Divided identities in the niche

To maintain a stem cell niche at a fixed size, the niche exerts control over both cell differentiation (how many cells functionally leave the niche) and cell division. G. Venugopala Reddy and Elliot Meyerowitz (Caltech, Pasadena, CA) now show that, in the growing shoots of plants, these two control points are separable in both time and space.

Their model was the shoot apical meristem (SAM). The SAM has a central zone (CZ) of stem cells plus various surrounding cells that make the transcription factor WUSCHEL. Although WUSCHEL creates a signal that promotes stem cell identity back in the CZ, it also induces these stem cells to produce CLAVATA3 (CLV3), an extracellular ligand that keeps WUSCHEL repressed centrally. This forces the inducing ring to keep its distance from the induced stem cells.

Reddy and Meyerowitz turned on an RNAi-inducing transcript that knocked down CLV3. As others have found, the end result was more WUSCHEL activity, and thus more promotion of stem cell identity and an expanded CZ and SAM. Unfortunately, says Reddy, any such terminal phenotype is the sum of changes in multiple interacting components of a network. The function of individual genes, he suggests, is best understood via a transient perturbation that allows changes in cell identities and cell division to be observed as the phenotype develops.

To see this kind of dynamics, the pair used GFP-labeled cell markers and transient induction of the CLV3 RNAi. Soon after this treatment the CZ expanded, via the dedifferentiation or respecification of surrounding cells. Only later was there an increase in the division rate of cells more distant from the stem cell area, but still within the SAM.

The separable effects point to a complicated system of signaling. WUSCHEL is no doubt part of the answer, but real-time analysis of a few more genes will probably be necessary to understand fully how plants keep a group of stem cells at a buffered and consistent size. JCB

Reference: Reddy, G.V., and E.M. Meyerowitz. 2005. Science. doi:10.1126/science.1116261.

ClpX eats randomly

ClpXP is an ATP-powered eating machine. Its ring of six ClpX ATPase modules feeds substrates to the ClpP protease. The ClpX subunits have been suggested to fire all at once or in a sequential, piston-like sequence. But now Andreas Martin, Tania Baker, and Robert Sauer (MIT, Cambridge, MA) find that the hexamer functions even if only one subunit can hydrolyze ATP. They suggest that the order of firing of the subunits may be not deterministic but probabilistic—all the better to manhandle half-digested protein substrates whose features protrude haphazardly.

Martin wanted to test the models by mixing and matching functional and non-functional ClpX subunits. But the six subunits are identical, so coexpression of wild-type and mutant subunits would yield only messy mixtures. “If you were going to make headway you needed to connect the subunits” into a single unit, says Sauer. Martin set to work, but “progress was not encouraging,” says Sauer. “After six months there were only insoluble proteins. I was encouraging him to think of other projects, but he refused to give up.” As a last attempt Martin deleted a nonessential part of ClpX. The deletion—which was done “because we could,” says Sauer—worked.

The resulting construct of six linked subunits could be reassorted at will. Amazingly, activity per functional subunit barely decreased as more mutants crept in. Hexamers with only two active subunits had almost a third of the activity and ATP efficiency of a fully active hexamer. With only one active subunit the hexamers fell down further in activity assays, but still showed impressive digestive powers.

If random mixtures of mutant and wild-type subunits can function so efficiently, Sauer figures that the fully wild-type hexamers probably operate by a random firing of their subunits. This would allow the hexamer to keep processing a protein no matter which ClpX subunit the substrate happened to bump up against. JCB

Reference: Martin, A., et al. 2005. Nature. doi:10.1038/nature04031.

Without CLV3 (bottom), stem cell niches overgrow and lineages are larger.
**From error to tetraploid**

Anaphase is not the point of no return, say Qinghua Shi and Randall King (Harvard Medical School, Boston, MA). If cells make errors in anaphase chromosome segregation, they mount a rescue operation. The final stages of cytokinesis shut down, producing tetraploid cells that might even be able to regenerate functional diploid cells.

