The centrosome is an actin-organizing centre

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Microtubules and actin filaments are the two main cytoskeleton networks supporting intracellular architecture and cell polarity. The centrosome nucleates and anchors microtubules and is therefore considered to be the main microtubule-organizing centre. However, recurring, yet unexplained, observations have pointed towards a connection between the centrosome and actin filaments. Here we have used isolated centrosomes to demonstrate that the centrosome can directly promote actin-filament assembly. A cloud of centrosome-associated actin filaments could be identified in living cells as well. Actin-filament nucleation at the centrosome was mediated by the nucleation-promoting factor WASH in combination with the Arp2/3 complex. Pericentriolar material 1 (PCM1) seemed to modulate the centrosomal actin network by regulating Arp2/3 complex and WASH recruitment to the centrosome. Hence, our results reveal an additional facet of the centrosome as an intracellular organizer and provide mechanistic insights into how the centrosome can function as an actin-filament-organizing centre.

The functional coherence between cell internal architecture and cell microenvironment depends on the accurate orchestration of cytoplasmic and peripheral polarities. This requires a tight coordination of microtubules and actin filaments in space and time. It is ensured by common signalling pathways co-regulating the two network dynamics. In addition, several crosslinkers support the physical interaction of microtubule plus ends with actin filaments at the cell periphery. However, it is worth considering that such a crosstalk could also occur at the cell centre, where microtubule minus ends are connected to the centrosome. Indeed, unexplained, but recurrent, observations have highlighted the influence of the actin network on centrosome positioning.

In highly adherent cells, disassembly of actin filaments dampened centriole motion and inactivation of ROCK-dependent acto-myosin contractility increased inter-centriolar distance and centriolar exploration towards the cell periphery. In poorly adherent polymorphonuclear leukocytes, actin disassembly blocked the splitting of centrioles that was associated with cell spreading in response to PKC activation. Similarly, at the onset of mitosis, actin filaments seemed to be involved in the splitting of duplicated centrosomes in various systems ranging from early Drosophila embryos to mammalian cultured cells. Ciliogenesis is another example of the close association between the centrosome and actin filaments. It starts by centrosome migration from the centre to the periphery of the cell, where it attaches to the cortical-actin network. Actin filaments not only bind the centrosome to the cell cortex through focal-adhesion-like and stress fibre-like structures but also regulate centrosome migration to the edge of the cell. Similarly, when cytotoxic T lymphocytes encounter a target cell, reorganization of the actin network seems to promote centrosome migration to the cell cortex where it will promote the assembly of the immune synapse.

The converse has also been observed and various forms of actin-network reorganizations have been described in the vicinity of centrosomes. In early Drosophila embryos, centrosomes organize and position actin-based interphase caps around them. On a different note, the inhibition of acto-myosin contractility around the sperm centrosome directs a cortical flow that further determines the one-cell stage Caenorhabditis elegans embryo axes. The interaction of centrosomes with actin filaments seems a general feature of mitosis. In a number of examples, the inhibition of acto-myosin contractility around the sperm centrosome directs the formation of mitotic spindle poles involved in spindle assembly and orientation in frog embryonic cells and mammalian cultured cells. Actin-network disassembly also seems to occur next to centrosomes as they reach the target cell cortex during immune synapse formation.

Several physiological functions have been attributed to centrosome–actin connections, notably the regulation of centrosome attachment to the actin networks surrounding the nucleus or spanning the cell cortex. However, and despite few examples

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Figure 1 Cytoskeleton filament assembly from isolated centrosomes. (a,b) Centrosomes were isolated from Jurkat cells expressing enhanced green fluorescent protein (EGFP)-centrin1 and seeded on glass coverslips. (a) The addition of purified tubulin dimers led to the assembly of dynamic microtubules. Time is in minutes. Images are representative of seven independent experiments. (b) The addition of purified actin monomers led to the assembly of radial arrays of actin filaments. Time is in minutes. Images are representative of 12 independent experiments. (c–e) Centrosomes were isolated from: Jurkat cells expressing dTomato–centrin1 (five independent experiments; c), non-modified Jurkat cells (five independent experiments; d) and from HeLa cells expressing EGFP–centrin1 (eight independent experiments; e). All centrosome preparations induced the growth of actin filaments in the presence of actin monomers. (f) The assembly of both microtubules and actin filaments from isolated centrosomes. Images are representative of five independent experiments. Scale bars, 10 μm.

of direct interaction between centrosomes and actin filaments15,17, microtubules were most often considered as necessary intermediates between centrosomes and the actin network. Proteomic analyses have systematically revealed the presence of actin and actin-associated proteins at the centrosome32–35. However, they were considered as contaminants because of the abundance of actin in the cytoplasm. Thus, clear evidence for a direct role of centrosomes in actin-filament assembly and organization has yet to be shown.

