordinates, corresponding to the goniometers' marks): each base faces and subtends a vertex solid angle of $4\pi/45$ steradians, then its extension $(4pr^2/45)$, in a cell with a diameter of 10 mm (radius: 5 mm; surface: approximately 314 mm^2) corresponds to about 7 mm^2 (a circle with a radius of 1.5 mm, or a square with a side of 2.6 mm). These dimensions together with the physical properties of the MTs (bending-resistance and rigidity: Gittes et al., 1993) give an idea about the interesting order of magnitude of the noise-resistance of this system and of its precision, possibly better than that of chemical gradients.
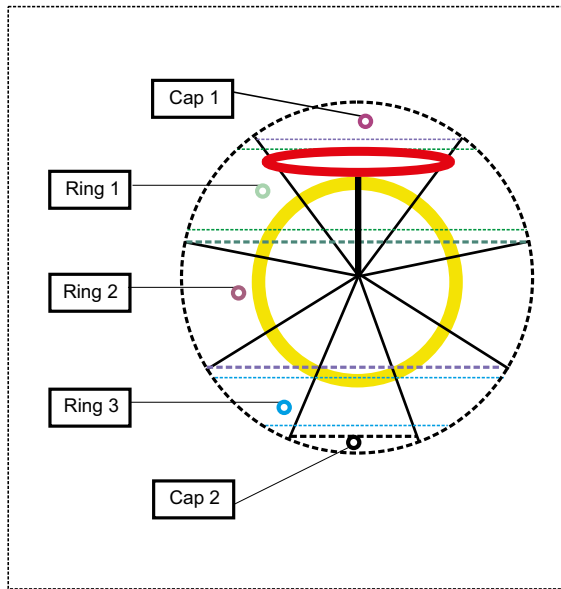
Figure 2 – Red circle: horizontal goniometer; Yellow circle: vertical goniometer. Grey dotted circle: centrosome surface. Because of the symmetrical disposition of its rays, the vertical goniometer divides the space in 2 caps and 3 rings (coloured boundaries) of different amplitude.
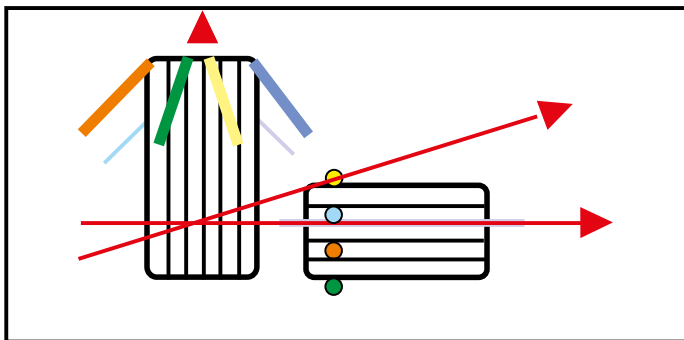

Figure 3 – Orthogonality of centrioles. Left: mother with distal and sub distal appendages; right: daughter with ribs; in red, the orthogonal axes of centrosome polarity (the angle between the two axes measures 90°: the apparent obliquity derives from perspective).

This theoretical analysis focuses the requirements of a spherical reference system based on two orthogonal goniometers with nine notches, which is what the centrioles precisely are: to satisfy the basic requirements of a spherical reference system, in order to recognize coded geometric signals (input) and translate them into their desired final locations (output), "cellular goniometers" must possess: 1) different

marks; 2) a constant ordered sequence of marks; 3) a start mark; 4) a controlled spatial orientation. Centrioles geometry is well defined: through the nucleation of microtubules (MTs) perpendicular to the centriole axis and with a centrifugal radial direction, each of the centrioles might transmit its own circumferential polarity first to the PCM and then to the whole cell cortex.

Does the Metazoan centrosome, with its two orthogonal centrioles built with 9-fold symmetry and capable to assemble robust MTs in oriented directions, possess all these requirements to be the cell spherical reference system?

Centriole/Basal bodies in Protists

"Among flagellates, the appendages are also varied, biochemically and morphologically; even microtubule appendages may have highly complex shapes as, for example, in *Physarum* or in *Ochromonas*" (Beisson and Jerka-Dziasdosz, 1999).

Many studies on Protists have demonstrated that the nine triplets of their centrioles/basal-bodies are different (not-equivalent) and arranged in an ordered sequence: the circumferential polarity in the arrangement of basal body triplets is accorded to the disposition of the cytoskeleton (Beisson and Jerka-Dziasdosz, 1999). Protists contain only centrioles, each at the base of each cilium or flagellum: they do not have centrosomes containing two orthogonal centrioles; only during the assembly of a new centriole, the nascent probasalbody grows perpendicularly to a pre-existing one. The biflagellate unicellular green alga *Chlamydomonas reinhardtii* has an ordered location of its organelles, cilia, oral apparatus, nucleus, chloroplasts, pyrenoid, eyespot, excretory vacuoles; this organization depends on the disposition of different cytoskeletal fibres (four cruciform rootlets: two thick, made up of 4 MTs, and two thin, made up of 2 MTs); the cell appears clearly polarized: an Apical-Basal axis from cilia to pyrenoid, a Dorsal-Ventral axis orthogonal to the first, from nucleus to oral apparatus; also a "Left-Right" axis of asymmetry (orthogonal to the other two axes) is established because of the asymmetric position of the eyespot. In Protists centrioles organize the cytoskeleton (Feldman et al., 2007): structural anomalies in centrioles cause disorders in the cytoskeleton (cruciform fibres lose their normal composition or their orthogonal disposition). *Chlamydomonas* has two apical flagella, whose movements are coordinated during planar 2D strokes, and during conical-helical 3D rotations. The axoneme of its flagella is composed by the classic nine MTs blades; these blades are not equivalent: electron microscopy has allowed the identification of each one of the nine doublets (cilia and flagella have "doublets", not triplets) each one showing its own morphological characteristic, that distinguishes itself from the other, and its own fixed location relatively to the others (Hoops and Witman, 1983; Bui et al., 2009); the two central MTs of the axoneme are also different (Wargo and Smith, 2003). Electron microscopy has highlighted that the circumferential asymmetry of the axoneme corresponds to an even more marked circumferential asymmetry of the basal body: it is possible to distinguish each one of its nine triplets and their orderly sequenced arrangement, determine the 180° rotation of one basal body compared to the second (O'Toole et al., 2003) and observe the connection between each triplet and particular fibers of the cytoskeleton: the striated fibres of the "distal connector", connect and fasten the triplets 9-1-2 of one basal body with the triplets 2-1-9 of the other, which are in front of them; the thick cruciform fibers are attached to the triplets N° 3 and 4,

the thin ones are attached to the triplet N° 8; the newly-forming procentriol is always in front of the triplet N° 9. Geimer and Melkonian (2004) have described an "acorn-like" structure in the inner distal part of the basal body (see in their article the Fig. 7 D), adhering in a highly asymmetric manner to the triplets 2-1-9-8-7, and another structure, shaped like the letter "V" in contact with the triplets 9, 5 and 4: "whereas the cartwheel is thought to nucleate the nine fold rotational symmetry of the microtubular triplets the acorn might play an equally important role imposing rotational asymmetry on the microtubular triplets, perhaps leading to the asymmetric assembly of basal-body-associated fibers and hence cellular asymmetry in general". Thus, the process through which centrioles are built appears composed by two different stages: the 9-fold symmetry is firstly established (cartwheel's task: Cottee et al., 2011); then rotational polarity is imposed (acorn's task: Geimer and Melkonian, 2004). The shape of any structure is the consequence of its molecular composition: in *C. reinhardtii* the polypeptide VFL1 coded by the gene *vfl1* (Variable number of FLagella), binds only to the triplet N° 1 (Silflow et al., 2001) confirming the biochemical nature of the circumferential asymmetry. In Metazoa are known different isomeres of α, β, γ, δ and ε tubulins and many families of proteins associated with centrioles, centrosome and MTs -kinesins, dyneins, dynactins, MAPs, CLAPs, nineins, etc.- each composed by numerous isomeres; also many different centrosomal non-coding RNAs have been described (Alliegro et al., 2006; Alliegro and Alliegro, 2008).

Another Protist, *Paramecium,* has similar characteristics (Beisson and Jerka-Dziasdosz, 1999): the high number of basal bodies (and cilia:4,000) is accompanied by their ability to self-organize and to connect with each other in about 70 regular rows, always with the same orientation of the triplets (in order to beat with coordination and synchronism), just using their circumferential asymmetry and structural differences between the triplets (Beisson, 2008); each new basal body arises at right angle from an old one, then straightens up and rotates to acquire a precise coordination with its complex cytoskeleton: the triplet N° 9 links to the "postciliary ribs", the triplet N°4 is attached to the "transverse ribbon", the triplets N° 5-6-7 are connected to the "kinetodesmal fibers" (Beisson and Jerka-Dziasdosz, 1999). This role of basal bodies in organizing the arrangement of cortex and cytoskeleton agrees with the movements of the daughter centriole described in many Metazoa (cortical cytasters: Salinas-Saavedra and Vargas, 2011) and in Mammals (Piel et al., 2000), where it appears to organize in detail specific peripheral cell sectors. Interventions of the centrioles/ basal bodies in a complicated cortical organization have also been described in *Trypanosoma* (Lacomble et al., 2010), in which a characteristic rotation of the new daughter centriole around the mother has been observed.

Chlamydomonas basal-bodies also regulate the length of the two flagella: Rosenbaum et al. (1969) observed that, following partial surgical ablation of one flagellum, the other is immediately reduced in length, then re-growth occurs simultaneously in both flagella until they reach the normal length: basal-bodies organize axonemal components, attentively regulating the activity of kinesins and dyneins through a highly sophisticated mechanism, the IntraFlagellar Transport (IFT).

Summarizing, in Protists each triplet shows its own morphology its fixed position and a molecular individuality has been proved for the triplet N° 1; furthermore, centrioles are univocally oriented in the cytoplasm, in respect to the cytoskeleton: all of the theoretical requirements of a "biological" goniometer are satisfied.
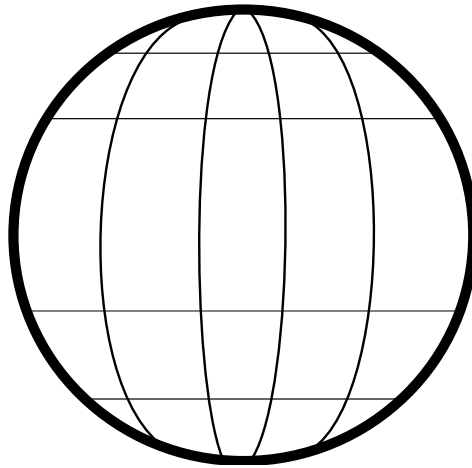
Figure 4 – Subdivision of the space: meridian (vertical) wedges (longitude), parallel (horizontal) caps and rings (latitude), oriented areas, each one with its own θ and φ angles.

A first model of the centrosome

"Centriole duplication is part of the mechanism by which the cytoskeleton of the daughter cell is patterned upon that of the mother. It is probably not correct to say that one centriole provides a template to make another (and there are instances of centrioles arising de novo), but some kind of copying appears to be involved". "Examples of self-organization have long been familiar to biochemists under the heading of self-assembly. Ribosomes, microtubules, microfilaments, virus particles, and lipid bilayers come to mind" (Harold, 2005).

Centrioles may act to keep PeriCentriolar Material components in a precise position throughout the cell cycle and so be useful in the control of the position of the axis of polarity and division." (Gueth-Hallonet et al., 1993).

"It seems that in Protists and in Metazoa the triplets of basal bodies are not-equivalent" (Beisson and Jerka-Dziasdosz, 1999).

Equivalent or not equivalent: that is the question. If Metazoan centriolar triplets are diverse and not equivalent like in Protists, centrioles may operate like "biological" goniometers also in Metazoa. Vorobjev and Chentsov (1982) presented interesting pictures of the appendages of the "mother" centriole in mammalian cells: each one is different in shape from the others and also different appear the nine ribs of the "daughter" centriole (Figs. 1c and 3c of their article). Studies on centrioles in Metazoa are very difficult because centrioles are embedded in the proteinaceous matrix of the PCM.

