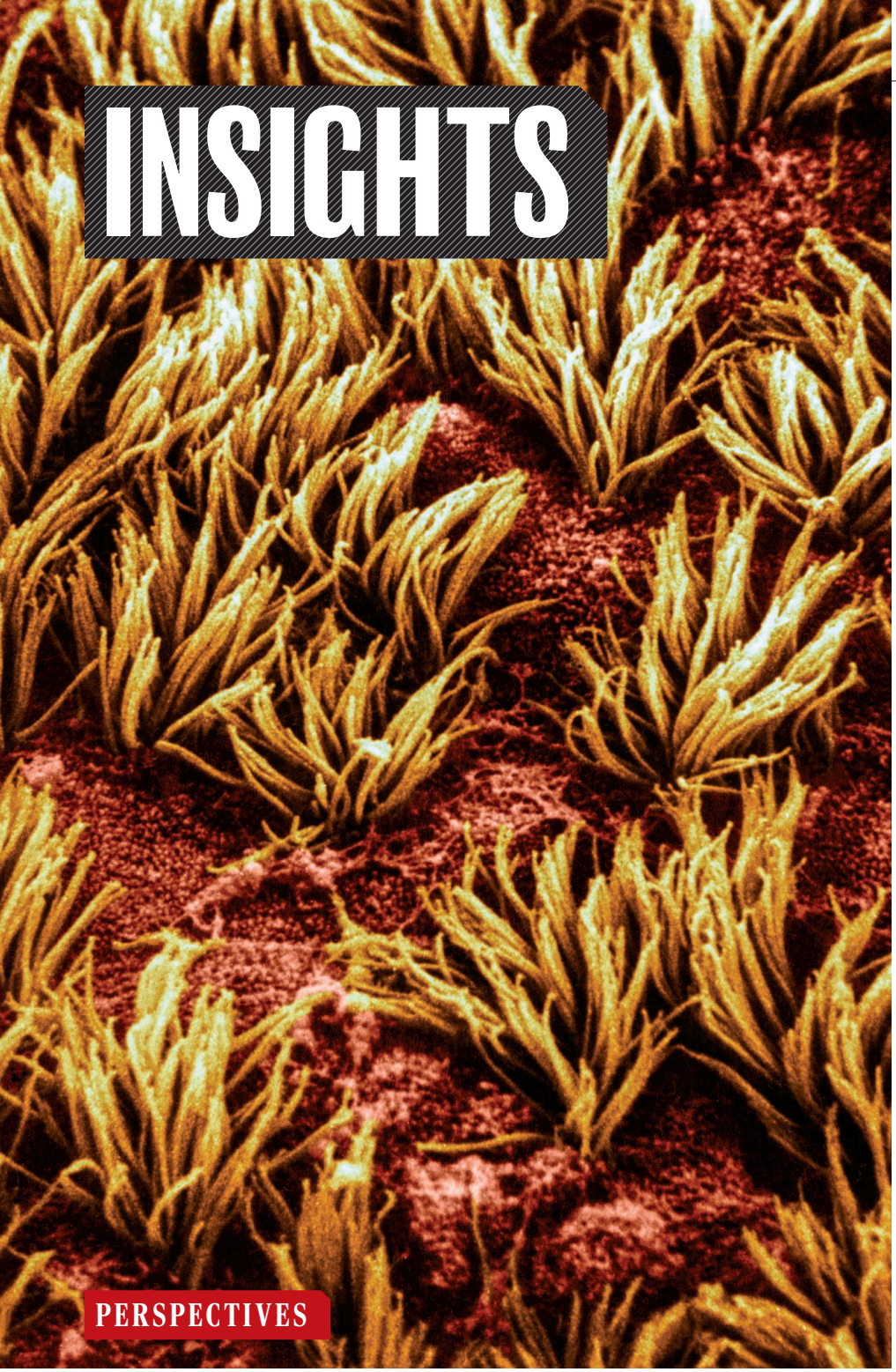

INSIGHTS

PERSPECTIVES

Multiciliated cells (yellow) line the human oviduct.

