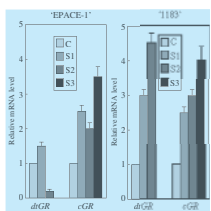


## GTPase ran in—and out

The DNA of eukaryotes, and especially of plants, is well known for containing a fifth base, 5-methylcytosine. The presence of  $m^5C$  does not affect the double helix itself but it is involved in epigenetic regulation of gene expression and in chromatin organization. These effects are mediated via methyl CpG-binding proteins, which in turn interact with other regulatory proteins. Our knowledge of these proteins in plants has been extended significantly by the work of **Yano *et al.* (Japan and Israel, pp. 1179–1187)**. They focused on a methyl CpG-binding protein in *Arabidopsis thaliana*, AtMBD5.

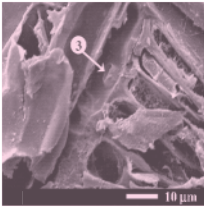
Using the yeast two-hybrid assay system and affinity pull-down techniques, they demonstrated an interaction within the *A. thaliana* proteome between AtMBD5 and a member of the Ran-GTPase family of regulatory proteins, AtRAN3. The cDNAs encoding these two proteins were spliced, respectively, to the sequences encoding the N-terminal and the C-terminal halves of yellow fluorescent protein (YFP) and the constructs were used to transform tobacco BY2 cells. Interaction of the two proteins *in vivo* was then detected by bimolecular fluorescence complementation (BiFC): fluorescence is only detected if the two halves of YFP are brought together, via protein–protein interactions, to recreate functional YFP. Use of BiFC plus differential fluorescent labelling facilitated the visualization of the two proteins at different cell cycle stages. In ‘interphase’, AtMBD5 was located in the nucleus, especially with heterochromatin (which is rich in  $m^5C$ ). AtRAN3 (presumed to be the active GTP-bound form) was also located in the nucleus and associated with AtMBD5 during this phase. At anaphase, however, the complex was not directly associated with chromatin but instead was located on the spindle. During telophase, AtMBD5 (and a proportion of the AtRAN3) re-associated with heterochromatin while the bulk of AtRAN3 was located at the growing cell plate. These results thus imply a role or roles for AtMBD5, in association with AtRAN3, in chromatin dynamics during cell division.



## Role for reductase in ROS resistance?

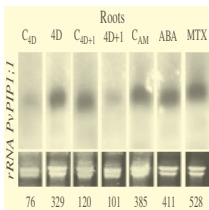
Water deficit is already the major abiotic factor that affects crop productivity worldwide and as the effects of global climate change become more intense, things can only get worse (misquoting a well-known song). Work on drought-response mechanisms, such as that carried out by **Contour-Ansel *et al.* (Université Paris 12, pp. 1279–1287)**, is therefore very important. The authors have focused on glutathione reductase (GR), an enzyme involved both in maintaining the pool of reduced glutathione (GSH) and in protection against reactive oxygen species (ROS). Drought-tolerant and non-tolerant

cultivars of cowpea (*Vigna unguiculata*) were exposed to drought by withholding water; leaves were desiccated by air drying. Changes in leaves of the levels of mRNAs encoding the cytosolic and the organellar GRs were assayed by RT–PCR. In the drought-tolerant cultivar, progressive drought of whole plants led to a decrease in the expression of the organellar GR and an increase in the expression of cytosolic GR. In the non-tolerant cultivar, expression of both genes increased. In air-dried leaves, the tolerant cultivar exhibited no overall change in expression of the organellar GR whereas the expression of cytosolic GR increased, as already seen in whole plants. In the non-tolerant cultivar, it was expression of the organellar form that increased. Application of ABA to leaves led eventually to an increase in expression of both genes in the tolerant cultivar but only of the organellar form in the non-tolerant cultivar. These results thus reveal a complex set of responses, but one feature particularly stands out: in the non-tolerant cultivar, expression of the organellar GR increases in response to withholding water from whole plants, to rapid desiccation of leaves and to application of ABA. It is therefore likely to be involved in protection against ROS. In the drought-tolerant cultivar, only ABA led to an increase in expression of organellar GR, suggesting that other mechanisms exist in this cultivar for protection against ROS.



