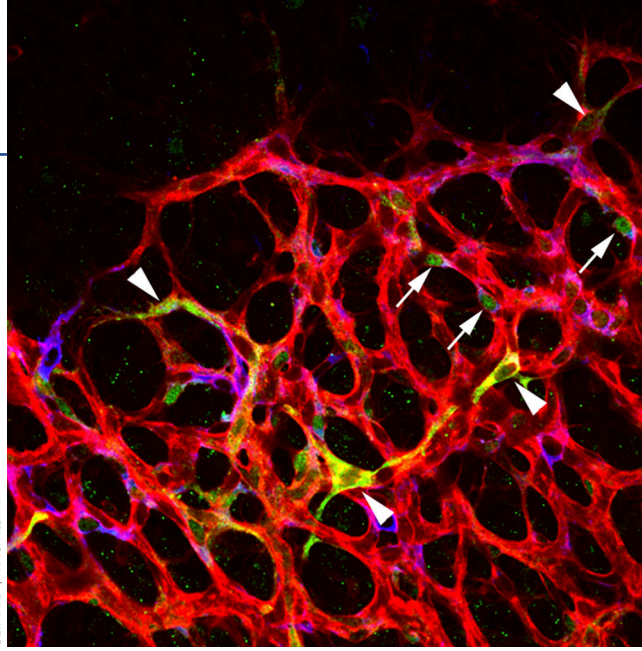
# Research Roundup

## How to make less webby webs

Forming a web of blood vessels requires both adventurous explorers and stable stay-at-homes. Notch, say two teams of researchers, helps endothelial cells to split up between these two fates. The explorers form new vessels even as the stay-at-homes maintain the integrity of existing vessels.

The explorers are called tip cells. Mats Hellström, Christer Betsholtz (Karolinska Institute, Stockholm, Sweden), Holger Gerhardt (Cancer Research UK, London) and colleagues found that inhibiting the Notch pathway in a mouse retina greatly increased the number of endothelial cells that had both tip cell markers and the tip cell habit of sprouting. The resulting webs of vessels were overly dense and disorganized; similarly Notch-inhibited and disorganized vessels were recently shown to be largely nonfunctional in mouse tumors.



GERHARDT/MACMILLAN

**Patches of Notch activity (green/yellow) ensure that not all blood vessel cells sprout.**

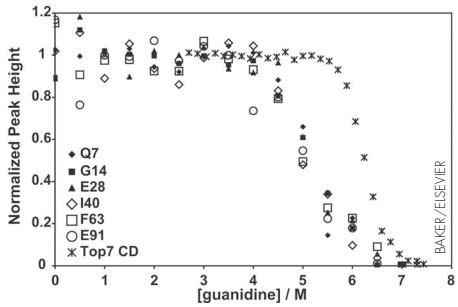
Arndt Siekmann and Nathan Lawson (University of Massachusetts Medical School, Worcester, MA) report similar results in zebrafish. Embryos lacking a Notch signaling component sent more than the normal number of endothelial cells into vessels sprouting from the dorsal aorta. The result was an excess of cells in the target vessel.

Notch is famous for its ability to help two neighboring cells distinguish themselves into two distinct fates—a process termed lateral inhibition. The simplest model in blood vessels would be that cells with the strongest Notch signal remain as the stay-at-home supporters of the originating vessel, whereas the neighbor with lower Notch signal becomes the wandering explorer.

Unfortunately for that hypothesis, says Gerhardt, the "patterning is not very neat." Cells deleted for Notch signaling were only somewhat more likely to have tip cell characteristics, and signs of Notch signaling were evident in both tip and non-tip cells. He suspects a "dynamic bilateral signaling event."

The details will have to await the isolation of downstream targets of Notch signaling, and investigations into possible posttranscriptional and posttranslational regulation of the pathway. One key fact is clear, however. "All endothelial cells respond to [the outgrowth inducer] VEGF," says Lawson. "Notch determines in what way they do so." JCB

Reference: Hellström, M., et al. 2007. *Nature.* doi:10.1038/nature05571.
Siekmann, A.F., and N.D. Lawson. 2007. *Nature.* doi:10.1038/nature05577.




**Unfolding Top7 loses secondary structure (x) after tertiary interactions, unlike the one-step unfolding of natural proteins.**

## Evolving folding

Naturally occurring small proteins fold in a single cooperative step. That is because evolution has selected for such behavior, say Alexander Watters, David Baker (University of Washington, Seattle, WA), and colleagues. They proved the rule by testing the exception: a computationally designed protein called Top7 with no evolutionary history and a far more complex folding strategy.

Previous tests relied on proteins that had been modified extensively but were still based on naturally occurring protein structures. These variants also folded rapidly, suggesting that cooperative folding might be intrinsic to any protein of a certain size and final stability.

The Washington group thought, however, that Top7 would make a more rigorous test substrate. They had computationally designed Top7 to be stable despite its completely novel fold and structure. Its folding, they now report, involves at least three distinct kinetic phases and one or more intermediate structures. A rapid collapse is followed by a slower process of internal rearrangement.

Top7, the authors suggest, may be too stable for its own good. It lacks the buried polar interactions that often destabilize nonnative conformations. Top7 also uses a lot of local interactions, explaining why fragments of Top7 are individually stable. These local structures may complicate or slow the folding of the protein as a whole, whereas natural proteins favor long-range interactions that lock the native structure into place.

The group's conclusions are based on one protein, which is why the paper "is in the theory section of the journal," says Baker. "It is definitely a speculation. One won't know until one sees further examples." It may be other groups that provide those examples, however, as Baker is now focusing on altering existing proteins to generate new functions. JCB

Reference: Watters, A.L., et al. 2007. *Cell.* 128:613–624.