ELSEVIER

Stomatal development: from neighborly to global communication

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Stomata are epidermal structures that are responsible for modulating the exchange of gases between the plant and the environment. Stomata are formed and patterned by asymmetric cell divisions. The number and orientation of these asymmetric divisions is informed by plant intrinsic signals acting locally (among epidermal cells) or at a distance (from mature to young leaves) and by plant extrinsic factors such as the quantity of light, water and CO_2 in the atmosphere. Recent studies have implicated a set of conserved cell surface receptors and intracellular signaling molecules in the perception and response to developmental cues. Complementary studies have probed the nature of environmental signals and how these signals are transduced from the site of perception to the cells in the stomatal lineage.

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Current Opinion in Plant Biology 2006, **9**:478–483

This review comes from a themed issue on
Cell signalling and gene recognition
Edited by Joseph Kieber and Takashi Araki

Available online 4th August 2006

1369-5266/$ – see front matter

DOI 10.1016/j.pbi.2006.07.001

Introduction

Stomatal development is a paradigm for studying the integration of genetic and environmental inputs in developmental decisions. Stomata are structures in the epidermis of aerial organs that function as conduits for the exchange of carbon dioxide (CO_2) and water vapor between the plant and the atmosphere. A stoma consists of a pore, flanked by two epidermal cells, that lies above an airspace in the mesophyll (Figure 1a). Stomata are separated from one another by intervening epidermal cells, a pattern thought to be a functional requirement for efficient gas exchange [1]. In *Arabidopsis*, this pattern is referred to as the one-cell spacing rule [1]. Stomatal density (stomata mm^{-2}) and stomatal index (number of stomata per total number of epidermal cells) vary with growth conditions, whereas the spacing pattern does not. This points to possible differences in the source of regulatory information for each parameter. For example, stomatal density is highly regulated by the environment, but spacing might be under the sole jurisdiction of developmental genes.

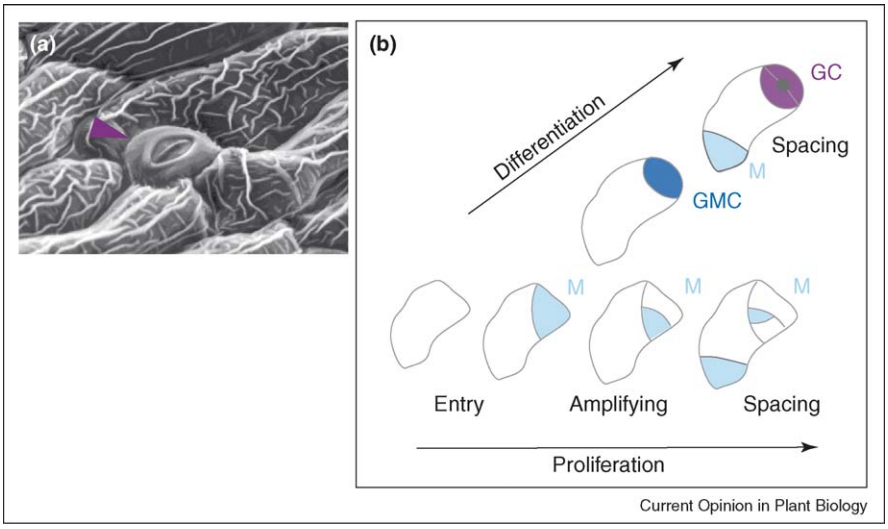
Stomatal development is characterized by an orchestrated series of asymmetric cell divisions followed by a single symmetric division (Figure 1b). 'Entry divisions' initiate the lineage by creating a small cell called a meristemoid and a larger sister cell. The sister cell may become an epidermal (pavement) cell or undergo an asymmetric division later. 'Amplifying divisions' occur in meristemoids. These divisions not only regenerate the meristemoid but also increase the number of larger daughter cells, and thus the total number of epidermal cells. 'Spacing divisions' resemble entry divisions in their outcome but have additional constraints because they occur in cells next to a stoma or precursor, and because their asymmetric division must be oriented so that the larger daughter cell intercalates between the stoma and the new meristemoid. The differentiation of stomatal guard cells requires that the meristemoid transits through its amplifying divisions and converts into a new cell type, the guard mother cell (GMC). The GMC divides only once, and its symmetric division creates the two guard cells of the stoma.

