Research Roundup

How to make a helix

Plant stems twist and turn with the help of aberrant microtubule structures, according to results from Siripong Thitamadee, Kazuko Tuchihara, and Takashi Hashimoto (Nara Institute of Science and Technology, Ikoma, Japan).

The insights come from studies of Arabidopsis thaliana, which normally grows straight. Hashimoto’s team isolated lefty1 and lefty2—which have left-handed helical growth—as suppressors of an existing right-handed helical growth mutant. The new mutants have an identical change in either TUA6 or TUA4—two of the plant’s α-tubulin genes.

The change is near the interface with β-tubulin. The disturbed interface may produce the altered angle of microtubules seen in the mutants. Cortical microtubules are normally found running directly across the cells, but the mutant microtubules form in a skewed right-handed helix. This should alter the direction of growth, as cellulose-forming enzymes are thought to use microtubules as train tracks to lay down new cellulose fibers, and these circumferential fibers then constrict lateral growth and force all growth in a perpendicular direction.

Although the microtubule bending might determine the direction in which helical growth twists, the extent of helical growth is probably determined by the instability of the aberrant microtubules. Hashimoto has previously shown that treatment with antimicrotubule drugs affects the growth of inner cells disproportionately, leading to more rounded growth in these cells. Normal growth continues in outer cells, however. To prevent these more elongated cells from getting too far ahead of the inner cells, the outer cells skew their growth pattern to form a helix.

Thus, two aspects of microtubule behavior determine both the direction and extent of helical growth.


Cadherins jump into the pool

Pools of motor neurons use cadherin combinations to sort themselves into discrete units, say Stephen Price, Thomas Jessell (Columbia University, New York, NY), and colleagues.

Differential expression of cadherins has been seen in the brain, so Jessell looked to see which cadherins are made in chick spinal cord. He studied defined motor pools, each of which innervates a single limb muscle, and found 15 cadherins, 7 of which were expressed in different subsets of the motor pools. Combinations of these cadherins could easily account for the 40 pools needed to innervate the 40 muscles in one limb.

Two of the pools—EF and A—were well suited to further analysis. These two pools shared expression of three different cadherins, but only the A pool expressed the additional MN-cadherin. When Jessell eliminated this difference in cadherin profile by expressing either MN-cadherin in the EF pool or a dominant–negative version of MN-cadherin in the A pool, the cells from the two pools intermixed. The effect was pool and cadherin specific.

But pool identity probably does not start with cadherin expression, or even with a geographical code. “The pools are not organized in any clear inside-out or dorsal-ventral pattern,” says Jessell. “I think it’s related to the birth date of the motor neurons.”

In this scheme, earlier-born neurons could instruct the identity of later-born neurons as they arise. The later-born neurons then migrate outwards through the older neurons, and it is here that cadherin expression may be important in keeping the two populations distinct as they slip past each other. Further subdivision of pools occurs after the initial migration, although Jessell does not yet know if cadherins are important in this second process.

Even the need for pools is a bit of a mystery. The motor neurons link to sensory neurons, which function perfectly well despite being jumbled and intermixed in various ganglia outside the spinal cord. Clustering may help the motor neurons to fire a coordinated movement signal, as the neurons in a pool are electrically coupled. Jessell plans to test this idea by globally disrupting all cadherin interactions. Existing evidence suggests that this will scramble the motor neuron pools but allow the individual cells to maintain the transcription factor mix that defines their identity.

Lipid bilayers should be a no-flipping zone for integral membrane proteins, with the hydrophobicity blocking any cross-membrane excursions. But now Mikhail Bogdanov, Phillip Heacock, and William Dowhan (University of Texas, Houston, TX) report that fully synthesized lactose permease (LacY) can reverse orientation when the lipid composition of the membrane is changed.

Dowhan focused on phosphatidylethanolamine (PE), which is the only zwitterionic lipid in Escherichia coli (the other lipids all have anionic head groups). He knew that PE was needed for LacY synthesis, the existing LacY flipped back into PE synthesis was induced in the absence of new lipid (the other lipids all have anionic head groups). He knew that PE was needed for LacY activity in vitro, and found that the same was true in vivo. Mutant E. coli lacking PE made a version of LacY that allowed facilitated but not active transport of lactose. This aberrant LacY had half of its 12 transmembrane domains in a conformation opposite to that of normal LacY. But when PE synthesis was induced in the absence of new LacY synthesis, the existing LacY flipped back into its native conformation, and could now do active transport.

The key to this change may lie in the weakly hydrophobic seventh transmembrane domain, which may flip out of the membrane or form a hairpin loop. Dowhan hopes to detect the direction of propagation of the change—where the flipping begins and ends—by blocking the process with a single large covalent modifier. He will also test whether the flipping requires reentry into the translocon machinery.

For LacY, the dependence on PE may be a structural accident. But other proteins function only in certain parts of the cell because of differing lipid environments. And for Dowhan, the result emphasizes the importance of lipids. “Everyone focuses on the proteins, and ignores the lipids, viewing them as a simple solvent,” he says. “But membrane protein sequences are written for a particular lipid environment.”


Melanoma cells send out microvesicles loaded with Fas ligand (FasL) to kill their would-be assassins, according to Stefano Fais (Istituto Superiore di Sanità, Rome, Italy), Licia Rivoltini (Istituto Nazionale dei Tumori, Milan, Italy), and colleagues.

The FasL hijacks a normal transport pathway for melanin, the pigment that melanosomes load into microvesicles and send to neighboring keratinocytes. In melanoma cells, these microvesicles, or exosomes, are also loaded with FasL, which can trigger apoptosis in Fas-expressing immune cells that might otherwise counteract tumor growth.

This mechanism of counterattack may operate in other tumors, as microvesicles are released from a number of cell types. In the case of melanoma cells, the new work clears up a controversy. Microvesicles explain how melanoma cells can have an apoptotic effect (the initial observation) without expressing FasL on their cell surface (the subsequent, seemingly contradictory observation).

Although purified microvesicles can kill cultured Fas-expressing cells, the same cells are not killed by coculture with melanoma cells. This suggests that, in vivo, some other microenvironmental factors may either provoke a higher level of microvesicle production or optimize the effects of the killer exosomes. Fais also hopes to determine how the Fas-expressing melanoma cells manage to avoid killing themselves with their own microvesicles.


The transcription–export link

Transcription is a congested business, with many different proteins crowding in to exert their influence. Now Katja Sträßer, Ed Hurt (University of Heidelberg, Germany), and colleagues have found that even nuclear export proteins get into the act, via an interaction with transcription elongation factors. The findings suggest that transcription, splicing and nuclear export are linked processes, and that there are many levels of regulation between these processes that remain to be discovered.

Hurt found that yeast protein Sub2, which with Yra1 connects directly to mRNA exporters, binds a collection of proteins that make up the THO complex. This complex promotes transcription by acting on the elongation step. The proposed connections were confirmed by synthetic lethal interactions, and with human homologues of the THO proteins. Hurt and colleagues named the combined complex the transcription–export or TREX complex, and found that proteins in the complex could be tracked by chromatin immunoprecipitation as they moved along a transcribed gene with RNA polymerase.

Mutants lacking a THO component had an mRNA export defect, but Hurt does not think that transcripts get posted directly from a gene into a nuclear pore complex. Transcription occurs throughout the nucleoplasm, and TREX factor binding at these sites may simply allow nuclear pores to capture mRNA–protein complexes after they diffuse away from these sites.