Calibrated mitotic oscillator drives motile ciliogenesis

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Cell division and differentiation depend on massive and rapid organelle remodeling. The mitotic oscillator, centered on the cyclin-dependent kinase 1–anaphase-promoting complex/cyclosome (CDK1-APC/C) axis, spatiotemporally coordinates this reorganization in dividing cells. Here we discovered that nondividing cells could also implement this mitotic clocklike regulatory circuit to orchestrate subcellular reorganization associated with differentiation. We probed centriole amplification in differentiating mouse-brain multiciliated cells. These postmitotic progenitors can redeploy and calibrate the mitotic oscillator to drive the orderly progression of centriole reorganization associated with differentiation. We investigated if multiciliated progenitors implemented molecular mechanisms of cell division to orchestrate subcellular remodeling associated with differentiation.

Motile cilia of multiciliated cells generate streams of vital fluids that promote respiratory, reproductive, and brain functions (I). Defects in motile ciliation or cilia number cause life-threatening diseases by perturbing cilia-based flow (I–4). However, the events leading to motile ciliogenesis and the mechanisms regulating cilia number are poorly explored. Organelle biogenesis and remodeling are common to cell division and differentiation. Centrioles are microtubule-based organelles that are duplicated in dividing cells to provide daughter cells with a full centrosome (5). In differentiating multiciliated cells, centrioles are amplified to nucleate patches of 30 to 300 cilia (6–9). The mitotic machinery (fig. S1A) participates in centriole number control (10, 11) and regulates centriole growth (12) and dynamics (5, 13–16) in dividing cells. How these steps, essential for motile ciliogenesis and cilia number control, are regulated in multiciliated progenitors is unknown. Centriole amplification follows a typical spatiotemporal pattern (9), prompting an analogy with centriole duplication. We therefore investigated if multiciliated progenitors implemented molecular mechanisms of cell division to orchestrate subcellular remodeling associated with differentiation.

Centriole amplification in mouse-brain progenitor cells is marked by three sequential phases delimited by two switchlike transitions (fig. S1B) (9). The A to G transition borders the amplification (A) phase, during which procentrioles form around centrosome-derived deuterosome platforms and the growth (G) phase, during which all procentrioles grow synchronously from these platforms. At the A to G transition, centrosomal

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**Fig. 1. Mitosis machinery is transiently activated in differentiating multiciliated progenitors.** (A and B) Immunostaining profiles of CDK1, p27kip1 (a CDK inhibitor), pH3, and pVim in cultured CEN2-GFP+ cells at different stages of cilia-nucleating centriole formation during postmitotic multiciliated-cell differentiation. (C) Horizontal bars illustrate immunoreactivity of mitosis markers relative to differentiation stages. C, centrosome stage (black); A phase, centriole amplification phase (gray); G phase, centriole growth phase (orange); D phase, centriole disengagement phase (green); and MBB, multiple basal body stage (black). The nucleus is outlined with dashed lines, and “x” marks GFP aggregates. Scale bars, 2 μm.
Centrioles separate and deuterosomes disperse along the nuclear membrane to assume a perinuclear distribution (fig. S2, A to D). The G to D transition borders the G and disengagement (D) phase, during which maturing centrioles detach from their growing platforms in a wavelike manner, the primary cilium resorbs, and microtubules reorganize (fig. S2, D to H, and movie S1). Disengaged centrioles then migrate and dock apically to become basal bodies and initiate cilia (movie S1). This precise spatiotemporal pattern of centriole amplification (fig. S2I) mirrors the centriole dynamics of dividing cells, where duplicated centrosomes separate along the nuclear membrane at the G2 to M transition of the cell cycle before disengaging at the metaphase to anaphase transition (5).

The G2 to M and metaphase to anaphase transitions are spatiotemporally coordinated by the mitotic oscillator, centered on the cyclin-dependent kinase 1–anaphase-promoting complex/cyclosome.