Three transcription factors have been identified that control the GMC to guard cell transition [2•,3•]. No genes that are required exclusively for entry divisions have yet been identified, but a set of genes that encode cell surface receptors is critical for oriented divisions and plays a role in modulating all of the asymmetric divisions in the stomatal lineage.

Receptors mediate cell–cell communication in epidermal pattern

ERECTA (*ER*) encodes a receptor-like protein that contains leucine-rich repeat (LRR) extracellular domains, a single transmembrane domain and a cytoplasmic kinase (a receptor-like kinase [RLK]). This protein has far-reaching effects on plant growth, developmental pattern and responses to biotic and abiotic stresses [4,5]. Many of the processes that are affected by loss of ER function are united in their requirement for cell proliferation. The expression of an ER protein without the kinase domain (ER-Δkinase) results in plant growth phenotypes that are more severe than those found in *er* null plants, suggesting that the truncated ER protein acts as a dominant negative, probably by forming unproductive heterodimers with other LRR-RLKs [6]. Good candidates to be partners in these heterodimers are ER's closest homologues *ERECTA-LIKE1* (*ERL1*) and *ERL2*. Both *ERL1* and

Figure 1



Stomatal structure and developmental pathway. **(a)** Scanning electron micrograph of closed *Arabidopsis* stoma (arrowhead) surrounded by sepal epidermal cells. **(b)** Diagram of divisions in the stomatal pathway. Young epidermal cells make entry divisions to create meristemoids (M). The meristemoid might continue dividing (amplifying divisions) or undergo differentiation to form a guard mother cell (GMC) and, via a symmetric division, a pair of guard cells (GCs). Spacing divisions can occur next to GCs, GMCs, or older Ms as indicated.

ERL2 can functionally substitute for *ER* in overall plant growth when expressed under the control of *ER* regulatory sequences [4]. *erl1* and *erl2* single mutants and *erl1*;*erl2* double mutants are indistinguishable from wild-type at the whole-plant level, but *erl1*;*erl2*;*er* triple mutants are dwarfed and sterile [4,7••].

A closer examination of the *er*;*erl1*;*erl2* mutants revealed a striking stomatal overproliferation and spacing phenotype in all organs that normally make stomata [7••]. The phenotypes of the *er*;*erl1*;*erl2* mutant suggest that the three LRR-RLKs are redundantly required for stomatal development. However, careful studies of the phenotypes of all single and double combinations of mutated *ER* family genes revealed subtle differences in each gene's function during asymmetric stomatal lineage divisions. Only the triple mutant exhibits defects in patterning, suggesting that activity of any of the ER family members is sufficient to orient the spacing divisions [7••].

The excitement surrounding the *ER* findings derives not only from the dramatic effect of these genes on stomatal development, but also because previous studies with the *TOO MANY MOUTHS* (*TMM*) gene hinted at the involvement of an LRR-RLK in stomatal development. TMM encodes an LRR receptor-like protein (RLP) that lacks an intracellular kinase domain (LRR-RLP), suggesting that *TMM* participates in signal transduction through interactions with an LRR-RLK partner [8].

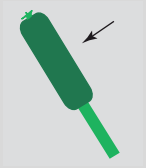
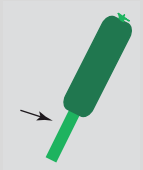
In contrast to *er* phenotypes, the phenotypes of *tmm* mutants are exclusively related to stomatal development. The primary mechanism for the suite of *tmm1* phenotypes appears to be a failure of neighbor cells to respond to positional cues: *tmm* mutants fail to orient spacing divisions and fail to inhibit entry divisions in cells adjacent to multiple stomata or stomatal precursors. *tmm* mutant cells also undergo fewer amplification divisions and produce a higher percentage of stomata relative to pavement cells [9].