"Is it possible to confirm this idea that the circumferential, morphological, structural and molecular asymmetry of centrioles can be inferred from Mammals ciliated epithelia? While the circumferential anisotropy of centrioles cannot be ascertained within the centrosome, its existence can be inferred from the properties they express during ciliogenesis, be it the formation of a primary cilium or of bona fide 9+2 cilia
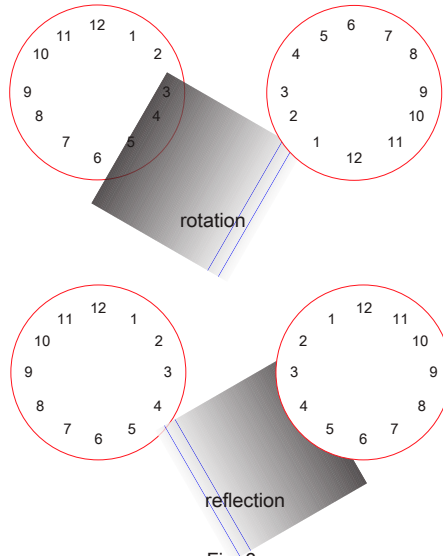
Figure 5 – Rotation vs. Reflection. Rotation: the sequence is unaltered. Reflection: the sequence changes from clockwise to counter-clockwise (or reverse).

in ciliated epithelia, some of which at least derive directly from the centrioles. As in Ciliates and flagellates, these basal bodies nucleate appendages of various molecular compositions (basal foot, striated rootlets, alarm sheets, etc, which anchor the basal body to the membrane and to the cytoskeleton) and these nucleations arise at specific sites of the basal body cylinder; in particular, the basal foot is located on triplets 5 and 6 corresponding to the side of the effective stroke of the cilium. What is remarkable is that basal feet develop before the basal bodies reach their membrane site and before they acquire their functional orientation "(Beisson and Jerka-Dziasdosz, 1999). It is not hazardous trying to answer to the previous question, following this way: the theoretical analysis of a spherical reference system based on two orthogonal goniometers gives significance and meaning to the rotational polarity of centrioles and indicates how a centrosome could operate; if centrosome behavior in Metazoa agrees to the functioning of a spherical reference system, it is possible to hypothesize that its geometrical role is very likely.

Centrioles and centrosome in Protists and Metazoa: biophysical and architectural considerations

"Among the conserved proteins involved in the biogenesis of the canonical 9-tripletcentriolar structures, Sas-6 and Bld10 have been shown to play central roles in the early steps of assembly and in establishment/stabilization of the ninefold symmetry." (Jerka-Dziadosz et al., 2010)

As already seen, centrioles are present in eukaryotic cells, but not in higher plants and most fungi; unicellular organisms have only centrioles/basal bodies, each one

independent, while in multicellular algae and in Metazoa two orthogonal centrioles are embedded in the PeriCentriolar Material that forms the centrosome.

Protists show cylindrical (or rather circumferential) symmetry, Metazoa show bilateral symmetry and a 3D architecture of their organs and limbs; Protists have cylindrical centrioles, Metazoa have two orthogonal cylindrical centrioles embedded in a spherical centrosome (Beisson and Jerka-Dziasdosz, 1999): the second centriole, orthogonal to the first, adds the capability to control a third dimension, indispensable to manage the arrangement of cells in multicellular organisms. The idea is that Metazoa have developed new pathways to adapt Protists' sophisticated molecular mechanisms in order to transmit the 9-fold symmetry and polarity of two orthogonal cylindrical centrioles to the spherical wedges and sectors of the centrosome (or rather of the PCM): so Metazoa could assemble a tool through which they can control, direct and organize their 3D anisotropic growth. In fact, to use a spherical reference instrument based on two orthogonal goniometers, a two-step process is necessary: firstly the longitude of a point (ϕ coordinate) must be found, then its latitude (θ coordinate) can be correctly identified, after a rotation of the vertical goniometer around its vertical axis that aligns it in correspondence of the found longitude ; this appears particularly complex and difficult (and frankly unlikely) in a cell; on the contrary, if a little sphere, like the centrosomal PCM (organized only once and for all in each cell), is built with its surface subdivided in 45 small areas (as seen in the above geometrical analysis) each one oriented and labelled by its molecular receptors that recognise the geometric (molecular) signals intended for its correspondent longitude and latitude (and, consequently, for the correspondent cytoplasmic pyramid) the problem of spatial orientation in the cell environment has a very ease solution. Like on a globe, parallels and meridians are marked, once for all, on its surface to facilitate the task of finding a point of given coordinates, so the 9-fold symmetry of two orthogonal cylindrical centrioles, transmitted and impressed on the centrosome surface once for all, permits an easy translation of molecular geometric signals (in which the intended coordinates are coded) into their correspondent localizations in the cytoplasm, only by the usual signal-receptor interaction. Obviously, this would happen while maintaining fixed and controlled the orientation of the centrosome in the cell, which would be in accordance with the described mobility of the daughter centriole in Vertebrate cells (Piel et al., 2000): after building the PCM surface, the mother centriole controls its fixe position and orientation, while the daughter centriole disengages and is more free in the cytoplasm.

A regular polygon of 9 sides (nonagon or enneagon) has 9 internal angles of 140° and is composed by 9 isosceles triangles (radially disposed) whose vertex (or central) angle measures 40° (Fig. 1b). Protists possess advanced biochemical mechanisms (with high predisposition for self-assembly) to assemble and build two angles: the right angle and the angle of 140°; the first is evident in the orthogonal arrangement of mother and daughter centrioles during duplication, and in the orthogonality between the cartwheel spokes and the MT blades of the centriolar wall; the second (and its supplementary of 40°) is evident in the 9 fold symmetry of cartwheel spokes and triplets; in the transition zone of *Chlamydomonas* basal bodies, where the axoneme is organized, there is an astonishing "stellate structure" (see Figs. 1 and 4 in Geimer and Melkonian, 2004) composed by 9 isosceles triangles (nonagon): any side of each triangle (the base, for example) crosses the correspondent side of the consecutive triangle

at 140°. Kitagawa et al. (2011) and van Breugel et al. (2011) have shown that the N-terminal domains of SAS-6 dimers (a conserved protein indispensable for centriole building) naturally interact between themselves at 140° (internal angle) to form curved oligomers, rings and left- or right-handed helices; SAS-6 has been found in every model organism: *Chlamydomonas*, worms, *D. rerio, Xenopus*, chicken, *H. sapiens*. "Intriguingly, when Drosophila SAS-6 is over expressed together with Ana2 (ortholog of SAS-5), the two proteins can assemble into well-ordered tubules that bear a striking resemblance to the cartwheel" (Cottee et al., 2011). Then, SAS-6 is a powerful and quasi-autonomous (self-assembling) tool to build angles of 140°. Guichard et al. (2010) have shown a 110-nm stalk that connects at 90° the central hub of the daughter procentriol to the mother centriole in human centrosome; *Chlamydomonas* Bld-10p, a component of the cartwheel-spoke tip, attaches the triplet to the spoke and maintains it orthogonal to the spoke (Hiraki et al., 2007). Picone et al. (2010) have identified dynamic centrosomal MTs that mediate homeostatic length control in Metazoa cells, like *Chlamydomonas* manages axoneme length (Rosenbaum et al., 1969). In humans, during the assembly of centrioles, a γ-TuRC-like structure at the proximal end of the A-microtubule is necessary for its nucleation, but the B- and C-microtubules "self-grow" bidirectionally, using, respectively, the A- and B-microtubules as templates; the distal end of the B- and C-microtubules remains curved while they grow longitudinally (Guichard et al., 2010). Geometrically, the management of 90° and 140° angles, together with the capability to control distances and the predisposition for self-assembly of rings and curved structures, are sufficient for the cylindrical (or, rather, prismatic) symmetry of two orthogonal centrioles to be transferred to a spherical (or, rather, polyhedral) shaped centrosome; some models (Piel et al., 2000; Bornens, 2002, 2012) show that centrioles, through the nucleation of MTs lying on planes passing through the axis of the centriole and through the triplet-blade from which MTs originate), having the same length but different tilt (like the radii of a circle) can transform the longitudinal rectilinear shape of their walls into a curved spherical surface (wedge); centrosome architecture reminds fullerenes: both manage an angle (140° in centrosomes, 109,5° in fullerenes) and build cylindrical or spherical structures taking advantage of their aptitude for self-assembly. Also the self-assembled "baskets" of clathrin triskelia (vesicles) have something in common with centrosomes: intriguingly, the N-terminal of the human Sas-6 shows 40% identity (65% positives) with a central domain of the clathrin light chain. Rodrigues-Martins et al. (2007) have shown that self-assembly is required in centriole formation: "centriole biogenesis is a template-free self-assembling process triggered and regulated by molecules that ordinarily associate with the existing centriole. The mother centriole is not a *bona fide* template but a platform for a set of regulatory molecules that catalyzes and regulates daughter centriole assembly". Song et al. (2008) arrive to similar considerations, finding a mutual cooperation between the PCM and the centrioles: "the PCM itself may direct the formation of the daughter centrioles". In mice, first embryonic divisions occur with unpredictable cleavage plane positions and blastomeres lack centrioles (they will appear only at the blastocyst stage) but possess PCM and γ-TuRCs to form spindles: evidently, PCM is autonomously self-assembled in each new cell until the stage of 64-blastomeres, when centrioles begin to be formed. Kubo et al. (1999) and Dammermann and Merdes (2002) observed that pericentriolar satellites, after recruiting proteins indispensable for anchoring γ-TuRCs to their centrosomal docking plat-

form (centrin, pericentrin, ninein, etc.), are transported to the centrosome by MT-linked dynactin. Centrosomes of Spisula, after chemical disassembling (1,0 M KI) lose γ-TuRCs, but maintain their PCM intact: after incubation with oocyte extracts containing γ-TuRCs, they recover: "This recovery process occurs in the absence of microtubules, divalent cations, and nucleotides" (Schnackenberg et al., 1998). Ou et al. (2002) found that the PCM is organized in an ordered and geometric fashion, showing a characteristic "centrosomal tube", closed at one (inner) end and open at the other end, with a diameter of 1.5 mm and a depth of 2 mm, dimensions that are larger than centrioles height and width but comparable to the dimensions of the centrosome: "A subset of PCM proteins have been shown to be arranged in a tubular conformation with an open and a closed end within the centrosome": "Microinjection of antibodies against either CEP110 or ninein into metaphase HeLa cells disrupted the reformation of the tubular conformation of proteins within the centrosome following cell division and consequently led to dispersal of centrosomal material throughout the cytosol (CEP250/c-Nap1, pericentrin, γ-tubulin and ninein)" (Ou et al., 2002). Alliegro and Alliegro (2008) have identified in *Spisula solidissima* several non-coding centrosomal RNAs: the importance of non-coding RNA in organizing protein complexes is today well known (spliceosomes, ribosomes, and nucleoli: Strachan and Read, 2010). Unlike fullerenes and clathrins vesicles, which are spherical layers, centrosomes do not build an accurately spherical surface: the PCM grow around centrioles (the core components of the centrosome) and organize perfectly oriented γ-TuRCs on an irregularly spherical surface; a model to explain how the "quasi-spherical" shape of the centrosome might be organized is conceivable (Fig. 6): a half ring of four SAS-6 dimers (25 nm diameter), lying on the plane containing the axis of the centriole and a triplet-blade, positioned near the proximal centriole pole, along the MT blade, can radiate four MTs (from the C-terminals of the SAS-dimers) starting with an angle of 40° between themselves, like cartwheel spokes (see Figs. 2 and 3 in Cottee et al., 2011); these MTs, like radii, are orthogonal to the centrosomal surface, and a molecular complex similar to *Chlamydomonas* Bld-10 (Hiraki et al., 2007) can assure the orientation of the correspondent γ-TuRCs (or their docking platforms), orthogonal to MTs and parallel to the local tangent plane. This can be the skeleton of a curved structure lying on the same plane: a similar process from each blade of the two orthogonal centrioles (near the proximal poles, then close to an ideal center of the centrosome) can constitute the structural basis of a "spherical" centrosome, or rather the platform on which centrosomal proteins are assembled. This is not far from the "hypothetical model of how centrioles could organize the centrosome matrix" (see Figs. 2 and 4 in Bornens, 2002). It seems that only one centriole is sufficient for organize the spherical PCM; indeed, a second centriole, orthogonal to the first, is indispensable to transmit correct "latitudinal" marks (or latitude receptors responsible for angle θ) to the parallel caps and rings (Fig. 2). In this geometrical model, as already hypothesized, cells use only mechanisms to manage 90° and 140° angles. So, it seems that Metazoa have empowered the sophisticated centriolar molecular apparatuses of Protists (capable to order and arrange new cell structures under the influence of other, already existing, structures: assembly of microtubules, centrioles duplication, assembly of axonemes and IntraFlagellar Transport) to spherically organize their centrosomes (the diameter of the centrosome is about only two times the height of centrioles, then not so different): like Protists build a circular cartwheel with 9-fold
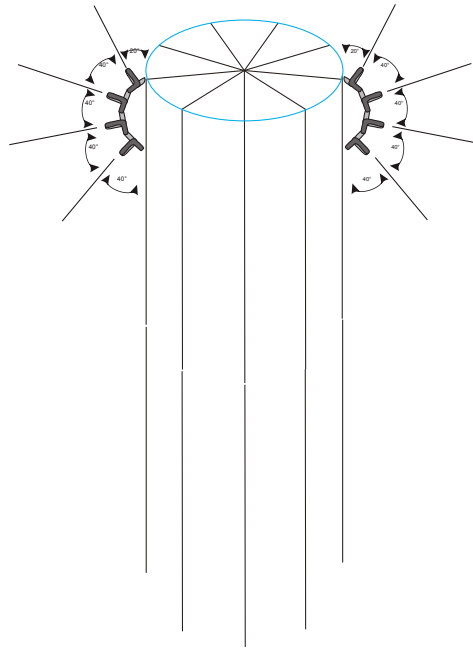
Figure 6 – From centriole to centrosome: four MTs lying on planes passing through the axis of the centriole and through the triplet-blade from which MTs originate and having the same length but different tilt (like the radii of a circle) can transform the longitudinal rectilinear shape of the centriolar wall into a curved spherical surface (wedge). A half ring (25 nm diameter) made up of four SAS-6 dimers (light and dark grey "T") lying on the plane containing the axis of the centriole and a triplet-blade, close to the proximal centriole pole, parallel to the MT blade, can radiate four MTs (from the C-terminals of the SAS-dimers), starting with an angle of 40° between themselves. Note that each MT is parallel to a spoke of the other orthogonal centriole (not drawn: compare with Fig. 1a).

