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Article Addendum

Ca^{2+}-Responsive cis-Elements in Plants

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KEY WORDS

calcium, signal transduction, gene expression, transcription factors, abscisic acid, stress responses

ABBREVIATIONS

CaM	calmodulin
TF	transcription factor
ABA	abscisic acid
ABRE	ABA-responsive element

Addendum to:

Rapid Transcriptome Changes Induced by Cytosolic Ca^{2+} Transients Reveal ABRE-Related Sequences as Ca^{2+}-Responsive cis Elements in Arabidopsis

Kaplan B, Davydov O, Knight H, Galon Y, Knight MR, Fluhr R, Fromm H

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ABSTRACT

External physical and chemical stimuli are transduced via second messengers, following primary interaction with specific membrane or soluble receptors. Ca^{2+} is an important second messenger in plants as in other eukaryotes, mediating responses to numerous environmental stimuli and affecting a multitude of cellular processes including gene expression. However, there is yet very little information concerning the cis-elements that mediate Ca^{2+}-responsive gene expression. In this article we discuss a recent investigation combining bioinformatics with experimental data, revealing DNA regulatory elements that convey specific cytosolic Ca^{2+} transients to the transcription machinery.

In response to environmental stimuli, including abiotic (cold, heat, salt, drought, light, touch) and biotic stresses, Ca^{2+} concentrations are transiently elevated, via an increased Ca^{2+} influx.[1] Ca^{2+} transients are transduced by Ca^{2+}-binding proteins, many of which contain an 'EF-hand' motif, which is a helix-loop-helix structure with high specificity for Ca^{2+} binding.[2] Ca^{2+} transducers include calmodulin (CaM) and CaM-like proteins (CMLs),[3,4] Ca^{2+}-dependent protein kinases,[5] calcineurin-B-like proteins (CBL),[6] potassium[7] and Ca^{2+}-channels.[8] These affect numerous downstream targets and cellular processes including metabolism,[9] cellular structures[10] and gene expression.[11,12]

The apparent complexity of these responses raises questions concerning the mechanisms underlying stimulus-response specificity and cross-talk with other cellular processes.[13-15] Studies of Ca^{2+} signaling in plants and animals suggest that the intracellular Ca^{2+} signals carry different elements of information including duration of the signals, amplitude, and frequency of oscillations.[16-19] These signals need to be decoded[17] and translated to the appropriate cellular responses. However, in spite of the obvious importance of Ca^{2+} signaling, little is known about the mechanisms mediating Ca^{2+}-responsive gene expression in plants. In the discussed paper, Ca^{2+}-responsive genes and cis-elements in *Arabidopsis* were characterized. The implications and open questions emerging from these studies are discussed.

The complexity of the Ca^{2+} signaling machinery and stress-induced cellular signaling makes it difficult to distinguish the effects of the Ca^{2+}-response per se from other effects evoked by the same stimuli that induce the Ca^{2+} signals. For example, ABA activates both Ca^{2+}-dependent and -independent pathways.[15, 20] In addition, subcellular compartmentalization of Ca^{2+} signaling is also a complex issue that needs to be addressed.[21] One approach to identify Ca^{2+}-specific responses is by induction of artificial Ca^{2+} transients with or without Ca^{2+}-signaling inhibitors (e.g., channel blockers, protein inhibitors). Artificial cytosolic Ca^{2+} transients may be achieved by repeatedly changing the ionic composition of the extra-cellular environment from a depolarizing to hyperpolarizing buffer.[16] This type of artificial manipulation revealed the physiological importance of the frequency of Ca^{2+} oscillations.[16] In addition, because several plant Ca^{2+} transporters are regulated by CaM,[4] exposure of plants to CaM antagonists may also alter their Ca^{2+} homeostasis. Indeed, using aequorin-based luminometry and photon imaging it was shown in the discussed study that CaM antagonists including TFP, W7, calmidazolium and SKF-7171, induced rapid transient changes in cytosolic Ca^{2+} in intact *Arabidopsis* and tobacco seedlings. These Ca^{2+} bursts peaked 30–100 sec after the addition of the CaM antagonists, lasting 5-10 min, with an elevation of at least 10-fold of cytosolic Ca^{2+} over the measured basal levels. The Ca^{2+} signals triggered by these antagonists were completely inhibited by the Ca^{2+}-channel blockers, lanthanum and gadolinium, but not by potassium channel blockers.

