Commentary

Stomatal function has an element of hysteresis

Plants are unable to move away from stressful habitats, thus they have evolved adaptive morphogenetic and cellular responses to fluctuating environmental conditions. Terrestrial plants experience a diverse array of stresses, including those that are abiotic, such as drought, widely fluctuating temperatures and excessive insolation; in addition, they are subjected to biotic stresses imposed by pathogens and animal grazers. Plants respond to these negative influences by increasing their stress tolerance levels. A history of stress exposure induces responses that increase the ability of individuals to cope with future stress. This is a process of hysteresis, which operates in the absence of any nervous memory system in plants (Bruce *et al.*, 2007). Thus, *Arabidopsis* plants with prior experience of drought acquire an enhanced ability to reduce water loss from leaves (Ding *et al.*, 2012), thereby indicating that stress induces a 'memory' that plastically changes plant behaviours. In this issue of *New Phytologist*, Virlouvet & Fromm (pp. 596–607) report that guard cells, which are responsible for stomatal movements, facilitate dehydration stress memory and maintain stomatal closure even after a period of relief from drought, possibly by increasing abscisic acid (ABA) levels and regulating gene expression (Fig. 1).

> *'The discovery of guard cell memory by Virlouvet & Fromm may provide a new model system for the study of hysteresis in plants.'*

Dehydration memory in guard cells

Stomatal pores are the apertures between a pair of guard cells in the plant epidermis; they regulate gas exchange between the leaf aerenchyma and the atmosphere. The stomatal opening allows CO_2 entry into leaves for carbon (C) fixation, and water vapour egress, which is the terminal phase in the transpirational stream of moisture uptake from the soil (Shimazaki *et al.*, 2007). Water vapour transit through the pores is the major driver of dehydration; stomatal closure during drought is crucial to the survival of homiohydric plants. Since Francis Darwin (1898) reported light-induced stomatal opening, the mechanism has been subject to intensive investigation. Of particular importance are molecular genetic experiments on the model plant *Arabidopsis* that have identified diverse signalling molecules, signalling pathways, and the mechanism of ion transport underlying rapid stomatal movements (Kim *et al.*, 2010). Nevertheless, few studies have examined the regulation of stomatal movements over protracted periods of time. Virlouvet & Fromm examined the effect of prior dehydration on stomatal opening after re-watering. Interestingly, stomata remained closed even after the plants were rehydrated, indicating that guard cells develop a dehydration stress memory to keep pores closed over protracted periods, thereby significantly reducing water loss from drought-recovered plant leaves (in comparison with controls).

Guard cells develop transcriptional memories

Plants are likely to improve tolerance capacities by memorizing prior stress. The mechanism is not well understood but probably involves transcriptional changes. Guard cells have a unique profile of gene expression for stomatal movement (Leonhardt *et al.*, 2004; Yang *et al.*, 2008), and the gene expression patterns of guard cells are changed by dehydration stress. Virlouvet & Fromm examined transcript levels of selected genes in guard cells prepared from plants exposed in sequence to dehydration followed by re-watering and a second round of dehydration. Some genes, including GORK, the outwardly rectifying K^+ channel involved in stomatal closure, were up-regulated by dehydration and maintained high expression levels even after the stress had been removed. Expression levels may increase further after a second bout of stress. Thus, guard cells have a stress memory that functions at the transcriptional level. Whether this transcriptional memory has a critical role in stomatal behaviour remains unclear, but this line of investigation has provided a new perspective on the gene expression patterns associated with guard cell function.

Abscisic acid (ABA) mediates the stomatal memory response

The phytohormone ABA is produced in response to drought and plays a crucial role in plant tolerance of this stressor (Kim *et al.*, 2010). The genetic analysis by Virlouvet & Fromm indicates that the memory response is mediated by ABA. Water loss by the ABA-deficient mutant *aba2* following a second bout of dehydration stress was not different from that of controls, indicating a failure of memory response in this mutant. Although the physiological action of ABA in the stomatal closing process has been investigated extensively, little is known of its role in plant stress memory responses. These new findings suggest a novel ABA function in plant adaptive responses to drought stress.