RESULTS

Isolated centrosomes promote the assembly of actin filaments

Isolated centrosomes were used to investigate a potential direct interaction between the centrosome and actin cytoskeleton. This initial in vitro approach was used in preference to an in vivo approach to mitigate potential artefacts arising from the presence of dense cytoskeletal networks surrounding the centrosome in living cells. Centrosomes were purified from the human T-lymphocyte Jurkat cell line, modified to express EGFP–centrin1, a core component of centrioles36. Preliminary tests to validate our cytoskeleton-assembly conditions revealed that the classical buffer for the study of centrosomes and microtubules in vitro, the Brinkley buffer, impaired the nucleation of actin filaments in the presence of regulatory proteins (Supplementary Fig. 1 and Supplementary Video 1). This limitation was overcome by the development of a new polymerization buffer, the TicTac buffer, fully compatible with actin and microtubule assembly (see Methods for buffer composition and Supplementary Fig. 1 and
Figure 2 Association of actin filaments with centrosomes in living cells. (a) Actin-filament staining with phalloidin (red) in Jurkat cells expressing EGFP–centrin1 (green). Images are representative of four independent experiments. (b) F–actin (phalloidin, red) and ninein (green) in HEK293T cells. Images are representative of two independent experiments. (c) F–actin (phalloidin, red) and ninein (green) in RPE1 cells, adherent and in solution. Images are representative of two independent experiments. Cells were fixed with paraformaldehyde (PFA). (d) Time series of Jurkat cells expressing EGFP–centrin1 (green) transfected with Lifeact–RFP to visualize the actin network (red). Image gamma was set to 0.65 to highlight the cytoplasmic network. Images are representative of six independent experiments. (e) The same as in d except after the addition of 10 μg/ml−1 cytochalasin D at t=0. Images are representative of three independent experiments. (f) DNA (blue) and F–actin (phalloidin, green) in pre-permeabilized and fixed Jurkat cells (left panel) expressing EGFP–centrin1 (red) and HEK293T cells (right panel) stained for ninein (red). Images are representative of five and two independent experiments for Jurkat and HEK293T cells respectively. Scale bars, 5 μm.

Supplementary Video 1). Centrosomes, isolated in TicTac buffer and seeded on glass coverslips in the presence of 30 μM purified tubulin dimers, resulted in the classical assembly of dynamic microtubules (Fig. 1a and Supplementary Video 2). Strikingly, when seeded in the presence of 1 μM purified actin monomers, isolated centrosomes generated large radial arrays of actin filaments (Fig. 1b
Centrosomes are associated with an actin meshwork in cells

In view of these results, we closely examined the localization of actin filaments in Jurkat cells. In fixed cells, phalloidin staining, as expected, an intense signal along the cell periphery emanating from the cortical-actin network. However, around the centrosome, a less intense cloud-like signal was consistently observed (Fig. 2a). Similar actin clouds were also observed in human embryonic kidney-derived cells HEK293T (Fig. 2b). Actin clouds could not be observed in highly adherent cell lines, such as human retinal pigment epithelial cells RPE1, in which the centrosomes were often seen close to bright actin bundles (Fig. 2c). However, an actin cloud could be detected at the centrosome when those cells were forced to detach on trypsin treatment (Fig. 2c).

These observations were further confirmed by time-lapse microscopy. The thick cortical-actin network formed dynamic protrusions deforming the roundish T lymphocytes. Meanwhile, centrosomes seemed to be continually associated with a cloud-like actin meshwork in the cytoplasm (Fig. 2d and Supplementary Video 5). This association did not seem to be disrupted by the addition of the actin polymerization inhibitor, cytochalasin D, which blocks actin-filament dynamics and induces network collapse on its most steady portions\(^ {37}\). Within a few minutes after the addition of cytochalasin D to T lymphocytes, actin networks fragmented into dense patches all around the cell periphery and at the centrosome (Fig. 2e and Supplementary Video 6). Furthermore, the association of the centrosome with the cloud-like meshwork of actin filaments was better observed in fixed T lymphocytes and HEK293T when the cells were treated with detergent before fixation; a process that removed the cortical-actin network and soluble cytoplasmic proteins, including actin monomers (Fig. 2f).

Centrosomes are genuine actin-filament nucleators

These last two experiments suggested that the pool of actin filaments at the centrosome was quite stable and may have resisted the actin-disrupting step during centrosome isolation from T lymphocytes. Indeed, phalloidin staining showed that centrosomes were bound to actin filaments after purification (Fig. 3a). It was unclear whether these filaments were already present before purification or whether they had aggregated during purification. Hence, the observed actin-filament assembly on isolated centrosomes may have been due to elongation of pre-existing filaments, rather than to de novo nucleation. Therefore, to distinguish between these two scenarios (elongation versus nucleation), network assembly was performed on purified centrosomes, first by incubating with red-fluorescent actin monomers and then by incubating with green-fluorescent actin monomers (colour-switch assay). In the elongation scenario, green-fluorescent actin monomers would be added only at the extremity of red filaments. In the nucleation scenario, actin monomers would form new additional filaments at the centrosome (Fig. 3b). Using the purified centrosomes from T lymphocytes, both scenarios were observed (Fig. 3c and Supplementary Video 7). Newly assembled filament segments were visible at the extremity of red filaments and at the centrosome (see green filaments at \( t = 0 \) in Fig. 3c).