symmetry orthogonally to the mother centriole, Metazoa arrange spherical rings and wedges around two orthogonal centrioles, by the only operational control of 90° and 140° angle. Like in *Chlamydomonas* rotational asymmetry of the microtubular triplets is supposed to be imposed by the "acorn" structure (Geimer and Melkonian, 2004), after the cartwheel has assisted the "semi self-assembly" of the 9-fold symmetry of the probasalbody, so in Metazoa the two orthogonal centrioles, after assembling a "quasi-spherical" centrosome, recruiting, attaching, orienting and anchoring γ-TuRCs to impose spherical polarity and asymmetry, cooperate to "label" γ-TuRCs. γ-tubulin rings are oriented and anchored by the participation of many proteins - ninein, centrin, pericentrin- each one present in several isoforms: the high number of these proteins confirms that the process of γ-TuRCs anchoring is not a simple docking process: it establishes also their sophisticated (and indispensable) spatial orientation, their labeling and the orientation of the microtubule they will nucleate. Like in Protists each centriolar triplet uses its own unique shape to recognize and bind its particular ligand (a cytoskeletal component), Metazoan γ-TuRCs have receptors ("labels") to recognize and bind their proper ligands (geometric signals composed by targeting

sequences of nucleotides or amino-acids, possibly linked to kinesins' light chains or heavy chain C-terminals) in order to nucleate MTs that drive targeted molecular complexes to the desired locations.

Whether centrosomes and MTs are involved in AP polarity formation in *C. elegans* embryos and in other organisms has been a subject of controversy (Cowan and Hyman, 2004; Motegi and Seydoux, 2007; Erkang et al., 2011). Tsai and Ahringer (2007) have cleared up this problem demonstrating that "polarity only occurs when a small, late-growing microtubule aster is visible at the centrosome; MTs deliver positional signals and are required for establishing polarity in many different organisms and cell types. In *C. elegans* embryos, posterior polarity is induced by an unknown centrosome-dependent signal". This is a fundamental milestone: the polarity of centrosomes and centrioles (necessarily rotational) organizes a polarized cytoskeleton in Metazoa. The properties of centrioles observed in Protists, taken together with the above considered findings on metazoan centrioles and centrosomes, allow me to pose the following hypotheses about Metazoan centrioles and centrosomes.

Hypotheses

- Centrioles have a circumferential ordered asymmetry as a consequence of the biochemical difference of their triplets.
- Centrioles are platforms for a set of regulatory molecules that assists and facilitates the semi-self-assembly of the PCM.
- Centrioles transmit to the PCM their circumferential asymmetry and impress their molecular not-equivalence.
- Each γ-TuRC (or its centrosomal docking platform) is oriented in accord to the tilt of the local tangent plane.
- Each γ-TuRC (or its centrosomal docking platform) receives from both centrioles the receptors corresponding to its own position and orientation.
- Each γ-TuRC (or its centrosomal docking platform) displays its receptors to recognise the signal (a molecular ligand with a particular targeting sequence) corresponding to its own orientation and to the intended location in the cytoplasm.
- The direction of centrosomal MTs depends on the orientation and tilt of the γ-TuRCs by which they are nucleated.
- Each signal has a 3D shape that recognises only the receptors of that γ-TuRC that is oriented in order to nucleate an MT with the desired direction: there is a precise correspondence between geometric signals, γ-TuRC's receptors, γ-TuRC's orientations and MTs directions.
- The centrosome is the cytoskeletal organizing center; it is polarized and its orientation in the cell is strictly controlled.
- The centrosome is a geometric interface that receives geometric coded signals (input), matches each one with the correspondent γ-TuRC's receptor (decoding) and nucleates oriented MTs (translation) to reach the required locations (output).
- Aster, cytoskeleton and cell cortex, during mitosis, receive from the centrosome the same spherical asymmetry (mapping or polarization).
- Centrioles are chiral structures as a consequence of their rotational asymmetry.

Discussion

To test the above hypotheses about the geometric role of the centrosome, the following discussion reviews and analyzes Metazoans centrosome behavior during several morphogenetic processes.

Cell Polarization

"Centrosomal microtubules induce cortical polarity in the *C. elegans* zygote" (Siegrist and Doe, 2070)

"Centrosomes direct cell polarity in *C. elegans* embryos"(Cowan and Hyman, 2004) and in other species (Sardet et al., 2007).

In the early *C. elegans* embryo, the correct functioning of Delta-Notch signaling pathway requires that the first cleavage planes are precisely located: therefore, in the absence of external cues (the zygote and the egg shell are not polarized before fertilization: Tsai and Ahringer, 2007), cell (and spindle) poles are positioned by an intrinsic autonomous control. Just after fertilization, the zygote acquires its AP polarity; the cue of this process is the PCM of the sperm centrosome: its laser ablation prevents polarity establishment (Tsai and Ahringer, 2007). The posterior pole is positioned by the physical association of the sperm centrosome to the cortex without contribution of MTs (Cowan and Hyman, 2004). The positioning of the anterior pole is different, because it must be oriented in three dimensions to control the proper positioning of the spindle pole (which has precisely three degrees of freedom, one for the distance from the posterior pole and two for the angles θ, between AP and DV axes, and ϕ, between AP and LR axes); the spindle axis of the first cleavage is always located in a particular and invariable position, always with the same values for θ and ϕ angles; this process is mediated by long astral MTs (Erkang et al., 2011) which carry, drive and deliver proper cortical signals (Tsai and Ahringer, 2007; Galli and van den Heuvel, 2008): embryology text books describe AP polarization like an asymmetric distribution of PAR (PARtitioning defective) proteins, PAR-3/PAR-6 anteriorly, PAR-1/ PAR-2 posteriorly; PAR-3 and PAR-6, positioned throughout the entire cortex at fertilization, suddenly contract into the anterior half: half zygote cortex is not a precise fixed point for the location of a cell (or spindle) pole. The role of MTs in precisely determining zygote polarity is fundamental to orient division planes. In fact, from the zygote (P_0; see Fig. 1 in: Gönczy P., Rose L.S., 2005) two daughter cells arise, anteriorly AB, larger, posteriorly P_1, smaller: P_1 inherits P granules (ribonucleoproteins destined to the germ "P" cells); AB divides into two daughters, ABa (anterior) and ABp (posterior); P_1 divides into EMS (anteriorly and ventrally) and P_2 (posteriorly and dorsally). Only ABp must contact P_2 in order that only ABp's Notch receptors (but not ABa's) are activated by P_2's Delta signals. The fate of ABa and ABp is decided immediately after their birth: both display Notch receptors but, in this stage of development, only in ABp this way of signaling is activated by P_2 Delta signals; so the progeny of ABp will follow a different fate from that of ABa (only some descendants of ABa will produce the pharynx). If the location of ABa and ABp is surgically inverted, ABa (which also expresses Notch receptors), receiving Delta ligands from P_2, follows the fate of ABp, while ABp, not receiving Delta signals, follows the fate of ABa; then, to avoid the contact between ABa and P_2, the spindle axis in AB must be

perfectly positioned on the sagittal plane. If, on the contrary, it shifts from the sagittal plane, ABa and ABp risk to be one on the left side and the other on the right side of the anterior surface of P_2, and P_2 could contact both of them: only during the next division, in fact, will both ABa and ABp generate such cells, two left, ABal ABpl, and two right, ABar ABpr, controlling attentively the shifting of the spindle axis in order to position left cells more anteriorly than the right ones (their different cell-cell contacts will permit again different cell fates: Gönczy and Rose, 2005); thus neither ABa nor its descendants contact P cells. In the absence of other cues, cell-cell contacts are a consequence of the orientation of the spindle axis, which is intrinsically autonomous and founded on a pre-existing, global, fine tuned polarization. How are the first cleavage planes (that depend on the orientation of the spindle poles, i.e. the location of centrosomes) correctly and precisely positioned? The oocyte starts from a not-polarized state, and acquires from the sperm centrosome not only a simple AP polarity, but a complete circumferential and spherical polarity, through which it can control the positioning of the sagittal plane and the lateral shifting of the spindle axis: the *C. elegans* zygote has two different spindles at the same time, meiotic, anastral and acentrosomal, and mitotic with astral MTs (Müller-Reichert et al., 2010). These structural differences of the spindles imply different functions: meiotic spindles have the sole task of dividing chromosomes; the mitotic spindle form different types of MTs: astral MTs that reach the entire cell cortex, polar anchoring MTs directed toward the cortical cell pole, and midzone MTs; only the last ones (linked to the centromeres or overlapping MTs from the other centrosome) and a bundle of polar MTs, directed from the centrosome (i.e. the spindle pole) to the cell pole, play a role in dividing chromosomes and pulling the spindle towards the cell poles; astral MTs are not useful for chromosome movements, as their plus-ends contact all around the cortex and their different directions are not oriented to pull in one fixed way. Astral MTs perform other tasks (Müller-Reichert et al., 2010): through them, DNA imposes, all around the cortex, the proper cell geometry, "maps" the cortex and controls attentively the mechanism that polarizes the whole cell (Schenk et al., 2010). The centrosome is the only (Vinogradova et al., 2012) cytoskeletal organizing center: to reach the forecast part of the cortex and deliver molecular polarity complexes (the "memory" of cell global polarity), its PCM (formed by the two orthogonal centrioles) support the necessary two degrees of freedom (the third being imposed by the distance from centrosome and cortex) with a precision whose order of magnitude (to be sufficiently resistant to the noise) is compatible with the dimensions of the structures that must be positioned, e.g. adherens junctions or new centrioles in mitosis (Siegrist and Doe, 2007).

Ascertain that in *C.elegans* a fine polarity is imposed by the centrosome is a fundamental milestone: Metazoan cells have a global "real" circumferential polarity, physically mapped in the cortex through molecular signals delivered by astral MTs, consisting of nine meridian sectors (as the blades of the mother centriole), possibly further subdivided (cytasters: Salinas-Saavedra and Vargas, 2011) to which a second polarization (the latitude, imposed by the daughter orthogonal centriole) is added to manage a third dimension. Anterior, posterior, dorsal, ventral, left and right poles are only some of the real poles whose molecular landmarks map the cell cortex: AP, DV and LR axes are only "theoretical" or "virtual" axes, used as references.

