Vertebrate primary cilia: a sensory part of centrosomal complex in tissue cells, but a “sleeping beauty” in cultured cells?

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Abstract

Primary cilium development along with other components of the centrosome in mammalian cells was analysed ultrastructurally and by immunofluorescent staining with anti-acetylated tubulin antibodies. We categorized two types of primary cilia, nascent cilia that are about 1 µm long located inside the cytoplasm, and true primary cilia that are several µm long and protrude from the plasma membrane.

The primary cilium is invariably associated with the older centriole of each diplosome, having appendages at the distal end and pericentriolar satellites with cytoplasmic microtubules emanating from them. Only one cilium per cell is formed normally through G₀, S and G₂ phases. However, in some mouse embryo fibroblasts with two mature centrioles, bicilates were seen. Primary cilia were not observed in cultured cells where the mature centriole had no satellites and appendages (Chinese hamster kidney cells, line 237, some clones of β-fibroblasts). In contrast to primary cilia, striated rootlets were found around active and non-active centrioles with the same frequency. In proliferating cultured cells, a primary cilium can be formed several hours after mitosis, in fibroblasts 2–4 h after cell division and in PK cells only during the S-phase. In interphase cells, formation of the primary cilium can be stimulated by the action of metabolic inhibitors and by reversed depolymerization of cytoplasmic microtubules with cold or colcemid treatments.

In mouse renal epithelial cells in situ, the centrosome was located near the cell surface and mature centrioles in 80% of the cells had primary cilium protruding into the duct lumen. After cells were explanted and subcultured, the centrosome comes closer to the nucleus and the primary cilium was depolymerized or reduced. Later primary cilia appeared in cells that form islets on the coverslip. However, the centrosome in cultured ciliated cells was always located near the cell nucleus and primary cilium never formed a characteristic distal bulb. A sequence of the developmental stages of the primary cilium is proposed and discussed. We also conclude that functioning primary cilium does not necessarily operate in culture cells, which might explain some of the contradictory data on cell ciliation in vitro reported in the literature.

Keywords: Centrosome; Centrioles; Primary cilium; Striated rootlets; Pericentriolar satellites; Microtubules

1. Introduction

Formation of primary cilium is one of the basic functions of the centrosome (Barnes, 1961; Sorokin, 1962). Primary cilium is a special structure forming on the centriole. It has 9+0 axoneme that means nine doublets of microtubules with no microtubule in the center. Primary cilium consists of two parts—a basal body and a membrane-bound ciliary axoneme that lacks the central doublet and dynein arms typical for motile cilia. The primary cilium is non-motile organelle that occurs singly on most cells in the vertebrate body that have been carefully examined, with the few exceptions being cells of myeloid and lymphoid origin (Wheatley, 1995). Primary cilia are frequently found in embryonic tissues and during early postnatal development (Fonte et al., 1971; Jurand, 1974; Sorokin, 1962; Tucker et al., 1992). Also many differentiated cells have primary cilia (Albrecht-Buehler, 1992; Bystrevskaia et al., 1992; Dahl, 1963; Dalen, 1981; Latta et al., 1961; Poole et al., 1985; Wheatley et al., 1994). There are few cell types where a primary cilium is not formed, e.g. nucleated blood cells, adipocytes, hepatocytes (Wheatley, 1969, 1982).
Primary cilia were found in different cultured fibroblasts (Albrecht-Buehler, 1977; Tucker et al., 1979a,b; Wheatley, 1971; Wheatley et al., 1994, 1995) where up to 87–88% of cells might be ciliated (Wheatley, 1982). In the epithelial cell lines percent of ciliated cells is lower—about 30% (Alieva and Vorobjev, 1989; Bystrevskaya et al., 1992; Rieder et al., 1979; Roth et al., 1988; Wheatley, 1971).