Shi and King started out looking for different chromosome missegregation rates in various cell lines. They noticed that the rate of missegregation was 45–166-fold higher in spontaneously arising binucleated cells than in cells with normal divisions. In time-lapse experiments, these aberrant cells did not delay in mitosis but reversed their division process just before cytokinesis completion. The thin, remaining bridge between dividing cells opened back up, yielding a binucleate cell.

Extrapolating from experiments using probes for four chromosomes, it is likely that there is a segregation error during virtually all divisions that produce new binucleates. Chromosomes were not always obstructing the division furrow, so the pair think it is possible that a dedicated signaling mechanism may detect segregation errors and put the brakes on cytokinesis.

The virtues of opting out of the diploid world depend on what happens to tetraploids. David Pellman (Harvard Medical School) has recently shown that tetraploids lacking p53 promote tumorigenesis, but tetraploids with p53 may not behave so badly. There may even, suggests King, be a pathway from tetraploids back to functional, nonaneuploid diploids. “If that were true,” he says, “there would be a full pathway for the resolution of errors.”

References: Fujiwara, T., et al. 2005. Nature. doi:10.1038/nature04217. Shi, Q., and R.W. King. 2005. Nature. doi:10.1038/nature03958.

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**NO link to destruction**

Two domains of cell behavior—protein modification by nitric oxide (NO) and ubiquitin-dependent protein destruction by the N-end rule pathway—have been united by Rong-Gui Hu, Jun Sheng, Alexander Varshavsky, and colleagues (Caltech, Pasadena, CA). They find that NO oxidizes NH$_2$-terminal cysteines on certain proteins, thus marking them for future destruction. NO could therefore act as a very spatially and temporally delimited source of a destruction signal.

Cysteine was already known to be destabilizing when present as an NH$_2$-terminal residue, and there were hints that its oxidation might be involved. The Caltech group now find that oxidation is essential to make the cysteine a substrate for arginyl transferase. The arginylated protein is then destroyed by the rest of the N-end rule pathway.

One substrate of this pathway is shown to be the GTPase-activating protein RGS4. It is no longer arginylated and is more abundant in vivo when either arginyl transferase is deleted or NO levels are reduced pharmacologically. Two other RGS proteins are also substrates, and ~30 uncharacterized proteins are candidates.

NO’s short half-life, says Varshavsky, gives you the possibility of regulating proteins through on a subcellular level. NO-related drugs for regulating blood vessels and heart function may become more specific if they could be targeted to the N-end rule’s part of NO signaling.

Reference: Hu, R.-G., et al. 2005. Nature. doi:10.1038/nature04027.

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**Is it DNA or protein?**

Single-stranded DNA (ssDNA) and protein can mimic each other, according to Elena Bochkareva, Lilia Kaustov, Cheryl Arrowsmith, Alexey Bochkarev (University of Toronto, Canada), and colleagues. They find structural evidence that ssDNA and a helix from p53 both bind to replication protein A (RPA) in the same position and the same manner—with negative groups poking out and aromatic groups buried in a cleft.

The binding may allow p53 to titrate ssDNA during its response to DNA damage. Before damage, p53 is bound to RPA and thus prevented from acting as a transcriptional activator. When RPA first responds to the ssDNA exposed by DNA damage, it has additional domains that are better specialized for binding ssDNA, so p53 probably comes along for the ride. This exposes p53 to two signals about damage intensity: local kinases and ssDNA itself. The Canadian group shows that ssDNA can compete with p53 for binding to the same site on RPA. Released p53 should be free to activate transcription.

Yet more regulation comes from the phosphorylation of another subunit of RPA. This modified form of RPA is even better than ssDNA at displacing p53 from RPA, although the phosphorylation takes a while to accumulate. A combination of these regulators must somehow communicate the amount of DNA damage to p53 so it can help the cell to choose between apoptosis and the induction of DNA repair.

Reference: Bochkareva, E., et al. 2005. Proc. Natl. Acad. Sci. USA. 102:15412–15417.