The occurrence of genuine nucleation was further confirmed by the addition of capping proteins before initiating network assembly with green monomers to block the elongation of red filaments. In this situation, actin-filament assembly was then strictly initiated at the centrosome (Fig. 3d and Supplementary Video 8).

Various molecular pathways could be involved in the assembly of actin filaments at the centrosome. They mostly differ in the polarity of the generated filaments. Formins and Ena/Vasp bind to filament barbed ends whereas the Arp2/3 complex binds to filament pointed ends\(^ {38}\). The addition of new monomers at the distal ends of filaments (Fig. 3c; that is, the new filaments grew away from their nucleation sites) without signs of filament displacement as they grew (Supplementary Fig. 2) suggested that filament assembly was governed by Arp2/3-based nucleation.

Actin-filament nucleation at the centrosome is Arp2/3-dependent

Several distinct immunostainings revealed the presence of Arp2/3 subunits on purified centrosomes (Fig. 4a and Supplementary Fig. 3a). In addition, mass spectrometry of isolated centrosomes revealed the presence of five Arp2/3 complex subunits (Arp2, Arp3, p20-Arc and p41-Arc; Supplementary Table 1). We could also observe, by immunostaining, the Arp2/3 complex on the two centrioles of fixed Jurkat cells (Fig. 4b), HEK293T cells (Fig. 4c) and RPE1 cells (Fig. 4d) as already reported\(^ {39}\). The specificity of the antibodies was confirmed by carrying out a competition assay using purified Arp2/3 complex as the antigen. The level of centrosomal Arp2/3 subunit signal was progressively reduced in the presence of increasing amounts of soluble Arp2/3 complexes (Supplementary Fig. 4). The exogenous expression of an EGFP–Arp3 construct\(^ {40}\) in HEK293T further confirmed the centrilinear localization of the Arp2/3 complexes (Fig. 4e).

The Arp2/3 complex inhibitor CK666 (ref. 41; see activity test in Supplementary Fig. 5a) was used to evaluate the contribution of the Arp2/3 complex activity on actin-filament nucleation at the centrosome. The proportion of centrosomes nucleating actin filaments in vitro was reduced in the presence of CK666 (33% ± 3% in the presence of dimethylsulphoxide (DMSO), 15% ± 3% in the presence of CK666, \( n = 3 \) independent experiments). The nucleation process was assessed by the increase of actin-fluorescence intensity at the centrosome over time (Fig. 5a) and by the amount of new filaments at the centrosome in the colour-switch assay (Fig. 5e and Supplementary Videos 9 and 10). It was also strongly diminished in the presence of CK666. In addition, the weaker filament nucleation from centrosomes isolated from CK666-treated cells could be...
Isolated centrosomes nucleate actin filaments. (a) Actin-filament staining with phalloidin (green) on isolated centrosomes (EGFP–centrin1 in red). Images are representative of four independent experiments. Scale bar, 2 μm. (b) Schematic representations of actin-filament nucleation and elongation hypotheses. (c) Time-lapse imaging of actin-filament assembly from isolated centrosomes in the presence of Alexa568–actin monomers (red) followed by addition of Alexa488–actin monomers (green). Bottom row shows green channel and top row the overlay. Growth of green actin filaments at the centrosome supported the nucleation hypothesis. (d) The same as in (c) except that capping protein is added 10 min before Alexa488–actin monomers to block red actin filaments. Here also, growth of green actin filaments at the centrosome supported the nucleation hypothesis. (e) The same as in (c) in the presence of 0.2 mM CK666. Actin nucleation activity was measured at the centrosome in the presence of DMSO (filled circles, mean of n=9 actin asters) or 0.2 mM CK666 (open squares, mean of n=17 actin asters); data show the results of a single experiment, representative of two independent experiments. Error bars represent standard deviation (s.d.). a.u., arbitrary units. (f) The same as in (d) in the presence of 0.2 mM CK666. DMSO and CK666 mean of n=15 and 21 actin asters respectively; data show the results of a single experiment, representative of three independent experiments. Error bars represent s.d. With or without the capping of older actin filaments, Arp2/3 inhibition reduced actin nucleation at the centrosome. Scale bars in (c–f), 10 μm.

Enhanced by the addition of 100 nM of purified Arp2/3 complex to the actin mix (Fig. 5b). This firmly demonstrated the existence of a genuine Arp2/3-complex-dependent nucleation of filaments at the centrosome. In contrast, formin inhibition using SMIFH2 (see activity test Supplementary Fig. 5b) had no effect on centrosomal actin nucleation (Fig. 5a).
In parallel, CK666 treatment of live T lymphocytes markedly reduced the concentration of Arp2/3 complexes (Fig. 5c) and the amount of polymerized actin that could be detected at the centrosome (Fig. 5d). Similar effects were observed in HEK293T (Supplementary Fig. 6a) and RPE1 (Supplementary Fig. 6b). Therefore, both the in vitro and in vivo results supported the involvement of the Arp2/3 complex in actin-filament nucleation at the centrosome.

