asymmetric positioning of the maturation spindle in *Chaetopterus* sp. oocytes: I. Anchorage of the spindle to the cortex and migration of a displaced spindle. Cell Motil. Cytoskeleton *11*, 83–96.
2. Svoboda, A., Bahler, J., and Kohli, J. (1995). Microtubule-driven nuclear movements and linear elements as meiosis-specific characteristics of the fission yeasts *Schizosaccharomyces versatilis* and *Schizosaccharomyces pombe*. Chromosoma *104*, 203–214.
3. Ding, D.Q., Chikashige, Y., Haraguchi, T., and Hiraoka, Y. (1998). Oscillatory nuclear movement in fission yeast meiotic prophase is driven by astral microtubules, as revealed by continuous observation of chromosomes and microtubules in living cells. J. Cell Sci. *111*, 701–712.
4. Theurkauf, W.E., and Hawley, R.S. (1992). Meiotic spindle assembly in Drosophila females: behavior of nonexchange chromosomes and the effects of mutations in the nod kinesin-like protein. J. Cell Biol. *116*, 1167–1180.
5. Gueth-Hallonet, C., Antony, C., Aghion, J., Santa-Maria, A., Lajoie-Mazenc, I., Wright, M., and Maro, B. (1993). gamma-Tubulin is present in acentriolar MTOCs during early mouse development. J. Cell Sci. *105*, 157–166.
6. Albertson, D.G., and Thomson, J.N. (1993). Segregation of holocentric chromosomes at meiosis in the nematode, Caenorhabditis elegans. Chromosome Res. *1*, 15–26.
7. Verlhac, M.H., Lefebvre, C., Guillaud, P., Rassinier, P., and Maro, B. (2000). Asymmetric division in mouse oocytes: with or without Mos. Curr. Biol. *10*, 1303–1306.
8. Yang, H.Y., McNally, K., and McNally, F.J. (2003). MEI-1/katanin is required for translocation of the meiosis I spindle to the oocyte cortex in C elegans. Dev. Biol. *260*, 245–259.
9. Yang, H.Y., Mains, P.E., and McNally, F.J. (2005). Kinesin-1 mediates translocation of the meiotic spindle to the oocyte cortex through KCA-1, a novel cargo adapter. J. Cell Biol. *169*, 447–457.
10. Li, S., Armstrong, C.M., Bertin, N., Ge, H., Milstein, S., Boxem, M., Vidalain, P.O., Han, J.D., Chesneau, A., Hao, T., *et al.* (2004). A map of the interactome network of the metazoan *C. elegans*. Science *303*, 540–543.
11. Clandinin, T.R., and Mains, P.E. (1993). Genetic studies of *mei-1* gene activity during the transition from meiosis to mitosis in *Caenorhabditis elegans*. Genetics *134*, 199–210.
12. Clark-Maguire, S., and Mains, P.E. (1994). Localization of the mei-1 gene product of Caenorhaditis elegans, a meiotic-specific spindle component. J. Cell Biol. *126*, 199–209.
13. Kurz, T., Pintard, L., Willis, J.H., Hamill, D.R., Gonczy, P., Peter, M., and Bowerman, B. (2002). Cytoskeletal regulation by the Nedd8 ubiquitin-like protein modification pathway. Science *295*, 1294–1298.

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Guard Cells: Transcription Factors Regulate Stomatal Movements

Recent work shows that transcription factors are necessary for stomatal movements in plants. Different members of the plant-specific R2R3-MYB transcription factor family are required for mediating stomatal opening in response to light and stomatal closure in response to darkness.

Julie Gray

Stomatal pores on the surface of plants allow gaseous exchange across the cuticle of leaves and stems. The apertures of stomatal pores are controlled by a pair of guard cells which regulate the uptake of CO_2 from the atmosphere and the loss of water vapor from the plant. To act as effective regulators of gas exchange, guard cells process information from simultaneous, often conflicting, signals, such as light intensity, atmospheric CO_2 concentration and various plant hormones, including the drought response hormone abscisic acid (ABA) [1].

Environmental signals, such as reductions in light intensity or water availability, bring about reductions in stomatal gas exchange by promoting stomatal closure and inhibiting stomatal opening. These are two distinct turgor-driven processes which involve the co-ordinated activation and inhibition of ion channels present on the membranes of the guard cells. Recently there have been major advances in our understanding of the cellular events that underlie guard cell signaling. In addition to ion channels, many signaling components have been identified that are involved in the control of stomatal aperture, including second messengers, protein kinases, protein phosphatases and phospholipases [2–5].