Although functions ranging from vestigial remnants to sophisticated sensory devices such as the rods and cones of the retina have been attributed to primary cilia, their function in a variety of cell types has not been ascertained (Poole et al., 1997; Wheatley, 1995). A sensory function of the primary cilium was proposed long ago (Poole et al., 1985; Roth et al., 1988; Schwartz et al., 1997); however, only recently have experimental findings tended to confirm this hypothesis. It was suggested that the primary cilium operates as part of a sensory pathway, translating extracellular information to the centrosome and the Golgi complex to facilitate secretory pathway, translating extracellular information to the extracellular matrix (Poole et al., 1997). Thus the primary cilium serves an antenna, displaying specific receptors to the body in diﬀe­rent tissues, e.g. kidney primary cilium display polycystin-2, which forms part of a Ca2+ channel initiating a signal that controls cell diﬀer­entiation and proliferation (Pazour et al., 2002a,b; Somlo and Ehrlich, 2001). Kidney primary cilium also serve as mechanosensors that, when bent, initiate a Ca2+ influx that spreads throughout the cell and to the neighbouring cells (Pazour and Witman, 2003; Praetorius and Spring, 2001). Primary cilium in the other cell types specifically display diﬀerent tissue-specific receptors, including those for somatostatin (Handel et al., 1999) and serotonin (Brailov et al., 2000) in neuronal cilia.

Since primary cilium serves as an antenna enhancing speciﬁc external signals, it is natural to assume that centrosome at the base of primary cilium could be involved in the signal transduction pathway. The major function of the centrosome is formation of radial microtubule array, and activity of the centrosome in the organization of microtubules is variable. We suggest that increased formation of microtubules on the centrosome could be induced by initiation of the signal transduction from primary cilium and examined correlation between centrosome activity and frequency of primary cilium formation.

In general, sensory function of the primary cilium in tissue (in situ) seems to be arguable and clarified, but several questions regarding ciliogenesis and cilia remain to be answered. It is very diﬃcult to inquire into these questions with tissues in situ, but much more easy to make experiments using cultured cells. But the primary cilia functions are absolutely unclear in cultured cells, when tissue lost its 3D organization and cultural medium well provided for constancy of environment and surroundings. However, it might be possible that in cultured cells, primary cilium has no special function or its function is not permanent. The percentage of ciliated cells in vitro is different and can diﬀer from one passage to another (Alieva et al., 1999). The highest percentage of ciliation has always been observed among non-proliferating cells (Tucker and Pardee, 1979; Wheatley, 1995). In the current study, we have analyzed the details of primary cilium structure in several cell types, changes in the primary cilium during cell culturing and spreading on the substrate in vitro, primary cilia formation during the cell cycle, and the reaction of primary cilia to diﬀerent metabolic inhibitors.

2. Materials and methods

2.1. Cell cultures

Epithelial PK (pig kidney) cells, Chinese hamster kidney cells, and 1­-fibroblasts were cultured at 37 °C and 5% CO2 on medium 199 supplemented with 10% bovine serum and gentamycin. HeLa cells were grown at 37 °C and 5% CO2 in medium 199 supplemented with 10% fetal calf serum and gentamycin. 3T3 cells, rat kangaroo PTK1 cells and primary rat embryo fibroblasts (REF) were grown at 37 °C and 5% CO2 in DMEM/F-12 HAM (Sigma, USA) supplemented with 10% fetal calf serum and gentamycin.

Primary cultures of mouse fibroblasts (MEF) and renal epithelial cells were obtained from 15–19 day mouse foetuses according to the standard protocol. Cells were cultured at 37 °C and 5% CO2 on medium 199 supplemented with 10% fetal calf serum and gentamycin and analysed in the first passage.

2.2. Drug and cold treatments

All drugs used were added to the culture medium from stock solutions at the following final concentrations: FCCP—20 µM; 2,4-dinitrophenol (DNP)—800 µM; sodium azide—20 mM, ouabain—1 mM, calcium ionophore A23187—20 µM. Colemid was added at a final concentration of 10 µg/ml. Cold treatment was performed as described elsewhere (Vorobjev and Chentsov, 1983). Recovery from colemid treatment was performed by triple change of the culture medium to the fresh one. Recovery from the cold treatment was by transfer of the Petri dishes with cells from the ice-bath to the 37 °C incubator.