WASH promotes actin-filament nucleation at the centrosome

The Arp2/3 complex is constitutively inactive in the cytoplasm. Therefore, actin nucleation triggered by the Arp2/3 complex required the presence of an actin nucleation-promoting factor at the centrosome. One potential candidate is WASH; a potent nucleation-promoting factor that has been shown to be associated with the centrosome. Indeed, KIAA1033, a WASH subdomain, was present in centrosome mass spectrometry analysis (Supplementary Table 1) and we could detect WASH on isolated centrosomes (Fig. 6a and Supplementary Fig. 3c) as well as in fixed cells (Fig. 6b). The concentration of WASH was not higher on the centrosome than on the numerous endosomes in the cell cytoplasm. However, linescan analysis of fluorescence distribution showed that WASH was systematically localized at the centrosome (Fig. 6c). Two lines of evidence...
In Jurkat cells (Fig. 7d), PCM1 knockdown was used to investigate the role of PCM1 in actin-filament nucleation. PCM1 siRNA inhibition led to a significant decrease in the number of asters that could nucleate actin filaments, as determined by the percentage of p34-Arc-positive asters that also contain actin filaments (Fig. 7d). These results suggest that PCM1 is a key regulator of actin-filament nucleation at the centrosome. Indeed, PCM1 knockdown was found to reduce the number of asters that could nucleate actin filaments, and this effect was even more pronounced in the presence of CK666, which inhibits Arp2/3 complex activity (Fig. 7d). These findings indicate that PCM1 is a critical factor in the regulation of actin-filament nucleation at the centrosome.

**PCM1 regulates actin-filament nucleation at the centrosome**

To identify centrosomal components involved in the Arp2/3 complex and WASH at the centrosome, we examined the biochemical composition of the various structures purified during centrosome isolation and determined their ability to nucleate actin filaments (Fig. 7a). Interestingly, we found that 70% of actin asters contained actylated tubulin and PCM1 (Fig. 7b). This confirmed that centrioles, which are made of actylated microtubules, are the main scaffolds for actin-filament nucleation. Interestingly, most of the remaining asters did not contain actylated tubulin but were positive for PCM1 (Fig. 7b). In addition, the amount of PCM1 was correlated to the quantity of the Arp2/3 complex per spot (Fig. 7c). These results suggested that PCM1 could be a good candidate for actin regulation at the centrosome.

Indeed, PCM1 is a key regulator of centrosome composition. Downregulation of PCM1 reduced the amount of Arp2/3 complex and WASH at the centrosomes of Jurkat cells (Fig. 7d) and RPE1 cells (Supplementary Fig. 7a–e). It also diminished the associated cloud-like meshwork of actin filaments that was detected using the detergent treatment before the fixation protocol in Jurkat cells (Fig. 7d). Furthermore, centrosomes that were isolated from siRNA-treated Jurkat cells contained a lower amount of Arp2/3 complex and WASH at the centrosomes of Jurkat cells (Fig. 7d). These results pointed to a central role for PCM1 in centrosomal actin regulation.

PCM1 is the main component of centriolar satellites, which are small cytoplasmic granules that are transported on microtubules by dyneins towards the centrosome, where they accumulate and modulate centrosome composition. Both nocodazole treatment, to depolymerize microtubules, and ciliobrevin D treatment, to inhibit
**Figure 6** Implication of the WASH complex in actin nucleation at the centrosome. (a) Immunostaining of WASH (green) on isolated centrosomes (EGFP–centrin1 in red). Images are representative of two independent experiments. Scale bar, 2 µm. (b) RPE1 cells were fixed in cold methanol and stained for ninein (left panel) and WASH (central panel). Right panel shows the overlay of WASH (green), ninein (red) and DNA (blue). Images are representative of three independent experiments. Scale bars, 5 µm. (c) Linescan analysis of fluorescent distribution of WASH (red line) at the centrosome (ninein, black line; mean of n=31 cells, data are pooled from two independent experiments). Error bars represent s.d. (d) Inhibition of WASH on isolated centrosomes with blocking antibodies (bottom row). Control experiments performed without antibody (no ab; top row). Data show the results of a single experiment, representative of two independent experiments: n=25 and 27 actin asters for no antibody (green circles) and WASH antibody (WASH ab; red squares) respectively (mean ± s.d.). Scale bar, 5 µm. (e) Actin nucleation activity of centrosomes isolated from WASH1-siRNA-treated cells (bottom row) or control cells (top row). Data show the results of a single experiment, representative of two independent experiments: n=20 and 26 actin asters for the control (green circles) and WASH1 siRNA (red squares) condition respectively (mean ± s.d.). Scale bar, 5 µm.