Controlling axes orientation, precisely repositioning and realigning them (e.g. during limbs formation) is a very important topic in Metazoa development (Kondo, 1992):

Drosophila leg imaginal discs are divided along two orthogonal axes (Wingless, Decapentaplegic and Hedgehog gradients: Emlen D.J., Allen C.E. (2003), Baker, 2011) into different domains; only a little number of cells at the intersection of these domains form the distal pole tip of the newly forming leg; imaginal disc cells organize concentric rings corresponding to the segments of the adult leg (see Box 1 in: Emlen D.J., Allen C.E., 2003), and position the tip of the distal pole near to the center, but with a controlled asymmetry (off-centering) in respect to the external ring: so the future tilt of the proximal-distal (PD) axis of the forming limb is determined; its orientation in respect to the three axes of the body is the consequence of the controlled off-centring of the distal pole that orients the correspondent extroflection tilt of the imaginal disc: only finely and rotationally polarized cells can perform this task of precisely translating a fine-rotational-tuned 2D (planar) arrangement of concentric rings into a 3D limb, growing according to a definite orientation of its PD axis. The Proximal-Distal axis has a particular orientation in the different limbs of the same organism: in the ipsilateral wing, antenna, eye and legs of *D. melanogaser* it has different tilt angles. Also the orientation of Dorsal-Ventral and Anterior-Posterior axes are redefined in primordial buds for limbs: in Cephalopods, tentacles has a common PD axis, but each tentacle orientates differently from the others its own DV and AP axes; in Primates, the ipsilateral arm and leg show differently oriented PD, DV and AP axes. Of extraordinary importance for the evolution of Humans has been the repositioning, in the hand, of the thumb axes, properly oriented in order to build an opposable finger.

Apical constriction and adherens junctions

"Ninein, γ-TuRCs and MTOCs are released from the centrosome and move bidirectionally along microtubules" (Vinogradova et al., 2012).

"Ninein is released from the centrosome, translocated with the microtubules, and is responsible for the anchorage of microtubule minus-ends to the apical sites." (Mogensen et al., 2000)

"Actomyosin Contractility and microtubules drive apical constriction in *Xenopus* bottle cells" (Lee and Harland, 2007).

"Microtubules remodel actomyosin networks" (Waterman-Storer et al., 2000)

Apical constriction is a powerful morphogenetic instrument, used for bending tissues (e.g. gastrulation), to form and lengthen cylindrical structures (e.g.: neurulation, convergent extension), processes subjected to a precise geometry. Apical constriction occurs in apical-basal polarized cells: the nucleus in a basal position, a network of microfilaments at the apical cortex, apical tight junctions and sub-apical adherens junctions (AJ), a particular organisation of the whole cytoskeleton (Bellet et al., 2009). Apical constriction is sustained by F-actin and NM-myosin microfilaments that can be arranged in various configurations (Andrew and Ewald, 2010). Karr and Alberts (1986) wrote: "More surprisingly, our results suggest that the centrosome, presumably by virtue of its microtubule-organizing activity, can also act as an important organizing centre for actin". Sigrist and Doe (2007):"Microtubules deliver RhoGEF2 to the apical cortex, where it induces Rho1-dependent myosin II contractility during gastrulation"; "microtubules are also used to induce cadherin cortical polarity in epithelial cells". Centrosomal MTs control the remodelling of AJs (Foethke et al., 2009) and govern the arrangement of the actomyosin bundles interacting with RhoA kinase (Lee

and Harland, 2007; Takesono et al., 2010). This function, performed by centrosomal MTs, is crucial because apical constriction may be carried out with different geometry to obtain different morphogenetic results; all the participating macromolecular complexes show high operational directionality consequent on asymmetrical and anisotropic arrangements: thus different effects are created, constriction of the whole apical surface (blastopore bottle cells), constriction limited to only one edge of the apical surface of several neighbouring cells (rosette formation), constriction, in several aligned cells, involving two opposite edges of the apical surface, perpendicularly to a cylinder axis (morphogenesis of tubes). In order for these processes to be performed correctly, several mechanisms intervene: i) centrosomal MTs control the direction of the actomyosin microfilaments (Waterman-Storer et al., 2000); ii) centrosomal MTs regulate the geometry of the contacts of microfilaments bundles with AJs (Bellet et al., 2009); iii) regulation of the asymmetrical arrangement of AJs, by means of cadherin turnover, conveyed by vesicles recycled through endocytosis and repositioned in new specific positions (Ivanov et al., 2006); iv) control of the cell polarity complexes that map out the region involved (Sigrist and Doe, 2007; Rauzi et al., 2010). Various components of the catenin family form bridging links between intramembrane cadherins and specific components of the cytoskeleton (Kadir et al., 2011): in particular, p-120 catenin is linked to the MTs, while β and α catenin are linked to actin and to other microfilament associated proteins, including α-actinin, vinculin and formin (Lee and Harland, 2007; Sawyer et al., 2010). From the study of tracheal development in *Drosophila*, a process of tube morphogenesis where the architecture of *tracheae* is achieved through cell migration and convergent extension, it has emerged that cytoskeleton reorganisation occurs by repositioning new MTOCs (MicroTubule Organizing Centres), which are transferred from the centrosome to the expected locations in the cortex, under the guidance of the gene *tracheless*, by a two-stage process: the MTOC components are firstly released from the centrosome under the control of the protein Spastin and secured to the MTs, to be conveyed towards the membrane area which they are destined to; then, the trans-membrane protein Piopio keeps them anchored to the membrane (Moss et al., 2007; Brodu et al., 2010). Similar processes have also been described in other types of Vertebrates epithelial cells (Ou et al., 2002; Lopez and Jansen, 2004; Salman et al., 2005; Zallen, 2007).

These highly directional mechanisms contribute in a crucial manner to the dynamic cell functions and constitute a cellular basis for growth anisotropy (Waterman-Storer et al., 2000); in Metazoan tissues, cell motility is no longer entrusted to the cilia and only partly to the pseudopods, but based above all on the dynamics of cell-cell contacts and on apical constriction (Lee and Harland, 2007). These movements are managed by genes that control the correct disposal of AJs and polarity protein complexes by means of signaling pathways whose geometrical information is decoded by the centrosome (Bellet et al., 2009). A study on the MTs of cochlear cells has been conducted by many authors (Mogensen et al., 2000; Piel 2000; Bornens 2002); "Ninein seems to play an important role in the positioning and anchorage of the microtubule minus-ends in these cochlear supporting epithelial cells"; "evidence from studies on cochlear epithelial cells suggests that centrosomal nucleation is retained in these cells and that a microtubule release and capture mechanism is responsible for the construction of the apical cell surface associated non-radial microtubule arrays"; "ninein is released from the centrosome, translocated with the microtubules, and is respon-

sible for the anchorage of microtubule minus-ends to the apical sites." (Mogensen et al., 2000). MTOCs (centrosomal and extracentrosomal) have not only a controlled location and anchoring in the cell, but they must be correctly oriented in order to nucleate MTs in the proper direction. The demonstration that MTOCs originate in the centrosome and are conveyed by MTs from the centrosome towards the forecast extra-centrosomal cortical position is fundamental because underlines the geometric role of the centrosome: "MTOC components are first released from the centrosome by the activity of the MT-severing protein Spastin, and then anchored apically through the transmembrane protein Piopio. We further show that these changes are essential for tracheal development, thus stressing the functional relevance of MT reorganization for morphogenesis" (Brodu et al., 2010); "the Golgi itself functions as an unconventional MTOC; laser ablation of the centrosome did not alter already-formed Golgi complexes; acentrosomal cells fail to re-assemble an integral complex upon nocodazole washout. Our data indicate that centrosomal microtubules complement Golgi self-organization for proper Golgi assembly and motile cell polarization" (Vinogradova et al., 2012).

An in-depth review summarizing the role of centrosomal MTs has been carried out by Siegrist and Doe (2007):
1. MTs regulate cortical polarity and actin dynamics in neuronal growth cones
2. MTs induce cortical Rac1 activation and lamellipod formation during cell migration
3. MTs induce apical cortical polarity in Drosophila epithelia and neuroblasts
4. MTs induce cadherin clustering in Drosophila epithelia
5. MTs are used to induce cadherin cortical polarity in epithelial cells.
6. Nuclear localization signal peptides induce molecular delivery along microtubules (Salman et al., 2010)

Planar cell polarity

"Wnt /PCP signaling shapes the MT cytoskeleton by biasing the intracellular position of the centrosome". (Sepich et al., 2011).

Planar cell polarity (PCP) is one of the most important morphogenetic mechanisms because it supports the coordinated movement of an entire population of cells, like an epithelial sheet; it is closely connected to the architecture of the cytoskeleton, acts on apical-basal polarised cells and orients the proximal and distal poles of their apical surface through cortical bundles of MTs (Harumoto et al., 2010). The asymmetrical arrangement of the Planar Polarity "Frizzled" receptors (positioned on the distal side of the apical surface) and "Strabismus" ("Van Gogh" in Vertebrates, located proximally) precedes the appearance of planar polarity signals (Strutt, 2009; Vladar et al., 2009), is based on a pre-existing cell geometry (all cells must be apical-basal polarized and, above all, circumferentially orderly polarized) that is due to centrosomal MTs. Planar polarity components act on the MTOCs, changing, contemporaneously in all the cells involved, their orientation through centrosome repositioning: " Wnt / PCP signaling shapes the MT cytoskeleton by biasing the intracellular position of the centrosome and possibly dependent organelles. In turn, Wnt/PCP signaling requires MT function so it can respond to global AP positional information by enriching Wnt/ PCP components at anterior or posterior cell edges and mediate polarized cell movement behaviours underlying convergence and extension" (Sepich et al., 2011); the

centrosome supports, with precision and directionality, PCP signaling through the accurate positioning of receptors for PCP signals. The relationship between planar polarity and MTs in AJ remodelling has already been highlighted (Harris and Peifer, 2009). The orientation of the mitotic division plane induced by planar polarity signals is driven to enlarge and bend the sheet according to predetermined directions: centrosomes are positioned and reoriented by special cortical markers (Peyre et al., 2011); for example, the division of vertebrate neuroepithelial cells occurs by following a characteristic procedure: the spindle is shaped with the axis randomly oriented; in metaphase a rotation aligns the spindle axis parallel to the epithelial sheet plane; this is due to the presence in the cortex of a narrow equatorial band made up of NuMa (Nuclear and Mitotic Apparatus) proteins, LGN (a G-protein regulator) and Ga_i (Guanine nucleotide exchange factor). The localization of these complexes occurs in two steps: in interphase Ga_i and Ric-8A (resistance to inhibitors of cholinesterase-8) are localized on the centrosome together with γ tubulin (γ-TuRCs) and conveyed towards the cortex through centrosomal MTs (nucleated by centrosomal γ-TuRCs); in metaphase, astral MTs carry NuMA, LGN and dynein into locations where they can be anchored by the previously positioned Ga_i and Ric-8A (Woodard et al., 2010). The centrosome, through orientated MTs, attentively selected, positions and anchors first-ly the molecular motors (dynein) at the future cell poles: in metaphase, these motors, connected to select astral MTs, orient the spindle correctly. Sugiyama et al. (2011) illustrate the interaction between planar polarity, more correctly defined as Global Polarity Signaling, and centrosome/primary cilium in one of the most refined structures that exists, the lens.

Orientation of cleavage planes

"The preceding interphase aster centers and orients a pair of centrosomes prior to nuclear envelope breakdown, and the spindle assembles between these prepositioned centrosomes" (Wühr et al., 2010)

"Some characteristics of mouse early development could be linked to the absence of centrioles: (1) the absence of regular cleavage planes during early development; (2) the absence of any detectable axis of polarity within the blastomeres before the 8-cell stage; (3) the random position of the spindle relative to the axis of polarity within the blastomere during the fourth cleavage. Centrioles may act to keep PCM components in a precise position throughout the cell cycle and so be useful in the control of the position of the axis of polarity and division. This may become more important in differentiated cells, such as those found in the outer layer of the blastocyst" (Gueth-Hallonet et al., 1993).