2.3. Determination of the phase cell cycle

Determination of the stages of cell cycle was performed for individual cells using double H1 and C14 thymidine labelling as described elsewhere (Vorobjev and Chentsov, 1982). Cells were attributed to G0 period
when they did not incorporated labelled thymidine for at least 24 h.

### 2.4. Antibodies

Monoclonal antibodies C3B9 against acetylated tubulin were produced at the Laboratory of Keith Gull (Manchester, UK). Monoclonal antibodies against γ-tubulin were obtained at the Laboratory of I.A. Vorobjev and described earlier (Komarova et al., 1996). FITC-conjugated secondary antibodies were obtained from Sigma (USA).

### 2.5. Immunofluorescent studies

For immunofluorescent analysis, the cells were fixed using two techniques: (i) in 4% formaldehyde solution for 30 min; (ii) in 1% glutaraldehyde solution (Merck, Germany) in phosphate buffer for 30 min followed by a triple treatment with a NaBH₄ solution (2 mg/ml, 10 min each). In the latter case, prior to fixation the cells were lyzed in a mixture containing 1% Triton X-100 in microtubule-stabilizing conditions. The coverslips with the cells were taken out of the Petri dish, washed several times with phosphate buffered saline (pH 7.2) at 37 °C and then lyzed for 15 min in a solution containing 50 mM imidazole (pH 6.8), 5 mM MgCl₂, 1 mM EGTA, 0.1 mM EDTA, 35% glycerol and 1% Triton X-10 (Sigma). The preparations were included into a 2.5% solution of 1,4-diazabicyclo-[2.2.2]-octane (DABCO)(Sigma) on glycerol, examined under an Opton-3 photomicroscope (Opton, Germany) and photographed (film RF-3, Tasma, Russia).

### 2.6. Electron microscopy

Cells were fixed with a 2.5% solution of glutaraldehyde (Merck, Germany) in 0.1 M phosphate buffer (pH 7.2–7.4) for 30 min or lyzed in a mixture containing Triton X-100, in microtubule-stabilizing conditions, and then fixed with 1% solution of glutaraldehyde (Merck) in the same buffer for 30 min (Alieva and Vorobjev, 1995). Further preparation for electron microscopy was performed as described earlier (Alieva and Vorobjev, 1995). Serial ultrathin sections were made through the centrosome region and examined under HU-11B or H-700 electron microscopes as described elsewhere (Alieva et al., 1992).

### 2.7. Synchronization of cells

Mitotic cells were isolated by two techniques:

1. Mitotic cells of a rat fibroblast culture were accumulated by incubation of a monolayer culture in a medium containing nocodazole (0.1 μg/ml) for 4–6 h. Then the metaphase cells were shaken off, collected into 2-ml Eppendorf tubes and centrifuged at 1000 rpm three times 3 min each; during centrifugation the cells were washed with the medium without nocodazole. The sedimented metaphase cells were plated in a drop of the medium on to coverslips. 30 min after (upon complete attachment of the cells to the cover-slips) 3 ml of the medium was added to the Petri dishes.

2. Mitotic 3T3 cells were collected by shaking off normal metaphase cells and plating them on to coverslips as above but without nocodazole treatment. Thus, no mitostatic was used in this case and, by excluding its washout from the protocol, we reduced the time from the beginning of isolation to the time when cells were attached to coverslips.

### 3. Results

Electron microscopy and immunofluorescent study of the centrosome structure and appearance of primary cilia was analyzed in different cultured cells. The major part of this study was performed on kidney epithelial cells (PK cells and primary mouse embryo kidney cells), and in some studies we used other cell types.

Using serial EM sections we found nascent primary cilia in different cultured cell lines including fibroblasts and epithelial cells and also in primary cultures. The minimal cilium determined using serial ultrathin sections was about 0.5 μm long and hardly could be detected under the light microscope. However, detailed examination of the centrosome confirmed the absence of primary cilium in blood cells (monocytes and macrophages, lymphocytes and neutrophils). Also we failed to find primary cilium in those cells where two centrioles were of the same structure and capacity in formation of cytoplasmic microtubules. These were epithelioid Chinese hamster kidney cells and l-fibroblasts.