**dyneins, reduced the concentration of the Arp2/3 complex and WASH at the centrosome in Jurkat cells (Fig. 7f) and RPE1 cells (Supplementary Fig. 7f). Despite being unable to detect the Arp2/3 complex and WASH on satellites by immunofluorescence, these results suggest that they are involved in the recruitment of the Arp2/3 complex and WASH to the centrosome.**
Figure 7 Regulation of actin-filament assembly at the centrosome by PCM1.
(a) Immunostaining of PCM1 (green) and acetylated tubulin (ac-tub; white) on isolated centrosomes in the presence of actin (red). Scale bar, 10 μm.
(b) Quantification of aster proportion depending on the composition of the nucleation centre (42 asters, 1 experiment). (c) Amount of p34-Arc plotted against amount of PCM1 on isolated centrosomes. The Pearson correlation coefficient (r) and P value measure the correlation between the two variables. (d) Knockdown of PCM1 in Jurkat cells. Left panels: methanol-fixed cells, ninein (red), p34-Arc (green) and DNA (blue) (top) and γ-tubulin (red), WASH (green) and DNA (blue) (bottom). Top right: cells treated with detergent before PFA fixation and stained for γ-tubulin (red), F-actin (phalloidin, green) and DNA (blue). Top row: control siRNA. Bottom row: PCM1 siRNA. Scale bars, 5 μm. Graph: fluorescence intensity at the centrosome of F-actin, p34-Arc and WASH. "**P < 0.0001. (e) Left: centrosomes isolated from PCM1-siRNA-treated cells (bottom row) or control cells (top row) in the presence of actin and stained for γ-tubulin. Scale bar, 5 μm. Centre: ability of isolated centrosomes to nucleate actin calculated as ratio of the number of actin asters divided by the number of γ-tubulin spots. Error bar represents s.d. "**P < 0.01. Right: fluorescence intensity of p34-Arc and WASH. "**P < 0.0001. (f) Representative staining of Jurkat cells incubated with DMSO (top panel), nocodazole (central panel) and ciliobrevin D (bottom panel) and stained for DNA (blue), ninein (red), p34-Arc (green) and WASH (white). Scale bars, 5 μm. Graph: p34-Arc and WASH amount at the centrosome. "**P < 0.0001. Red bar indicates the mean. Unpaired t-test with Welch’s correction was used to generate P values.
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DISCUSSION

These results have identified a genuine actin-filament nucleation process at the centrosome. They shed some light on numerous previous works reporting the unexplained presence of actin-binding proteins at the centrosome. Indeed, actin nucleators\textsuperscript{21,39,42,48}, focal adhesion-associated proteins\textsuperscript{15,48–51}, actin regulatory proteins\textsuperscript{52–57} and various myosins\textsuperscript{49,26,58,59} were described to localize, at least transiently, to the centrosome. Together these data reveal the centrosome as a true actin-organizing centre. The convergence of actin filaments and microtubules at the centrosome contributes to the view that it acts as a spatial integrator of various biochemical signals and cytoskeleton networks.

The high density of actin filaments throughout the cytoplasm precluded the clear delimitation of a specific centrosomal actin network in cells. Nonetheless, centrosome-associated actin filaments were clearly detected in live cells, in agreement with our \textit{in vitro} observations. Electronic or sub-diffraction optical microscopy may shed light on the actual architecture of the centrosomal actin network. In addition, further description of the precise localization of the Arp2/3 complex and WASH at the centrosome would be required to progress in this direction. At first glance, the Arp2/3 complex seems to localize to the proximal end of centrioles, where the pericentriolar material is highly structured\textsuperscript{60,61}. This suggested that actin filaments might interact there with the pericentriolar material and influence centrosome architecture and function.

Unravelling the molecular mechanism supporting the recruitment of the Arp2/3 complex and WASH to the centrosome is a critical step to further understand how the assembly of the centrosomal actin network is regulated. We found that PCM1 was strongly involved in this mechanism but the operating mechanism remains to be elucidated. To our surprise, we could not detect any enrichment of the Arp2/3 complex or actin filaments on PCM1-positive centriolar satellites in cells although \textit{in vitro} 20\% of the spots nucleating actin filaments were positive for PCM1 and negative for acetylated tubulin, strongly suggesting that they were centriolar satellites. In addition, the potential role of satellites in centrosomal actin assembly was further reinforced by the observation that microtubules and dyneins were involved in the recruitment of actin nucleators to the centrosome. This raised the question of whether satellites could actually nucleate actin filaments, which would be hidden in a strong cytoplasmic background, or whether they simply carry part of the actin nucleation machinery to the centrosome. In any case, the implication of intracellular trafficking in the modulation of actin nucleation at the centrosome is of great interest as it opens a full spectrum of regulation in space and time.

Interestingly, actin nucleators could be detected at the centrosome of all tested cell types although actin filaments were observed only in the less adherent cells. Cell adhesions are central and powerful hubs in the nucleation of actin filaments. Competition between actin nucleators\textsuperscript{62} for actin monomer has recently appeared as a central regulatory process in actin-network assembly. The centrosome may thus be considered as a poor actin nucleator, from which actin filaments grow only when cell adhesion is low. This should not be considered as a weakness but instead as an increased sensitivity to external changes. Indeed, this interesting hypothesis implies that any change in cell adhesion, such as during cell mitosis or contact-induced polarization, would affect actin-filament assembly at the centrosome.