Fig. 2. CDK1-APC/C axis is conserved in differentiating multiciliated progenitors and can trigger mitosis. (A) Representative immuno- and histone H3 phosphorylation relative to cycling progenitors. Error bars represent mean ± SD. (B) pH3 quantifications in differentiating progenitors with pharmacologically induced mitosis compared to control cycling or differentiating progenitors. Error bars represent mean ± SD. (C) CEN2-GFP+ and histone H2B-RFP+ dynamics during proTAME-induced mitosis starting from G phase (RFP, red fluorescent protein). Mitosis entry (prophase-like nucleus) occurs during D phase (13:20) followed by metaphase-like (13:30 to 14:00) and anaphase-like (14:20) states. Arrowheads indicate micronuclei after mitotic exit (16:20). Time in hours:min.

(CDK1–cyclin B1–APC/C) axis (cyclin B1 and CDC20 are mitotic coregulators) (17–19). By immunostaining differentiating progenitors in vitro and in vivo, we observed the expression of mitosis-specific regulators (17) and the presence of mitotic phosphorylations (20) (Fig. 1 and figs. S3 to S6), suggesting a transient exit from quiescence and mitosis machinery reactivation. The sequential profile of mitotic phosphorylations (Fig. 1C) suggested the involvement of G2 to M and metaphase to anaphase regulators in A to G and G to D transitions, respectively. To confirm the sequential activity of mitosis regulators in differentiating progenitors, we performed short incubations with pharmacological modulators of the CDK1–APC/C axis (fig. S1C). We used phosphorylated histone H3 [pH3 (pSer10)] and phosphorylated vimentin [pVim (pSer55)] as mitotic machinery–activity readouts. Inhibition of the G2 to M CDK1 inhibitors Wee1-like protein kinase (WEE1) and membrane associated tyrosine/threonine kinase 1 (MYT1) (17) with PD 166285 increased pH3 phosphorylation in G- and D-phase cells (Fig. 2A and fig. S7, A to C). Massive pH3 phosphorylations also appeared in G- and D-phase cells, some of which showed prophase-like chromosome condensations (Fig. 2, A and B, and fig. S7C, red dots). Coinhibiting WEE1-MYT1 and APC/C with PD 166285 and proTAME (21) further augmented pVim and pH3 phosphorylations and increased prophase-like figure incidence in D-phase cells (Fig. 2, A and B, and fig. S7, A to C). Metaphase- and anaphase-like figures appeared in this condition (Fig. 2, B and C). Inhibiting APC/C alone with proTAME increased pH3 and pVim phosphorylations and triggered prophase- to anaphase-like figures only in D-phase cells (Fig. 2, A to C, and fig. S7, A to C). Differentiating progenitors with mitosis-like events presented hyperphosphorylations comparable to those observed during mitosis in cycling progenitors (Fig. 2D and fig. S7D). Cotreating cells with the CDK1 inhibitor RO-3306 abolished hyperphosphorylations and mitotic events (fig. S7, C and E). Thus, CDK1 is controlled and calibrated by WEE1-MYT1 at the A to G transition and by APC/C at the G to D transition, confirming the proposed parallel between mitotic G2 to A transition and G1 to S progression.

