FEBS Letters 581 (2007) 1251–1254

Minireview

Clockwise or anticlockwise? Turning the centriole triplets in the right direction!

Rustem Uzbekov[a,*], Claude Prigent[b]

[a] *Cell Cycle Group, Department of Electron Microscopy, A.N. Belozersky Institute of Phisico-Chemical Biology, M.V. Lomonosov Moscow State University, Moscow, Russia*
[b] *CNRS UMR 6061, Genetique et Developpement, Universite de Rennes 1, IFR140, Faculte de Medecine, 2 av du Pr Leon Bernard, 35043 Rennes cedex, France*

Received 26 January 2007; revised 19 February 2007; accepted 28 February 2007

Available online 6 March 2007

Edited by Ulrike Kutay

Abstract Centrosomes are small cytoplasmic macromolecular assemblies composed from two major components, centrioles and pericentriolar material, each with its own complex architecture. This organelle is of interest because it plays a role in a number of fundamental cellular processes and defects in these processes have recently been correlated with variety of human disease. Increasingly, what is known about the structure of this organelle has been overshadowed by the increasing wealth of information on its biochemistry. In this short review, we highlight some of the common centriole structural errors found in the literature and define a set of rules that define centriole structure.

Keywords: Centrosome; Centriole; Microtubules; Cell cycle

1. Introduction

Before starting this minireview we must clarify the terms ''centriole'' and ''centrosome''. The ''centrosome'' is a cytoplasm organelle containing two ''centrioles'' (two cylinders made of nine triplets of microtubules) surrounded by a pericentriolar material.

The centrosome was discovered in the XIX century around 1870 by Flemming [1–3]. Its ultrastructure and its behaviour during cell cycle progression were investigated very early by electron microscopy. As early as 1970 a detailed description of many aspects of centrosome morphology was already available [4–6]. Since its discovery, the centrosome has attracted many scientists from various fields such as microscopy, cell biology, molecular biology, biochemistry, pathology, etc. However, after more than 100 years of extensive studies, many aspects of this organelle remain a mystery; this is the case for its mechanism of centrioles duplication, for instance. During the last few years, modern biochemical methods have been used to identify centrosomal proteins, giving some new insights into centrosomal functions. But because modern biology focuses more on the functional aspects of the centrosome than on structural aspects, cell biologists tend to ignore the structure of the centrosome. Incorrect models of centrosomes that have spread in scientific papers and in well-known textbooks currently used for teaching basic biology illustrated this. The presence of these errors prompted us to write this short note. Let us take for instance two of the most popular textbooks in Cell Biology: Molecular Cell Biology (MCB), Third (1995) and Fourth (1997) edition (Ed. by J.E. Darnell) and Molecular Biology of the Cell (MBC), Fourth edition, 2002 (Ed. by B. Alberts). In all cases, a ''family portrait'' of 4 centrioles taken from McGill et al. [7] was used to illustrate centriolar morphology. In MBC, the figure legend describes the illustration as ''a new-replicated centriole'', while in MCB the phase of the cell cycle was identified as S-phase. However, these centrioles cannot be from an S-phase cell, because procentrioles and mother centrioles are (1) disconnected and too far away from each other and (2) their axes have lost the perpendicular orientation characteristic of S-phase. This picture might rather correspond to centrioles from a G1 polyploid cell. To understand the origin of the mistake, one must return to the original paper from McGill et al. [7] which running title is indeed ''Abnormal centrioles in mammalian cells''. The centrioles used for this illustration were not taken from normal cells, but from cells incubated for 4 h in 10 μg/ml propidium iodide, a treatment reported to disturb centrosome organization as well as cell cycle progression [7]. However, the most common error lies in the structure of the two centrioles and particularly on the direction in which the centriolar triplets are curling. In the 1960s it was shown that if one observed a centriolar cylinder from its proximal end, triplet curling (vector from internal microtubule ''A'' to external microtubule ''C'') was oriented anticlockwise. This orientation remains unchanged in all kinds of centrioles [6,8]. Misorientation first appeared in 1976 when Krstic published a scheme in which triplets were oriented clockwise [9]. Later the same mistake spread to several other publications [10–12]. A correction was made in the 1995 MBC textbook edition, which shows the triplets in the correct orientation. A similar error reappeared in the first version of the textbook from Pollard and Earnshaw [13], in which triplets in mother and daughter centrioles curl in different directions. For a mature mother centriole the direction is anticlockwise as it should be, but for daughter centriole (and the scheme) the direction is clockwise when it should be anticlockwise (their figure 37-9).

*Corresponding author.
E-mail addresses: Rustuzbekov@aol.com (R. Uzbekov), Claude.prigent@univ-rennes1.fr (C. Prigent).

0014-5793/$32.00
doi:10.1016/j.febslet.2007.02.069

Also a confusion is made between proximal and distal ends of centrioles (Fuller et al., 1995 in their Fig. 3) [15]. This error in the orientation of the centriole triplets seems to emanate from the legend of the centrosome structure describing the sections "numbered 1–5 from proximal to distal ends", whereas in fact the numeration was from the distal to proximal ends (Fig. 2 in Tournier and Bornens [14]).