In all cell types analyzed where the primary cilium were found, their fine structure was conservative—forming on the centriole cylinder. It has axoneme of 9+0 formula, which means nine doublets of microtubules with no microtubules in the center. The length of individual doublets was different, and in apical cross-sections primary cilia have fewer of them (between 7 and 3).

Formation of the primary cilium was always restricted to the mature centriole. In cells with two centrioles there is only one primary cilium; but in cells in G₂ period where only one of four centrioles was mature also only one cilium formed. However, in some primary cultured murine fibroblasts with two pairs of centrioles, two cilia were found (Fig. 1). This is in accord with the data on primary cilia cycle in PtK₁ cells: cells released from a continuous taxol treatment contained two normal centriole pairs, and one or both mature centrioles possess primary cilium (Jensen et al., 1987).
3.1. Primary cilium in renal epithelium

To compare primary cilia in situ and in cultured cells we used renal epithelium from mouse embryo. In the renal duct cells centrosome is located near the cell apex and far away from the nucleus. Two centrioles had structure typical for the centrosome in interphase cells: one mature centriole had nine appendages at its distal end and often (14 out of 18 cells examined on serial sections) formed primary cilium that was several microns long and directed into the duct lumen. Cilium had axoneme 9+0 and often formed a bulb at the distal end (not shown). The daughter centriole was located in the vicinity of the mature one but had no appendage and no pericentriolar satellite. Both centrioles were embedded in the fine fibrillar matrix.

In isolated and suspended renal cells the centrosomes were located near the cell nucleus and the percentage of ciliated cells decreased.

Renal epithelial cells spread slowly on the coverslip and after several hours formed islets among fibroblasts. During cell spreading centrosome was always located in the vicinity of cell nucleus and 30 min after seeding with the same probability it was under the nucleus above it or aside. Nascent primary cilia were found in 30% of cells. These cilia were embedded into the cytoplasm and never came to the cell surface. In the single epithelial cells that had no contacts with neighbours the same picture retained up to 24 h after seeding—primary cilium was found in 30% of cells, it was very short and did not appear on the cell surface.

Fig. 1. Electron micrograph of serial sections (1, 3, 6 and 9) showing the fine structure of the centrosome and two ciliated active centrioles in MEF cell. A–D—two active centrioles with primary cilia (on the sections 1, 3 and 6) and two nonactive centrioles (on the sections 1, 6 and 9). A—active centriole; N—nonactive centriole, PC—primary cilium. Bar=0.5 µm.
In the cells that form islets centrosome and primary cilia changed. In the internal cells of the islet cilia first appeared on the cell surface 6 h after seeding. When cell islets were completely stretched on the coverslip location of centrosome and primary cilia in internal cells and marginal cells of the islets became different. In the marginal cells centrosome was always located aside of the nucleus between nucleus and free cell edge. 70% of the marginal cells had short primary cilia (with axoneme 0.5–1 µm long), but was always embedded into the cytoplasm. In the internal cells centrosome was also close to the cell nucleus but located near the plasma membrane of the upper cell surface. 80% of cells in the islets had primary cilia that was usually found on the upper cell surface. This cilia was 2–5 µm long, but has no bulb on its distal end. Thus primary cilia restored in the renal cells inside the islets, but these cilia were of different fine structure from the original ones.

The next question was about behavior of the primary cilium during the cell cycle. It was shown that primary cilium is lost when cell enters mitosis (Jensen et al., 1987; Rieder et al., 1979), and we addressed the question when it appears after completion of cell division. Addressing this question we used two approaches: first, cells were individually selected at different stages of the cell cycle and second, mitotic cells were collected, subcultured and examined at different time point after subculturing.

3.2. Primary cilium in the cell cycle

In some cultured cells structure of mature centriole is different from regular one described elsewhere (Vorobjev and Chentsov, 1980, 1982). The difference is in the absence of appendages at the distal end and pericentriolar satellites. Consequently, cytoplasmic microtubules radiate in the same way from both centrioles in such centrosome. We observed these “incomplete” centrioles in t-fibroblasts and Chinese hamster kidney cells, line 237. In both cultures we failed to find primary cilium (no cilium for 80 cells in each culture).

