Text by Mitch Leslie
mitchleslie@comcast.net



# CLAMPing down on microtubules in migratory cells

Par complex and "sperm" protein crucial for embryonic cell relocation.

A little-known microtubule-binding protein promotes cell migration during development. Werner et al. find that the protein, CLAMP, stabilizes microtubules at the front of crawling cells and enables the cells to fit in when they reach their new home (1).

Embryonic cells and cancer cells often travel far from their birthplace and may have to traverse barriers to reach their destinations. During *Xenopus* development, two types of cells that are born in an interior layer of the embryo—multiciliated cells and ionocytes—perform a maneuver called radial intercalation. They head for the embryo's surface, where they force aside cells in the outer layer and then interlock with their new neighbors (2).

Migrating cells often relocate their centrioles to a position between the nucleus and the front end of the cell, and they typically stabilize microtubules aligned with the axis of movement. Proteins of the Par complex—which include Par3, Par6, and aPKC—stimulate cell migration, and previous studies suggested that they promote centrosome relocation and microtubule stabilization (3, 4). Werner et al. delved further into the functions of the Par proteins during radial intercalation.

The researchers engineered embryos to manufacture a dominant-negative version of Par3 that blocks the protein's function. Multiciliated cells contain numerous centrioles that help form cilia. But in multiciliated cells expressing dominant-negative Par3, centrioles failed to gather at the front, apical side of the cells. Moreover, 80% of the cells could not manage radial intercalation. A morpholino that depletes cells of Par3 produced the same effect, suggesting that the Par complex is essential for radial intercalation.

Stable microtubules are also necessary, the researchers found. They observed that these microtubules concentrated at the apical end of migrating multiciliated cells and that dosing embryos with nocodazole, which breaks down microtubules, drastically reduced the number of cells reaching the outer layer.

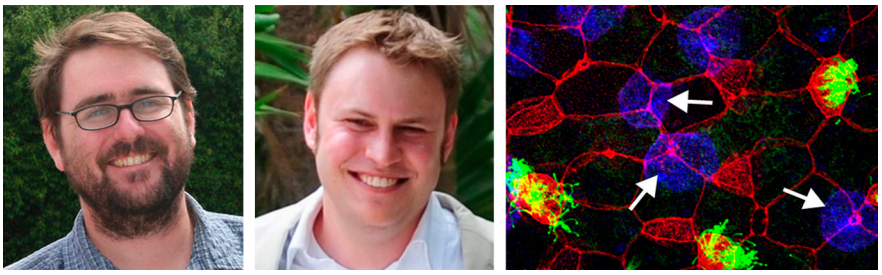
Further experiments showed that the Par complex was necessary for microtubule stabilization. To find out how the Par complex interacts with microtubules, the researchers went fishing for binding partners of aPKC. They reeled in CLAMP, a microtubule-binding protein mainly studied in sperm flagella. Werner et al. showed that CLAMP stabilized microtubules in vitro and in cells. For example, a morpholino that depleted cells of CLAMP reduced microtubule stability in cells of the embryo's outer layer. It had an even larger effect on multiciliated cells that were in the process of intercalating, cutting the amount of acetylation—a marker of microtubule stability—by 60%. Losing CLAMP didn't prevent multiciliated cells from differentiating, but they remained trapped in the interior of the embryo.

> "This protein is really important for stabilizing microtubules in a subcellularly restricted manner."

The discovery of CLAMP's role raises a further question: how does the protein preferentially stabilize microtubules in the correct part of the cell? Werner et al. noticed that Par complexes amass in the apical portion of multiciliated cells, and they determined that CLAMP also localizes there. This finding suggests that the Par complex helps position CLAMP so that it can act on microtubules at the leading edge of a migrating cell.

The study reveals that CLAMP has a bigger influence than researchers suspected. "This protein is really important for stabilizing microtubules in a subcellularly restricted manner," says senior author Brian Mitchell. When researchers think about the cytoskeleton in a migrating cell, they tend to think of actin, he says. "In this context, it turns out that microtubules are also critical." One question that remains to be answered, he adds, is how cells regulate CLAMP to stabilize microtubules at the right time. It's also unclear if cancer cells depend on CLAMP as they seek out new homes and barge through cellular barriers.

## Focal Point



PHOTOS COURTESY OF JENNIFER MITCHELL (MITCHELL) AND ELIZABETH ROSS (WERNER)

**Brian Mitchell (left), Michael Werner (center), and colleagues (not pictured) determined how the Par complex and the microtubule-stabilizing protein CLAMP allow internal cells to move to the outer layer of the embryo. The image on the right shows the result of depleting CLAMP with a morpholino. Arrows indicate multiciliated cells (blue) that migrated to the embryo's surface but failed to integrate with the cells already there (red).**

1. Werner, M.E., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201312045.
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3. Schmoranzer, J., et al. 2009. *Curr. Biol.* 19:1065–1074.
4. Chen, S., et al. 2013. *Dev. Cell*. 24:26–40.