

## ER membrane puts up barriers in *C. elegans*

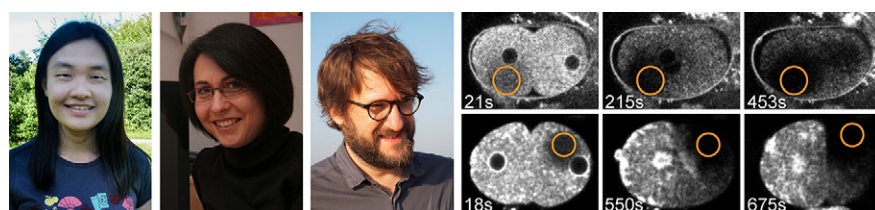
Compartmentalization of ER membrane might ensure that fate determinants end up in different cells of embryo.

A *C. elegans* embryo organizes its ER membrane into two distinct domains, Lee et al. reveal (1). The researchers suggest that these regional differences could promote the asymmetric division of the embryo.

Fate-determining factors such as PIE-1 and MEX-5 are unevenly distributed in the single-celled *C. elegans* embryo, an imbalance that remains until the cell divides into a larger anterior blastomere and a smaller posterior one (2). Yet the embryo's cytoplasm is continually mixing, raising the question of how these factors concentrate in different areas of the cell. One possibility is that the ER polarizes and takes the fate-determining molecules along for the ride. In yeast and mouse neural stem cells, researchers have found that the ER membrane is not homogeneous. Diffusion barriers created by sphingolipids and lamin produce unique membrane domains, which end up in different daughter cells after division (3, 4). But researchers haven't determined whether the ER in the single-celled *C. elegans* embryo shows this same asymmetry.

Lee et al. tracked the movements of molecules inside the ER with a technique called fluorescence loss in photobleaching (FLIP). Using a GFP-containing reporter molecule that remains within the ER lumen, the researchers repeatedly photobleached the anterior or posterior end of the embryo and followed the resulting dark patch. To find out whether the molecule could travel freely, the team compared its movement along the anterior-posterior axis to its movement in a direction that was perpendicular to that axis. The molecule moved at the same speed in both directions, suggesting that no diffusion barriers obstruct the ER lumen.

The scientists next tested whether such impediments are also absent from the ER membrane. When Lee et al. tracked the diffusion of two markers within the membrane, they found that the photobleaching shadow



(Left to right) Zuo Yen Lee, Monica Gotta, Yves Barral, and Manoël Prouteau (not pictured) observed the diffusion of marker molecules in the ER of *C. elegans* embryos undergoing their first division. When the researchers followed a molecule that stays within the ER lumen (top row), the dark patch produced by fluorescence loss in photobleaching (orange circle) spread across the embryo, suggesting diffusion barriers are absent. But when the scientists followed a molecule found in the ER membrane (bottom row), the dark patch remained on one side of the embryo, indicating that a barrier blocked the molecule's movement. The researchers think that this diffusion barrier might help polarize the *C. elegans* embryo to aid the asymmetric inheritance of fate determinants.

PHOTOS COURTESY OF ANDRZEJ SIWA-GONZALEZ (LEE), RUTH KROSCHEWSKI (BARRAL), AND YVES BARRAL (GOTTA).

**"This result opens the possibility that the ER plays a more important role in organizing the cell."**

tended to stay on the side of the embryo where it originally formed, implying that the membrane proteins encountered obstacles. The team then asked whether the reporter molecules moved as readily in the anterior-posterior direction as in a perpendicular direction. They discovered that the markers traveled at the same speed along both axes until shortly before the nuclear envelope breaks down at the start of mitosis, when exchange along the anterior-posterior axis slowed, suggesting that at this time something starts to interfere with diffusion between the two future daughter cells of the embryo.

The researchers ruled out two hypotheses for the sudden slowing. One potential explanation is that the ER breaks somewhere along the anterior-posterior axis. However, when the researchers scrutinized cells that were just about to dissolve their nuclear envelopes, the ER network was still intact. The second hypothesis involves the transformation that the ER undergoes during mitosis. ER tubules in mitotic cells become longer and thicker, clusters of tubules at the edge of the cell enlarge, and sheet-like structures form near the mitotic spindle. Lee et al. analyzed two mutants in which these alterations don't occur, and found that diffusion of the marker

molecules in the anterior-posterior direction slowed shortly before nuclear envelope breakdown. Thus, the mitotic reshaping of the ER doesn't account for the molecules' restricted movement.

The explanation that best fits the researchers' data is that a diffusion barrier appears just before nuclear envelope breakdown and divides the ER membrane into separate compartments. The team's measurements suggest that the section of ER that will fall in the larger, anterior daughter cell after division is bigger than the section of the ER that will end up in the smaller, posterior daughter cell. The polarity protein PAR-3 was required to position the boundary between the two compartments, but what creates the diffusion barrier remains unclear.

"The ER is much more structured than we thought before," says senior author Yves Barral. "This result opens the possibility that the ER plays a more important role in organizing the cell." The next step, he says, is to test whether polarization of the ER leads to polarization of fate-determining factors. To do that, researchers need to identify mutants in which ER polarization fails and then measure whether the distribution of the fate-determining factors is altered.

1. Lee, Z. Y., et al. 2016. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201601047>
2. Rose, L., and P. Gönczy. 2014. *WormBook*. Dec 30: 1–43.
3. Clay, L., et al. 2014. *eLife*. 3:e01883.
4. Moore, D.L., et al. 2015. *Science*. 349:1334–1338.