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Article Addendum

Arabidopsis MPK3, a Key Signalling Intermediate in Stomatal Function

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Addendum to:

Guard Cell-Specific Inhibitin of Arabidopis MPK3 *Expression Causes Abnormal Stomatal Responses to Abscisic Acid and Hydrogen Peroxide*

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ABSTRACT

Regulation of stomatal aperture is of critical importance to plants to balance gas exchange and water loss, and also to control ingress of bacterial pathogens. MAP kinase signal transduction pathways are mediators of biotic and abiotic stress, and have been indicted in the control of stomatal movements. Cell-specific antisense was used to down-regulate *MPK3* gene expression in Arabidopsis guard cells, resulting in ABA insensitivity during inhibition of stomatal opening, but a normal ABA response in promotion of closure assays. This response is similar to that of the heterotrimeric G protein alpha subunit mutant *gpa1*, as is the imposition of ABA insensitivity during stomatal closure by butyrate treatment, suggesting that MPK3 and GPA1 are in the same ABA signal transduction pathway and adding further evidence for parallel signalling pathways during ABA-induced closure. By contrast, antisense plants were less sensitive to H_2O_2 in both promotion of closure and inhibition of opening assays, although H_2O_2 production in response to ABA was not affected. Regulation of stomatal aperture by PAMPs has recently been shown to be an important plant defense mechanism; since MPK3 is also activated by such pathogen elicitors, we postulate that in addition to a signalling role in guard cell movements, MPK3 is involved in the active prevention of bacterial infection through stomata.

In our recent work we provide evidence for the involvement of a mitogen-activated protein kinase (MAP kinase) signal transduction cascade in the control of stomatal movements in *Arabidopsis thaliana*.[1] This was achieved by using the guard cell-specific *KST1* promoter[2] to direct the antisense mediated downregulation of a specific MAP kinase gene in Arabidopsis guard cells. Previous work (for example inhibitor studies)[3,4] have strongly indicated that MAP kinases play a role in stomatal physiology but as yet there has been no good evidence for the identity of the MAP kinase(s) involved (there are 20 *MPK* genes encoding MAP kinases in Arabidopsis).[5] We chose to focus on *MPK3* for several reasons; this gene is known to be expressed in guard cells,[6] and MPK3 activity is induced by both abscisic acid (ABA) and H_2O_2,[7] both of which promote stomatal closure.

Antisense inhibition of *MPK3* expression in guard cells partially abolished stomatal sensitivity to ABA in inhibition of opening assays, but not in the promotion of closure, thus providing further evidence of the existence of different pathways for ABA signal transduction, depending on the initial state of the stomatal pore opening.[8] One of the effects of ABA is to increase cytosolic pH, and when this was prevented in *MPK3* antisense lines with 1 mM sodium butyrate, promotion of closure by ABA was now reduced as compared to controls, indicating that in promotion of closure, the absence of MPK3 can be compensated for by other signaling components, whilst in inhibition of opening, it can not. The stomatal phenotype of the *MPK3* antisense lines in response to ABA closely resembles that of the *gpa1* G protein alpha subunit mutant,[9] thus suggesting that MPK3 might act in the same ABA signaling pathway as this protein.

We also found that *MPK3* antisense plants are reduced in sensitivity to H_2O_2, both in promotion of stomatal closure and in inhibition of stomatal opening, thus arguing that H_2O_2 acts further downstream than the compensating mechanism for ABA-induced promotion of closure (as revealed by the butyrate treatment). Induction of H_2O_2 synthesis by ABA was not found to be significantly altered in the *MPK3* antisense plants, showing MPK3 to act downstream of H_2O_2. While it remains to be demonstrated that exogenous and endogenous H_2O_2 are equivalent in their effects on guard cells, it seems probable that a MAP kinase cascade acts downstream of H_2O_2 in guard cells, whose synthesis is induced in this cell type in response to several stimuli which promote stomatal closure, such as pathogen elicitors,[10] ABA,[11,12] darkness[13,14] and ozone.[15] However, much more work is needed to

confirm and analyze the interactions of all the potential upstream and downstream signaling components of this pathway, in particular the role of nitric oxide needs to be explored.

MPK3 has also been shown to act in a MAP kinase cascade downstream of the receptor for the bacterial elicitor flagellin, FLS2.[16] Recent work[17] highlights the important role of stomata in innate immunity against pathogens. The authors show that flagellin is one of the pathogen-associated molecular patterns (PAMPs) which promote stomatal closure in Arabidopsis, thus preventing pathogen entry through stomata. Thus it seems conceivable that MPK3 acts in signaling downstream of flagellin in promotion of stomatal closure. In this context it is of interest that Arabidopsis heterotrimeric G protein components are also implicated in plant defense signalling.[18] Additionally, reactive oxygen species such as H_2O_2 have been proposed as systemic signals generated in response to pathogens, as these molecules are relatively stable in the apoplast due to its weak redox buffering capacity.[19]

Our work also highlights the usefulness of employing tissue specific promoters to study the role of signalling components in plants. In the specific case of *MPK3*, Menke et al.[20] have reported that they failed to recover Arabidopsis plants with a significant reduction in its expression after transforming them with an RNAi construct targeted against the coding region. Thus, targeting RNAi or antisense construct expression to particular tissues allows a cleaner assessment of the role of the gene product in a particular location, whilst avoiding problems of non-viability through global down-regulation of key genes.

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