Virlouvet & Fromm demonstrated that the ABA-activated Snf1-related protein kinases (SnRK2s) mediate the signalling pathway of

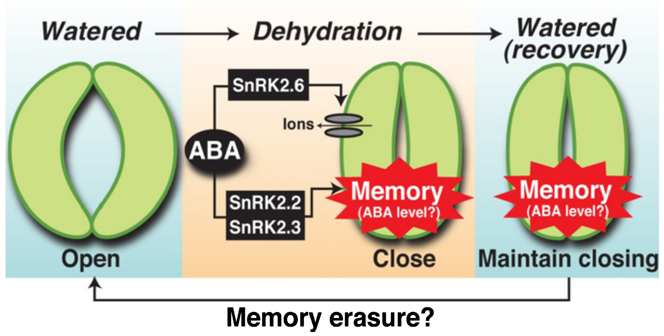

Fig. 1 Schematic of dehydration-induced stomatal guard cell memory. Dehydration probably induces memory through increases in abscisic acid (ABA) levels. SnRK2.2 and SnRK2.3 are important for the stomatal memory response; SnRK2.6 promotes stomatal closure through the phosphorylation of ion channels. The stress memory maintains stomatal closure even after relief from stress. Prepared from data in Virlouvet & Fromm (this issue of *New Phytologist*, pp. 596–607).

the stomatal memory response. Earlier work indicated that SnRK2.6 (OST1) has a critical role in ABA-induced stomatal closure, and that SnRK2.2 and SnRK2.3 have partially redundant minor roles in guard cells (Fujii *et al.*, 2007; Fujita *et al.*, 2009). The *snrk2.2 snrk2.3* double mutant had a reduced memory response in water loss assays, but the *snrk2.6* mutant retained memory function (Virlouvet & Fromm). Thus, the SnRK2.2 and SnRK2.3 protein kinases are the most important for stomatal memory function. SnRK2s mediate almost all ABA signalling, including ion transport, reactive oxygen species (ROS) production and the regulation of transcription factors through phosphorylation of a range of substrates (Furihata *et al.*, 2006; Geiger *et al.*, 2009; Lee *et al.*, 2009; Sirichandra *et al.*, 2009; Takahashi *et al.*, 2013). The guard cell memory response is likely to be recognized as a new form of ABA signalling.

Mechanism of guard cell memory

The most important issue remaining to be clarified is the underlying mechanism of memory formation in guard cells. Transcriptional memory may be formed by epigenetic changes. Ding *et al.* (2012) demonstrated that dehydration stress increases histone trimethylation levels in some drought-responsive genes located in *Arabidopsis* leaves; these changes were maintained even after removal of the stressor. Thus, transcriptional memory in leaves is probably underpinned by histone modifications. However, the effect of guard cell transcriptional memory on stomatal aperture regulation has yet to be examined.

Virlouvet & Fromm demonstrated that the rate limiting ABA biosynthetic genes *NCED3* and *AAO3* are expressed at high levels in guard cells after dehydration and during the subsequent recovery period; they therefore postulated that residual ABA maintains stomatal closure after recovery from drought. Alternatively, changes in guard cell signalling capacity may maintain stomatal closure. Guard cells are highly specialized components of the stomatal structure; they are endowed with a comprehensive mechanism for regulating gas exchange. The activities of many signalling components in these cells are regulated by reversible modifications, such as phosphorylation. The accumulation of signalling components or their modification may facilitate the guard cell memory response. Identification of the molecular mechanism of guard cell memory is an important issue for progress in plant physiology.

Perspective

How does stomatal memory contribute to the physiological performance of plants? Virlouvet & Fromm postulated that the mechanism facilitates adaptation to future dehydration stress by closing stomata and reducing water losses from homiohydric plants. However, the opening of stomata should be advantageous when water supplies are adequate because increased gas exchange facilitates C accretion and, hence, the growth performance of plants competing with one another for limiting resources. Bet-hedging for best performance over the long term probably requires selection of the most appropriate time for erasing memory. The factors that weaken or strengthen stomatal memory should be identified as they are likely to be crucial for plant growth and survival. Guard cells have long been used as models in investigations of intracellular signalling (Kim *et al.*, 2010). The discovery of guard cell memory by Virlouvet & Fromm may provide a new model system for the study of hysteresis in plants.

