Stomatal pores, surrounded by the pairs of guard cells, regulate plant gas exchange. Correct stomatal regulation is crucial for plant survival under various stress conditions. We have recently utilized the air pollutant ozone (O₃) to study stomatal signaling and showed that application of O₃ induces rapid decrease in stomatal conductance. Here we have addressed the recovery of stomatal conductance and show that after exposures of plants to high O₃ pulses stomatal conductance recovered faster, reaching higher, “overshooting” values than were the pre-exposure values. We propose the hypothetical mechanism for this phenomenon and discuss it in the frames of current stomatal signaling models.

Rapid progress in understanding structural and molecular mechanisms of the core abscisic acid (ABA) signaling pathway and subsequent stomatal closure (reviewed in ref. 1) has been achieved by using a variety of mostly in vitro technologies and approaches. Data on early induction of stomatal response by a brief ABA pulse in vivo is almost absent, largely due to difficulties in rapid removal of ABA from intact guard cells. Application of O₃, an air pollutant efficiently utilized to study stomatal signaling, lacks this disadvantage and allows monitoring stomatal responses to brief, clean-cut, strictly dosed pulses of this powerful oxidant in planta. Application of O₃ for 1 min to intact Arabidopsis rosette triggered a Rapid Transient Decrease (RTD) in stomatal conductance which, after lasting for 8–10 min, was followed by a 3–4 times slower recovery. The entire RTD, lasting for up to 40–50 min, is a conserved response in plants; to date it is found to be present in about 90 Arabidopsis ecotypes/mutants and also in tobacco and birch (unpublished results). Absence of RTD in protein phosphatase ABI1 and ABI2 mutants (abi1-1 and abi2-1) which are unable to form complex with PYR/PYL ABA receptors, in protein kinase OST1 and in guard cell plasma membrane anion channel SLAC1 mutants, indicates that O₃-triggered signal propagates through the same phosphatase/kinase pair as does the signal triggered by ABA. Results of mostly proteomic, pharmacological and electrophysiological studies allow to suggest that the most likely reason for the rapid stomatal closure during RTD is the ABI1, ABI2 and OST1 mediated alterations in a battery of plasma membrane ion channels, including the outward-rectifying anion channel SLAC1 and the depolarization-activated K⁺ channel GORK1 which after their sequential activation result in efflux of osmotica, turgor loss and stomatal closure.

Physiological background of the recovery during RTD which takes place also under continuous exposure to ozone is less understood. To study this process further we exposed whole rosettes of intact 22–25 day old Arabidopsis plants to different O₃ concentrations for 3 min as described earlier and observed that after exposures to high concentration O₃ pulses stomatal conductance recovered faster and reached higher values than were the pre-exposure values. We term this phenomenon “overshooting”.

Ozone concentration of 70 nl l⁻¹ did not induce RTD (Fig. 1A). At higher
Concentrations of O₃ induced intense decrease in stomatal conductance within 4–6 min after application. This was followed by rapid stoppage of the closure, a brief transition period and a sluggish, almost linear recovery where the pre-exposure value of stomatal conductance was reached about 30 min after the onset of O₃ (Fig. 1A). The rates and extents of the O₃-induced stomatal closure, as well as rates of reopening were concentration dependent. Continuation of the linear increase in stomatal conductance after reaching the pre-exposure value resulted in almost two-fold higher values at 50 min after the onset of 385 nl l⁻¹ of O₃.

Overshootings were dependent on ozone concentration (Fig. 1B) and on the extent of the initial decrease in stomatal conductance (Fig. 1C). Both dependencies were exponential indicating a presence of threshold at 150–200 nl l⁻¹ of O₃ and at 20% of initial O₃-induced decrease in stomatal conductance, respectively.

What could be the reason and mechanism explanation for described O₃-induced "overshooting" in stomatal conductance? The protein kinase OST1 is required for induction of rapid closure phase of the O₃-triggered RTD.³ Besides phosphorylating SLAC1,⁵⁶ OST1 has been shown to phosphorylate also the inward-rectifying K⁺ channel KAT1 resulting in its inhibition.⁶ Inhibition of K⁺ uptake, which allows faster membrane depolarization and stomatal closure, has been shown to occur under various stresses.⁷ Presumably, H⁺-ATPase activity and proton pumping, tightly coupled to K⁺ uptake via channel energization⁸ are also suppressed by O₃. It has been shown that in depolarized guard cell, plasma membrane proton pumping may precede volume and turgor increase.⁹ We speculate that in the O₃-triggered, SLAC1- and GORK-mediated stomatal closure, when ion efflux and turgor loss proceed at high rates, reactivation of H⁺-ATPase and proton pumping and associated recovery of K⁺ uptake are induced to avoid guard cell plasmolysis.¹⁰ Guard cells begin to regain turgor and stomata reopen. At the same time outward-rectifying ion channels are transiently locked (inactivated) as stomata become completely insensitive to repeated O₃-pulses during recovery phase.³ This interpretation is supported by our

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**Figure 1.** Ozone-triggered rapid decrease in stomatal conductance is followed by recovery to higher "overshooting" values. (A) Typical asymmetric time patterns of stomatal conductance after exposure of 22–25 day old Arabidopsis plant leaf rosettes to different concentrations of ozone as described in Kollist et al.² In (B and C) O₃-induced "overshooting" is plotted against O₃ concentration and O₃-induced decrease in stomatal conductance, respectively.
observation that the recovery in stomatal opening is heavily suppressed in kincless mutant where the inward rectifying K⁺ current is abolished. In addition, peak densities of inward K⁺ currents (2–4 μA/cm² membrane) are shown to be much lower than those for outward anion and K⁺ currents (17–20 μA/cm²). This could be a reason why stomatal reopening is much slower than the initial O₃-induced closure. Our findings (Fig. 1) suggest that the faster and deeper the O₃-triggered turgor loss, the faster and extensive is its recovery. The "overshootings" suggest plasma membrane hyperpolarization and predict a viable oscillation-like stomatal behavior where the system tends to restore the initial equilibrium. Longer experiments are needed to address whether such an oscillating response exists in Arabidopsis elicited by O₃.

Taken together, our data suggest the presence of a "security" mechanism in plant guard cells which avoids the excessive dehydration and precipitous turgor loss by reswitching the reaccumulation of osmotica ultimately leading to stomatal opening. Molecular mechanism(s) linking feedback from low turgor to activation of plasma membrane proton pumping and subsequent ion uptake are obscure. Irrespective of mechanism(s), our data indicate that stomata tend to recover from stress the faster the stronger has been the perturbation at its onset. Undoubtedly, rapid O₃-induced transient decrease in stomatal conductance is one of countless expressions of the Le Chatelier's principle having numerous wordings like: "any change in status quo prompts an opposing reaction in the responding system," or paraphrased on the basis of our results—the stronger the stimulus (O₃ concentration) the stronger the response ("overshooting").

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