Analysis of transcriptome changes 1-hr post stimulus revealed 230 Ca^{2+}-responsive genes, of which 162 were upregulated and 68 downregulated. Considering that the

chip used in this study represented only 25% of *Arabidopsis* genome, the total number of upregulated genes might be ~650, comprising some 2.3% of the whole genome, and a total of 280 downregulated genes, comprising 1% of the genome, for this specific Ca^{2+} response. The upregulated genes contained more TFs, post-transcriptional regulatory proteins, and signaling-related genes, whereas the downregulated genes included more transporters, specifically aquaporins, and defense-related genes, particularly peroxidases. Thus, although the induced cytosolic Ca^{2+} burst was artificial, it allowed the identification of genes responding to a cytosolic Ca^{2+} burst, in the absence of an applied environmental stress. However, many of the upregulated genes were found to be known early stress-responsive genes, such as the touch-responsive genes *TCH2 (CML24)*, *TCH3* (*CML12*) and *TCH4*-like, the cold-induced genes *COR47* and *KIN2* and the dehydration early-responsive genes ERD1, *ERD6, ERD10, ERD13, ERD14* and *ERD15*.

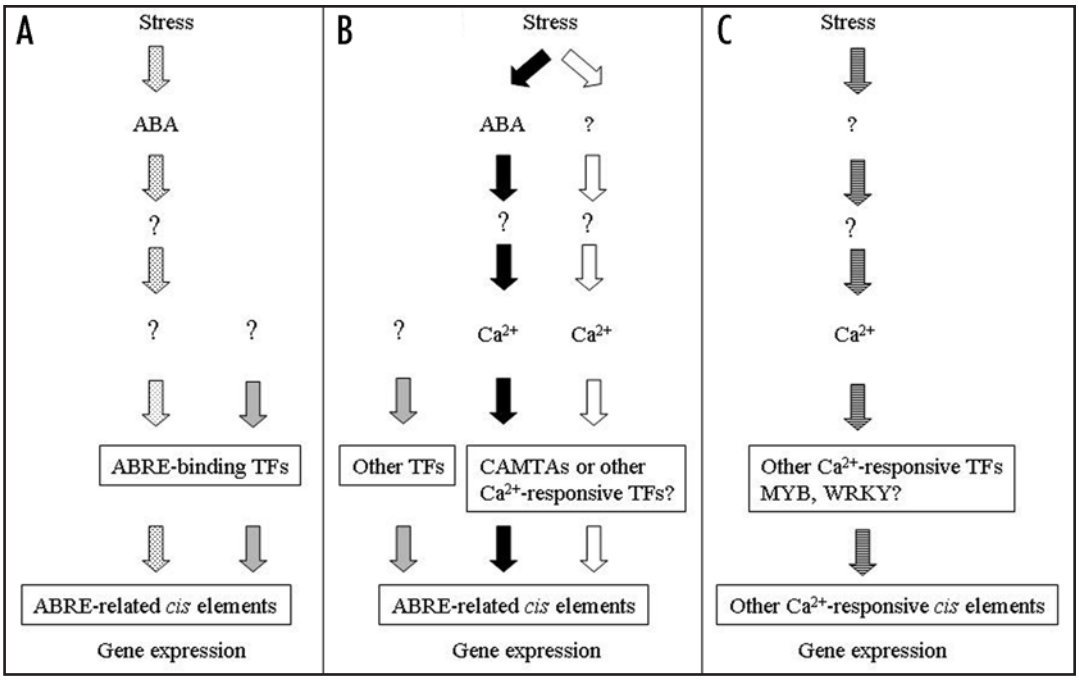

Figure1. Possible transduction pathways of stress signals to Ca^{2+}-responsive and/or ABA-responsive cis elements. (A) ABA signaling, independent of Ca^{2+} (dotted arrows); (B) Ca^{2+} signaling induced by ABA (black arrows), or other stress induced factors, independent of ABA (white arrows), coinciding at ABRE-related cis-elements; (C) Stress-induced Ca^{2+} signaling acting at cis-elements different from ABREs (stripped arrows); Gray arrows: other signaling pathways not involving ABA or Ca^{2+}; Question marks indicate unknown signaling intermediates.