Fig. 3. CDK1-APC/C axis tunes centriole number and controls centriole amplification in postmitotic differentiating multiciliated progenitors. (A and C) Box (25 to 75%) and whisker (10 to 90%) plots of A (gray), G (orange), and D (green) phase durations in differentiating CEN2-GFP+ progenitors incubated 22 to 24 hours with DMSO (controls); RO-3306, BI 2536 (PLK1 inhibitor), and PD 166285; and proTAME with or without apcin. Lines indicate medians, and crosses indicate means. (B) Quantification of final centriole number in differentiating CEN2-GFP+ progenitors at the D phase after 72 hours of incubation with DMSO (control), CDK1 inhibitors (RO-3306 and CQP74514A), or BI 2536; or after 24 hours of incubation with DMSO (control) or PD 166285. Error bars represent mean ± SD. (D and E) Mitosis machinery is active, yet calibrated, in terminally differentiating progenitors of multiciliated cells to orchestrate the massive production of cilia-nucleating centrioles (D) while avoiding the mitotic commitment threshold (E). Deregulating the calibration alters centriole number, maturation, and motile ciliation and can drive the differentiating progenitor into abnormal mitosis. *P = 0.018; **P < 0.006; ***P ≤ 0.0009; ****P < 0.0001; ns, not significant.
M–metaphase to anaphase transitions and multiciliated A to G–D to transitions. To validate the dormant mitotic capacity of differentiating progenitors, we live imaged centriole and chromosome dynamics with APC/C inhibition. Differentiating cells entered mitosis after the G to D transition, as suggested by immunostainings (Fig. 2E; fig. S8, A and B; and movie S2). Mitosis was characterized by cyclin B1 immunostainings (Fig. 2E; fig. S8, A and B; and movie S6). The G to D transition was significantly affected. It was slightly delayed, and cells that failed to initiate D phase regrouped their deuterosomes (Fig. 3C and fig. S14B). As expected, motile cells passing the G to D transition underwent mitosis (fig. S11, C and D), and movie S6). In cells with a D phase spared from mitotic entry, D-phase duration increased in a dose-dependent and synergistic manner (Fig. 3C and movie S6) and led to partial motile ciliation (fig. S14, D and E), as with CDKI and PLK1 inhibition. This suggests that CDKI activates APC/C{Cdc20}. Together with PLK1, APC/C triggers the G to D transition and controls synchronous centriole disengagement required for functional migration, docking, and ciliation. In return, APC/C dampens CDKI activity, thus preventing mitosis in differentiating progenitors.

This study reveals that the CDKI-APC/C mitotic oscillator is summoned after progenitor cell division to drive terminal differentiation instead of proliferation (Fig. 3D). Postmitotic progenitors redploy the robust mitotic clocklike regulatory circuit to drive the orderly progression of centriole production—number control, growth, and disengagement—and provide multiciliated cells with a sized patch of centrioles competent for motile ciliation. This finding aids understanding of the development of multiciliated cells and motile cilia–powered flows crucial for organism homeostasis.

Although centrosome duplication in cycling cells is coupled to cell division (10–16), multiciliated progenitors dampen the mitosis machinery to drive centriole dynamics but avoid nuclear division (Fig. 3E). This is consistent with studies in mammalian cycling cells showing that CDKI couples nuclear and cytoplasmic events at mitotic entry (23, 24) and with studies in Drosophila showing the uncoupling of nuclear–cytoplasmic events by experimentally dampening CDKI (25–27). Thus, calibration of the mitosis machinery to uncouple cytoplasmic from nuclear processes exists physiologically in mammalian cells. This mechanism can be used by postmitotic progenitors to impose timing and directionality in the control of cytoplasmic events such as organelle remodelling associated with differentiation. By contrast, this kind of calibration could allow cycling cells to undergo pathological centriole amplification linked to cancer and microcephaly (5, 26–30).

REFERENCES AND NOTES


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SUPPLEMENTARY MATERIALS

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Calibrated mitotic oscillator drives motile ciliogenesis

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Taming mitosis for differentiation

The mitotic oscillator consists of molecular switches known to drive cell division forward. This conserved clocklike regulatory circuit has not previously been implicated in cellular processes other than division. Multiciliated cells generate motile cilia-powered flows that are essential for brain, respiratory, and reproductive functions. Al Jord et al. found that the mitotic oscillator was activated in a calibrated fashion in terminally differentiating progenitors of multiciliated cells (see the Perspective by Levine and Holland). The oscillator function was used to drive massive production of cilia-nucleating centrioles while avoiding mitotic commitment. Thus, mammalian postmitotic progenitors can recruit and calibrate the mitotic oscillator to impose timing and directionality of cellular differentiation instead of proliferation.

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