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A tidal wave of signals: calcium and ROS at the forefront of rapid systemic signaling

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Systemic signaling pathways enable multicellular organisms to prepare all of their tissues and cells to an upcoming challenge that may initially only be sensed by a few local cells. They are activated in plants in response to different stimuli including mechanical injury, pathogen infection, and abiotic stresses. Key to the mobilization of systemic signals in higher plants are cell-to-cell communication events that have thus far been mostly unstudied. The recent identification of systemically propagating calcium (Ca^{2+}) and reactive oxygen species (ROS) waves in plants has unraveled a new and exciting cell-to-cell communication pathway that, together with electric signals, could provide a working model demonstrating how plant cells transmit long-distance signals via cell-to-cell communication mechanisms. Here, we summarize recent findings on the ROS and Ca^{2+} waves and outline a possible model for their integration.

Cell-to-cell communication

The primordial leap(s) from unicellular to multicellular life initiated an exciting evolutionary race for a highly sophisticated and efficient cell-to-cell communication network. In plants, cell-to-cell communication occurs via at least three different routes: (i) symplastic – between different cells within the same tissue, or among tissues, that are cytoplasmically connected via plasmodesmata; (ii) vascular – between different groups of cells or tissues that are connected via the phloem or xylem vessel systems; and (iii) apoplastic – between adjacent cells within a tissue, or over longer distances such as between different tissues, but via the extracellular space. Such plant communication systems are also defined by their range. When different cells within the same tissue, for example a root branch or a leaf, communicate with each other it is generally referred to as local communication or local response, and when different cells within a specific tissue communicate with the entire plant it is typically referred to as systemic communication or systemic response.

Systemic responses in plants have been further divided based on their functions into: systemic acquired resistance (SAR) responses that are typically initiated by pathogens (e.g., virus, bacteria, or fungi), systemic wound responses that are activated by insects or mechanical injury; systemic acquired acclimation (SAA) that is triggered by abiotic stresses (e.g., high light, UV, heat, cold, salinity), systemic metabolic responses that are produced by changes in the level of sugars, phosphate, or other metabolites; and systemic developmental responses that are used to coordinate growth and development such as the control of stomatal distribution. The ultimate goal of these systemic signaling pathways is thought to be activating response mechanisms in remote systemic tissues, for example pathogen defenses during SAR or acclimation mechanisms during SAA. From an evolutionary point of view systemic signaling mechanisms improve the ability of the organism to prepare all of its tissues to an upcoming challenge that may initially only be sensed by a few local tissues or cells. Among the many different messengers that have been proposed to mediate cell-to-cell communication in plants are: electric signals, RNA molecules, different volatiles such as methyl salicylate and methyl jasmonate, different peptides and protein molecules, different metabolites such as salicylic acid (SA), jasmonic acid (JA) and azelaic acid, hormones such as auxin and abscisic acid (ABA), and redox and reactive oxygen species (ROS) [1–4].

Recent studies have highlighted the importance of rapid systemic responses for the acclimation of plants to abiotic stresses focusing on two known players of this complex network of cell-to-cell communication, namely the ROS wave and electric signals [2,5–7]. A new and

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mostly unexpected player, namely the calcium (Ca^{2+}) wave, now joins these elements of the systemic communication machinery [8]. In this paper we will attempt to integrate the findings from these different studies and propose a new model for rapid cell-to-cell communication in plants.

The ROS wave and its biological role

Perhaps among the most evolutionary-conserved of messengers, ROS are used for cell-to-cell communication by unicellular and multicellular organisms. Because ROS can be charged (e.g., superoxide radical), uncharged (e.g., hydrogen peroxide), or lipophilic (e.g., lipid peroxides), they can be confined to certain subcellular compartments or easily travel through membranes. In addition, because their levels are controlled by a delicate balance between their production and scavenging they do not need to be stored in a particular compartment and could be rapidly generated and/or removed anywhere in the cell or the apoplast. For example, a burst of ROS production is initiated in plant or animal cells in response to many different biotic or abiotic stimuli, and ROS produced during this burst by respiratory burst homolog (RBOH or NOX) proteins was shown to diffuse from the site of production to adjacent cells in plants and animals [9,10]. These ROS have an important biological role in the activation of local defense or acclimation mechanisms.