Bioinformatic analysis of the 5' upstream regions of 162 of the upregulated genes revealed a DNA element associated with Ca^{2+}-responsive upregulated gene expression ($p < 10^{-13}$). The sequence motif [C/A)ACG(T/C)G(T/G/C)] includes the ABRE consensus (ACGTG) and the ABRE-CE core (ACGCG), reported as a functional equivalent of the classical ABREs.[22] The analysis also showed that overrepresentation of the ABRE-related motifs occurred exclusively in the upregulated genes. Subsequently, to verify the bioinformatic predictions, a tetramer of the classical ABRE cis-element was tested and found sufficient to confer transcriptional activation in response to cytosolic Ca^{2+} transients, suggesting that ABREs function as Ca^{2+}-responsive cis-elements at least in some promoter combinations and Ca^{2+} signaling pathways. However, this does not imply that every ABRE-related promoter sequence is a Ca^{2+}-responsive cis-element, or that every cytosolic Ca^{2+} signal is transduced by an ABRE-related regulatory element. Ca^{2+} signals are also associated with the rapid oxidative burst leading to the formation of ROS,[23] which plays a role in both biotic and abiotic signaling.[24] In this context it is interesting to note that CaM antagonists (e.g., W7), which induced the cytosolic Ca^{2+} transients in the discussed manuscript, also induced rapid and transient ROS (Fluhr R, personal communication).

Therefore, the results in the discussed paper raise several questions; How Ca^{2+} signals are transduced to the transcription machinery at the ABRE cis-elements? Are these Ca^{2+} signals transduced directly to TFs containing EF-hands, or through TFs that respond to a Ca^{2+}-binding protein (e.g., CaM and CMLs), or through other Ca^{2+}-responsive proteins like kinases, or phosphatases? Do these Ca^{2+} transduction pathways involve components of ABA-signaling, and if not, how do these Ca^{2+}-transduction pathways interact with ABA-signaling pathways operating at the same DNA regulatory elements? How do these Ca^{2+} signaling pathways interact with ROS-signaling in relation to ABA responses? Are these Ca^{2+} signals transduced by downstream ROS signals? Figure1 depicts some of these possibilities and open questions.

Independently, a novel family of Ca^{2+}-dependent CaM-binding TFs, designated CAMTAs (or AtSRs) has been characterized in plants[12,25,26] and in other multicellular organisms.[27,28] In plants, these TFs may function as a link between Ca^{2+} signaling and ABRE-related cis-elements. The DNA-binding specificity of CAMTAs was shown to match both the ABRE-CE core sequence (ACGCGT/G/C) and the classical ABRE (ACGTGT), though with somewhat lower affinity[12] (Finkler A, Fromm H, unpublished results). Interestingly, Choi et al.[12] showed that CAMTA-dependent activation of gene expresssion via a synthetic CAMTA-binding site is inhibited by Ca^{2+}/CaM. Putative candidates for activation or repression by CAMTAs via ABRE-related cis-elements are members of the *DREB1* family.[29] Analysis of *DREB1* promoters revealed a number of motifs that correspond to classical ABRE (ACGTG), ABRE-CE motif (CCGCGT or ACGCGG), and ICEr1 motif (CACATG). *DREB1A, B* and *C*—all contain in their promoters the ABRE core sequence (ACGTG) with various flanking sequences. The variations in the flanking sequences may impose different affinities for CAMTA binding (Finkler A, Fromm H, unpublished results), with possible implications on fine- tuning of *DREB1* expression.

In summary, different approaches may reveal other Ca^{2+}-responsive cis-elements and Ca^{2+}-responsive TFs in plants. In this context, it should be mentioned that a CaM-binding MYB transcription factor was found to enhance salt tolerance in *Arabidopsis*,[11]

and *Arabidopsis* WRKY group IId TFs also bind CaM.[30] Thus, certain Ca^{2+}-transduction pathways in plants operate through CaM-binding TFs to modulate gene expression. We found that the Ca^{2+}/CaM-responsive CAMTAs bind to the same Ca^{2+}-responsive cis-elements that were identified in the discussed study, suggesting a link between Ca^{2+}-responsive TFs and ABRE-related cis-elements. This link merits further investigations in the context of ABA, ROS signaling and stress responses.

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