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Plant Development: YODA the Stomatal Switch

Dispatch

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The appearance of stomatal pores during plant evolution is believed to have been a crucial step in land colonisation. A recent screen for genes involved in stomatal development has identified for the first time a mutant plant with no stomata; the results implicate a MAP kinase cascade in stomatal development.

Stomata are small pores on the surfaces of leaves that control the exchange of gasses between the interior of the plant and the atmosphere [1]. They make a significant contribution to global water and carbon cycles, as an estimated 440 x 10^{15} g CO_2 and 32 x 10^{15} kg of water vapour pass through stomatal pores annually [2]. Stomatal pore opening and closure are regulated by plant hormones and environmental signals. The presence of these control systems allows the plant to conserve water during periods of drought and to maximise CO_2 uptake during the day. It has been convincingly argued that the acquisition of stomata is a key element in the evolution of advanced terrestrial plants [3], as they allow the plant to inhabit different environments while maintaining control over their water content. Stomatal development and patterning are also influenced by environmental factors, and the identity of some of the components involved in controlling these processes is beginning to emerge [4–6].

In this context, recent work from Bergmann, Lukowitz and Somerville [7] is of great interest, as these authors have shown that a newly characterised mitogen activated protein (MAP) kinase kinase kinase known as YODA is an important negative regulator of stomatal development. Of equal significance, however, is their observation that plants engineered to express a constitutively active YODA have no stomata and die before they reach reproductive maturity. Although further work is required before it is possible to reach definitive conclusions, this result is potentially very important because it suggests that the possession of stomata is vital to plant survival and as such lends support to Raven's suggestion [3] that the acquisition of these structures was a key element in the evolution of advanced terrestrial plants.

In the *Arabidopsis* epidermis, stomatal development starts with the initiation of a meristemoid mother cell. When this cell divides asymmetrically the smaller product is known as the meristemoid and this, after several asymmetric divisions, is eventually converted into a guard mother cell, which divides symmetrically to form the pair of guard cells that gate the stomatal pore [4–6]. At least three rules govern stomatal development and patterning. First, their development follows the series of asymmetric divisions described above; second, a patterning rule ensures that stomata are separated from each other by at least one non-stomatal epidermal cell; and third, the number of stomata that form, relative to the non-stomatal epidermal cells, is subject to control by environmental factors. The isolation of *Arabidopsis* mutants that carry lesions in stomatal development and patterning has provided significant insights into the signal transduction pathways that underlie these processes [4,5]. *Arabidopsis* mutant screening also formed the basis of the work which allowed Bergmann *et al.* [7] to uncover the role of a putative MAP kinase kinase kinase encoded by the *YODA* gene (*YDA*) in stomatal development.

Bergmann *et al.* [7] carried out a visual screen for aberrant stomatal patterning and out of the 19 mutants recovered, six were alleles of *YDA* [8]. This gene had recently been identified as a site of mutations that affect early embryo development. After fertilization, the *Arabidopsis* zygote undergoes an asymmetric division to form a small apical cell that goes on to form the embryo and a larger lower cell that develops into an extra embryonic structure known as the suspensor. In *yda* mutants, the suspensor does not form and the cells of the lower cell lineage are incorporated into the embryo.

On the basis of these observations Lukowitz *et al.* [8] concluded that the putative MAP kinase kinase kinase encoded by the *YDA* gene acts as a switch that promotes extra-embryonic fate. From what we know of MAP kinase cascades, it seems reasonable to conclude that a MAP kinase signalling cassette is involved in this example of the control of post asymmetric division cell fate. The six *yda* alleles identified by Bergmann *et al.* [7] also cause defects in post-embryonic development; additionally, they result in plants developing with extreme overproduction and clustering of stomata in the epidermis of the cotyledons and hypocotyls. Most *yda* seedlings fail to survive on transfer to soil, but those that do were found to be dwarfed and characterized by compact leaves filled with clusters of stomata.

