

# COMMENTARY

# Controlling the direction of division

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# **Abstract**

Quyn and colleagues report that gut stem cells have a biased spindle orientation and asymmetric retention of label-retaining DNA. These features are lost in mouse and human tissues when the microtubule binding protein Apc is mutated. In the developing kidney, Apc acts downstream from primary cilium signaling to influence spindle orientation when noncanonical Wnt signaling predominates. Do gut stem cells also have primary cilia?

The direction in which a cell divides can be of paramount importance for tissue development and differentiation. This is certainly true for stem cells, where the two daughters of a division often have different fates - one perhaps remaining in the stem cell population, while the other goes forward in differentiation toward a functional end state.

A recent article by Quyn and colleagues in Cell Stem Cell [1] (with an accompanying preview by Yamashita [2]) explores the directional bias of division in gut epithelial stem cell compartments using multiphoton microscopy to determine spindle orientation in three dimensions. In the intestinal crypt and colon, the majority of stem cells divide perpendicularly to the apical surface. The division orientation correlates with asymmetric retention of label-retaining DNA, which stays with the basal daughter. In Quyn and colleagues' procedure, label-retaining DNA marks the oldest DNA in the nucleus [1]. Presumably, the perpendicular division eventually initiates differentiation - with the oldest DNA remaining with the stem cell daughter, and the other daughter moving into the transit-amplifying compartment. These results support Cairns' hypothesis whereby stem cells retain unreplicated DNA strands to minimize mutations [3]. Spindle orientation becomes less stringent as the cells reach the transit-amplifying compartment.

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Strict spindle orientation in the stem cell compartment evidently depends at least in part on microtubule binding proteins such as Apc, the product of the adenomatous polyposis coli gene. The preference for perpendicular spindle orientation and asymmetric label retention are both lost in precancerous mouse and human tissues where even one copy of the Apc gene is mutated. In addition, cell shape in the stem cell compartment of the mouse intestinal crypt becomes less regular.

Apc is a multifunctional protein that is well known for its role in canonical Wnt signaling, where it forms part of a multiprotein complex involved in degradation of βcatenin. The protein also localizes in part to the centrosome and plays a role in stabilizing microtubule-plus ends; for example, in astral microtubules of the spindle. Further, Apc loss has been implicated in chromosome instability (reviewed in [4]). These features make Apc a molecule of importance for mitotic orientation and function, not only in the gut stem cells but also generally where spindle orientation, mitotic stability and perhaps DNA replication asymmetry are critical for normal morphogenesis. For example, Apc modulates mouse embryonic stem cell differentiation [5] and prevents cystic kidney neoplasia [6], probably by controlling Wnt signaling.

How Wnt signaling could affect the development of the kidney throws an interesting light on the role of spindle orientation in tissue differentiation in general [7]. The canonical Wnt signaling pathway is necessary for initial tubulogenesis in the developing kidney. To prevent cystic kidney formation, however, this pathway must be regulated. This regulation is performed through the primary cilium. An organelle found on many types of cells of the body, as well as on embryonic stem cells in culture, the primary cilium grows at the apical surface above the cell centrioles – the older of which becomes the ciliary basal body – during the G<sub>1</sub> phase of each cell cycle (reviewed in [8]). In cells that divide, the primary cilium is resorbed before division, at which time the duplicated centrioles migrate to the poles of the forming mitotic apparatus.

Several important receptors and signaling molecules are sequestered in the primary cilium, and some of these molecules are influential in converting canonical Wnt signaling to noncanonical signaling in the planar cell polarity pathway, which probably determines the spatial orientation and organelle positioning within the cell. Each cell in the developing kidney tubule normally grows a primary cilium. The mitotic spindle of the developing kidney tubule cell that can grow a primary cilium usually becomes oriented parallel to the long axis of the tubule, while spindles are more randomly oriented in mutants that lack cilia. The uniform longitudinal orientation leads to normal tubule elongation, while the random orientation is conducive to cyst formation.

We can speculate that Apc functions downstream of ciliary signaling in a mitotic orientation in situations where planar cell polarity signaling predominates. The Apc truncation used by Quyn and colleagues, however, does not alter cell polarity as defined by PAR-3 and should not interfere with the normal regulation of Wnt signaling [1]. Moreover, we have few ideas about how planar cell polarity actually produces mitotic orientation in vertebrates: what cell orientation marker proteins are really important in setting the position of the poles? In the kidney tubule, normal spindle orientation correlates with the physiological direction of flow measured by bending the primary cilium – which suggests that signals leaving the cilium, perhaps such as Vangl2 [9], interacting with centrosomal or basal body proteins such as Apc, influence the final positioning of the mitotic apparatus poles to be formed in the future. The basal body itself may contain positional clues, such as left-right orientation with regard to the apical surface of the cell.

Do gut stem cells have primary cilia? It is generally thought that fully differentiated, mature enterocytes do not have primary cilia, but researchers have just begun to look for them seriously at deeper levels in the crypt or in the intervilllus region of the developing intestine before the crypts are formed. M Saqui-Saloes and JL Merchant (personal communication) have recently found that gastric epithelial cells of mice have primary cilia, with

most ciliated epithelial cells located at the base of the glands, slightly below the proliferative zone. Some ciliated cells were stained with stem cell markers, supporting the conclusion that gut stem cells are present in the stomach. If intestinal stem cells also have primary cilia, the uniform orientation of the spindle apparatus as found by Quyn and colleagues may be the result of the same type of signaling as in the developing kidney, flowing from the cilium to noncanonical Wnt signaling.

#### Competing interests

The author declares that he has no competing interests.

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