Finally, how actin-filament nucleation at centrosomes contributes to physiological events involving the modulation of centrosome architecture, centrosome positioning or the interaction of the centrosome with the cell cortex or nucleus is an exciting field open to investigation.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Supplementary Information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

J.S., L.B. and M.T. conceived and supervised the project. E.F. and J.S. performed the experimental work. J.G. and C.G. defined the TiTac buffer. Y.C. performed the mass spectrometry analysis. F.F., J.S., L.B. and M.T. analysed the data. M.T. wrote the manuscript, which was edited by E.F., J.S. and L.B.

COMPETING FINANCIAL INTERESTS

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METHODS

Cell culture. Jurkat cells (obtained from X. Gidrol laboratory), immortalized human T lymphocytes, were cultured in RPMI 1640, HeLa cells (obtained from M. Bornens laboratory), transformed human epithelial cells, and HEK293T cells, human embryonic kidney cells, in DMEM and RPE1 cells (obtained from A. Khodjakov laboratory), immortalized retinal pigment epithelial cells, in DMEM/F-12 (Gibco) at 37°C and 5% CO₂. All media were supplemented with 10% fetal bovine serum and penicillin/streptomycin/antimycotic (Gibco). All cell lines were tested monthly for mycoplasma contamination. We did not attempt to authenticate them.

Actin-network disruption was performed by adding 10 μM cytochalasin D (Sigma-Aldrich). Microtubules depolymerization was performed by adding 1 μM nocodazole (Sigma-Aldrich) for 1 h at 37°C and 5% CO₂ and for 30 min at 4°C. Cytoplasmonic dynein inhibition was performed using 50 μM cilobrevin D (Calbiochem) for 2 h.

For the Arp2/3 complex inhibition experiments, cells were incubated with 0.2 mM CK666 (Sigma-Aldrich) for 1 h. Control experiments were performed using DMSO.

Plasmids. The plasmid pDTomato-centrin1 was created by excising the EGFP coding sequence from pEGFP-centrin1 with the restriction enzymes Age I and BsrGI, which was amplified by PCR using Phusion DNA polymerase (Thermo Scientific) and for cells stably incorporating the EGFPcentrin1 sequence. After 2 weeks of selection, several z-stacks were acquired to observe the EGFP-Arp3 distribution. Fixation was performed by incubating cells with PFA for 20 min, washing 3 times with PBS and incubating cells with cold methanol for 3 min. Staining was performed as previously described.

Cells were treated with nocodazole (0.2 μM) and cytochalasin D (1 μg ml⁻¹) followed by hypotonic lysis. Centrosomes were collected by centrifugation onto a 60% sucrose cushion and further purified by centrifugation through a discontinuous (70%, 50% and 40%) sucrose gradient. The composition of the sucroses solutions was based on an ad hoc buffer (TicTac buffer), in which the activity of tubulin, actin and actin-binding proteins is maintained: 10 mM Hepes, 16 mM Pipes (pH 6.8), 50 mM KCl, 1 mM MgCl₂, 1 mM EGTA. The TicTac buffer was supplemented with 0.1% Triton X-100 and 0.1% β-mercaptoethanol. After centrifugation on the sucrose gradient, supernatant was removed until about 5 ml remained in the bottom of the tube. Centrosomes were stored at −80°C after flash freezing in nitrogen liquid.

For cell filamentization assay, cells were fixed with 4% paraformaldehyde (PFA) for 20 min, incubated with quenching agent NH₄Cl for 10 min and blocked with antibody blocking buffer (PBS supplemented with 1% BSA, PBS–BSA) for 30 min. Permeabilization was performed with 0.2% Triton X-100 for 1 min. Primary and secondary antibodies were diluted in PBS–BSA and incubated on cells at room temperature for 1 h and 30 min respectively. After washing, Alexa-647–phalloidin (200 nM) was incubated for 20 min. DNA was labelled with a 0.2 μg ml⁻¹ solution of 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) (Sigma). The coverslips were air-dried and mounted onto glass slides using Mowiol mounting medium. Arp2/3 staining and WASH staining were performed by fixing cells with methanol at −20°C for 3 min and blocking with PBS–BSA for 30 min. Primary and secondary antibodies, diluted in PBS–BSA, were incubated for 1 h and 30 min respectively. DNA labelling and coverslip mounting were performed as previously described. In-solution RPE1 staining was performed by detaching the cells with trypsin (Life Technologies), pelleting and then fixing them with PFA (final concentration 4%). Cells were incubated on poly-l-lysine-coated coverslips for 30 min and permeabilized with 0.2% Triton X-100 for 1 min. Cells were stained as previously described. HEK293T cells expressing EGFP-Arp3 were stained under a microscope. Before fixation, several z-stacks were acquired to observe the EGFP-Arp3 distribution. Fixation was performed by incubating cells with PFA for 20 min, washing 3 times with PBS and incubating cells with cold methanol for 3 min. Staining was performed as previously described.

Cell pre-extraction was carried out by pelleting Jurkat and HEK293T cells, previously detached with trypsin, and by resuspending in 1% Triton X-100 for 20 min. Fixation was performed in solution by adding PFA (final concentration 4%). Cells were incubated on poly-l-lysine-coated coverslips for 30 min and stained as previously described.