Cleavage planes are orientated by intrinsic (nuclear) or extrinsic (signaling pathways) cues; Wühr et al. (2010) have modeled cleavage plane determination in the large cells of fishes and frogs embryos: "Current models for cleavage plane determination propose that metaphase spindles are positioned and oriented by interactions of their astral microtubules with the cellular cortex, followed by cleavage in the plane of the metaphase plate. In early frog and fish embryos, where cells are unusually large, astral microtubules in metaphase are too short to position and orient the spindle. Rather, the preceding interphase aster centers and orients a pair of centrosomes prior to nuclear envelope breakdown, and the spindle assembles between these prep-

ositioned centrosomes". The sperm aster (linked to the zygote membrane after entering) only centers and orients (coordinate φ and θ) the two centrosomes and the two nuclei (female and male) for next mitosis, then it disappears without participating in mitosis; two new little asters are formed around each centrosome, without changing their orientation; these asters until anaphase, have MTs too short to anchoring to the membrane and orienting centrosomes for next division; when, in anaphase-telophase, they reach the cell cortex, they divide the zygote, link to the cell membrane and, only in this stage, centrosomes (already duplicated in advance) can be oriented for next mitosis (author's immunofluorescence photos clearly show the two new centrosomes already aligned according to the next division plane, in both the sisters cells during cytokinesis); in these large cells the process of centrosome duplication is performed long before cells enter S phase, because there is not enough time, at the onset of mitosis, for the new centrosomal MTs to reach the cell cortex for positioning the molecular motors in the correct forecast locations in order to orient and pull centrosomes: centrosomes duplicate during previous mitosis and orientate themselves exploiting spindle MTs that, in anaphase, are enough long to reach the cell cortex; after being positioned and orientated, they assemble the new spindle that fix the new division plane. This is particularly important, because shows the real task of the centrosome in mitosis: the old centrosome positions and orientates the new one ("Centriole duplication is part of the mechanism by which the cytoskeleton of the daughter cell is patterned upon that of the mother": Harold, 2005); then, after being correctly located in accord with DNA instructions, they orientate the cleavage plane; in these large cells, centrosomes utilize an already existing (labeled) aster to acquire their forecast locations in order to drive the next division plane according to morphogenetic instructions. Also in brown algae, which show a wide range of sizes and forms and, unlike high plants, have centrioles and centrosomes but lack cortical MTs, cytokinesis is not synchronized with centrosome duplication; it starts long after the end of telophase and the division plane is determined by the position of the centrosomes which, after mitosis, change their location and find the new position, pulled by Mt operating motors, previously placed near the cortex through MT-depended transport (Katsaros et al., 2006).

Vorobjev and Chentsov (1982) studied the movements of the mother centriole: "of particular interest is the phenomenon of orientation of mother centrioles towards the spindle axis in metaphase and anaphase and towards the substrate plane at the beginning and at the end of interphase"; *in vitro* "during interphase, a centriole is oriented perpendicular to the substrates in the periods of complete rearrangement of the microtubule system (leaving mitosis and entering mitosis); during mitosis, centrioles are pole-oriented when chromosomes form a metaphase plate and are in the course of their separation. Thus, the orientation of centrioles may be associated with that of other intracellular structures". Karr and Alberts (1986) described the 90° movement of centrosomes around the nucleus in *Drosophila* syncytial embryos during prophase and their return, in telophase, to the premitotic position. Also Jonsdottir et al. (2010) observed that centrioles are mobile during mitosis and that their movements are not the same in different cell lines: during cell division centrioles move along the nuclear membrane or along MTs, frequently, but not always, near to the intercellular bridge where they are not indispensable for completion of abscission. As already said, a two-step movement orientates the mitotic spindle in neuroepithelial cells (Peyre et al., 2011): before metaphase, the three axes -x, y, z- of the spindle are randomly orient-

ed; first, all cells align their spindle parallel to the apical surface (planar orientation: angle θ), then, through a rotation (different from cell to cell, in order to enlarge the epithelial sheet in any direction) cells align the spindle axis in the "x y" plane (angle φ). The dynamics of this "xy"-rotation is not equal and cells change the direction of their 2xy" axis at least once during metaphase.

Cell migration and convergent extension

"An intact centrosome is required for the maintenance of polarization during directional cell migration" (Wakida et al., 2010).

"Like blood neutrophils, dHL60 cells respond to a uniform concentration of attractant by polarizing in apparently random directions. How each cell chooses its own direction is unknown. We now find that an arrow drawn from the center of the nucleus of an unpolarized cell to its centrosome strongly predicts the subsequent direction of attractant-induced polarity" (Xu et al., 2007)

Cell migration is another fundamental morphogenetic process in which the essential role of centrosomal MTs has been highlighted; by intervening pharmacologically on the MTs dynamics (taxol, colchicine, nocodazole) the formation of the characteristic polarity of migrating cells (a posterior uropod and an anterior lamellipod) is impeded (Eddy et al., 2002). Depolymerisation of the MTs array causes uncoordinated cortical contractions; the accumulation of the characteristic uropod proteins is present in sites independent of the direction of chemotactic stimuli (Schliwa et al., 2002). Wakida et al. (2010) have demonstrated the essential role of the centrosome and centrosomal MTs during migration: centrosome laser-ablation produces a variation in shape (less asymmetry and more homogeneity), polarization persistently disappears, both the array of MTs and the network of actin microfilaments are modified profoundly, and cell migration is consequently dramatically modified; the speed of the movements is slightly reduced but the orientation is seriously affected, so that the direction of the movements is random. When cells are subject to external cues (morphogens or chemo attractants) the centrosome is re-orientated (again making use of geometrical properties of asters, PCM and centrioles) to modify cell geometry by changing the polarization of the cytoskeleton, in order to define a protrusive front and a retracting rear; MTs extend in the direction of the free edge and contribute to connect the centrosome to the nucleus. Without "wheels" to turn, migrating cells (in which maintaining a programmed and coordinated polarity, like in tissues, is not necessary) centrosome drive the cell through the re-orientation and re-positioning of the entire cytoskeleton in accord with the new front-rear axis, in order to correctly deliver the molecular machinery of pseudopods. During convergent extension, cues for centrosome reorientation are cadherin-mediated cell–cell interactions that "induce nucleus and centrosome off-centering toward cell–cell contacts, and promote orientation of the nucleus–centrosome axis toward free cell edges" (Dupin et al., 2009).

Filopods, cytonems and morphogens

MTs-based filopods and cytonems have been found during development in many phyla and in different tissues, as in sea urchin archenteron (Miller et al., 1995) and fly imaginal discs (Gibson and Schubiger, 2000). Filopods and cytonems are cellular

extensions that mediate sensory functions and precise long range signaling: through them, cells can reach with high precision their targets. This is a mechanism that adds a long range 3D precision to morphogens signals, whose gradients cannot reach high local accuracy. Filopods have been described also in Vertebrates (Schober et al., 2007): in mouse fibroblasts, the directional role of centrosomal MTs in organizing actin bundles in filopods has been investigated; their sensorial activity, associated with centrosome geometry, detects 3D spatial information: a cell can identify an extracellular location relatively to its own position and orientation. In *Drosophila,* filopodia extensions are involved in different signaling pathways during disc evagination (Roy et al., 2011): Bryant's (1981) experiments showed that, in the imaginal discs of *D. melanogaster* and in the early limb buds of Vertebrates, cell-cell contacts are mediated by a geometrical circumferential polarization of cells: few cells can establish the precise planar location of the limb Distal pole, off-centering its tip in respect to the disc's boundary, in order to orientate the tilt of the PD axis (Kondo, 1992). Filopods and cytonemes have been studied in limb buds; the role of centrosomal MTs in recruitment and control of actin microfilaments, already examined in apical constriction, is particularly important in filopods: MTs empower actin microfilaments giving filopods the capability to orientate and regulate their own length (Picone et al., 2010), fundamental for sensorial functions and morphogens transport.

In chemical signaling pathways and morphogens gradients, the background noise is often high in relation to the dimensions of the macromolecular complexes involved (Lander et al., 2009): the centrosome, with two circumferential polarities orthogonal to each other has the possibility of identifying in a three-dimensional cortex, by means of MTs, precise spatial localizations, characterized by extensions that are too small to be easily managed by extracellular chemical gradients; the dynamics and the constantly variable geometry of AJs (Kadir et al., 2011), apical constriction (Huang et al., 2011), philopods and pseudopods (Cernuda-Morollòn, 2010) are difficult to explain without such a geometrical and precise tool.

Tube morphogenesis

Cylindrical structures are widespread in all multicellular organisms: in plants, they make up a large part of the organism, and in Metazoa digestive, circulatory, respiratory and urogenital apparatuses are basically cylindrical, as are long bones and other organs (cochlea, semicircular canals, glands, dental roots, etc.). In Vertebrates, cylindrical structures reach degrees of high complexity: an example is the renal parenchyma. The formation of tubes takes place through a set of geometrical processes: formation of the tube outline, lengthening, bending, bifurcation or opening of lateral derivations. The initial formation of a cylinder uses apical constriction (Andrew and Ewald, 2010): the contraction of cortical actin bundles parallel to each other and located on opposite sides of the apical surface allow directional shortening of the apex perpendicularly to the tube axis (wrapping: e.g. neurulation). Constriction in only one side, simultaneously in several adjacent cells, leads to the formation of rosettes (budding: e.g. invagination of tracheae in *Drosophila).* Tubes are lengthened through convergent extension, through extra-cellular matrix fibre tension, suitably oriented with regard to the tube axis (Keller, 2006) and through the orientation of the mitotic plane. All these phenomena follow the same procedures already examined in

the description of apical constriction (Sawyer et al., 2010) and AJ turnover (Lee and Harland, 2007). In Metazoa, the tubes fork and bend, supplying spectacular aspects of morphogenesis: nephrons histology and the bilateral bending and orientation of inner ear semicircular canals in Mammals illustrate the sophisticated morphogenetic control of cylindrical structures. Recent surveys on the extra-cellular matrix have revealed the close relationship between MTs and receptors (integrins) linking the cell membrane to the fibres of the extra-cellular matrix (Pulina et al., 2011). Migrations of groups of cells and the dragging of others, anchored to the first by AJs, direct the orientation and bending of the entire structure: time coordination of these events in all the cells concerned is controlled by dozens of receptors for the different signaling pathways involved. So, a geometric architectural plan, structured on several levels allows surprising results to be achieved: firstly, positioning and orienting of the actomyosin bundle (apical constriction) shapes the primitive tube outline; then the control of growth (orientation of the division plane, tension of extra-cellular matrix fibres, management of the convergent extension by means of programmed directional remodelling of AJs) guides planned elongation of tubes. Bending (geometry of AJs and extra-cellular matrix fibres) and orientation of the cytodieresis plane generate the final form (Keller, 2006).

Left-Right

"What sub cellular component is responsible for the crucial orientation event that defines "leftward"? One likely possibility is that the coordination of the 3 axes is performed by a cytoskeletal organizing center such as the centriole or basal body". "The sharp midline separation suggests that the first cell cleavage in *X. laevis* may produce L and R halves that inherit differential chiral information." (Vanderberg and Levin, 2009).

"The development of handed asymmetry requires a special mechanism for consistently specifying a difference between left and right sides" (Brown and Wolpert, 1990).

"An intrinsically chiral structure, perhaps the centrosome, serves as a template for directing polarity in the absence of spatial cues. Such a template could help to determine left–right asymmetry and planar polarity in development" (Xu et al., 2007).

Cilia are considered the initiators of asymmetry: the flow generated by the primary cilia of Hensen's node cells induces asymmetry in many internal organs (heart, liver); the cilium has a basal body: a centriole. Do centrioles play a role in left-right patterning, as supposed by Xu et al. (2007) and Vanderberg and Levin (2009)? Brown and Wolpert (1990) hypothesized a molecule or a cellular structure, named "F", with intrinsic enantiomorphism (reversible or rather reflectable into a mirror image) , capable to add a Left-Right axis perpendicularly to the AP and DV axes: doubtless, centrioles are the only organelles whose components (supposed not-equivalent), if assembled with inverse circumferential sequence (clockwise / counter clockwise), can generate enantiomorphous organelles, right- or left-handed; in addition, the centrosome is strictly connected to the cytoskeleton and organizes cell geometry and polarity; the centrosome is the unique cytoplasmic organelle existing in only one copy; furthermore centrioles and centrosomes have a characteristic unique process of duplication: after mitosis each one of the two new daughters-sisters cells inherits from the mother an old centriole (epigenetic, or rather, non-genetic transmission of information).

