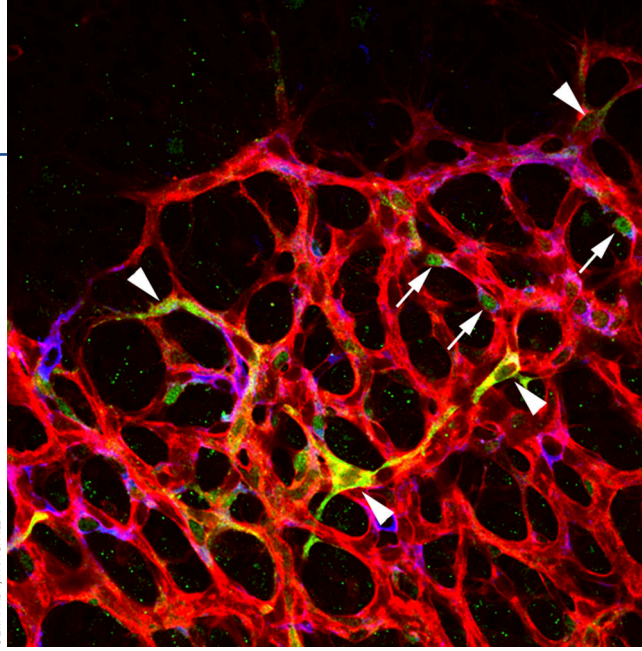
Research Roundup

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.

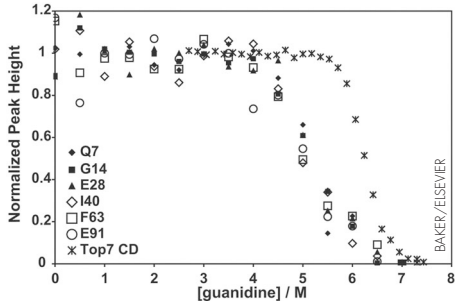
Arndt Siekmann and Nathan Lawson (University of Massachusetts Medical School, Worcester, MA) report similar results in zebrafish. Embryos lacking a Notch signaling component sent more than the normal number of endothelial cells into vessels sprouting from the dorsal aorta. The result was an excess of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

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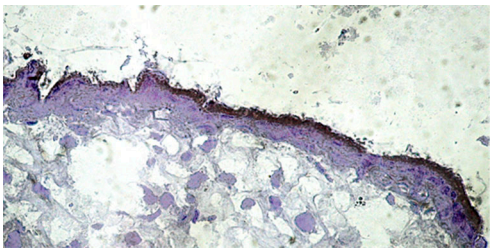

MANTOVANI/NAS

D6 (black) protects placenta from invading chemokines.

Chemokine blockade

A fetus must be sheltered from any inflammatory battle taking place in the mother. Now, Yeny Martinez de la Torre, Alberto Mantovani (University of Milan, Italy), and colleagues report that a decoy receptor in the placenta captures potentially dangerous pro-inflammatory chemokines from the mother. This scavenging suppresses inflammation and prevents fetal loss.

Decoy receptors such as the D6 receptor bind many inflammatory chemokines without activating intracellular signaling. Instead, the receptor and chemokine are internalized and the chemokine is destroyed.

The Italian group confirmed that D6 is expressed in the placenta, specifically on the apical side of syncytial trophoblasts. This is the side looking at the maternal blood and thus "a strategic location at the very interface between mother and fetus," says Mantovani.

To test the function of D6, the team injected pro-inflammatory LPS. The response was greater in mice lacking D6: several inflammatory chemokines built to higher levels in the circulation and placenta; more macrophages and T lymphocytes invaded the placenta; and there was more fetal loss.

Fetal loss may occur after a deadly positive feedback between inflammation and blood clotting in the placenta. In the mice lacking D6, this can be prevented with an infusion of anti-chemokine antibodies. A similar blocking approach may be possible in some humans who suffer from recurrent fetal loss, although it is not yet clear whether changes in D6 function are implicated in any of these individuals. JCB

Reference: de la Torre, Y.M., et al. 2007. *Proc. Natl. Acad. Sci. USA*. doi:10.1073/pnas.0607514104.

Pass the cycloheximide

Worms with reduced protein production capacity live longer, according to Popi Syntichaki, Kostoula Troulinaki, and Nektarios Tavernarakis (IMBB, Foundation for Research and Technology, Heraklion, Greece). The reduction appears to save energy—energy that can be put to work in fixing life-threatening damage.

Protein translation rates decrease with age. Tavernarakis wondered if increased translation might increase turnover of damaged proteins and thus slow aging. But, he says, "what we found was the opposite."

The group knocked down the levels of *IFE-2*, one of the five isoforms of the eIF4E translation initiation factor in worms. The development and eating patterns of the worms were normal, but they had an extended lifespan.

Other longevity-promoting pathways, such as the insulin and caloric restriction pathways, may modulate the eIF4E pathway (e.g., caloric restriction may reduce protein synthesis by down-regulating eIF4E). But *ife-2* knockout was additive with mutation of these other pathways, suggesting that a simple linear relationship is unlikely.

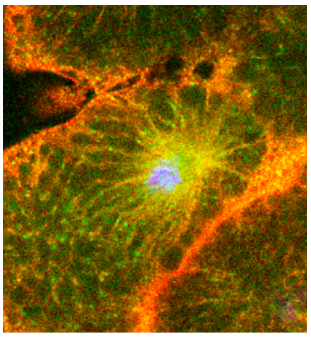
Animals with less eIF4E had higher ATP levels and were more able to resist oxidative damage. "If we reduce the rate of protein synthesis we allow the cell to invest some extra energy in maintenance and repair functions," says Tavernarakis. "By repairing damage the cells can now survive for longer."

This benefit is only relevant in the soma. In the germline, by contrast, translation is already near a minimum and a further reduction in eIF4E activity was lethal. "The germline invests in repair and maintenance but not so much in building," says Tavernarakis. "If we make our soma look a little more like our germline…then we might prolong the life of the soma." JCB

Reference: Syntichaki, P., et al. 2007. *Nature*. doi:10.1038/nature05603.

Motoring to a signaling check-up

The Smad2 signaling protein is shuttled back to the membrane to check up on its receptor, according to Julie Batut, Michael Howell and Caroline Hill (Cancer Research UK, London). The check-ups ensure that intracellular signaling accurately reflects the activation status of the membrane-bound receptor.


HILL/ELSEVIER

Smad2 (green) motors along microtubules (red).

Smad proteins are phosphorylated by and act downstream of TGF-β receptors. Once Smad2 has helped activate transcription, the group previously found, it is shuttled out of the nucleus.

They now discover that this discarded Smad2 is dragged back to the TGF-β receptor by the microtubule motor kinesin. Smad2 phosphorylation and nuclear accumulation was prevented by microtubule poisons and an antikinesin drug, even in the presence of a constitutively activated receptor. This result held true in frog and zebrafish embryos and mammalian cells. Unphosphorylated Smad2 coimmunoprecipitated with a kinesin light chain.

Long-range transport of Smad2 by kinesin has not yet been demonstrated. Hill also hopes to find a motor that transports active Smad2 from receptor to nucleus. Smad and STAT signaling pathways may be particularly suited to undertake such journeys, as in these two cases a single protein first interacts at the membrane and then acts in the nucleus. In these cases, "I think diffusion is never enough," says Hill. "With cells we tend to assume that things are swimming around in a soup, but I think everything is directed." JCB

Reference: Batut, J., et al. 2007. *Dev. Cell.* 12:261–274.