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Cell cycle proteins moonlight in multiciliogenesis:

Proteins that control cell cycle progression also drive multiciliated cell differentiation

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Multiciliated cells (MCCs) are a specialized population of postmitotic cells that are decorated with tens to hundreds of hairlike protrusions, termed motile cilia, that beat back and forth to direct fluid flow across an epithelium (1). MCCs line the respiratory tract, brain ventricles, and reproductive tracts of vertebrates and play a crucial role in tissue homeostasis; defects in the formation or movement of motile cilia can cause fertility defects, chronic respiratory infections, and/or a buildup of fluid in the brain. Despite their importance to human health, the pathways controlling the production of motile cilia in differentiating MCCs remain poorly understood. On page 803 of this issue, Al Jord *et al.* (2) shed light on this question by showing that multiciliated progenitor cells implement components of the mitotic cell cycle machinery to coordinate events that are required for motile ciliation and cellular differentiation, while avoiding cell division (mitosis).

Each motile cilium is nucleated by a centriole-based structure, termed a basal body. In proliferating cells, centriole biogenesis is tightly coupled to cell cycle progression to ensure that only one new centriole forms adjacent to each of the two existing centrioles (3). Centriole duplication begins at the G₁ to S phase transition of the cell cycle in a process that requires the activity of cyclin-dependent kinases (CDKs). A second kinase, polo-like kinase 1 (PLK1), acts at the G₂ to M transition of the cell cycle to catalyze steps required for centriole maturation and licensing of a new round of centriole duplication (see the figure). These regulatory transitions place strict limits on centriole duplication and maturation in dividing cells. Aberrations in centriole number and function can promote mitotic errors and have been linked with several human diseases, including developmental disorders and cancer (4, 5).

In contrast to the strict numerical control of centriole number in cycling cells, multiciliogenesis relies on the mass production of centrioles in interphase to produce hundreds of basal bodies that serve as the foundation for producing motile cilia. This raises the question of how MCCs coordinate the massive production of centrioles in the absence of distinct cell cycle transitions. To address this, Al Jord *et al.* used fixed and live cell imaging to detail the stepwise progression of centriole biogenesis in differentiating brain MCCs. They observed that during the centriole amplification stage, new centrioles are constructed on the surface of specialized structures called deuterosomes (see the figure). Deuterosomes are composed of multiple proteins required for centriole biogenesis and are nucleated from an existing centriole (1). Centrioles then grow upon the deuterosomes to reach their final length. After the growth phase, centrioles disengage from the deuterosome in synchrony and migrate to the apical plasma membrane, where they dock and nucleate motile cilia.

Notably, Al Jord *et al.* observed that differentiating MCCs displayed low-level increases in several mitotic-specific phosphorylation events and transient expression of multiple proteins that play important roles during mitosis. This suggested that temporal activation of proteins required for mitosis could be involved in controlling the stepwise process of centriole amplification in MCC progenitors. To explore this further, the authors used pharmacological inhibitors in differentiating MCC progenitors to probe the requirement for CDK1 and PLK1, two kinases that normally act to control events occurring in late G₂ and mitosis of the cell cycle. Inhibition of CDK1 or PLK1 delayed the amplification phase of centriole biogenesis in MCCs, leading to an increase in the production of deuterosomes and ultimately centrioles. Centriole growth and disengagement stages were also hindered in cells with lower CDK1 activity, resulting in partial motile ciliation. In G₂ cells, CDK1 activity is negatively regulated by the activity of two kinases, Wee1-like protein kinase (WEE1) and membrane-associated tyrosine- and threonine-specific Cdc2-inhibitory kinase (MYT1, also known as PKMYT1). Increasing CDK1 activity, by inhibiting WEE1 and MYT1, accelerated progression through each stage of multiciliogenesis, resulting in a corresponding decrease in the production of deuterosomes, centrioles, and motile cilia. CDK1 activity is therefore calibrated to control the orderly progression of centriole amplification, growth, disengagement, and ciliation in differentiating MCCs.

In cycling cells, the activity of CDK1, in complex with cyclin B1, is controlled to allow for timely progression through mitosis. As CDK1 activity increases during entry into mitosis, CDK1–cyclin B1 phosphorylates and inactivates WEE1 and MYT1. At the same time, CDK1 promotes the full activation of the anaphase-promoting complex with specificity determined by CDC20 (APC/C^{CDC20}) that is inhibited in interphase cells (6). APC/C^{CDC20} then targets cyclin B1 for degradation at anaphase to inactivate CDK1 and allow for mitotic exit (see the figure). Notably, inhibiting the activity of the APC/C^{CDC20} in MCCs increased CDK1-dependent phosphorylation events and drove MCCs into mitosis. Multiciliated progenitors therefore fine-tune the activity of CDK1 to control centriole biogenesis, whilst avoiding commitment to cell division.

Like CDK1 and PLK1 inhibition, reducing APC/C^{CDC20} activity also delayed centriole disengagement. This suggests a model in which CDK1 activates the APC/C^{CDC20}, which, in collaboration with PLK1, then acts to promote the synchronous disengagement of the centrioles (see the figure). Meanwhile, APC/C^{CDC20} also dampens CDK1 activity by targeting cyclin B1 for destruction, thereby preventing mitotic entry. Together, this points toward a surprising role of APC/C^{CDC20} in interphase in promoting centriole biogenesis and terminal differentiation, rather than cell division. The finding that APC/C^{CDC20} can function outside of mitosis adds to a growing body of literature showing that molecules that regulate cell division can also act during interphase to help control cell fate (7).

The new findings by Al Jord *et al.* demonstrate that, like cycling cells, PLK1, CDK1, and APC/C^{CDC20} activity coordinate the timing of centriole biogenesis in differentiating MCC progenitors. The biochemical changes required for multiciliogenesis are reminiscent of those that promote cell cycle transitions in proliferating cells. What remains unclear, however, is how PLK1 and CDK1 coordinate the amplification, growth, and disengagement phases of centriole biogenesis. In the future, it will be important to identify the key targets of PLK1

and CDK1 and establish how phosphorylation of these substrates coordinates centriole amplification.

An additional area of investigation is to examine the role that other CDK-cyclin complexes play in multiciliogenesis. Given that centriole duplication in cycling cells relies on the activity of CDK2 during S phase of the cell cycle, it would not be surprising if MCCs also use CDK2 activity to drive centriole amplification. In light of this, it would be interesting to examine the role of cyclin O, which can bind CDK2 and is specifically expressed in MCCs, where it functions to promote deuterosome formation and centriole amplification (8, 9). Interestingly, cyclin O is encoded within a conserved genomic locus that contains multiple key regulators of MCC formation, including the CDC20 paralog CDC20B. It is therefore tempting to speculate that cyclin O and CDC20B are specifically expressed in MCCs to help regulate CDK activity and promote differentiation. Recent studies have shown that mutations in cyclin O and proteins that specify MCC cell fate cause respiratory tract disease by reducing the production of motile cilia (10, 11). A better understanding of the molecular machinery that controls multiciliogenesis will therefore have important implications for human health.

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Centriole biogenesis in cycling cells **Centriole biogenesis in multiciliogenesis**

Figure. Stepwise progression of centriole biogenesis in proliferating and multiciliated cells
 Multiciliogenesis requires the cell cycle machinery. During amplification, deuterosomes are formed on the side of the daughter centrioles, producing multiple procentrioles. Once mature, centrioles detach, form distal appendages, and migrate to the apical plasma membrane, where they form basal bodies that nucleate cilia.