It is clear from the phenotypes of mutations in *TMM* and *ER*-family members that all four of the proteins encoded by these genes play roles in stomatal development. It is not clear whether these proteins are part of a single signaling complex or whether some can signal independently. The phenotypes of loss-of-function mutations in *tmm* and the *er*-family are similar but not identical. ER-family proteins primarily modulate entry divisions and amplifying divisions whereas TMM's preeminent role is in spacing divisions.

Combinations of *tmm1* mutants with single and multiple mutants in the *ER*-family result in complex stomatal development phenotypes. Many of the published genetic interactions between *tmm* and the *er*-family mutants are consistent with the affected proteins being part of one physical complex, but the phenotypes in several organs can also be explained by TMM acting independently of the ER family ([7••,8]; Table 1). One of the issues confounding the interpretation of the function of these genes is that different methods and different stages of tissue development were examined by the groups studying mutants in which *TMM* and the *ER*-family were affected.

Table 1

Summary of phenotypes in stomatal signaling mutants

	tmm	*er*	*erl1*	*erl2*	*er erl1 erl2*	*yoda*	Other
Cotyledon 	Increased SI, clusters	Decreased SI	Wildtype	Wildtype	Greatly increased SI, large clusters	Greatly increased SI, large clusters	
Stem 	No stomata	Wildtype	Slight increase in SI	Slight increase in SI	Small clusters	Small clusters	*tmm* epistatic to single *er* family mutants only
Silique 	Decreased SD, clusters	Some arrested	Slight increase in SI	Wildtype	Greatly increased SI, large clusters	ND	*er tmm* double mutants show no differentiation of stomata, neomorphism
Pedicle 	Gradient	Decreased SI	Slight increase in SI	Wildtype	Greatly increased SI	ND	

TMM, YODA and the ER family have different roles in different tissues. Examples of some of these roles are summarized in the table. Each row represents a tissue, each column a mutant phenotype. ND, no data; SD, stomatal density; SI, stomatal index.

If ER/ERL1/ERL2 and TMM do function together, what is the physical nature of their interactions? Animal receptor tyrosine kinases dimerize, and ligand binding induces phosphorylation of the receptors and the activation of downstream signaling pathways. Genetic and protein interaction studies suggest that plant LRR-RLKs probably share some of these activities [10]. TMM and the ER-family proteins contain paired cysteine residues in their extracellular domains that might be responsible for dimerization [10]. Mechanistic models that are consistent with the genetic interaction data and the coincident expression patterns of *TMM*, *ERL1* and *ERL2* in stomatal lineage cells would suggest that TMM and ER-family members interact with each other in both competitive and cooperative modes [7••]. Many interaction models can be tested by careful genetic-dosage studies and by looking at *in planta* physical interactions among the proteins. Discovering physical connections among these four proteins will have a significant impact not only in the field of stomatal development but in the field of plant receptor signaling. Previous experiments with the LRR-RLK–LRR-RLP pair encoded by the CLAVATA1 and CLAVATA2 proteins suggested that heterodimerization activated or stabilized the complex [10]. If TMM inhibits members of the ER-family by forming heterodimers, this will demonstrate a novel mode of plant receptor interaction, and also provides a mechanism by which specificity can be granted to broadly used LRR-RLKs.

Regardless of whether the ER family and TMM form a single signaling complex or several, loss of these proteins results in the disruption of multiple downstream cellular behaviors, including transcription, cytoskeletal organization and targeted secretion. Intracellular signaling is required to transduce signals from the cell periphery to these numerous targets. A mitogen-activated protein (MAP) kinase signaling pathway was implicated in stomatal development by the discovery of mutations in the MAP kinase kinase kinase (MAPKKK) gene *YODA* that profoundly alter stomatal density and spacing [3•]. The phenotype of the *yoda* mutant is similar to that of the *er*;*erl1*;*erl2* triple mutant; it includes a severe reduction in overall plant height and internode length and excess production of guard cells [3•,7••]. Studies of developing cotyledons suggest that the asymmetric divisions that are compromised in *tmm* and *er*;*erl1*;*erl2* are also affected in *yoda*. Cells in the epidermis of *yoda* cotyledons exhibit excessive entry divisions, fail to orient spacing divisions and fail to prevent the division of neighbor cells that contact two cells of the stomatal lineage.