Although, until recently, the role of transcription factors in regulating stomatal apertures had not been directly investigated, there was some evidence indicating that changes in gene expression patterns were involved in controlling stomatal movements. For example, the application of transcriptional inhibitors inhibits stomatal opening under some conditions [6], and RNA processing has been implicated in ABA-induced stomatal closure [7,8]. A guard cell expressed transcription factor has been reported [9], and the ectopic expression of ABI3 — a transcription factor involved in ABA-regulated seed dormancy — has effects on ABA signaling in guard cells [10]. Furthermore, it is clear that changes in gene expression are associated with stomatal movements. A decade ago, ABA-induced changes in guard cell gene expression were reported by Taylor *et al.* [11], and since then many other detailed reports have followed [12–14]. But it has not been established whether such changes are required during changes in stomatal aperture.

Two papers published very recently in *Current Biology* [15,16], demonstrate the involvement of two R2R3-MYB transcription factors in the regulation of stomatal apertures, implicating gene expression as an addition level of control in the proposed intracellular guard cell signaling network that controls stomatal aperture [1].

Plant genomes encode a comparatively large number of putative transcription factors. But even in the case of the most intensively studied of the model species, *Arabidopsis thaliana*, the function of only ~5% of these transcription factors has been determined by detailed phenotypic analysis of the corresponding mutants [17]. The MYB family is one of the largest groups of plant transcription factors, of which the major

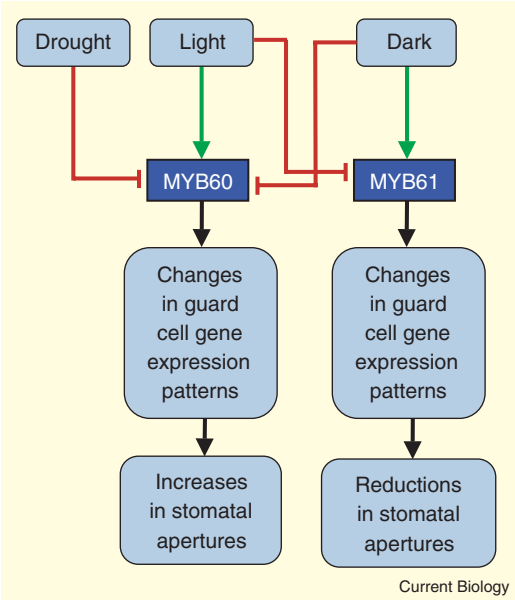

Figure 1. Model to illustrate how the expression of *MYB60* and *MYB61* potentially mediate light, dark and drought-induced alterations in stomatal apertures.

Green arrows and red bars, represent induction and inhibition of expression of guard cell transcription factor genes, respectively.

subgroup, the R2R3-MYBs, has 125 members and is specific to plants [18]. It has been proposed that this R2R3-MYB subgroup evolved to regulate plant-specific processes such as responses to plant hormones, cell identity, and responses to environmental stimuli [17,19].

As reported in the new papers [15,16], two R2R3-MYB type transcription factors, AtMYB60 and AtMYB61, are expressed in guard cells, and play opposite roles in the control of stomatal apertures [15,16]. The expression of *AtMYB60* is environmentally regulated in guard cells [15]. Stimuli which usually cause reductions in stomatal aperture, including darkness or ABA, strongly down-regulate the expression of this gene, whereas light treatment, which usually causes increases in stomatal apertures, increases *AtMYB60* gene expression. Expression of this gene does not, however, always correlate with stomatal movement, as increasing atmospheric CO_2 concentration, which typically brings about stomatal closure, had little effect on *AtMYB60* transcript levels.

To investigate the role of this gene product further, Cominelli *et al.* [15] studied the stomatal responses of an *atmyb60* mutant, and found that light-induced opening of stomata was impaired in these plants, but that stomatal opening induced by reduced atmospheric CO_2 concentration was unaffected. Furthermore, stomatal closure induced by ABA or dark was unaffected by the *atmyb60* mutation. These results suggest that in wild-type plants AtMYB60 is specifically involved in regulating light-induced opening of stomata, and that ABA may mediate reductions in stomatal apertures, at least in part, by inhibiting *AtMYB60* gene expression (Figure 1).

As AtMYB60 specifically regulates the stomatal opening response to light, then it might be expected that other transcription factors will regulate stomatal responses to other environmental stimuli, and this is exactly what is reported by Liang *et al* [16] for AtMYB61. In contrast to *AtMYB60*, *AtMYB61* is expressed only in guard cells in the dark, under conditions when stomatal pores are usually closed, and barely or not at all in the light (Figure 1). Again the *AtMYB61* gene expression pattern is very specific, as other stomatal closure stimuli, such as ABA or drought, do not induce *AtMYB61* expression.