A new hypothesis

The mirror symmetry of two objects consists in the sign of only one of the three coordinates: any point P (with coordinates x, y, and z) belonging to one object, is symmetrical to the point P' belonging to the other object whose coordinates are -x, y, z; in a spherical reference system, only the sign of the coordinate ϕ changes. The idea that the inverse polarity of the mother centriole can be the basis of the bilateral symmetry of Metazoa appears attractive and tempting: the centrosome is the cytoskeletal organizing center, therefore cells whose mother centriole has an inverted polarity build their asters and cytoskeletons with the same inverted polarity, and so is for the disposition of cell landmarks, polarity complexes, receptors and signaling molecules: all the morphogenetic processes (planar polarity, migration, tube lengthening and bending, convergence and extension, etc.) can be carried out symmetrically, because all cues stimulate cells that, possessing an inverted disposition of the cytoskeleton and landmarks, respond through mirror symmetric executions of morphogenetic instructions. Thus, the same inverted circumferential polarity is transmitted to tissues and organs that develop bilaterally symmetric. Moreover, to reverse the polarity of a polygon (like the *Chlamydomonas* "acorn" shaped structure, described by Geimer and Melkonian, 2004), only a simple, easy reflection (Fig. 5) is necessary and sufficient. In *Lymnaea peregra*, a snail, a maternal cytoplasmic molecular complex is responsible of the left- or right-handed spiral cleavage during the generation of the first blastomeres (Andreuccetti et al., 2010), suggesting that homozygous defective mothers do not form a cytoplasmic factor able to repress the reversal of centriole polarity in the zygote: in fact it is quite surprising that only one defective gene is able to completely reverse (with perfect mirror symmetry) in many and many cells their arrangement and orientation of cleavage planes. The centrosome (or rather its mother centriole) frankly is the only cellular organelle that possesses all the characteristics to be the "chiral structure" which determines the left-right pattern (Xu et al., 2007) or the "cellular component" that specifies the difference between left and right sides (Vandenberg and Levin, 2009): invertible sequence of triplets, heritable transmission of the mother centriole (only once per cell cycle duplicated), management of cytoskeleton polarity, uniqueness (one cell-one centrosome); it appears very difficult, if not impossible, to find another structure or another process which is capable to concretely realize the precise reversal (reflection) of only one coordinate in all the cells of many different organs.

In plants there is neither bilateral symmetry nor left-right polarity. Unlike animals, plants and trees clearly do not show any general bilateral symmetry; only leaves and flowers present a shape that "seems" (locally) to be nearly symmetric, but 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 (Tsukaya, 2005; Efroni et al., 2010). 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 (Almeida and Galego, 2005): 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 ana-

tomical, histological and developmental foundations; the two cotyledons (in dicots) have different shapes too; other symmetries frequently appear, with several reflection planes. It is known that the apparent bilateral symmetry of zygomorphous flowers has an advantage in attracting pollinator insects: flowers have reached their quasi-symmetry, modifying their plans for building laminae and layers. Bilateral symmetry means that two (eventually distant) structures (the hands or the ears) show anatomical, histological and cellular mirror symmetry; in the two mammalian cochleae, the general shape is clearly symmetric: furthermore also symmetric is the disposition of membranes (basilar, tectorial, Reissner's), of cells (Corti's, Deiters', Koelliker's) and of their cytoskeletal MTs. The two hands are clearly symmetric: however a single finger can show (like in zygomorphous flowers) its own "apparent" plane of symmetry, but anatomy, histology and embryology do not support this idea. In *C.elegans*, morphogenesis of the intestine shows that the two first cells (Ea and Ep) divide transversally and generate their bilateral counterparts: from now up, right and left cells will have mirror symmetric shape, polarity, dimensions, movements, orientation of cleavage planes, and cell-cell adhesions (McGhee, 2007); on the contrary, during vulva formation, the first divisions occur longitudinally and an "apparent" anterior-posterior bilateral symmetry appears; however asymmetry is detectable between the anterior and posterior halves: the anterior one develops faster than the posterior and cells do not show signs of differentiation, and have different dimensions, different cell-cell contacts and different cell adhesions (Sharma-Kishore et al., 1999). As already said, centrioles are present in eukaryotic cells, but not in higher plants and most fungi; unicellular organisms have only centrioles/basal bodies, each one independent, while in algae and Metazoa two orthogonal centrioles are embedded in the PeriCentriolar Material that forms the centrosome (the review by Bornens, 2012, is interesting in this regard).

An accurate bilateral symmetry and a definite left-right polarity have been established in Metazoa.

Drosophila right and left wings reproduce, mirror-like, the same shape, edge curvature, compartments and arrangement of *tracheae* and their anastomoses; *Ultrabithorax* mutants, instead of halters, develop a pair of additional wings that are built with the same bilateral symmetry; a mutation in the *apterous* gene causes a phenotype without wings: inserting in the mutant fly embryo the human ortholog gene LHX2, the normal wild-type phenotype is rescued, with two bilaterally symmetric wings (Strachan and Read, 2010); it is clear that upstream the same genes and the same signals act, but downstream there are two different ways, left- and right-handed, of carrying out instructions; development confirms that wings originate from symmetrical primordia, the imaginal discs. The bilateral symmetry of Metazoa has clear anatomical, histological and developmental foundations. Metazoa possess centrosomes, plants do not. Obviously, the inevitable variability of biological processes (due to several cues, especially when enormous numbers of cells are involved) can originate very little differences between left and right: our faces, frankly bilaterally symmetric, can show a slight (controlled) asymmetry (but our legs have the same precise -bilaterally symmetric- length!).

Bilateral symmetry in Metazoa is more marked than it may appear: it is evident in the unpaired bones, cranium, vertebral column, pelvis and thoracic cage, but also the unpaired internal organs, originated from symmetrical primordial, keep this symme-

try until, during development, asymmetric movements, rotations, twists and developmental adaptations are superimposed in only one half of the body. Metazoa are often described as superficially symmetric but interiorly asymmetric: my personal opinion is that in Metazoa bilateral symmetry is a fundamental basic property of their locomotive system and of their sensorineural apparatus, which drives locomotion movements: bilateral symmetry is the simplest and the most efficient way to drive and control the direction of movements and to localize perceived signals (differential stimulation of two equal bilateral effectors or receptors); without bilateral symmetry, for animals the control of their balance would be a very difficult problem; this seems to be the reason of the extraordinary evolutive success of bilateral symmetry in mobile organisms (the asymmetry of high brain functions, like language or face perception, is superimposed to the basic sensorial bilateral symmetry). The initial symmetry of internal organs is therefore only a consequence of the establishment of a general symmetry plan, but does not have functional significance: in fact Vertebrates have developed and adopted asymmetry in many internal organs to solve, for example, the not-elementary problems of the anatomy and physiology (fluid dynamics) of the great vessels.

In mathematics (Savriama and Kingelberg, 2011) the bilateral symmetry of three-dimensional objects consists, as already said, in the opposite sign (+/-) of only one of the three coordinates: when a plane of symmetry is chosen ("z y" for example), any point P of coordinates "x, y, z" is symmetrical to the point P' which has coordinates "-x, y, z"; in a spherical reference system, only the sign of the coordinate φ changes. If one of two instruments composed of two orthogonal goniometers has the goniometer responsible for the angle φ assembled with inverted polarity (inverted sequence of marks), it carries out each instruction relating to coordinate φ symmetrically. Bilateral symmetry has strictly geometrical bases: the mirror symmetry of our ears (pinna, middle ear with its little bones, semicircular canals and cochlea with their inner structures) is incredible, but the symmetry consists in nothing but the sign of only one coordinate of each point. Centrosomes, with inverted polarity of the mother centriole, are geometrical interfaces which receive the same input (signal with information for the value of the coordinate φ) and translate it with bilateral symmetry (output); during organogenesis, their inverted polarity, transmitted to the aster, cytoskeleton and cell cortex and acquired by descendant cells, organizes and forms symmetric organs. Bryant (1981: for review see also: Gilbert, 2010, Baker, 2011) proposed the "Polar Coordinate Model of Positional Information in the Developing and Regenerating Limb": in this model, cells are supposed to have a circumferential value and adjacent cells "sense" neighbouring signals. If developing tissues or cells normally not-adjacent are juxtaposed, duplications arise: Bryant's polar coordinate model forecasts the orientation of duplicated limbs; the graft of a left limb bud (or a left regeneration blastema) on the contralateral right stump, causes three areas of re-growth that produce three limbs; a central left limb, composed by the transplanted left cells, that maintain their circumferential value (inverted regarding right stump cells) and the characteristic left-handed tilt and orientation of the three axes; other two abnormal external right limbs growth, composed by stump right cells, conserving the AP and DV axes proper of the right limb, but with the PD axis rotated respectively of +/- 90° (cells try to normalize circumferential cell-cell contacts inducing the arising of correctly polarized cells interpolated between graft and stump cells); cells that are differently patterned (left/right) differently respond to the same morphogenetic stimu-

lus: they build the forecast structures, but through their own polarity; the orientation of PD axis in limb buds depends on the spatial position (orientation of circumferential values) of the neighbouring cells from which the blastema is induced. Bryant's "shortest intercalation rule" shows that the juxtaposition of surface receptors (circumferentially ordered) "senses" incorrect cell-cell contacts and stimulates the correct differentiation and intercalation of cells with correctly oriented rotational polarity. Bryant's model completely agrees with the two statements: i) cells possess a circumferential global polarity, imposed by the centrosome which organizes the cytoskeleton; ii) inverted polarity, imposed by the mother centriole, is the base of left-right bilateral symmetry. Bryant's model indicates that the "chiral structure" that determines the left-right pattern (Xu et al., 2007) and the "cellular component" that specifies the difference between left and right sides (Vandenberg and Levin, 2009) must possess a "rotational" chirality, not a simple generic enantiomorphism: this can confirm that only the mother centriole has such properties.

Germ cells and, consequently, every zygote has a mother centriole built with "default" rotational polarity: in the *Xenopus* zygote, Danilchik et al. (2006) filmed *in vivo* a circumferential asymmetry: rotational equatorial cortical movements (pharmacologically induced) are oriented in a single fixed ("default") direction in the zygote and in parthenogenetically activated oocytes: after mitosis, the two first blastomeres show similar equatorial cortical movements but with opposite direction between them; a left-right (symmetric) polarity (left- and right-blastomere) had been established. Every zygote has the same "default" pattern: in fact *situs viscerum solitus* is the same in every adult, not 50% as expected in case of random left/right patterned zygotes.

Breaking of bilateral symmetry. During the early stages of development, cilia of cells in the Hensen node (or its functional equivalent in other phyla) rotate unidirectionally, producing a flow that activate genes encoding transcription factors only in the cells of the left part of the organism that are reached by this flow. Thus many internal organs in Vertebrates are asymmetrical: heart, great vessels, digestive apparatus and its accessories, lungs, spleen, cerebral cortex. *Situs viscerum solitus* (well conserved during evolution) is established: the spleen and the heart on the left, the liver on the right. Studies carried out on the Zebra fish (*Danio Rerio*) have filmed the rotation and migration events that occur during heart early development and that are superimposed on the original bilateral symmetry (Okabe et al., 2008; Smith et al., 2008). In these organs, originated with bilateral symmetry, during a certain phase of development different processes are performed that involve only one half of the organism (Vanderberg and Levin, 2009): heterochelia, the difference in shape between the right and left chelae in Crustaceans, is one example of this; one of the two chelae is larger, is used in courtship and has a more suitable shape for cutting, while the other specializes in grasping. It appears that there is a common "default" program for both bilateral primordia: later, during development, in the left half this "default" program is silenced and replaced by a modified program, reserved uniquely to the left half (left-reserved program), that conserves the left-handed modality of execution. Errors in this phase cause "heterotaxia": in the rare cases of cardiac right isomerism, two both right atria develop (the "default" program is not silenced in the left half and the left-reserved program is not activated), while in left cardiac isomerism two both left atria develop (the "default" program is silenced also in the right half and the left-reserved program is activated in the right half too): surprisingly, in both cases, the

two atria are mirror-images of each other (bilateral symmetry) (Hildreth et al., 2009); this can confirm that cells, determined (circumferentially polarized by the mother centriole) to be right, transmit their polarity to aster and cytoskeleton, and execute any morphogenetic program in right-handed modality, while left cells translate the same instructions in left-handed modality. Also the already examined early spiral cleavage in snails is a reserved unilateral program, restricted to "default" cells.

