

## Regulation of stomatal opening by the guard cell expansin *AtEXPA1*

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**S**tomatal movement is strictly regulated by various intracellular and extracellular factors in response environmental signals. In our recent study, we found that an Arabidopsis guard cell expressed expansin, *AtEXPA1*, regulates stomatal opening by altering the structure of the guard cell wall. This addendum proposes a mechanism by which guard cell expansins regulate stomatal movement.

Stomatal movement is the most popular model system for cellular signaling transduction research. A complicated complex containing many proteins has been proposed to control stomatal responses to outside stimuli. The known regulation factors are primarily located in the nucleus, cytoplasm, plasma membrane and other intracellular organelles.<sup>1,2</sup> Although the cell wall structure of the stomata is different from that of other cells,<sup>3,4</sup> the presence of stomatal movement regulation factors in the cell wall has seldom been reported in reference 5. In our previous work, we found that extracellular calmodulin stimulates a cascade of intracellular signaling events to regulate stomatal movement.<sup>6</sup> The involvement of this signaling pathway is the first evidence that cell wall proteins play an important role in regulation of stomatal opening. Cell wall-modifying factors constitute a major portion of cell wall proteins. However, the role of these factors in the regulation of stomatal movement is not yet known.

Expansins are nonenzymatic proteins that participate in cell wall loosening.<sup>7-9</sup> Expansins were first identified as “acid-growth” factors because they have much higher activities at acidic pHs.<sup>10,11</sup>

It has been reported that expansins play important roles in plant cell growth, fruit softening, root hair emergence and other developmental processes in which cell wall loosening is involved.<sup>7-9,12,13</sup> Wall loosening is an essential step in guard cell swelling and the role of stomatal expansins was investigated. *AtEXPA1* is an Arabidopsis guard-cell-specific expansin.<sup>13,14</sup> Overexpressing *AtEXPA1* increases the rate of light-induced stomatal opening,<sup>14,15</sup> while a potential inhibitor of expansin activity, *AtEXPA1* antibody, reduces the sensitivity of stomata to stimuli.<sup>14</sup> We showed that the transpiration rate and the photosynthesis rate in plant lines overexpressing *AtEXPA1* were nearly two times the rates for wild-type plants (Fig. 1). These in plant data revealed that expansins accelerated stomatal opening under normal physiological conditions. In addition, the increases in the transpiration and photosynthesis rates strongly suggested the possibility of exploiting expansin-regulated stomatal sensitivity to modify plant drought tolerance. Compared with the effect of hydrolytic cell wall enzymes, the destruction of cell wall structures induced by expansins is minimal. In addition, it is very difficult to directly observe the changes in the guard cell wall structure caused by expansins during stomatal movement. Our recent work showed that, in *AtEXPA1*-overexpressing plants, the volumetric elastic modulus is lower than in wild-type plants,<sup>14</sup> which indicates the wall structure was loosened and that the cell wall was easier to extend. Taken together, our data suggest that expansins participate in the regulation of stomatal movement by modifying the cell walls of guard cells.