Infra-red thermography showed that *atmyb61* mutant plants were approximately 0.5°C cooler than wild-type plants, suggesting that *atmyb61* stomata are more open than wild-type. Direct measurements of stomatal aperture confirmed that dark-induced stomatal closure is impaired in *atmyb61* plants [16]. These results indicate that the expression of *AtMYB61* in the dark is necessary for dark-induced reductions in stomatal aperture, but not for ABA-induced reductions in stomatal aperture (Figure 1).

Together, these results identify, for the first time, transcription factors that are important for the environmental regulation of stomatal apertures. As the roles of AtMYB60 and AtMYB61 appear to be so specific for the regulation of light-induced opening and dark-induced closure, respectively, this suggests that other transcription factors will be identified that are involved in regulating the stomatal opening and closing responses to other environmental variables such as atmospheric CO_2 concentration. These findings also provide additional evidence for the proposal that the control of stomatal movements is regulated by a dynamic and complex signaling network [1], with regulation at many levels including protein phosphorylation and gene expression, which in turn exert effects on guard cell turgor and increases or decreases in stomatal apertures.

The identification of transcription factors regulating stomatal movements is of additional importance because the control of plant water relations is an attractive target for the production of drought tolerant plants. It is therefore significant that *atmyb60* mutant plants, and plants overexpressing *AtMYB61*, both show reductions in stomatal apertures and reduced rates of stomatal gas exchange, and that *atmyb60* plants have enhanced drought tolerance.

References

1. Hetherington, A.M., and Woodward, F.I. (2003). The role of stomata in sensing and driving environmental change. Nature *424*, 901–908.
2. Assmann, S.M., and Wang, X.Q. (2001). From milliseconds to millions of years: guard cells and environmental responses. Curr. Opin. Plant Biol. *4*, 421–428.
3. Schroeder, J.I., Allen, G.J., Hugouvieux, V., Kwak, J.M., and Waner, D. (2001). Guard cell signal transduction. Annu. Rev. Plant Physiol. Plant Mol. Biol. *52*, 627–658.

4. Hetherington, A.M. (2001). Guard cell signaling. Cell *107*, 711–714.
5. Vavasseur, A., and Raghavendra, A.S. (2005). Guard cell metabolism and CO_2 sensing. New Phytol. *165*, 665–682.
6. Fukuda, M., Hasezawa, S., Nakajima, N., and Kondo, N. (2000). Changes in tubulin protein expression in guard cells of Vicia faba L. accompanied with dynamic organisation of microtubules during the diurnal cycle. Plant Cell Physiol. *41*, 600–607.
7. Hugouvieux, V., Kwak, J.M., and Schroeder, J.I. (2001). An mRNA cap binding protein, ABH1, modulates early abscisic acid signal transduction in Arabidopsis. Cell *106*, 477–487.
8. Li, J., Kinoshita, T., Pandey, S., Ng, C.K.Y., Gygi, S.P., Shimazaki, K., and Assmann, S.M. (2002). Modulation of an RNA-binding protein by abscisic-acid-activated protein kinase. Nature *418*, 793–797.
9. Yang, S.H., Berberich, T., Sano, H., and Kusano, T. (2001). Specific association of transcripts of tbzF and tbz17, tobacco genes encoding basic region leucine zipper-type transcriptional activators, with guard cells of senescing leaves and/or flowers. Plant Physiol. *127*, 23–32.
10. Parcy, F., and Giraudat, J. (1997). Interactions between the ABI1 and the ectopically expressed ABI3 genes in controlling abscisic acid responses in Arabidopsis vegetative tissues. Plant J. *11*, 693–702.
11. Taylor, J.E., Renwick, K.F., Webb, A.A., McAinsh, M.R., Furini, A., Bartels, D., Quatrano, R.S., Marcotte, W.R., Jr., and Hetherington, A.M. (1995). ABA-regulated promoter activity in stomatal guard cells. Plant J. *7*, 129–134.
12. Aghoram, K., Outlaw, W.H., Bates, G.W., Cairney, J., Pineda, A.O., Bacot, C.M., Epstein, L.M., and Levenson, C.W. (2000). Abg1: a novel gene up-regulated by abscisic acid in guard cells of Vicia faba L. J. Exp. Bot. *51*, 1479–1480.
13. Shen, L.M., Outlaw, W.H., and Epstein, L.M. (1995). Expression of an mRNA with sequence similarity to pea dehydrin (Psdhn-1) in guard cells of Vicia faba in response to exogenous ABA. Physiol. Plant. *95*, 99–105.
14. Leonhardt, N., Kwak, J.M., Robert, N., Waner, D., Leonhardt, G., and Schroeder, J.I. (2004). Microarray expression analyses of Arabidopsis guard cells and isolation of a recessive abscisic acid hypersensitive protein phosphatase 2C mutant. Plant Cell *16*, 596–615.
15. Cominelli, E., Galbiati, M., Vavasseur, A., Conti, L., Sala, T., Vuylsteke, M., Leonhardt, N., Dellaporta, S.L., and Tonelli, C. (2005). A guard-cell-specific MYB transcription factor regulates stomatal movements and plant drought tolerance. Curr. Biol. *15*, 1196-1200.
16. Liang, Y.-K., Dubos, C., Dodd, I.C., Holroyd, G.H., Hetherington, A.M., and Campbell, M.M. (2005). AtMYB61, an R2R3-MYB transcription factor controlling stomatal aperture in *Arabidopsis thaliana.* Curr. Biol. *15*, 1201-1206.
17. Riechmann, J.L., Heard, J., Martin, G., Reuber, L., Jiang, C., Keddie, J., Adam, L., Pineda, O., Ratcliffe, O.J., Samaha, R.R., et al. (2000). Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. Science *290*, 2105–2110.
18. Stracke, R., Werber, M., and Weisshaar, B. (2001). The *R2R3-MYB* gene family in *Arabidopsis thaliana.* Curr. Opin. Plant Biol. *4*, 447–456.
19. Chen, W., Provart, N.J., Glazebrook, J., Katagiri, F., Chang, H.S., Eulgem, T., Mauch, F., Luan, S., Zou, G., Whitham, S.A., *et al*. (2002). Expression profile matrix of Arabidopsis transcription factor genes suggests their putative functions in response to environmental stresses. Plant Cell *14*, 559–574.