In all other cases primary cilium was one per centriole pair and it was associated with the mature centriole. In some cells in primary fibroblast culture (MEF) there were four centrioles and they had two primary cilia (Fig. 1).

In the overall population of PK cells (in regular culture conditions) about 10% of centrosomes had primary cilium. Analyzing individual cells at different periods of the cell cycle we found that in PK cells primary cilium is absent in G1 period. It first appears only in S-phase (in 10% cases—3 of 28 cells) and became more frequent in G2 (37%—6 of 16 cells). These cilia were always short (with axoneme about 1 µm long) and usually embedded into the cytoplasm. In cells shifted to G0-period (incubated without change of cultured medium for 3 days) cilia were present in 40% (25 of 62) of cases. These cilia were 2–3 µm long or more and always came out to the cell surface (Figs. 2 and 3). Thus in PK cells primary cilia appears after rather long period after cell goes into the interphase—G1 period in these cells lasts for >5 h. To determine formation of the primary cilia more precisely we used epithelial cells and fibroblasts synchronized in mitosis and analyzed primary cilia formation by immunofluorescent staining of cilia with antibodies against acetylated tubulin (Wheatley et al., 1994, 1996).

3.3. Formation of the primary cilium after mitosis

Since primary cilium might be rather short and hardly visible under the light microscope, to validate the method of immunostaining we first compared frequency of primary cilia in epithelial and fibroblast cell cultures using EM serial sectioning and immunostaining (Table 1). The two approaches appear to be in a very good accord with each other.

We further analyzed formation of primary cilium after mitosis in two types of cultured fibroblasts. In primary culture of rat embryo fibroblasts mitotic cells were accumulated using low dose of nocodazole (0.1 mg/ml for 4 h). One hour after mitotic cells were collected and plated on to the coverslip telophase cells and pairs of cells connected with residual body were observed. No primary cilium was found at this time. First cilia appeared 2 h after cell plating and later on their percentage slightly increased (Table 2). Taking into account that nocodazole might perturb formation of primary cilium in the next set of experiments we used 3T3 cells where mitotic cells could be collected by shaking without treatment with nocodazole or other mitotic poison. In mitotic cells plated on the coverslip no cilium was found 1 h after plating, low percent of ciliated cells appeared 2 h after plating, and after 4 h of culturing percentage of ciliated cells was the same as in control culture. Occasionally 4 h after seeding primary cilia were formed in both sister cells, but mainly it appeared only in one cell from the pair.

Formation of primary cilium after cell division was found to be a rather long process and percentage of ciliated cells was different from one cell type to another. The next question is whether primary cilium can form in response to different treatments.

3.4. Primary cilia and centrosome activity

It was shown that a variety of drugs, including calcium ionophore A23187, uncouplers of oxidative phosphorylation DNP, FCCP, treatment with sodium azide and inhibitor of K+/Na+ ATPase ouabain can “activate” the centrosome, causing an increase in the number of microtubules associated with mature centriole and radiating from it. Under the action of these drugs, certain changes of the mature centrioles...
orientation were revealed by statistical analysis and the frequency of appearance of different centrosome components—pericentriolar satellites, striated rootlets etc. (Alieva et al., 1992; Alieva and Vorobjev, 1995).

Using stereoscopic analysis we did not find any change in the three dimensional structure of primary cilium in PK cells after all treatment used. But the axonemes of cilia had appeared after any treatment were much more shorter that in normal cells, and such cilia never came to the cell surface.

As a result of drug treatment mature centriole usually became perpendicular to the substrate surface (Alieva and Vorobjev, 1994, 1995). This nonrandom orientation of the mature centriole correlates with increase in number of pericentriolar satellites on the mature centriole and cytoplasmic microtubules radiating from the centrosome. Besides active mature centrioles changed their localization inside the cell—they came between cell nucleus and the upper cell surface and were not located at the lateral side from the nucleus as in control cells.