Remarkable is the importance of gap junctions between left- and right-patterned adjacent cells: the pioneering experiments on frogs of first embryologists (Wilhelm Roux in 1888 and Oskar Hertwig in 1893) beyond a century ago showed that if one of the two first blastomeres is burnt off and the other continues to sense its membrane receptors (gap junctions), only a half embryo develops; on the other hand, if the two first cells are separated, each cell cannot "sense" the other cell through the gap-junctions and two complete organisms arise (Andreuccetti et al., 2010). "When separated at the 2-cell stage, Newt embryos exhibit 89% incidence of organ laterality reversal in one of the twins". (Takano et al 2007; Vanderberg and Levin, 2009). "The sharp midline separation suggests that the first cell cleavage in *X. laevis* may produce L and R halves that inherit differential chiral information" (Vanderberg and Levin, 2009); however, L and R cells with chiral information are (during the very first stages of development) able to "reset" and restart from an "original" condition: in fact, 16-cells-blastulae of sea urchin, artificially divided into two halves (one Ventral and one Dorsal) produce two complete larvae, the Ventral one conserving Animal-Vegetal and Dorsal-Ventral axes, and the Dorsal one that maintains only the original A-V axis, but acquires a new repositioned D-V axis and reverse L-R polarity (probable imperfect division of the blastopore); the graft of the Spemann organizer shows that the blastopore possesses L-R inductive capability, being able to induce receptor cells to form duplicated ectopic structures, bilaterally symmetric along a new midline, from neural tubes to complete heads (Andreuccetti et al., 2010). In these experiments the blastopore appears to control the positioning of the midline and to possess an unique role in managing L-R polarity (symmetry conservation or symmetry breakage induction); so, normally, it can maintain the natural L-R patterning with a fixed midline or can intervene to "reset and restart" imperfect blastulae in order to save very early splitting-events (twins). This is a powerful mechanism that greatly improves the survival of defective blastulae and the success of the reproduction. The blastopore, unpaired positioned on the midline, its bilateral symmetry, inducing activity and developmental connection with the Hensen node (or its equivalent) shows its important role in L-R symmetry and in symmetry breaking mechanisms; in Mammals, during gastrulation, left-right patterned cells from the epiblast primitive node (on the midline) invaginate and, directed from the midline to the Left or Right embryo half, replace the previous hypoblast cells (likely not left-right determined) to form the embryo endoderm; a second wave of entering left-right patterned cells forms the mesoderm, many cells moving laterally and some directed forwards to form the notochord on the midline, whose inducing activity is well known (neural plate, somites, intermediate and lateral plate mesoderm, prechordal plate: all these structures are formed through bilaterally symmetric morphogenetic processes), as well known is the role of nodal cells in L-R symmetry breaking (unidirectional flow of perinodal fluid due to unidirectional rotation of nodal cells primary cilium). Primordial germ cells (containing a "default" mother centriole) arise in the posterior region of the epiblast,

on the midline, close to the posterior end of the primitive streak, and likely subjected to its inducing activity. In Bryant's "Polar Coordinate Model of Positional Information in the Developing and Regenerating Limb" it appears that cells try to normalize circumferential cell-cell contacts, by inducing the arising of correctly polarized cells that interpolate between graft and stump cells, indicating a left-right pattering inducing activity also in limbs buds and in regenerating blastemas (probably triggered by signals from gap- junctions as seen in the experiments of Roux and Hertwig).

Left-right determination is an epigenetic process that has something in common with sex chromosomes and sex determination: in the two sexes the majority of organs have the same shape but the morphogenetic programs of the characteristic genital organs of one sex are strictly reserved to and expressed in this one only and silenced in the other one. Similarly, in the two halves of the organism, many organs are the same (symmetric) while others are characteristic and strictly reserved to one half only (chelae, spleen, bicuspid and tricuspid valve, left and right ventricle). In both cases this means that only in one sex or in one half of the body, access is allowed to that part of the genome which is reserved only to that sex or that half. The link between alterations of the normal functioning of sex chromosomes and left-right patterning is interesting: it is present in CHILD syndrome (König et al., 2000; see Fig. 2 in Vanderberg and Levin, 2009) and in gynandromorphism (see the astonishing image by Palmer, 2010). It is also useful to remember that teratomas, when derived from germ cells, occur in testes or ovaries, when derived from embryonic cells, occur on the midline.

A theoretical model of the centrosome

The centrosome plays the role of a geometrical, noise-resistant tool, through which DNA firstly maps (polarizes) the cell cortex and then finds any desired cellular location through the nucleation of oriented MTs; the centrosome is the cytoskeletal organizing center; its γ-TuRCs are not equivalent: each one is marked by its own receptor and oriented in accord to its position on the centrosome (parallel to the tangent plane at that point); γ-TuRCs nucleate MTs with correspondent direction (perpendicular to the tangent plane). The centrosome appears a geometric interface that receives coded signals (input) and returns correspondently oriented MTs (output): different signals (ligands), showing a particular targeting sequence, each one intended for a particular cortex location, are recognized by the correspondent receptors (decoding and translation are carried out by the usual ligand-receptor interaction) of those γ-TuRCs precisely oriented to nucleate an MT with the requested orientation; each γ-TuRC (or its centrosomal docking platform) displays its receptors to bind the correspondent ligand; the join between signals and γ-TuRCs receptors induces and triggers the nucleation of MTs, oriented to reach the desired locations. There is a biunivocal correspondence between signals and receptors and between coded spatial information (signal targeting sequences) and γ-TuRC's orientation. So any molecular complex, equipped with such geometrical targeting signals, can reach its forecast location.

Like all instruments, it is possible for the centrosome not to be used in a certain process: the task of separating chromosomes in mitosis and meiosis can be performed by the centrosome or be carried out in its absence by other redundant mechanisms, because this is only one of its accessory functions (Müller-Reichert et al., 2010, Riparbelli and Callaini, 2011).

Orientation of γ-TuRCs

The orientation of γ-TuRCs (or their centrosomal docking platforms) is the consequence of the semi-self-assembly process that forms the PCM around the two orthogonal centrioles: each docking platform, containing some equal γ-TuRCs (same orientation and receptors), is surrounded by four similar platforms (positioned at "North, South, East, West"): any angle between the planes of two adjacent platforms measures 140°, like each internal angle of a 2D regular nonagon and like the angle between the planes tangent to two consecutive MTs bundles on the centriolar wall; the two centrioles form an organizing platform for a set of regulatory molecules that catalyze and regulate PCM assembly: the biochemical management of 140° and 90° angles (Guichard et al., 2010; Cottee et al., 2011), the capability to control distances (Rosenbaum et al., 1969; Picone et al., 2010) and the intrinsic aptitude to order and arrange new cell structures under the influence of other, already existing, structures, the predisposition of some PCM components for semi-self-assembly in circular rings (Kitagawa et al., 2011; van Breugel et al., 2011) allow the cylindrical (or, rather, prismatic) shape of the wall of the two orthogonal centrioles to be transformed into a spherical shaped PCM, providing, according to the tilt (angles ϕ and θ) of the local tangent plane, the proper orientation of γ-TuRCs. (Figs. 2, 3, 6): as already seen, a half ring of four SAS-6 dimers lying on the plane containing the axis of the centriole and a triplet-blade can radiate, from the centriolar wall, four MTs (starting from the C-terminals of the SAS-dimers with an angle of 40° between themselves), having the same length but different tilt (like the radii of a circle) and terminating with a molecular complex like the *Chlamydomonas* Bld-10p that maintains γ-TuRCs orthogonal to the MTs (Hiraki et al., 2007): so, the longitudinal rectilinear shape of the centriolar wall can be transformed into a curved spherical surface. The surface of the centrosome is not really spherical, but the orientation of each γ-TuRC must correspond to the local curvature of both mother and daughter centriolar walls.

Labelling of γ-TuRCs

The attribution of address-receptors is carried out by the two orthogonal centrioles. Each centriole, through the nucleation of MTs perpendicular to its wall (or rather, lying on planes containing the axis of the centriole and the triplet-blade from which MTs originate) and through MT-operating molecular motors (kinesins), transmits its polarity to γ- TuRCs. The particular eccentric configuration of the two centrioles polarizes the centrosome: one vertical wedge is the "0°" mark (see "An input-output spherical reference system based on two orthogonal goniometers") used to orient the centrosome; the centrosome surface is subdivided into 45 small areas: in each one there are some γ-TuRCs (Schnackenberg et al., 1998) with the same orientation and the same address-receptors. Receptors are composed by two different molecules: one provided by the mother centriole relative to the ϕ coordinate (9 different receptors), the other (θ coordinate) received from the daughter centriole ("0°" mark, 4 different "left" marks and 4 corresponding "right"). Geometric signals join their correspondent γ-TuRC receptors and trigger the nucleation of MTs with the desired direction. Geometric signals can be present in kinesin-like-proteins, molecules whose heavy chain C-terminals together with their light chains are present in about 50 different types: they can bind diverse centrosomal γ-TuRC receptors and different cargo.

Centrosome orientation

During fertilization the sperm centrosome enters the oocyte and immediately maps the cortex of the zygote by its aster, whose MTs carry and deliver cortical landmarks: the mother centriole anchors the centrosome to the cell cortex (through the aster) and/or to the nuclear membrane; the distal appendages of the mother centriole provide the correct correspondence between aster and cell polarity. During mitosis centrioles and centrosomes are oriented in order to conserve and transmit the same coordinate polarity: "Centriole duplication is part of the mechanism by which the cytoskeleton of the daughter cell is patterned upon that of the mother" (Harold, 2005).

The centrosome can change its position (under a strictly genomic control) reorienting its "0°" wedge relatively to the pre-positioned cortical landmarks; migrating cells change their global polarity, firstly repositioning centrosomes and, then, changing asters and cortical landmarks to build a newly oriented cytoskeleton (see below: "Three pathways for centriole duplication").

Chirality of the mother centriole

Only the mother centriole can be polarized with inverted circumferential sequence (clockwise/counter-clockwise), to generate a rotationally enantiomorphous organelle. Centrosomes with inverted polarity of the mother centriole are geometrical interfaces which receive the same input (signals with the information for the value of the coordinate ϕ) but translate them symmetrically (output); during organogenesis, they transmit their intrinsic chirality to each symmetrical organ. As already said, only the mother centriole can be assembled with inverted polarity, on the contrary, the daughter centriole has always the same rotational polarity, in every cell.

Difference between longitudinal and latitudinal mapping

The fineness in the polarization impressed by the two centrioles is different: the mother centriole divides the centrosome (and the cell) into 9 equal meridian wedges, the daughter into two polar caps and three parallel sectors (rings) of different amplitude (because of the eccentric position of the daughter centriole in respect to the mother). The circumferential relationships between cells placed on the same plane, side by side, are therefore favoured (more finely defined), while apical-basal polarity is less defined. This is logical in epithelia, and in Planar Polarity mechanisms that guide the morphogenesis of laminar tissues by controlling the lateral contacts of cells.

Precision and Noise-Resistance. The level of precision is ensured by MTs rigidity and bending resistance. At the cortex, centrosomal MTs can distinguish positions at a distance of 3mm: the cortex is subdivided by centrosomal geometry into 45 small areas, each subtending its vertex solid angle of $4\pi/45$ steradians; the surface of one area has an average extension ($4pr^2/45$) in a cell with a diameter of 10mm (5 mm radius, 314 mm^2 surface) of about 7 mm^2 (a circle with a radius of 1,5 mm). A finer local organisation may be performed by cortical shifting of the daughter centriole (cytasters).

Canonical or default mother centriole

The "original" centrosome is the one that fertilizes the oocyte, or the one *de novo* assembled during parthenogenesis: its "mother" centriole possesses "canonical" or "default" circumferential polarity: in each species the zygotes and germ cells have a

mother centriole with this circumferential "default" polarity. In fact every zygote has the same "default" pattern and *situs viscerum solitus* is the same in every adult, not 50% as expected in case of random left/right patterned zygotes.

The mechanism that assembles a mother centriole with inverted polarity is strictly controlled by signals from neighbouring cells on the midline (or of regenerating blastemas, where and when they exist) and by DNA instructions (in Mammals, primordial germ cells arise in the posterior region of the epiblast, on the midline and very close to the posterior end of the primitive streak): it absolutely needs a pre-existing mother centriole that works like a platform organizing the inverse circumferential polarity of the newly-forming centriole; normally it happens only once during development: only in case of separation of the very first blastomeres, the mechanism can be reset to start again from a quasi-zygotic initial condition (monozygotic twinning): "splitting of mammalian embryos results in subtle asymmetries initiated very early and not reversed by later embryonic events"(Vanderberg and Levin, 2009).