Constitutive activation of *YODA* eliminates stomata [3•]. This effect is dosage dependant and heterozygous *CA-YODA*/+ plants do not have dramatically affected stomata. However, *CA-YODA* can dominantly suppress the stomatal proliferation and patterning defects of *tmm1* [3•]. Suppression of *tmm-1* by *CA-YODA* is consistent with *YODA*'s acting downstream in a common signaling pathway, but does not rule out the possibility that *YODA* participates in an independent and parallel signaling pathway in young epidermal cells. A physical or biochemical connection between the YODA MAPKK kinase and the stomatal regulators at the membrane will be needed to rule out this second possibility. Obtaining definitive *in vivo* interaction data might be challenging. Even in the intensively studied plant defense response LRR-RLK/MAPK pathways, it is not clear whether the LRR-RLK directly phosphorylates and activates the MAPKKK or whether this activation requires an intermediary protein or protein complex [10].

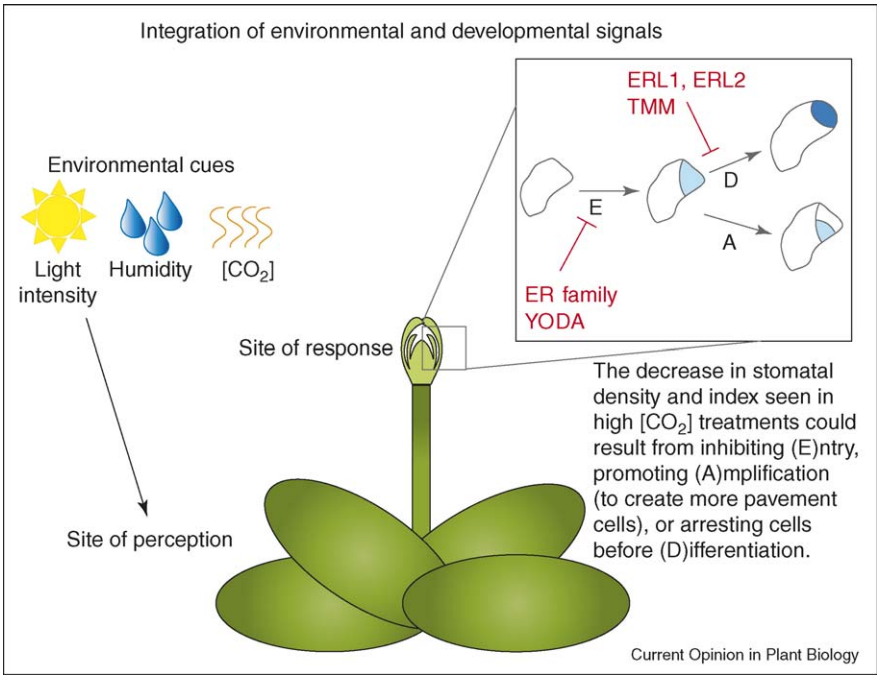
Environmental influences: direct and mediated through signals from old to new

The experimental focus on the 'developmental' genes that act within the epidermis to control cell identity and division behavior will identify many of the signaling pathways and cell autonomous factors that are required for stomatal development and pattern. However, signals from underlying tissues and from the environment clearly influence the stomatal density and index. Long-range signals could act by modulating the activity of genes such as *TMM*, *YODA* and members of the *ER* family or they could impinge directly on the cell-cycle machinery and other downstream targets (Figure 2).