These changes came along with increase in the percentage of primary cilia (Fig. 4). Thirty min after introduction of different inhibitors (A23187, ouabain, DNP, sodium azide), the frequency of primary cilia

Fig. 2. Electron micrograph of serial sections showing the fine structure of the centrosome and primary cilium (cross section) in PK cell situated in G0 period. A–E—active centriole in the region of cilium appearance (sections 1, 3, 4, 5 and 8), S—pericentriolar satellites, A—appendages. F–H—nonactive centriole (sections 1, 5 and 7). Bar=0.2 µm.
increased from 60 to 100% compared to the control level. Formation of the additional cilia was rather rapid—even 5 min treatment with uncouplers of oxidative phosphorylation led to increase in the frequency of primary cilia (Fig. 5).

3.5. Primary cilia and striated rootlets

Since the action of drugs lead to a number of changes in the centrosome, we analysed the correlation between different centrosome activities separately. Of particular interest was correlation between formation of primary cilia and striated rootlets.

Striated rootlets are one of the odd components of the centrosome in mammalian cells. Striated rootlets were described in different types of non-ciliated cells (Lauiveryns and Boussauw, 1973; Nadezhdina et al., 1979; Sakagushi, 1965; Vorobjev and Chentsov, 1977), however, they remain largely unexplored. We found that the number of rootlets in the centrosome is unstable and
they run more frequently from mature, but also from immature (daughter) centriole. In 10% of cells striated rootlets run from both centrioles. Under the action of some metabolic inhibitors, the frequency of association of striated rootlets with immature centrioles increased. Under the action of all metabolic inhibitors used in the present study the frequency of striated rootlets changed. However, it was not correlated with the frequency of primary cilia under the same treatment (Fig. 4).

According to the function of striated rootlets in basal apparatus ("anchors" of cilia and flagella; Hard and Rieder, 1983), the regulation of cilia and flagella motility (Hyams and Borisy, 1975; Salisbury and Floyd, 1978) and signal-transforming function (Kleve and Klark, 1980), the possibility is that those of primary cilia serve the same functions as centrosomal striated rootlets, for example, the anchorage of primary cilia. But we found no correlation between the appearance of striated rootlets in the centrosome and cillum formation, and conclude that in cultured cells, striated rootlets may have lost their function and are atavistic.

3.6. Primary cilia under the action of microtubule-depolymerizing agents

Primary cilia contain acetylated tubulin in its axoneme, and thus might be expected to be more resistant to microtubule-depolymerizing treatments. However, we found that in PK cells primary cilia disassemble after cold treatment (2 h at 0°C), after colcemid treatment (1 µg/ml, 4 h) and under high-pressure treatment (2000 atm, 2 h). In the latter case, however, the cilium did not disappear, but its axoneme was depolymerized while membrane protrusion attached to the distal end of the mature centriole remained (data not shown).

It is interesting to note that 45 min after release from cold and colcemid treatments, rudimentary primary cilia with axonemes of 0.5–1 µm long appeared in the majority of cells, while they were present in <10% of control PK cells. This formation of rudimentary cilia seems to be a non-specific reaction of the centrosome related to the burst in the formation of cytoplasmic microtubules on the centrosome, as happens during recovery of microtubule array (Vorobjev and Chentsov, 1983).

Summarizing the results obtained we conclude that formation of the primary cilium after mitosis usually takes several hours, and they never appear in 100% of interphase cells. However, under experimental treatments formation of additional cilia in the interphase cells is more rapid process and might take 5–30 min.
4. Discussion

4.1. Three hypotheses for the primary cilium function

Since the discovery of primary cilium in 1898 (Zimmerman, 1898) three major hypotheses for their function have been put forth. The first is that primary cilia are vestigial organelles inherited from an ancestor whose cells had motile cilia, and now are of no purpose in multicellular organisms (Sorokin, 1962). The second is that they are involved in control of the cell cycle (Tucker and Pardee, 1979). And the third is that primary cilia are sensory organelles (Barnes, 1961; Munger, 1958; Poole et al., 1985; Wheatley et al., 1996).

