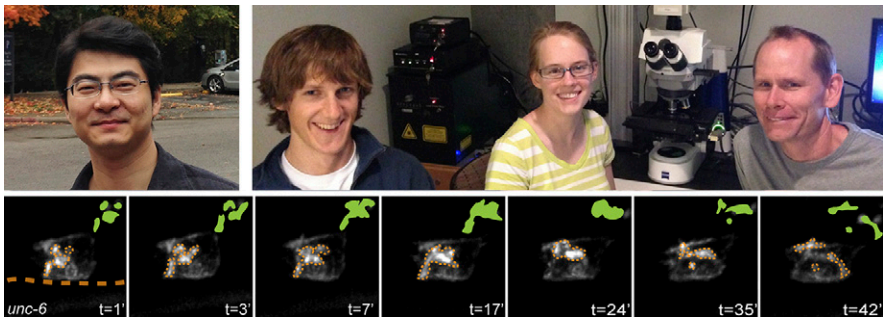
Text by Ben Short
bshort@rockefeller.edu

Netrin puts an end to the anchor cell's vacillations

The guidance cue orients cell invasion by stabilizing the oscillations of its receptor UNC-40.

Focal Point



PHOTOS COURTESY OF ZHENG WANG AND DAVID SHERWOOD

(Top, left to right) Zheng Wang, Kaleb Naegeli, Lara Linden, David Sherwood, and colleagues (not pictured) describe how netrin polarizes its receptor UNC-40 in order to direct the invasion of the *C. elegans* anchor cell. In the absence of netrin, UNC-40 is active and clusters at the cell surface. UNC-40 clusters undergo cycles of assembly and disassembly at random positions around the cell (see time-lapse series, bottom). Netrin stabilizes clusters at the basal surface of the cell to promote the formation of an invasive protrusion that penetrates the underlying basement membrane.

The extracellular ligand netrin guides a variety of cell movements, including axon outgrowth and cell invasion. Inside the target cell, the netrin receptor polarizes toward the source of netrin and recruits effector proteins that generate F-actin and drive membrane protrusion. How netrin polarizes its receptor has been unclear, but Wang et al. now show that, in the *C. elegans* anchor cell, the netrin receptor UNC-40 undergoes cycles of clustering and dispersal all over the cell cortex until netrin stabilizes clusters that are oriented in the correct direction to initiate cell invasion (1).

During *C. elegans* development, a specialized uterine cell called the anchor cell invades through its underlying basement membrane to contact the vulval epithelium on the other side (2). The netrin ligand UNC-6 guides this process by accumulating in the basement membrane so that UNC-40 polarizes at the basal surface of the anchor cell to drive the formation of an actin-rich invasive protrusion (3, 4). David Sherwood and colleagues at Duke University were studying this process when they noticed that removing either the netrin ligand or its receptor had different effects on F-actin assembly (1). "In the absence of ligand, we saw F-actin clusters in different domains of the anchor cell," Sherwood explains. "When we removed the receptor, we saw a reduction in F-actin at the cell–basement membrane interface, but it wasn't mispolarized."

The ectopic actin clusters formed in netrin-deficient worms colocalized with UNC-40 and its actin-generating effector proteins, suggesting that, in the absence of its ligand, the netrin receptor is active but polarized randomly around the cell. Sherwood and colleagues, led by graduate student Zheng Wang, were puzzled by their observations until they received inspiration from a completely different model of cell polarization. In the presence of low concentrations of mating pheromone, the yeast polarity protein Cdc42 undergoes cycles of clustering and dispersal at the cell cortex. High pheromone concentrations or steep gradients then stabilize Cdc42 clusters that are oriented toward the chemoattractant (5, 6).

"We realized that we needed to use time-lapse imaging to see what was going on with the mispolarized UNC-40 clusters in the ligand mutants," Sherwood remembers. "And, sure enough, we saw that the clusters formed and broke down, and formed and broke down again." This oscillatory behavior indicates that UNC-40 cluster assembly is promoted by a positive feedback mechanism and is linked to a negative feedback mechanism that induces receptor dispersal.

To investigate how netrin influences the receptor's oscillations, Wang et al. ectopically expressed the ligand so that it accumulated at the anchor cell's apical surface instead of in the underlying basement membrane. "The oscillations stopped, and UNC-40 stably polarized toward the source of netrin," Sherwood says. "That implies that netrin's interaction with the receptor somehow antagonizes the negative feedback loop that would otherwise break the UNC-40 cluster apart."

> **"When this oscillator is perturbed, the cell cannot robustly polarize."**

Moreover, when the source of netrin shifted its position over time, UNC-40 quickly reoriented to polarize the anchor cell in a different direction. This, explains Sherwood, may be one key advantage of the oscillatory system. "It's an exquisitely sensitive system. The positive feedback mechanism likely allows cells to rapidly polarize, and, wherever netrin is, it may draw the receptors there because they're no longer subject to negative feedback. If we remove netrin, the negative feedback mechanism might also allow polarity to be rapidly diminished and relocated."

To test the importance of UNC-40 oscillatory clustering, Wang et al. examined worms lacking *madd-2*, which encodes a conserved TRIM family member protein that was known to somehow potentiate UNC-40 signaling. In the absence of MADD-2, UNC-40 clustered much more slowly, and the clusters lasted for longer times before disassembling. "And the receptor no longer polarized consistently toward netrin," says Sherwood. "So when this oscillator is perturbed, the cell cannot robustly polarize."

Sherwood and colleagues now want to examine the mechanisms underlying UNC-40 clustering and dispersal and to investigate how the receptor's oscillations might differ in other cell types that polarize in response to netrin.

1. Wang, Z., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201405026.
2. Hagedorn, E.J., and D.R. Sherwood. 2011. *Curr. Opin. Cell Biol.* 23:589–596.
3. Ziel, J.W., et al. 2009. *Nat. Cell Biol.* 11:183–189.
4. Hagedorn, E.J., et al. 2013. *J. Cell Biol.* 201:903–913.
5. Dyer, J.M., et al. 2013. *Curr. Biol.* 23:32–41.
6. Bendezu, F.O., and S.G. Martin. 2013. *Curr. Biol.* 23:42–47.