Daughter centriole maturation

Before duplication, mother and daughter centrioles separate and each behaves like a mother, acting as a platform for the assembly of a new daughter; this presupposes that the (old) daughter centriole, before separating from its mother, matures, losing its "daughter" polarity (ribs) (the "0°" mark, 4 "left" and 4 symmetric corresponding "right" marks) and acquiring the same polarity of the mother centriole (distal and subdistal appendages in ordered sequence; "0°" mark and 8 different ordered marks): the acquisition of this correct circumferential polarity is also the key of bilateral symmetry. So the mother centriole is something more than only a simple platform: in *Chlamydomonas*, the "acorn", the structure that "confers rotational asymmetry on both basal bodies and probasalbodies" (Geimer and Melkonian, 2004), located at the distal end of the nascent probasalbody, appears rotationally oriented in accord with the circumferential polarity of the mother centriole, which then plays a very managing role in forming the correct rotational polarity of the new-arising basal body and in the alignment of both (mother and daughter) "0°" marks (see below: "Three pathways for centriole duplication").

Three pathways for centriole duplication

There are three different ways of centrioles duplication:

i) the classical pathway, that utilizes the mother centriole as a platform, begins with the process of daughter formation: so, the daughter centriole acquires its particular symmetrical circumferential polarity (Fig. 2; see also the paragraph "Orientation of the second goniometer"), with its "0°" spoke (triplet) aligned with the corresponding mother "0°" mark (Fig. 1) and its four "right" triplets with four different marks (+40°; +80°; +120°; +160°, clockwise measured from the "0°" mark) equal to its four "left" triplets (the "left" triplet positioned at 320°, or rather -40°, from the "0°" has the same mark of the "right" triplet positioned at +40°; the "left" triplet positioned at 280°, or rather -80°, from the "0°" has the same mark of the "right" triplet positioned at +80°, and so on). After building the centrosome, the daughter centriole, no longer useful in the centrosome, matures: this is indispensable for a correct transformation of the daughter centriole into a new "young" mother with the same rotational polarity of its "old" mother: during this process of daughter centriole mat-

uration, cells with left- or right-handed mother centriole impose the same rotational polarity to the transforming (from daughter to mother) centrioles. At the next mitosis, this new mother centriole is placed in the cell by the old mother centriole (that positions and orientates the cleavage plane, according to DNA instructions) and maintains its own orientation according to cell global polarity (before mitosis), so that the new arising cells conserve the same global polarity of the parent cell. As described by Karr and Alberts (1986) and Jonsdottir et al. (2010), centrosomes can leave their interphase position to assume a new position in mitosis, recovering in telophase their previous position and orientation, memorized in the cell cortex through landmarks positioned (and easily recognizable) by centrosomal γ-TuRCs receptors.

ii) *de novo* formation (parthenogenesis, basal bodies assembly in multiciliated epithelial cells) produces only "default" mother centrioles. This mechanism can be performed in the absence of the mother centriole, taking advantage of the aptitude of SAS-6 dimers for self-assembling. The main difference between these two ways consists in the phase of maturation of the old daughter centriole into a new (left or right) mother centriole, that is absent during the process of "*de novo*" formation.

iii) The third pathway builds a mother (never a daughter) centriole with inverted rotational polarity (first blastomeres LR patterning): this is a property of somatic cells only; germ cells cannot assembly mother centrioles with inverted polarity. This pathway utilizes the mother centriole as a platform, but inverts polarity (perhaps through the reflection of the equivalent of the "acorn-like" structure described by Geimer and Melkonian, 2004, controlled by the mother centriole): so, in "midline blastomeres" that have been separated surgically (Hertwig's experiments) or naturally (monozygous twinning) the mother centriole, under DNA instructions (activated by signals from gap junctions or from primary node cells, or from neighbouring regeneration blastema cells), controls that the correct inverted polarity is transmitted. Depending on the genetic and epigenetic mechanisms characteristic of the species, different and curious phenotypes appear: for example, Newt embryos derived from the separation of the two first blastomeres, as already seen, exhibit 89% incidence of organ laterality reversal in one of the twins (for this reason zygotes always start with "canonical" polarity and embryos have *situs solitus*); in Humans, identical twins show the normal *situs solitus* but inverted hair whorls: "in monozygotic twins, such hair whorls are mirror images, revealing that splitting of mammalian embryos results in subtle asymmetries initiated very early and not reversed by later embryonic events" (Vanderberg and Levin, 2009).

Echinoderms and *Drosophila*: exceptions to the hypotheses?

Echinoderms (Bilateria) larvae are clearly bilaterally symmetric (images can be seen in any embryology text), but adult organisms (starfish) show a characteristic radial symmetry that appears in contrast with the hypothesis about LR patterning; furthermore, during development the left side of the body grows very much more than the right one.

The relationship between larval bilateral structure and juvenile radial symmetry in some species has been described and the different contribution supplied by the left and right sides of the larva has been established (Morris, 2007, Morris et al., 2009; Ziegle et al., 2009; Vellutini and Migotto, 2010). "There is a bilateral plane of symmetry through the podia, the mouth, the archenteron and the blastopore. This adult

bilateral plane is thus homologous with the bilateral plane of bilateral metazoans and a relationship between the radial and bilateral body plans is identified ". " At the level of the five podia, this bilateral plane passes through podium D and between podia B and A, on the evidence that podia B and A form from the right and left lateral walls of the archenteron" (Morris, 2007).

Rather than a radial symmetry, this *phylum* seems to have adopted an unusual circular metamerism (different from the longitudinal metamerism, like segments in *D.melanogaster*. or somites in Vertebrates) and to have enormously amplified the left-right asymmetry. Of the three axes, AP, DV and LR, clearly present in the larva, only the DV axis (named as oral-aboral) remains, while the metamerism is arranged along the previous AP axis, which loses the usual cranial-caudal polarity to acquire a characteristic circular arrangement (without head nor tail). So the evident larval bilateralism, masked by the dominant development of the left half, disappears. Echinoderms do not seem to contradict the precedent hypotheses.

Also Cnidaria and Ctenophora are radially symmetric, but they are quite different: they are Radiata, not Bilateria and have only two germ layers, ectoderm and endoderm (Diploblasts); they have centrioles and cilia, but the behaviour of their centrosomes is quite different than in Bilateria (Bornens, 2012): Ctenophora fertilization is naturally polyspermic and many centrioles are utilized contemporaneously in the same cell; Cnidaria zygotes lack cytasters (Salinas-Saavedra and Vargas. 2011); the characteristic of these ancestral not Bilaterian centrioles appears more similar to that of Protistan basal bodies.

Drosophila: the mutant defective for the centriolar DSas-4 protein (Basto et al., 2006) develops up to the adult stage, and is almost completely lacking in centrioles, at least in the brain, starting from the third larval instar: (the lack of evident centrioles does not exclude the presence of functional quasi-centriolar structures, considering, above all, the aptitude of many PCM components for self-assembly; in mice first embryonal divisions occur in the absence of centrioles). Large stocks of maternal DSas-4 allow centrioles to be constructed during previous stages. Images of the adult fly (see Fig. 4 in Basto et al., 2006) show an individual with monstrous deformities; the shape, the tilt and the anomalous curvature of the wings certainly impede flight, just as the abnormal angle between coxa and body cannot allow walking movements. The authors say that flies "could not hold their wings or legs in a normal position". Such pathological anatomy of the appendices and organism *in toto,* instead of proving the dispensability of centrioles in development, confirms and underlines the importance of well operating centrosomes for a precise implementation of geometry (axes formation, apical constriction, AJ remodelling, etc.): centrosomes are not indispensable in mitosis (redundant mechanisms can intervene), but they are irreplaceable in carrying out geometric functions (Mirth and Akam, 2002; Simoes et al., 2006; Pope and Harris, 2008; Taylor and Adler, 2008).

Drosophila is manifestly mirror symmetric: however, in embryos, first divisions are syncytial and appear in contrast with previous hypotheses about left-right patterning. All syncytial mitotic divisions are absolutely not random, but strictly controlled by the centrosomes that remain united to their own nuclei (if centrosomes are damaged, development stops immediately): the first 4 divisions (Baker et al., 1993) generate nuclei that remain radially equidistant from the center and form a sphere; then, during cycles 4-6, the nuclei distance themselves along the AP axis, transform-

ing the sphere into an ellipsoid whose marginal nuclei are symmetrically equidistant from the cortex. During stages 7-10 a symmetric migration towards the cortex occurs; Baker et al. (1993) have proposed that "cortical migration is driven by microtubule-dependent forces that repel adjacent nuclei". A network of interdigitating microtubules (like the interchromosomal MTs of the metaphasic mid-zone) forms between yolk centrosomes (not-migrating) and peripheral migrating centrosomes: this geometric network of MTs pushes nuclei to the cortex (Kotadia et al., 2011) while their final ordered positions appear to be due to astral MTs population (unlikely chromosomal "midzone" MTs, astral MTs do not overlap with inverted polarity: boundary MTs of different asters go parallel with their plus ends pointing towards the same direction: Wühr et al., 2010); "in *Drosophila* the movements of nuclei to the embryo cortex are mediated by forces acting on the centrosomes rather than on the nucleus itself. Asters are presumably the main target of such forces. It is then conceivable that MTs, nucleated on either side of the centrosome or which display different characteristics, are nucleated under the influence of opposite sides of the centriolar shaft, just as different appendages arise from basal bodies. The situation in *S. cerevisiae* gives some support to the idea: the spindle pole body, functional equivalent of the centrosome, displays a marked structural and functional bipolarity with an intranuclear spindle and an aster of cytoplasmic microtubules. Like in Metazoa, defective astral microtubules lead to defective nuclear positioning and defective budding. The biochemical and physiological differences between the two microtubule arrays are already well documented. Different gamma-tubulin binding complexes interacting with the inner or outer plate respectively, are involved in the nucleation of the two microtubule arrays" (Beisson and Jerka-Dziasdosz, 1999). The ordered separation of nuclei, the controlled asymmetry of their arrangement, in anterior-posterior direction, and their division into two halves (Right and Left) has been highlighted in a study based on the different beginning and speed of mitosis N° 14, defining domains in which cells start to divide at the same time (Foe, 1989); 25 left and 25 right domains with different form and extent are evident: "all the domains described also occur as pairs. Whether paired or not, every domain is bilaterally symmetric." A clear midline is evident both dorsally and ventrally (see Figs. 1A, 1B, 1C, 3A, 3B, 3C in Foe, 1989). Surprisingly, domains appear arranged, according to the theoretical model of the centrosome, in longitudinal right and left wedges, intersecting two polar caps and three parallel rings: it appears that each half cortex of the embryo is mapped by the corresponding centrosome half; some domains are metameric and show an interesting link between centrosomal geometry and cleavage geometry: the extension of each domain is an autonomous genetic program that establishes how many cells must occupy each domain and, through the geometric structure of the centrosome, the proper location is reached. A particularly more defined subdivision (12 domains) concerns the procephalon, which is the most anatomically complicated; also the dorsal-ventral patterning appears well defined: the large ventral zone where *twist* and *snail* are expressed is subdivided in four right and four left domains, named by Foe 10, 14, N, M. Then, the preblastodermic syncytial development in *Drosophila* is not so unusual: it is a bilateral symmetric blastula quite similar to that of other animals (*Xenopus*, sea urchin, etc.) but with blastomeres without a membrane. *Drosophila* is not an exception to the hypothesis about left-right patterning.

Conclusion

Centrosome's behavior in Metazoa agrees to the functioning of a spherical reference system: than it is possible to hypothesize that its geometrical role is very likely. The centrosome is not an independent actor in anyone of the processes in which it operates; it is only a tool equipped with a particular three-dimensional structure and a good noise resistance; it allows DNA to "map" the cell, distribute and localize molecular complexes with the necessary precision in order to build the 3D architecture of Metazoan organs and organisms. Many previous suppositions about centrioles and centrosomes, through the present theoretical analysis of a spherical reference system based on two orthogonal goniometers and through a review of centrosome behavior in Metazoa, agree with this novel (hypothetical) operating way of the centrosome: because of the 9-fold symmetry of its centrioles, their rotational polarity and their orthogonal arrangement (resulting in the semi-self-assembly process that forms a spherically polarized PCM around the "catalyzing platform" set up by the two centrioles) it plays the role of a geometric instrument, a cellular spherical reference system that works like an interface that receives (input) coded signals (different ligands, each one intended for a particular cortex location), recognizes them through the receptors of its γ-TuRCs, decodes and translates them through the nucleation of MTs, correctly oriented (according to γ-TuRCs tilt) to reach the desired locations (output); thus it is capable to recognize and decode morphogenetic instructions, or, more generally, geometric signals, and translate them into their expected real locations in the cell. Finally, the centrosome possesses an intrinsic rotational chirality: the inverse polarity of the mother centriole appears to be the basis of the bilateral symmetry of Metazoa, a fundamental basic property of Metazoan locomotive system and of their sensor neural apparatus which drives locomotion movements.

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