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Yohei Takahashi[1] and Toshinori Kinoshita[1,2]**

[1]Division of Biological Science, Graduate School of Science, Nagoya University, Nagoya 464-8602, Japan;
[2]Institute of Transformative Bio-Molecules (WPI-ITbM), Nagoya University, Nagoya 464-8602, Japan
(*Author for correspondence: tel +81 52 789 4778; email kinoshita@bio.nagoya-u.ac.jp)

References

Bruce TJ, Matthes MC, Napier JA, Pickett JA. 2007. Stressful "memories" of plants: evidence for possible mechanisms. *Plant Science* **173**: 603–608.

Darwin F. 1898. Observations on stomata. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **190**: 531–621.

Ding Y, Fromm M, Avramova Z. 2012. Multiple exposures to drought 'train' transcriptional responses in Arabidopsis. *Nature Communications* **3**: 740.

Fujii H, Verslues PE, Zhu JK. 2007. Identification of two protein kinases required for abscisic acid regulation of seed germination, root growth, and gene expression in Arabidopsis. *Plant Cell* **19**: 485–494.

Fujita Y, Nakashima K, Yoshida T, Katagiri T, Kidokoro S, Kanamori N, Umezawa T, Fujita M, Maruyama K, Ishiyama K *et al.* 2009. Three SnRK2 protein kinases are the main positive regulators of abscisic acid signaling in response to water stress in Arabidopsis. *Plant & Cell Physiology* **50**: 2123–2132.

Furihata T, Maruyama K, Fujita Y, Umezawa T, Yoshida R, Shinozaki K, Yamaguchi-Shinozaki K. 2006. Abscisic acid-dependent multisite phosphorylation regulates the activity of a transcription activator AREB1. *Proceedings of the National Academy of Sciences, USA* **103**: 1988–1993.

Geiger D, Scherzer S, Mumm P, Stange A, Marten I, Bauer H, Ache P, Matschi S, Liese A, Al-Rasheid KA *et al.* 2009. Activity of guard cell anion channel SLAC1 is controlled by drought-stress signaling kinase-phosphatase pair. *Proceedings of the National Academy of Sciences, USA* **106**: 21425–21430.

Kim TH, Bohmer M, Hu H, Nishimura N, Schroeder JI. 2010. Guard cell signal transduction network: advances in understanding abscisic acid, CO_2, and Ca^{2+} signaling. *Annual Review of Plant Biology* **61**: 561–591.

Lee SC, Lan W, Buchanan BB, Luan S. 2009. A protein kinase-phosphatase pair interacts with an ion channel to regulate ABA signaling in plant guard cells. *Proceedings of the National Academy of Sciences, USA* **106**: 21419–21424.

Leonhardt N, Kwak JM, Robert N, Waner D, Leonhardt G, Schroeder JI. 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.

Shimazaki K, Doi M, Assmann SM, Kinoshita T. 2007. Light regulation of stomatal movement. *Annual Review of Plant Biology* **58**: 219–247.

Sirichandra C, Gu D, Hu HC, Davanture M, Lee S, Djaoui M, Valot B, Zivy M, Leung J, Merlot S *et al.* 2009. Phosphorylation of the Arabidopsis AtrbohF NADPH oxidase by OST1 protein kinase. *FEBS Letters* **583**: 2982–2986.

Takahashi Y, Ebisu Y, Kinoshita T, Doi M, Okuma E, Murata Y, Shimazaki K. 2013. bHLH transcription factors that facilitate K^+ uptake during stomatal opening are repressed by abscisic acid through phosphorylation. *Science Signaling* **6**: ra48.

Virlouvet L, Fromm M. 2014. Physiological and transcriptional memory in guard cells during repetitive dehydration stress. *New Phytologist* **205**: 596–607.

Yang Y, Costa A, Leonhardt N, Siegel RS, Schroeder JI. 2008. Isolation of a strong Arabidopsis guard cell promoter and its potential as a research tool. *Plant Methods* **4**: 6.

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