When the *yda* phenotype was investigated in detail, it was found that, compared to wild type, more *yda* epidermal cells had entered the stomatal fate pathway. This indicates that just as in embryos, *YDA* plays a role in cell fate choice in the leaf epidermis. To investigate the role of *YDA* further, Bergmann *et al.* [7] made transgenic plants expressing a gene (Δ*N-YDA*) encoding a form of the YDA MAP kinase kinase kinase designed to be constitutively active. Seedlings homozygous for Δ*N-YDA* totally lacked stomata, supporting the idea that YDA acts as a switch that negatively regulates stomatal fate. The authors also used genetic means to 'place' YDA in the stomatal development pathway relative to other gene products that affect stomatal development. The *TOO MANY MOUTH* (*TMM*) and *STOMATAL DENSITY AND DISTRIBUTION* (*SDD1*) loci encode a leucine-rich repeat receptor-like protein and a putative

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subtilisin-like serine protease, respectively [9,10]. Mutations in these genes result in plants that exhibit an increase in stomatal number, and current models to account for these phenotypes are based on the idea that the SDD1 protein generates a peptide signal that is recognized by TMM receptor [5]. As indicated in Figure 1, genetic analysis places YDA downstream of both these gene products.

Finally, Bergmann *et al.* [7] used whole-genome transcript analysis to identify further genes involved in the control of stomatal patterning and development. They reasoned that transcripts up-regulated in *yda* plants (mostly stomata) and down-regulated in Δ*N-YDA* plants (no stomata), compared to wild-type, would be good candidate regulators of stomatal patterning and development. Indeed, expression of three genes known to be involved in stomatal development, *TMM*, *SDD1* and *HIC* (involved in the control of stomatal development by atmospheric CO_2 concentration [11]), followed this expression pattern [7]. Analysis of genes that clustered with these three known stomatal genes revealed 109 that are strong candidates for involvement in the control of stomatal patterning and development. This was confirmed by selecting one of these genes known as *FAMA*, which encodes a putative transcription factor; when plants homozygous for a *T*-DNA insertion in *FAMA* were analysed it was found that they too lack stomata [7].

The impact of the work from Bergmann *et al.* [7] is likely to be profound. They have identified over 100 candidate genes involved in guard cell development and patterning which are set to be a rich source for uncovering the intricacies of these developmental pathways for a considerable time. In addition, they have provided a tantalizing glimpse into the possible significance of the acquisition of stomata for the evolution of advanced terrestrial plants [9]. Of course, because YDA is implicated in multiple developmental responses, it is not possible to conclude that Δ*N-YDA* plants fail to reach reproductive maturity because of their lack of stomata. But the fact that *fama* plants are small and sterile certainly adds weight to this possibility.

The issue should be resolved in the near future as Bergmann *et al.* [7] are currently constructing plants in which Δ*N-YDA* is expressed only in the epidermis (D. Bergmann, personal communication). Phenotypic analysis of these plants might result in the production of key data supporting Raven's suggestion [3] that the acquisition of stomata is key to the success of the advanced terrestrial plants. Even though a tobacco MAP kinase kinase kinase had previously been implicated in the control of guard cell development [12], the identification of YDA MAP kinase kinase kinase and the fate switch it controls is a major advance. Indeed, it is an advance that prompts many new questions. We need to know whether YDA functions in the same (as in Figure 1) or a separate pathway to TMM and SDD. If they are in the same pathway how does TMM feed into the MAP kinase signalling cassette? Might FAMA be a phosphorylation target for the putative MAP kinase signalling cassette? And how do components involved in the environmental control of stomatal development, such as HIC, interact with the YDA stomatal development pathway? Finally, we urgently need to identify the other components, such as the receptor-like kinase that one assumes interacts with TMM to inactivate YDA and activate stomatal development, and the MAP kinase kinase and MAP kinase scaffold proteins that will presumably be responsible for controlling the specificity of YDA interactions in the cells of the zygote and stomatal precursors [13].