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Division-Plane Positioning: Microtubules Strike Back

Two groups have recently developed physical techniques to manipulate the position of the nucleus in fission yeast. Their studies reveal how microtubules confine the nucleus to the cell center, and indicate how the position of the cleavage plane during cell division is coordinated with that of the nucleus.

Manuel Mendoza, Caren Norden and Yves Barral

During cell division the cleavage plane must be positioned correctly between the segregating chromosomes. Animal cells solve this problem by specifying the division plane during mitosis. The mitotic spindle dictates the site of furrowing, ensuring that the contractile ring cleaves the cell into two halves, each containing a complete set of chromosomes [1]. At first sight, the fission yeast *Schizosaccharomyces pombe* appears to use a different mechanism. Fission yeast cells are rod-shaped and divide in the middle. There is a tight correlation between the position of the interphase nucleus and that of the division site. The fission yeast nucleus is maintained at the cell middle during interphase, and mutants that exhibit abnormal nuclear positioning often divide off-center [2].

These and other observations have strongly suggested that, in fission yeast, the position of the interphase nucleus determines that of the cleavage plane. Thus, to understand how the position of the cleavage plane is set, we first need to determine how the nucleus is maintained in the center of the cell during interphase. The answer has long been thought to lie with microtubules. But in the absence of techniques for manipulating the position of the nucleus, the exact role of microtubules has been difficult to address.

Interphase microtubules in *S. pombe* are organized in four to six bundles which span the long axis of the cell. These bundles are anchored by their minus ends at multiple points on the nuclear membrane, so that the highly dynamic plus ends are oriented toward the cell tips [3,4]. Microtubules that contact the cell tips buckle under tension, generating forces capable of deforming the nuclear membrane. It has been suggested that the combined pushing forces of microtubules at opposite cell tips maintain the nucleus in the cell center [4]. Two recent papers [5,6] describe elegant physical approaches to displacing the nucleus of *S. pombe* cells. The results of these studies confirm the role of microtubule pushing forces in nuclear positioning, and bring further insight into the mechanism of cleavage plane specification in fission yeast.

Tolic-Nørrelykke *et al*. [5] used optical tweezers to trap a naturally occurring lipid granule in the fission yeast cytoplasm. By pushing the granule against the nucleus, they could displace it by almost 1 μm in an interphase cell (fission yeast cells are 7–12 μm in length). In most cases, the nucleus returned to the cell center after release from the optical trap, and cells placed the division site in the middle. Visualization of GFP-labeled microtubules showed that the