### Forbs frustrate parasite penetration

Plant-on-plant parasitism often involves very specific host–parasite interactions. However, other parasites are generalists, invading a range of hosts with varying degrees of ‘success’. A wide host range prompts the question posed by **Cameron *et al.*, at Aberdeen (pp. 1289–1299)**: what prevents the parasite invading species outside its host range? The authors worked with *Rhinanthus minor*, a root parasite of grasses and legumes but not of non-leguminous perennial dicots (‘forbs’), studying its interaction with two grasses (*Cynosurus cristatus*, *Phleum bertolonii*), one legume (*Vicia cracca*) and two forbs (*Leucanthemum vulgare*, *Plantago lanceolata*). Seedlings of target plants and parasite were grown together and interactions were monitored. When *R. minor* formed a parasitic relationship with either of the grasses or with *V. cracca*, there was a significant increase in parasite biomass and in the number of flowers formed, compared with ‘free-living’ individuals. In the abortive relationships with forbs, parasite performance was greatly impaired, even compared with free-living individuals. Cytological and histological examination of the host–parasite interface showed that, in successful invasions, haustoria encompassed the host root; host tissues were damaged mechanically and possibly also enzymically. Formation of a vascularized penetration peg led to invasion of the host’s vascular system, sometimes with the formation of specific conduits (oscula) between host and parasite. None of this happened in the unsuccessful attempts to invade the two forb species: the parasite was stopped before it could encompass the root and invade the vascular system. There was also some evidence for a hypersensitive reaction, indicated by host cell death at the host–parasite interface. Examination by Fourier-transform infra-red microspectroscopy of the invasion site in host and non-host species indicated that the latter synthesized lignin. This was visible in light micrographs as a darkly staining layer. Thus it is containment of the parasite, possibly accompanied by a hypersensitive reaction, that prevents the parasitism of these two forbs by *R. minor*.



### Probing PIP patterns in *Phaseolus* plants

The importance of drought for crop productivity worldwide is again emphasized, this time in a paper by **Aroca *et al.* (La Jolla, CA and Pisa, pp. 1301–1310)**, reporting an investigation of the effects of drought on water relations and on aquaporins in *Phaseolus vulgaris*. Aquaporins are proteinaceous pores in membranes that permit the diffusion of water across those membranes; PIP1 and PIP2 are classes of aquaporins located in the plasma membrane. From the authors’ extensive study we concentrate here mainly on the effects of drought. After 4 d of withholding water, the water potential of the rooting medium had dropped from  $-0.23$  to  $-0.63$  MPa. Leaf water status was unaffected but the transpiration rate was reduced by nearly 80 %. Re-watering the rooting medium restored its water potential and led to a return to control transpiration rates in the leaves. Drought also affected root hydraulic conductance ( $L$ ), which was reduced to approx. 50 % of control values. Interestingly, in plants re-watered after drought,  $L$  remained at the value observed in droughted plants. Study of PIPs showed that drought led to increased expression of *PIP2.1* (assayed by Northern blotting of mRNA) and increased levels of PIP1 and PIP2 proteins in leaves; levels of PIP proteins fell again after re-watering. In roots, droughted plants showed up-regulation of *PIP1.1*, *PIP1.2* and *PIP2.1* (no other PIP genes were assayed) plus decreased levels of PIP2 protein (correlating with the decrease in  $L$ ). Protein and mRNA returned to control levels after re-watering. Interestingly, drought treatment did not lead to an increase in ABA concentration in leaves or roots. However, topical application of ABA to well-watered plants had, in leaves, a similar effect to water deficit, but in roots, opposite effects: increased  $L$ , elevated levels of PIP1 and maintenance of PIP2 proteins. Paraphrasing the authors’ words, this is a very good start for analysis of how *PIP* genes are regulated by drought.

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