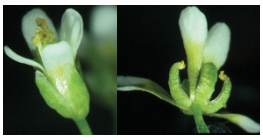
## Setting boundaries



Pattern development in plants and animals begins with the division of groups of cells into distinct domains. The cells within each group acquire different developmental fates as a result of precise gene expression patterns. MicroRNAS (miRNAs), which are involved in regulation of gene expression, often act within an RNA-induced silencing complex (RISC) to direct cleavage of their target messenger RNA (mRNA). In plants, most mRNA targets encode transcription factors that determine cell fate. In two recent reports the Bartel and Laufs groups show that the *Arabidopsis* miRNA164 (miR164) directs the expression of certain CUP-SHAPED COTYLEDON (CUC) transcription factors, and that this regulation is essential for normal embryonic, vegetative, and floral pattern formation. The CUC transcription factors specify the boundaries between different organs in the plant. Sequence complementarity suggested that *CUC1* and *CUC2* mRNAs could be targeted for degradation by miR164. Indeed, the expression levels of both genes were increased in *Arabidopsis* with impaired miRNA pathways. Both papers show that over-expression of miR164 recapitulates the phenotype of the *cuc1 cuc2* double mutant, which includes fusion of floral organs. The Bartel group shows that expression of miR164-resistant versions of *CUC1* mRNA causes alterations in *Arabidopsis* development, including misshapen leaves, and changes in flower petal and sepal numbers. Using similar over-expression experiments and by examining mutants with reduced miR164 levels, the Laufs laboratory shows that disruption of *CUC2* regulation also leads to abnormal organ growth. Together, the data suggest that miR164 directs degradation of *CUC1* and *CUC2* mRNAs, which limits the expansion of the domain boundaries and thereby controls the separation of different organs. The identification of miR164 as an important regulator of a subset of domain-defining genes identifies another posttranscriptional regulatory mechanism that controls pattern formation in plants. (*Curr. Biol.* **14**, 1035–1046; *Development* **131**, 4311–4322, 2004) *EJ*

## Channeling ammonia

Ammonia/ammonium transport is essential for nitrogen metabolism across all species. In bacteria, ammonia serves as a nitrogen source in amino acid synthesis and must be acquired from their extracellular environment through ammonium transporters (Amt). In humans, the related family of proteins transport ammonia and carbon dioxide across cell membranes and regulate pH while also preventing ammonium toxicity. Stroud and colleagues have solved the crystal structure of AmtB from *Escherichia coli*, an Amt protein that resides in the bacterial inner membrane, in the presence and absence of ammonium sulfate. Because the presence of ammonia/ammonium causes no dramatic conformational changes to the structure, the authors suggest that AmtB simply provides a passage for its substrate. AmtB forms a three-fold symmetric trimer with three channels present in the assemblage. Each AmtB monomer forms a channel comprised of 11 transmembrane helices that form a right hand helical bundle around the channel opening. The channel has an overall hourglass shape, narrowing to a constricted nonpolar region at the membrane midplane. This region is flanked on both sides by hydrophobic stretches. The authors propose that, because AmtB does not conduct water, it must strip waters of hydration and a proton from an ammonium ion in order for ammonia to pass through the channel. The nonpolar region is suitable for the passage of uncharged ammonia, but not ammonium. This creates a highly selective channel that is able to discriminate between the similarly sized ammonium and potassium ions. The structure of AmtB provides insight into the mechanism by which gases can cross membranes. (*Science* **305**, 1587–1594, 2004) *MM*

*Research highlights written by Rosemary Clyne, Hwa-ping (Ed) Feng, Evelyn Jabri and Michelle Montoya*

## All Rab'd up

The Rab5 GTPase is a regulator of endosome biogenesis and endocytosis. As with other GTPases, Rab5 function is activated by guanine nucleotide exchange factors (GEFs). The Rabaptin-5 effector interacts with the Rabex-5 GEF and this complex catalyzes Rab5 guanine nucleotide exchange and promotes endosome fusion. Rabex-5 is highly selective for the Rab5 GTPase subfamily. Lambright and colleagues have defined the region of Rabex-5 containing guanine nucleotide exchange activity and determined its crystal structure. The Rabex-5 catalytic core consists of a tandem arrangement of two structural domains: an N-terminal helical bundle and a conserved domain homologous to the yeast Vps9 protein. The authors show that the tandem Rabex-5 architecture confers specificity for the Rab5 subfamily through a small non-acidic residue preceding an invariant aromatic residue in the Rab GTPase. Although the Rabex-5 Vps9 fold is different from that of other GEFs, it resembles the structure of the Sec7 domain that interacts with Arf GTPases. The Vps9 and Sec7 domains may share a similar mechanism of GTPase activation. Knowing the structural determinants of Rabex-5 activity paves the way for studies to characterize the downstream cellular events. (*Cell* **118**, 607–617, 2004) *RC*

## Inserting proteins

**Mitochondria produce the bulk of ATP in the cell. The biogenesis of these organelles and their proper maintenance depends on proteins synthesized in the cytoplasm or inside the matrix being correctly transported to their target locations. In addition to the translocases of the outer and inner membranes that are central to the transport process, other proteins are also involved in the insertion of membrane proteins. For example, in yeast, several complexes important for respiratory growth, such as the cytochrome *c* oxidase, are in the inner membrane, and assembly of these complexes requires Oxa1p. Oxa1p is an integral inner membrane protein with five transmembrane helices; its N-terminal domain is in the intermembrane space and its C-terminal domain is in the matrix. To determine the regions of Oxa1p that may be important for function, Bonnefoy and colleagues introduced point mutations or large deletions into various parts of Oxa1p. Cell-based and biochemical assays revealed that point mutations near the beginning of the first transmembrane helix, deletion of the fourth and fifth transmembrane helices, or the combined deletion of two regions in the matrix severely affects the function of Oxa1p. In addition, the authors showed that overexpression of another inner membrane protein, Oms1p, can partially suppress the phenotypes of the various Oxa1p mutants. Oms1p contains motifs that are characteristic of methyltransferases, and mutations of a highly conserved aspartate residue and its neighbor abolish the suppressor activity of Oms1p. Based on these results, the authors suggest that the methyltransferase activity is important, but further studies will be necessary to understand the detailed molecular mechanism of Oms1p's function and its role in mitochondrial membrane protein assembly. (*J. Biol. Chem.* 8 September, 2004 doi:10.1074/jbc.M404861200) *HPF***