**Key words:** expansin, stomatal movement, *AtEXPA1*, guard cell, wall loosening

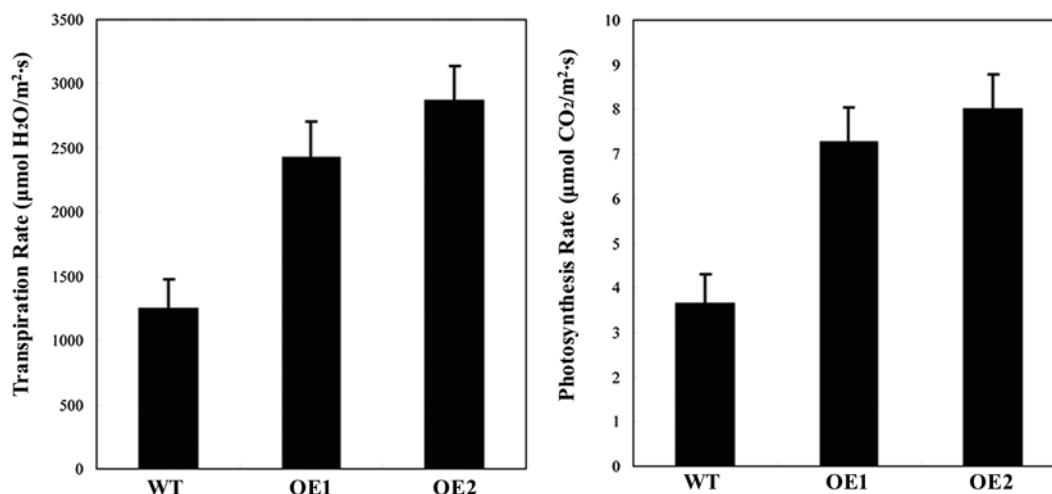
Submitted: 02/12/11

Accepted: 02/12/11

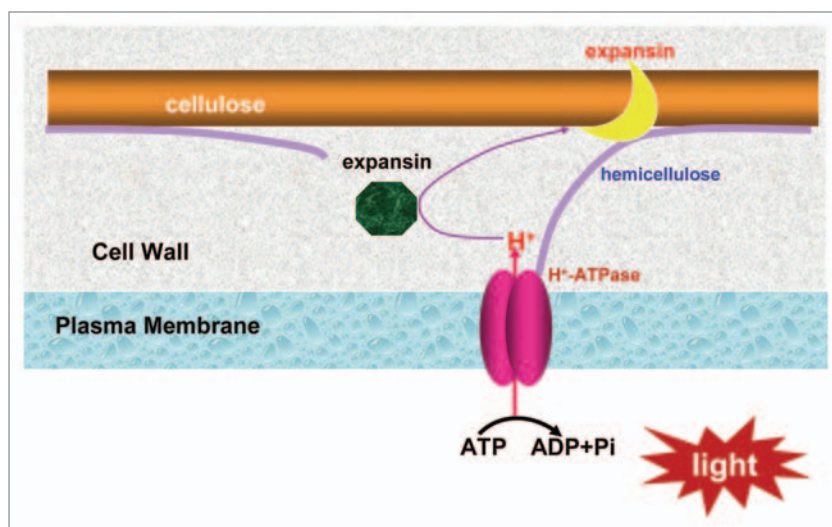
DOI: 10.4161/psb.6.5.15144

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Addendum to: Zhang XQ, Wei PC, Xiong YM, Yang Y, Chen J, Wang XC. Overexpression of the Arabidopsis  $\alpha$ -expansin gene, *atexpa1*, accelerates stomatal opening by decreasing the volumetric elastic modulus. *Plant Cell Rep* 2010; 30:27–36. PMID: 20976459; DOI: 10.1007/s00299-010-0.



**Figure 1.** Effects of *AtEXPA1* overexpression on transpiration rates and photosynthesis rates. The transpiration rate (left) and photosynthesis rate (right) of wild-type and transgenic *AtEXPA1* lines were measured at 10:00 AM in the greenhouse after being watered overnight. The illumination intensity was 180  $\mu\text{mol}/\text{m}^2\cdot\text{s}$ . Bars represent the standard error of the mean of at least five plants per line.



**Figure 2.** Model of how guard cell wall expansins regulate stomatal opening. Environmental stimuli, e.g., light, activate guard cell plasma membrane  $\text{H}^+$ -ATPases to pump  $\text{H}^+$  into the extracellular wall space. The accumulation  $\text{H}^+$  acidifies the cell wall and induces the activation of expansin. The active expansin disrupts non-covalent bonding between cellulose microfibrils and matrix glucans to enable the slippage of the cell wall. The wall is loosened coincident with guard cell swelling and without substantial breakdown of the structure.

It is well known that the activation of proton-pumping ATPase ( $\text{H}^+$ -ATPase) in the plasma membrane is an early and essential step in stomatal opening.<sup>16</sup> The action of the pump results in an accumulation of  $\text{H}^+$  outside of the cell, increases the inside-negative electrical potential across the plasma membrane and drives potassium uptake through the voltage-gated, inward-rectifying  $\text{K}^+$  channels.<sup>17-19</sup> The main function of the  $\text{H}^+$  pump is

well accepted to create an electrochemical gradient across the plasma membrane; however, the other result is the acidification of the guard cell wall, which may also contribute to stomatal opening. A possible mechanism responsible for this effect is as follows. Expansins are in an inactive state when the stomata are in the resting state. Stomatal opening signals induce wall acidification and activate expansins. Then, the expansins move along

with cellulose microfibrils and transiently break down hydrogen bonding between hemicellulose and the surface of cellulose microfibrils,<sup>20,21</sup> facilitating the slippage of cell wall polymers under increasing guard cell turgor pressure. The guard cell then swells and the stomata open (Fig. 2).

Although our results indicate that *AtEXPA1* regulates stomatal movement, the biochemical and structural mechanism by which *AtEXPA1* loosens the cell wall remains to be discovered. It remains to figure out the existing of other expansins or coordinators involving in this process. In addition, determining the roles of expansins and the guard cell wall in stomatal closing is another main goal of future research.

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