CELL BIOLOGY

Cell cycle proteins moonlight in multiciliogenesis

Proteins that control cell cycle progression also drive multiciliated cell differentiation

By **Michelle Levine** *and* **Andrew Holland**

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 G_1 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 G_2 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

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PHOTO: SPL/SCIENCE SOURCE

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 G_2 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 G_2 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.

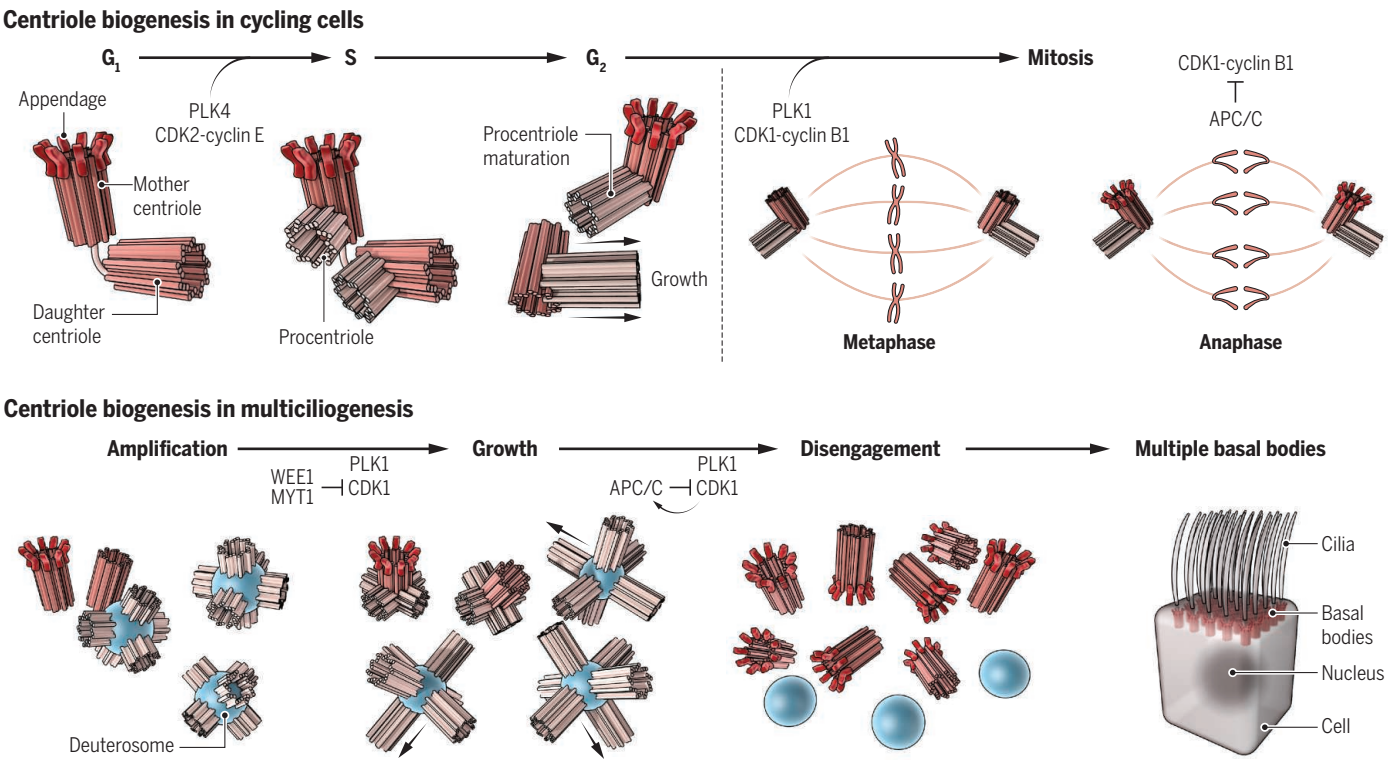
In cycling cells, the activity of CDK1, in complex with cyclin B1, is controlled to allow for timely progression through mitosis. As CDK1 activity increases during entry into mitosis, CDK1–cyclin B1 phosphorylates and inactivates WEE1 and MYT1. At the same time, CDK1 promotes the full activation of the 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 reminis-

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.