Ecophysiologists have long noticed a correlation between stomatal density and environmental characteristics, and have used this correlation to estimate past global CO_2 concentrations [11]. These studies tracked changes over hundreds of years. Because plants continuously generate organs, they might also be capable of responding to environmental change within one lifetime. Dicotyledonous plants such as *Arabidopsis* generate new leaves in a tightly packed arrangement around the meristem. Entry of protodermal cells into stomatal lineages precedes the emergence of the leaf into the open atmosphere. Therefore, leaves that are capable of altering their epidermal development are not capable of accurately measuring the surrounding environment. A cuvette system designed to manipulate the environment around single leaves of a plant revealed that it was the CO_2 concentration around mature leaves that determined the stomatal density of emerging leaves [12].

If mature leaves perceive the environment, there must be a long-range signal that transmits this information to young leaves. Recently, several labs have used modified cuvette systems to identify or test candidates for the mobile signal and to describe the cellular basis of altered

Figure 2



Integration of environmental and developmental signals. Cuvette studies indicate where the environment must be perceived and developmental studies indicate which cells are capable of responding. The site of action of developmental genes is noted in the stomatal lineage to suggest where crosstalk between signaling pathways might occur.

stomatal density in response to environmental change. Studies of maize [13•], *Arabidopsis* [14•], and poplar [15•] suggest that environmental perception by old leaves and response by new leaves are universal, but the details of the response can vary among species. In general, elevated CO_2 levels near mature leaves increased the size of new leaves but decreased the stomatal density of these leaves. Shading mature leaves decreased new leaf size but also decreased stomatal density. Shading and high CO_2 concentrations produce the same stomatal density effect in new leaves despite having opposite effects on carbohydrate production, effectively ruling out most models that invoke photosynthetic rates or sugar production as a signal that controls stomatal density. In poplar, the parameter that directly correlates with stomatal density response of new leaves is the rate at which water and CO_2 pass through stomata (i.e. stomatal conductance) [15•] of mature leaves. Stomatal conductance is itself a measure of multiple inputs, including light, humidity and CO_2 concentration. It is not obvious how this information would travel long distance, but it does make sense to integrate multiple inputs in the mature leaf to transmit a summed picture of the atmosphere to the new leaf.

The cell size and morphology of both guard and pavement cells can be affected by environmental treatments, suggesting several cellular mechanisms by which stomatal density decreases. Possible scenarios include altered expansion of pavement cells, altered number of entry divisions or rounds of asymmetric proliferative divisions in the stomatal lineage, and arrest (or transdifferentiation) of meristemoids or GMCs. The present studies focus on the final stomatal density, stomatal index and arrangement of stomata in responding leaves. Time-course studies would allow hypotheses about which developmental steps or genes might be targets of environmental regulation. Some evidence already points to the expansion of pavement cells and the division of stomatal lineage cells being under independent control [13•,15•].

Crosstalk in the system: roles of ERECTA in environmental signaling

The stomatal development and ecophysiology fields were recently united by the discovery of *ER* as a major quantitative trait locus (QTL) underlying differences in transpiration efficiency among *Arabidopsis* ecotypes [16••]. Transpiration efficiency is the ratio of carbon fixation though photosynthesis to water loss through transpiration — parameters that are tightly linked to the behavior of stomata. Given the role of *ER* in stomatal development and the implication that ER might have different partners for different developmental processes, what is the mechanism by which *ER* controls both development and transpiration efficiency? It is possible that the ER kinase acts with one set of partners for its stomatal development roles and a unique set of partners in a signaling pathway that coordinates transpiration and photosynthesis. It is also possible that changes in the transpiration efficiency of *er* mutants are an indirect consequence of ER's developmental roles (i.e. altered plant morphology, leaf thickness and stomatal density). Genetic or protein interaction screens to find ER's partners or to find downstream targets that are unique to each process might distinguish the direct and indirect models of *ER* action.