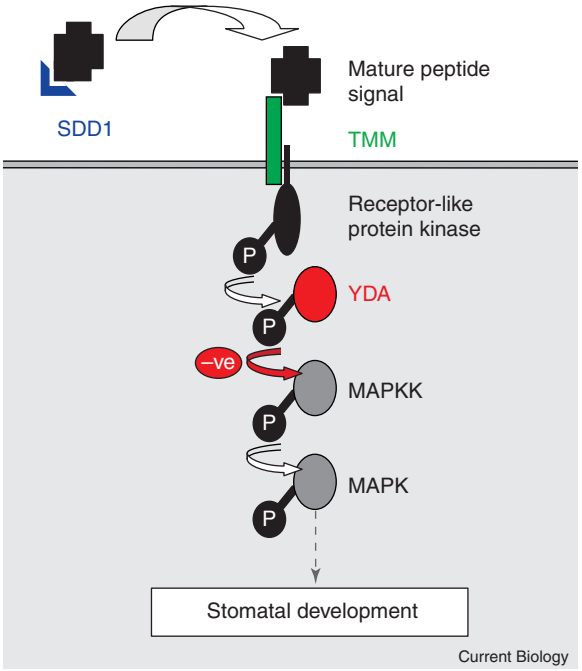

Figure 1. Hypothetical scheme linking components known to be involved in the control of stomatal development.

Genetic analysis places the MAPKKK YDA downstream of TMM, a leucine-rich repeat receptor-like protein, and SDD1, a subtilisin-like protease. Coloured components represent gene products known to affect stomatal development. Black components are predicted to be present on the basis of knowledge gained from other better characterised signalling pathways. Although this model for signalling during stomatal development assumes that all components shown act in the same pathway, it is quite possible, on the basis of our current knowledge, that some components act in separate signalling pathways.

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. Ciais, P., Denning, A.S., Tans, P.P., Berry, J.A., Randall, D.A. Collatz, C.J., Sellers, P.J., White, J.W.C., Trolier, M., Meijer, H.A.J., *et al.* (1997). A three-dimensional synthesis study of dO18 in atmospheric CO2. I. Surface fluxes. J. Geophy. Res. — Atmosphere *102*, 5857-5872.
3. Raven, J. (2002). Selection pressures on stomatal evolution. New Phytol. *153*, 371-386.
4. Bergmann D.C. (2004). Integrating signals in stomatal development. Curr. Opin. Plant Biol. *7*, 26-32.
5. Nadeau, J.A. and Sack, F.D. (2003). Stomatal development: cross talk puts mouths in place. Trends Plant Sci. *8*, 294-299.
6. Bird, S.M. and Gray, J.E. (2003). Signals from the cuticle affect epidermal cell differentiation. New Phytol. *157*, 9-23.
7. Bergmann, D.C., Lukowitz, W. and Somerville, C.R. (2004). Stomatal development and pattern controlled by a MAPKK kinase. Science *304*, 1494-1497.
8. Lukowitz W., Roeder, A., Parmenter, D. and Somerville C. (2004). A MAPKK kinase gene regulates extra-embryonic cell fate in *Arabidopsis*. Cell *116*, 109-119.

9. Nadeau, J.A. and Sack, F.D. (2000). Control of stomatal distribution on the Arabidopsis leaf surface. Science *296*, 1697-1700.
10. Berger, D. and Altmann, T. (2000). A subtilisin-like serine protease involved in the regulation of stomatal density and distribution in Arabidopsis thaliana. Genes Dev. *14*, 1119-1131.
11. Gray, J.E., Holroyd, G.H., van der Lee, F., Bahrami, A.R., Sijmons, P.C., Woodward, F.I., Schuch, W., and Hetherington A.M. (2000). The HIC signalling pathway links CO_2 perception to stomatal development. Nature *408*, 713-716.
12. Nishihama, R., Ishikawa, M., Araki, S., Soyano, T., Asada, T. and Machida, Y. (2001). The NPK1 mitogen-activated protein kinase kinase kinase is a regulator of cell-plate formation in plant cytokinesis. Genes Dev. *15*, 352-363.
13. Ptashne M. and Gann, A. (2003). Imposing specificity on kinases. Science *299*, 1025-1027.