GRAPHIC: C. BICKEL/*SCIENCE*

cent 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. ■

"...multiciliated progenitor cells implement components of the mitotic cell cycle machinery to coordinate events that are required for motile ciliation..."

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NEUROENDOCRINOLOGY

Linking smell to metabolism and aging

The olfactory system can have direct effects on energy homeostasis

By **Jennifer L. Garrison**[1,2] *and* **Zachary A. Knight**[3]

The sense of smell, or olfaction, allows animals to survey the chemical landscape of the outside world and use this information to guide behavior. Olfactory cues are particularly important for the regulation of feeding, but how odor perception influences other aspects of energy homeostasis remains poorly understood. Recent work has begun to uncover some of these connections, revealing an unexpected role for olfaction in the control of metabolism and longevity.

The idea that olfaction and metabolism could be connected goes back at least to the work of Ivan Pavlov, who showed that odors and other sensory cues associated with food could trigger hormonal and autonomic responses in anticipation of a meal (*1*). These "cephalic phase" responses, such as salivation and secretion of gastric acid, are thought to help prepare the body to accommodate the rapid influx of nutrients that occurs during eating. But how chronic changes in odor exposure affect metabolism has been less clear.

To address this question, a recent study manipulated the olfactory system in the mouse and measured the effect on energy homeostasis (*2*). By ablating olfactory sensory neurons in adult mice, they generated mice that had a reduced ability to smell. These hyposmic mice exhibited normal food intake and body weight on a regular diet, but were resistant to obesity caused by a high-fat diet. This leanness was due to both reduced food intake and, surprisingly, increased energy expenditure. Mechanistic studies revealed that this enhanced energy expenditure was caused by an increase in the activity of brown adipose tissue, a specialized thermogenic organ that functions to dissipate heat in mammals.

To test this idea further, the authors used complementary manipulations to generate mice with enhanced smelling ability. These mice exhibited increased body weight in the absence of any change in food intake (*2*). Together, these observations reveal that the olfactory system can regulate body weight via direct effects on energy expenditure, rather than solely through changes in food intake as has traditionally been assumed. Other recent studies have also suggested connections between olfaction and metabolism, although the exact relationship remains unclear (*3*, *4*).

What are the implications of these findings? It is well established that metabolism is a critical determinant of not only body weight, but also aging. Thus, we might predict that loss of smell could influence life span, and this has indeed been demonstrated in invertebrates: Disrupting olfactory neuron function, either through mutation or laser ablation, extends life span in both worms and flies (*5*, *6*). In these simpler organisms, the smell of food decreases life span, but only when the animals are calorie restricted (*7*). These data support the idea that olfactory perception may alter how an organism uses energy, and suggest the intriguing possibility that modulating smell could be a viable strategy for anti-aging interventions.

The mechanisms by which the olfactory system influences metabolism are unknown, but one possibility is suggested by the recent discovery that food odors can regulate "hunger neurons" in the hypothalamus (*8*–*10*). These cells, known as agouti-related protein (AgRP) neurons, are activated by food deprivation, and their activity powerfully influences food intake, energy expenditure, and peripheral metabolism. Traditionally, AgRP neurons were thought to be regulated exclusively by nutrients and hormones that circulate in the blood, but it is now appreciated that these cells also respond rapidly to sensory cues associated with food. Indeed, the smell of food alone can inhibit the AgRP neurons of a hungry mouse within seconds (*8*–*10*). How chronic disruption of this olfactory input would affect physiology has not been tested, but it is possible that such manipu-

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Cell cycle proteins moonlight in multiciliogenesis

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