There has been virtually no experimental evidence in support of any of these hypotheses, but the first one, atavistic hypothesis, is out of interest now. The importance of primary cilium at least in vertebrate visual and olfactory system is now evident (Brailov et al., 2000; Handel et al., 1999; Pazour et al., 2002b; Pazour and Witman, 2003; Poole et al., 1985; Praetorius and Spring, 2001; Roth et al., 1988; Schwartz et al., 1997; Somlo and Ehrlich, 2001) and diseases based on the defects of primary cilium, for example poly cystic kidney disease (PKD) were described (Hughes et al., 1995; Mochizuki et al., 1996; Nauli et al., 2003; Ong and Wheatley, 2003; Pazour et al., 2002; Somlo and Ehrlich, 2001; Yoder et al., 2002).

The first experimental evidence that primary cilia have a function came from studies of a mutant mouse, which has an insertion mutation in the Tg737 gene and, as a result, develops PKD (Moyer et al., 1994) because it was unable to assemble normal primary cilia (Pazour et al., 2000). Later it was shown that the mouse Tg737 protein is homologous to the intraflagellar transport protein IFT88 (Pazour et al., 2000), which is involved in the bidirectional movement of particles along ciliary and flagellar microtubules and is essential for ciliary and flagellar assembly (Pazour et al., 2000).

According to the second hypothesis primary cilia are involved in control of the cell cycle (Ho and Tucker, 1989; Tucker and Pardee, 1979). There is indirect evidence that primary cilia abandon liver epithelial cells in G1 and G0 periods may play some role in the inhibition of cell proliferation due to external signalling (Richards et al., 1997). However, this possibility remains largely unexplored.

During the last years, a growing body of evidence provided strong support for the hypotheses that primary cilia have sensory function—sensory transduction (Roth et al., 1988; Schwartz et al., 1997) or mechanoreceptors (Praetorius and Spring, 2001; Whitfield, 2003) and mechanosensors (Schwartz et al., 1997). They were shown to carry on specific receptors and relaying signals from their receptors to the cell (reviewed by Pazour and Witman, 2003). However, in all these studies the investigators dealt with normal tissue rather then cultured cells. We assume that the primary cilia could keep potential to perform sensory functions in cultured cells—at least in some part of cell cultures that descend from renal tissue, such as PK cells.

4.2. The primary cilium is a sensory part of centrosomal complex in tissue cells but it is “sleeping beauty” in cells cultured in vitro

Analyzing primary culture of renal epithelial cells we show that after explantation, when tissue loses its 3D organization (probably, with some of special tissue functions) and cultural medium provides constancy of surroundings, and cells round up primary cilium disappears. Later on when cells spread on the substrate primary cilium appears in some of them often being very short with axoneme inside the cytoplasm and located near the cell nucleus. Meanwhile the substantial length of primary cilium as far as the cilium rigidity and bending are the central and main for cilia functions. In mammals, the cilia are typically about 5 microns long, but have been observed to be as long as 30 microns on cells from kangaroo rat kidney (Wheatley and Bawser, 2000). The reversible, large-angle bending of the primary cilium upon exposure to fluid shear force was experimentally characterized in vitro, the cilium was then mathematically modeled as a cantilevered beam and the flexural rigidity of the primary cilium was calculated (Schwartz et al., 1997). It was shown that the removal of primary cilium abolished flow sensing (Praetorius and Spring, 2001) and bending the primary cilium opens Ca2+ sensitive intermediate-conductance K+ channels (Praetorius et al., 2003).

Primary cilium is generally recognized as a sensor with specific receptors receiving external signals (Brailov et al., 2000; Handel et al., 1999; Pazour et al., 2000; Pazour et al., 2003) and we suggest that it transduces them through the centrosome on to radial microtubule system and enhance microtubule related transport between Golgi zone and other cytoplasmic components. For normal function primary cilium requires continuous external signalling. Treatment of cultured cells with different inhibitors mimics such signallising and thus stimulates formation of the primary cilia. However, in the absence of subsequent signals these cilia do not maturate.