Conclusions

Signaling is required at several levels to ensure the correct specification, pattern and function of stomata. Proteins that are elements of canonical signal-response pathways, ER, TMM and YODA, are essential for many aspects of stomatal pattern, but many questions about their activities and interactions remain. Do ER, TMM and YODA work in a concerted signaling pathway? If so, to what do they respond and what are the ultimate downstream targets of their activity? Do any of these proteins serve as nodes for the integration of developmental and environmental signals?

Whole-genome and proteome approaches might hold the greatest promise for filling gaps in the pathway of stomatal development. For example, many more genes that are expressed in and required for the stomatal lineage might be identified through transcriptional profiling of cells that are isolated at each stage of stomatal development [17], by profiles of mutants that affect stomatal development [3•] or through a combination of both approaches. Many resources are available that can be used to identify the MAP kinase components downstream of YODA [18,19], and a recently developed protein array has already proven to be useful in a high-throughput screen for targets of plant MAP kinases [20•].

Acknowledgements

I wish to thank members of my laboratory for discussions and comments on the manuscript, and Fred Sack and Keiko Torii for our ongoing conversations about stomatal development. Research in the lab is supported by the US National Science Foundation (NSF-IOB-0544895), a Terman fellowship and Stanford University funds.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Nadeau JA, Sack FD: **Stomatal development in *Arabidopsis***. In *The Arabidopsis Book*. Edited by Somerville CR, Meyerowitz EM. Rockville, MD: American Society of Plant Biologists; 2002:1-28.

2. • Lai LB, Nadeau JA, Lucas J, Lee EK, Nakagawa T, Zhao L, Geisler M, Sack FD: **The *Arabidopsis* R2R3 MYB proteins FOUR LIPS and MYB88 restrict divisions late in the stomatal cell lineage**. *Plant Cell* 2005, **17**:2754-2767.

After cells are committed to enter the stomatal lineage through the action of signaling genes, a cell differentiation process that requires a change in division potential ensues. Two MYB-type transcription factors, MYB88 and FOUR LIPS (FLP), are required to stop excessive divisions of the guard mother cell and to ensure the creation of a normal pair of guard cells.

3. Bergmann DC, Lukowitz W, Somerville CR: **Stomatal**
• **development and pattern controlled by a MAPKK kinase**. *Science* 2004, **304**:1494-1497.
Characterization of loss-of-function and constitutively active forms of the MAPKK kinase YODA implicates MAP kinase signaling as part of a switch between guard cell and pavement cell development. The authors also describe a transcriptional profiling approach to identify new stomatal regulators by comparing gene expression between plants that lack stomata and those with excess stomata.

4. Shpak ED, Berthiaume CT, Hill EJ, Torii KU: **Synergistic interaction of three ERECTA-family receptor-like kinases controls *Arabidopsis* organ growth and flower development by promoting cell proliferation**. *Development* 2004, **131**:1491-1501.

5. Lease KA, Lau NY, Schuster RA, Torii KU, Walker JC: **Receptor serine/threonine protein kinases in signalling: analysis of the ERECTA receptor-like kinase of *Arabidopsis thaliana***. *New Phytol* 2001, **1**:133-143.

6. Shpak ED, Lakeman MB, Torii KU: **Dominant-negative receptor uncovers redundancy in the *Arabidopsis* ERECTA leucine-rich repeat receptor-like kinase signaling pathway that regulates organ shape**. *Plant Cell* 2003, **15**:1095-1110.

7. Shpak ED, McAbee JM, Pillitteri LJ, Torii KU: **Stomatal patterning**
•• **and differentiation by synergistic interactions of receptor kinases**. *Science* 2005, **309**:290-293.
Shpak *et al.* perform detailed genetic and phenotypic characterization of single to quadruple mutant combinations of *TMM* and the *ER*-family genes. This paper is important in pointing out that closely related genes might have both redundant and unique functions, and it suggests several novel (and testable) mechanisms by which LRR-RLKs could interact.

8. Nadeau JA, Sack FD: **Control of stomatal distribution on the *Arabidopsis* leaf surface**. *Science* 2002, **296**:1697-1700.