Competition assay in cells. We tested the specificity of the anti-Arp2 and anti-p34-Arc antibodies by performing a competition assay with blocking antigen (purified Arp2/3 complex). Briefly, primary antibodies were incubated with an increasing quantity of purified Arp2/3 complex (0, 12, 24 and 48 pmol) in TicTac–BSA buffer for 1 h. RPE1 cells were cultured on clean coverslips and fixed with methanol at −20°C for 3 min. Cells were stained with antibody/purified Arp2/3 complexes as previously described by substituting the PBS–BSA buffer with the TicTac–BSA buffer.

Isolation of centrosomes. Centrosomes were isolated from Jurkat (non-treated, CK666-treated or transfected with siRNA) and HeLa cells by modifying a previously published protocol45. In brief, cells were treated with nocodazole (0.2 μM) and cytochalasin D (1 μg ml⁻¹) followed by hypotonic lysis. Centrosomes were collected by centrifugation onto a 60% sucrose cushion and further purified by centrifugation through a discontinuous (70%, 50% and 40%) sucrose gradient. The composition of the sucroses solutions was based on an ad hoc buffer (TicTac buffer), in which the activity of tubulin, actin and actin-binding proteins is maintained: 10 mM Hepes, 16 mM Pipes (pH 6.8), 50 mM KCl, 1 mM MgCl₂, 1 mM EGTA. The TicTac buffer was supplemented with 0.1% Triton X-100 and 0.1% β-mercaptoethanol. After centrifugation on the sucrose gradient, supernatant was removed until only about 5 ml remained in the bottom of the tube. Centrosomes were stored at −80°C after flash freezing in nitrogen liquid.

Protein expression and purification. Tubulin was purified from fresh bovine brain by three cycles of temperature-dependent assembly/disassembly in Brinkley Buffer 80 (BRB80 buffer: 80 mM Pipes pH 6.8, 1 mM EGTA and 1 mM MgCl₂) according to ref. 66. Fluorescent tubulin (ATTO-565-labelled tubulin) was prepared according to ref. 67. Actin was purified from rabbit skeletal-muscle actin powder. Monomeric Ca-ATP-actin was purified by gel-filtration chromatography on Sephacryl S-300 at 4°C in G buffer (2 mM Tris-HCL pH 8.0, 0.2 mM ATP, 0.1 mM CaCl₂, 1 mM NaCl, and 0.5 mM dithiothreitol (DTT)). Actin was labelled on lysis with Alexa-488, Alexa-568 and Alexa-647; Recombinant human profilin, mouse capping protein, the Arp2/3 complex, GST–pWA and mDia1 were purified according to previous works24,47.

Pyrene assay. For the bulk actin polymerization assay, pyrene fluorescence at 407 nm (λex = 365 nm) was monitored over time. Experiments were performed using Brinkley buffer (supplemented with 50 mM KCl and 2.7 mM ATP) or TicTac buffer (supplemented with 2.7 mM ATP) to study the activity of the Arp2/3 complex. Each assay contained 2 μM actin monomers (10% pyrene), 2 μM profilin, 30 nM Arp2/3 complex and 200 nM pWA. The elongation assay was performed with 2 μM actin monomers (10% pyrene) in PBS–BSA, were incubated stabilized with non-labelled phalloidin. CK666 and SMIF2H inhibitors were tested in an actin polymerization assay. The CK666 assay contained 2 μM actin monomers (10% pyrene), 50 nM Arp2/3 complex, 500 nM pWA and 60 μM CK666. SMIF2H
inhibitor was tested using 2 μM actin monomers (10% pyrene), 1 mM mDia1 and 0.2 mM SMIFH2.

**Western blotting.** Western blots were performed by fractionating proteins on SDS polyacrylamide gels. Membrane blocking was carried out using 3% BSA in PBS. Primary and secondary antibodies were diluted in PBS supplemented with 1% BSA and 0.1% Tween-20 and washing steps were performed with PBS supplemented with 1% BSA and 1% Tween-20.

**Proteomic analyses.** Proteomic experiments were performed as previously described. Briefly, proteins were stacked in the top of a 4–12% NuPAGE gel (Invitrogen) before R-250 Coomassie blue (Bio-Rad) staining and in-gel digestion using modified trypsin (Promega, sequencing grade). Resulting peptides were analysed by online nanoLC-MS/MS (UltiMate 3000 RSLCnano and Q-Exactive Plus, Thermo Scientific) using a 120-min gradient. Peptides and proteins were identified through concomitant searches against Uniprot (Homo sapiens) taxonomy, September 2014 version and the classical contaminants database (249 sequences, home-made) and the corresponding reversed databases using Mascot (version 2.5.1). The Proline software was used to filter the results (conservation of rank 1 peptides, peptide identification FDR < 1% as calculated on peptide scores by employing the reverse database strategy, minimum peptide score of 25, and minimum of 1 specific peptide per identified protein group) before performing a compilation, grouping and comparison of the protein groups from the different samples. Proteins from the contaminants database and additional keratins were discarded from the final list of identified proteins. Only proteins identified in both biological replicates (minimum 1 specific spectral count in each replicate) and 2 spectral counts in one of them were considered as reliable purified centrosome components.