It seems likely that formation of the primary cilium is related to the cell–cell contacts—in the cells without junctions cilium seems to be absent, or at least appear at the very low frequency. Summarizing, the primary cilium, being the sensory part of centrosomal complex in tissue cells appears to be “sleeping beauty” (quiescent) in some cultured cells.

Our data support the idea that foundation of primary cilium and its maturation are different processes. We suggest that in cultured cells primary cilium cannot...
complete its maturation and function in a regular way because of the loss of cell differentiation that occurs in homogeneous culture medium with low fluid current. Very often primary cilium is inside the cytoplasmic vesicle and does not come to the cell surface. However, inhibitory analysis demonstrates that formation of such rudimentary cilia is a subject for external regulation. Taking together our observations we suggest that formation of primary cilium is a multi-step process.

4.3. Formation of the primary cilium

Use of serial sectioning allowed us to determine initial steps of the formation of primary cilium. In cultured cells centrosome is typically located near the nucleus rather than near the cell surface, however, the mother centriole does not lose its capacity to form primary cilium. Initial step of the formation of primary cilium is association of the distal end of maternal centriole with concave–convex vesicle about 0.3 microns in diameter probably from the Golgi complex. This vesicle is in firm contact with centriolar appendages and then axoneme begins to grow into concave part of it forming a small bud. Doublet microtubules of the primary cilium grow directly from the distal part of triplets of centriolar microtubules without any intercept. However, microtubules of the axoneme always have lower density compared to the triplet microtubules. The next step is elongation of the axoneme that requires growth of the membrane vesicle. Primary cilium at this stage looks like a long cone. Later on membrane vesicle surrounding axoneme fuses with the plasma membrane and primary cilium comes to the cell surface. Often it is localized in the pit on the cell surface.

In proliferating epithelial and fibroblasts cells (that are not in G0) primary cilium is rather short (about 1 micron) and often does not appear at the cell surface. Formation of the primary cilium is not associated with repositioning of the centrosome that occurs in some types of epithelial cells in situ. Elongation of primary cilium occurs in epithelial cells that move out of the cell cycle, but does not happen in resting fibroblasts.

Formation of the primary cilium does not inhibit other activities of the centrosome—maternal centriole with and without cilium has satellites from which cytoplasmic microtubules emerge. In cultured epithelial cells that are out of the cell cycle primary cilium becomes rather long and could be determined using light microscope. It is usually located on the upper surface of the cell, meanwhile always near the cell nucleus. In fibroblasts primary cilia are only few microns long and could be found on the upper surface as well as aside of the nucleus or even under it. We conclude that formation of the primary cilium comes through the following consecutive steps (Fig. 6): initiation, elongation of the axoneme, fusion of the axonemal vesicle with the plasma membrane, and final development and specialization of the distal part of cilium. It is interesting that at the first stage of primary cilium formation it is possible to see changes in the spatial organization of appendages on the active centriole—they become strictly symmetric.

In cells cultured in vitro, the last step seems to be abolished and the primary cilium may remain incomplete, unable to perform its sensory function. The function of the primary cilium is performed only in completely differentiated cells and not in proliferating ones. This may explain different frequency of the presence of primary cilia in cultured cells.

It is interesting to compare formation of the primary cilium and striated rootlets. Both structures seem to have no specialized function in cultured cells. In epithelial cells striated rootlets are formed simultaneously
with primary cilia. However, in response to different treatments the frequency of their formation is different. More than that, there is no correlation between the presence of striated rootlets and primary cilium in a particular cell. Besides striated rootlets are occasionally formed in blood cells (Vorobjev and Chentsov, 1977) where formation of the primary cilium is inhibited.

Taken together, the above observations bring us to the conclusion that centrosome in vertebrate cells inherited a complete set of features from the cell center of flagellated single-cell ancestors. It is capable of formation of the radial microtubule array in the interphase cell, participates in the organization of spindle poles, forms cilium, and striated rootlets. Location of the centrosome close to the cell nucleus and to the Golgi complex emphasizes its central role in the spatial organization of cytoplasm. However, numerous functions of the centrosome in multicellular organism seems to be down regulated. Some attributes of the original cell center are kept permanently, while others appear only from time to time and/or function only in specialized cells.

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