Live imaging puts new light on stem cell division

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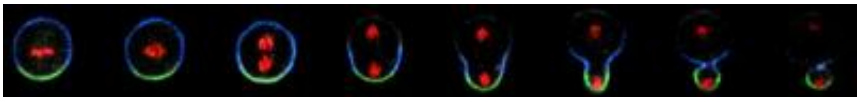

The asymmetrical division of a stem cell -- in this case a neuroblast of a fruit fly -- is shown, the result of research in the lab of Chris Doe at the University of Oregon. Credit: Courtesy of Clemens Cabernard

(PhysOrg.com) -- A long-held assumption about asymmetrical division of stem cells has cracked. Researchers at the University of Oregon report that the mitotic spindle does not act alone -- that cortical proteins help to position a cleavage furrow in the right location.

Their discovery, described in the Sept. 2 issue of the journal *Nature*, provides a new window on how stem cells divide to produce two unequal daughter cells: one that lives on as a new stem cell and other, smaller cell, that adopts a new function, in this case as a neuron.

A three-member team focused on drosophila (fruit flies) neural stem cells known as neuroblasts, long known for dividing asymmetrically. What is learned in these flies often applies to many other mitotic (dividing) cells in other organisms such as mammals, including humans.

"This addresses a fundamental question in cell biology, namely how a

cell knows where to place a cleavage furrow and thus divide in a symmetrical or asymmetrical fashion," said Clemens Cabernard, a postdoctoral fellow in the lab of Chris Doe, a Howard Hughes Medical Institute investigator in the UO Institute of Molecular Biology and director of the UO Institute of Neuroscience. Also on the team was Kenneth E. Prehoda, a UO biochemist and member of the Institute of Molecular Biology.

What the UO team found is that neuroblasts have two distinct dividing pathways that appear to work redundantly: one that is polarity induced and one that is spindle induced, Cabernard said.

Theories on cleavage furrow positioning during cell division have centered on the mitotic spindle, a network of fibers called microtubules.

One idea is that microtubules from spindle poles reach to the cortex, which delivers a positive or negative signal to determine the position of the cleavage ring. Another idea is that microtubule fibers from the center of the spindle reach out to the cortex resulting in the assembly of a cleavage ring (a complex consisting of several proteins, one of which is called myosin). A third model involves both. It was thought that asymmetrically dividing cells, such as drosophila neuroblasts, generate an asymmetric spindle and can position the cleavage ring in an asymmetric position, as opposed to symmetrically dividing cells that construct a symmetric spindle.

"We found a new mechanism in which a cleavage furrow can be placed at an asymmetric position," Cabernard said. "First, by way of a couple of experiments, we ruled out that the cleavage furrow is solely dependent on the position and symmetry/asymmetry of the mitotic spindle."

First researchers used mutants that lack astral microtubules, the microtubule fibers reaching out from the spindle poles towards the

cortex and watched with live imaging what happens to the cleavage furrow. A cleavage furrow still occurred in an asymmetric position. This has been seen before but not using the same markers, Cabernard said.

Next, researchers removed the entire spindle from the picture with targeted drugs. Usually cells stop dividing in this condition, but a genetic trick allows these cells to initiate cell division despite the lack of a mitotic spindle. Surprisingly, researchers found, the proteins involved in constructing a cleavage furrow became localized in an asymmetric fashion and positioned a cleavage furrow in an asymmetric position -- pretty much like in wild-type neuroblasts. "Although cell division could not be completed, the dividing point was correctly marked," Cabernard said. "This told us that there must be a mechanism independent of the spindle."

In a third set of experiments, the research team rotated the mitotic spindle of neuroblasts using genetic mutants and thus changed the position of any spindle-derived signal. Interestingly, the team found that two cleavage furrows now formed, but only one coincided with the new position of the mitotic spindle, strongly supporting the hypothesis that a spindle independent signal also is used. Further experiments revealed that a cortical protein, required for proper neural stem cell divisions in mice and humans, is necessary for the asymmetric positioning of the cleavage furrow.

One of the marker proteins watched closely in the experiments was myosin. When a cell starts the division process, Cabernard said, the spindle is symmetrical but the myosin markers segregated toward the basal side -- and is localized in an asymmetric fashion -- which becomes smaller and transforms into a neuron upon cell division.

Although this research addresses a basic question in cell biology, the findings have important implications. Asymmetric cell division in fly or

human stem cells is important to generate a number of differentiating cells while retaining a stem cell as a back up copy.

Previous work by Cabernard and Doe showed that if drosophila neuroblasts divide in a symmetric manner, which doesn't normally happen, two neuroblasts are generated. Thus, the researchers said, it is crucial for a stem cell to know where to place a cleavage furrow to produce all the required neurons. Similar results have been observed in neural stem cells in mice.

Provided by University of Oregon