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (http://www.proteomexchange.org) via the PRIDE partner repository with the data set identifier PXD003226.

**In vitro assays.** Experiments were performed in polydimethylsiloxane (PDMS) open chambers to add sequentially experimental solutions when needed. PDMS (Sylgard 184 kit, Dow Corning) was mixed with the curing agent (10:1 ratio), degassed, poured into a Petri dish to a thickness of 5 mm and cured for 30 min at 100 °C on a hot plate. The PDMS layer was cut to 15 mm × 15 mm and punched out using a hole puncher (ted Pella) with an outer diameter of 8 mm. The PDMS chamber and clean coverslip (20 mm × 20 mm) were oxidized in an oxygen plasma cleaner for 20 s at 80 W (Femto, Diener Electronic) and brought into contact.

Isolated centrosomes were diluted in TicTac buffer (10 mM HEPES, 1.6 mM Pipes (pH 6.8), 50 mM KCl, 1 mM MgCl₂, 1 mM EGTA) and incubated for 20 min. The excess of centrosomes was removed by rinsing the open chamber with large volume of TicTac buffer supplemented with 1% BSA to prevent nonspecific interactions (TicTac-BSA buffer).

Microtubules and actin assembly at the centrosome were induced by diluting tubulin dimers (labelled with ATTO-565, 30 μM final) and/or actin monomers (labelled with Alexa-488, or Alexa-568, or Alexa-647, 1 μM final) in TicTac buffer supplemented with 1 mM GTP and 2.7 mM ATP, 10 mM DTT, 20 μg ml⁻¹ catalase, 3 mg ml⁻¹ glucose, 100 μg ml⁻¹ glucose oxidase and 0.25% w/v methylcellulose. In addition, a threefold molar equivalent of profilin to actin was added in the reaction mixture.

Experiments based on the Brinkley buffer, the buffer usually used for centrosome and microtubules assays, were performed as previously described for the TicTac-BSA buffer. In the experiments with centrosomes isolated from PCM1 siRNA cells, a γ-tubulin staining at the end of the videos was necessary to label the centrosome, because PCM1 depletion impairs the recruitment of EGFP–centrin to the centrosome. Staining was performed as previously described, without prior fixation.

Centrosomes were considered positive for a given signal when their signal exceeded two times the background signal threshold.

**Image acquisition, processing and analysis.** Fixed cell images were captured on a confocal microscope (Leica SP2) using a ×40 1.25 N.A. objective lens, on a spinning-disc confocal microscope (Roper Scientific) equipped with an iLAsPulsed system, with a confocal head (Yokogawa) and an Evolve camera (EMCCD 512 × 512, pixel = 16 μm) using a ×60 N.A. 1.4 objective lens or on an Olympus BX61 upright microscope using a ×100 N.A. 1.4 objective lens and a Cool SNAP HQ2 camera (pixel = 6.45 μm). Live cell imaging was performed on a spinning-disc confocal microscope (Roper Scientific) using a ×60 N.A. 1.4 objective lens at 37 °C and 5% CO₂. Three-dimensional image processing was performed using ImageJ software. All of the images were deconvolved using a theoretical point spread function (PSF). All of the images show the centrosome plane, except in Fig. 4d,e where maximal intensity projections are shown.

Measurement of the actin amount around the centrosome in extracted cells was performed by measuring the integrated intensity of fluorescence in a 4-μm-diameter circle centred around the centrosome. Arp2, p34-Arc and WASP measurements were performed by measuring the integrated fluorescence intensity in a 3-μm-diameter circle centred around the centrosome. Data from separated experiments were normalized so that the average intensity in control cells was 1. Data from two distinct siRNA sequences against PCM1 (see above) were pooled.

Imaging of isolated centrosomes was performed with a total internal reflection fluorescence (TIRF) microscope (Roper Scientific) equipped with an iLAsPulsed system and an Evolve camera (EMCCD 512 × 512, pixel = 16 μm) using a ×60 1.49 N.A. objective lens. Aster proportion was calculated as the ratio of centrin1-positive actin asters divided by the number of centrin1 spots. For the centrosomes isolated from PCM1 siRNA cells, aster proportion was calculated as the ratio of the number of actin asters divided by the number of γ-tubulin spots. Data from separate experiments were normalized so that the average intensity in control experiments (DMSO or control siRNA) was 1. Actin nucleation activity was quantified by measuring the actin–fluorescence intensity integrated over a 2-μm-diameter circle at the centre of the actin aster and normalized with respect to initial intensity over time. Representative data for several experiments are shown.

TetraSpeck microspheres (Life Technologies) were used to calibrate fluorescence intensities on spinning-disc and TIRF microscopes.

**Statistical analyses.** Statistical analyses were performed with GraphPad Prism 5 (GraphPad Software). Unpaired t-test with Welch’s correction was used to determine statistical significance. In all of the graphics two-tailed P values were calculated and we reported the following Prism convention: NS (not significant; P > 0.05), “P < 0.05,” “P < 0.01,” “P < 0.001” and “P < 0.0001.”