Next common mistake regards the connection of the two centrioles by their distal ends (Fig. 8 in Fuller et al.) [15,16]. Centrioles are connected and oriented to each other by their proximal ends [17–19]. The origin of procentriole duplication that starts at the surface of the mother centriole cylinder imposed the orientation. The proximal end of the new centriolar cylinder always appears close to the proximal end of the mother centriole. Also, the distal end of the centriole always remains free for cilia formation. Drawing procentrioles with a diameter significantly smaller than the mother centrioles must also be avoided (Fig. 8g and h in Fuller et al.) [15]. The diameter of centrioles and procentrioles containing triplets remains practically identical during duplication. Centrosomes have been isolated from cells treated with nocodazole, cytochalasin, incubated in hypotonic and cold solutions and submitted to a series of centrifugations. Obviously, such treatments remove many associated proteins but unfortunately also induce morphological changes. This is particularly the case when one observes sub-distal appendages (pericentriolar satellites). In vivo the base of one sub-distal appendage always appears connected to 2–3 centriolar triplets. These connections impose the conical shape of the appendage with a spherical head (see Fig. 1 in Vorobjev and Chentsov) [10]. In contrast, sub-distal appendages observed on isolated centrioles have often lost this conical shape and look like cylindrical structures connected to only one centriolar triplet [17]. Schemes of centrioles pair sometimes contain two "mother" centrioles (Fig. 8 in Fuller et al.) [15] and (Fig. 9 in Andersen) [20] instead of one mother and one daughter centriole with a different morphology [10,18,21]. For example, in late interphase, after centrosome duplication and before mitosis, appendages are present only on a single mother centriole among the four centrioles, the two mother centrioles being different. Sub-distal appendages disappear during the first quarter of G2-phase [10]. During cell cycle progression, one cell, whether it contains one or two centrosomes, should contain only one centriole (the oldest) with sub-distal appendages from G1 to the middle of G2. After the middle of G2-phase none of the centrioles possess any sub-distal appendages [11,19,22–24]. ε-Tubulin was reported to localize on sub-distal appendages before centrosome duplication [25]. But because ε-tubulin immunostaining was observed on each duplicated centrosome, a short cut was used to draw G2 and mitotic centrosomes as each containing one centriole with sub-distal appendages [26]. Sub-distal appendages do not form on the second centriole but rather completely disappear from the older mother centriole during G2-phase. Replacement of sub-distal appendages by a mitotic halo during G2-phase was first reported in 1968 by Robbins et al. [23], and later confirmed with details by Vorobjev and Chentsov [10]. Therefore, in the second half of G2-phase both centrosomes look identical and sub-distal appendages construction restarts only during G1-phase of next cell cycle. Regarding distal appendages, they decorate the mother centriole in G1-phase. After centrosome duplication, distal appendages appear only during mitosis on one of the centrioles of the second centrosome, the future mother centriole. While even specialists in centrosome research sometimes ignore these fundamental morphological aspects of this organelle, we would like to present postulates of centrosomal structure illustrated by an updated scheme and selected electron microscopy images (Fig. 1), which hopefully will be useful to both new and established scientists interested in centrosome biology.

The best up-to-date morphological description of the centrosome is given in reviews by de Harven [5] Brinkley and Stubblefield [27], Fulton [6], and classical book of Wheatley [24], while in the most recent book "Centrosome in development and disease" (2004), largely describing all updated aspects of centrosome function, one does not find any structural scheme for the centrosome, which continues to intrigue a growing number of biologists.

2. Twelve principal postulates of centrosomal biology (see Fig. 1)

1. The centrosome and the basal body are two forms of the same organelle. The centrosome can produce cilia, and the basal body can function as a mitotic pole organizer. Centrosome usually contained two cylinders from nine MT triplets each – centrioles, pericentriolar matrix and some additional structures associated with centriolar cylinders. Basal bodies of cilia and flagella can contain one or two centriolar cylinders.
2. The centriole is a polar structure, with two functionally different ends. The distal end (plus-end of the centriolar triplet's microtubules) can be the site of cilia origin. The new centriolar cylinder (procentriole) usually starts to grow near the centriole surface, close to the proximal end (minus end of MT of triplets) or in rare cases directly from the proximal end of the centriole.
3. Centriolar polarity can be distinguished from the orientation of some of its components. Viewed from the proximal end, the triplets (in the direction internal MT "A" to external MT "C") are always twisted anti-clockwise while distal appendages twist in the opposite direction – clockwise (also viewed from proximal end). Pericentriolar material can be also positioned asymmetrically covering proximal but not distal end of centriole [19].
4. The external diameter of the distal part of the centriolar cylinder is smaller than the diameter of the proximal end. First, because the triplets change to duplets (the C-microtubule is usually shorter than A and B) and second because the angle of the vector that represents the triplets (and duplets) relative to the radius is decreasing.
5. In a centrosome from proliferating cells the two centrioles differ structurally and functionally. Only the more mature centriole (the mother) has appendages on the distal end; the daughter centriole has only electron dense ribs along the triplets (duplets). Also, only the mother centriole has sub-distal appendages. Microtubule nucleation sites are preferentially placed on or near mother centriole.
6. Mother and daughter centrioles are connected by their proximal ends. When a centriole functions as a basal body it's relationship to the daughter can either be completely absent as in ciliated epithelium or the daughter may lose its relationship to the basal body and move some distance away from it [28].