9. Geisler M, Nadeau J, Sack FD: **Oriented asymmetric divisions that generate the stomatal spacing pattern in *Arabidopsis* are disrupted by the *too many mouths* mutation**. *Plant Cell* 2000, **12**:2075-2086.

10. Torii KU: **Leucine-rich repeat receptor kinases in plants: structure, function, and signal transduction pathways**. *Int Rev Cytol* 2004, **234**:1-46.

11. Hetherington AM, Woodward FI: **The role of stomata in sensing and driving environmental change**. *Nature* 2003, **424**:901-908.

12. Lake JA, Woodward FI, Quick WP: **Long-distance CO_2 signalling in plants**. *J Exp Bot* 2002, **53**:183-193.

13. Driscoll SP, Prins A, Olmos E, Kunert KJ, Foyer CH: **Specification**
• **of adaxial and abaxial stomata, epidermal structure and photosynthesis to CO_2 enrichment in maize leaves**. *J Exp Bot* 2006, **57**:381-390.
Driscoll *et al*. [13•], Coupe *et al*. [14•] and Miyazawa *et al*. [15•] all use variations on the cuvette system of Lake *et al.* [12] to examine the mature to new leaf signals in response to environmental change. Each group found that mature leaves can sense environmental change and produce a response in new leaves, but that the amplitude and timing of that response varies among species.

14. Coupe SA, Palmer BG, Lake JA, Overy SA, Oxborough K,
• Woodward FI, Gray JE, Quick WP: **Systemic signalling of environmental cues in *Arabidopsis* leaves**. *J Exp Bot* 2006, **57**:329-341.
See annotation for [13•].

15. Miyazawa S, Livingston NJ, Turpin DH: **Stomatal development in**
• **new leaves is related to the stomatal conductance of mature leaves in poplar (*Populus trichocarpax* P. *deltoides*)**. *J Exp Bot* 2006, **57**:373-380.
See annotation for [13•].

16. Masle J, Gilmore SR, Farquhar GD: **The *ERECTA* gene regulates**
•• **plant transpiration efficiency in *Arabidopsis***. *Nature* 2005, **436**:866-870.
Using $\delta^{13}C$ discrimination as a method for estimating transpiration efficiency (TE), Masle *et al.* identified QTLs for this trait in *Arabidopsis* accessions and mapped a major QTL to the ER region. They showed that ER itself is responsible for altered TE. Some of the developmental defects, including changes in stomatal density, that are associated with mutation of ER and that could lead to altered TE were explored. However, no single morphological character is solely responsible for the effect.

17. Birnbaum K, Jung JW, Wang JY, Lambert GM, Hirst JA, Galbraith DW, Benfey PN: **Cell type-specific expression profiling in plants via cell sorting of protoplasts from fluorescent reporter lines**. *Nat Methods* 2005, **2**:615-619.

18. Asai T, Tena G, Plotnikova J, Willmann MR, Chiu WL, Gomez-Gomez L, Boller T, Ausubel FM, Sheen J: **MAP kinase signalling cascade in *Arabidopsis* innate immunity**. *Nature* 2002, **415**:977-983.

19. Liu Y, Zhang S: **Phosphorylation of 1-aminocyclopropane-1-carboxylic acid synthase by MPK6, a stress-responsive mitogen-activated protein kinase, induces ethylene biosynthesis in *Arabidopsis***. *Plant Cell* 2004, **16**:3386-3399.

20. Feilner T, Hultschig C, Lee J, Meyer S, Immink RGH, Koenig A,
• Possling A, Seitz H, Beveridge A, Scheel D *et al.*: **High throughput identification of potential *Arabidopsis* mitogen-activated protein kinases substrates**. *Mol Cell Proteomics* 2005, **4**:1558-1568.
This paper describes the creation of a protein microarray that contains about 1400 *Arabidopsis* proteins. This microarray was used to identify kinase substrates of MPK3 and MPK6. The validation of targets for these kinases though conventional kinase assays suggests that this high-throughput approach will have great utility in identifying possible targets of many plant signaling pathways.