Although certain ROS such as hydrogen peroxide are stable and can diffuse over long distances in different tissues, they are sensitive to ROS-scavenging enzymes such as peroxidases and catalases that are abundant in plant tissues. This ubiquitous scavenging activity led to the idea that ROS are not able to diffuse over long distances in plants and so are unlikely to represent a mobile element of systemic communication. Recently, however, a new mechanism for rapid, long-distance cell-to-cell communication utilizing ROS was described [5–7]. Application of different abiotic stresses such as high light, heat, salinity, cold, or mechanical injury to a particular tissue was found to initiate an enhanced production of ROS in the affected local tissue, as well as to trigger a systemic autopropagating wave of ROS production that traveled from the affected tissue to the entire plant at a maximal rate of approximately 8.4 cm/min (Figure 1A,B). This process was dependent on the presence of the respiratory burst homolog protein RBOHD (a superoxide-generating NADPH oxidase) and was accompanied by the accumulation of hydrogen peroxide in the apoplast of cells along the systemic path of the signal. The autopropagating nature of the signal suggested that each cell along its path activated its own RBOHD enzymes and generated ROS capable of triggering all adjacent cells to undergo the same process. This mechanism appeared to resemble the ROS-induced ROS-release (RIRR) response used to communicate between individual mitochondria in human muscle cells [11,12]. Because each cell along the path of the systemic signal actively produced ROS, the chances of this ROS being scavenged by, for example, peroxidases in the apoplast were lower. Thus, ROS such as hydrogen peroxide produced by the dismutation of the superoxide radical generated by RBOHD could now be used as a long-distance signal. External application of ROS failed however to trigger the ROS wave indicating that other mechanisms could be involved in its propagation (see below).

Recent functional analysis of the ROS wave demonstrated that it was required for the activation of SAA in systemic tissues of plants in response to local application of high light or heat stresses, indicating that the ROS wave has an important biological function in the acclimation response of plants to abiotic stresses [7]. Interestingly, although the ROS wave was required for the induction of SAA in systemic tissues in response to different stresses, it could not convey abiotic stress specificity to this response. This observation suggests that the main function of the ROS wave is to prime the systemic tissue for SAA activation and that other more abiotic stress-specific signals (e.g., signals specifying high light-, heat-, or wounding-related signals) are involved in mediating a stress-specific SAA. The ROS wave could therefore be viewed as an engine or an essential component of the signaling network that mediates the SAA of plants to abiotic stresses. At least during systemic responses to heat stress, the ROS wave was found to function in coordination with ABA in systemic tissues [7].

The recent functional analysis of the ROS wave has also identified an interesting link between ROS production by RBOH proteins and electric signals. Electric signals in plants have been known for many years. They manifest as changes in membrane potentials or electric currents that can rapidly travel for long distances in plants and were recently demonstrated to have an important role in the SAA of plants to high light stress [13]. It was found that, in mutants lacking the RBOHD protein required for the ROS wave, at least one type of systemic electric signal was compromised [7]. This link between the ROS wave and systemic electric signals further suggested that the ROS wave is essential for the propagation of rapid systemic signals in plants and that RBOH proteins could be viewed as the engines of systemic signaling. One of the most important aspects of RBOH proteins is that they link Ca^{2+} signaling with ROS signaling. A recent study has indeed demonstrated that Ca^{2+}-dependent protein kinase 5 (CPK5) is important for the propagation of the ROS wave in plants [14].

The Ca^{2+} wave and its biological role

As with ROS, changes in cytosolic Ca^{2+} are ubiquitous signals in biological systems [15] and long-range, systemic signals linked to Ca^{2+} changes have long been hinted at in the literature. For example, transport of hormones in the vasculature has been proposed to trigger Ca^{2+} changes at distant sites. Thus, ABA produced by the roots in response to water stress is thought to accumulate in the leaves, eliciting Ca^{2+}-dependent signaling responses in guard cells [16]. Likewise, Ca^{2+} itself being transported in the transpiration stream has been proposed to accumulate in the leaves, again triggering Ca^{2+}-dependent signaling cascades, in this case related to the Ca^{2+}-sensing receptor (CAS) sensory system of the plastid [17]. In tobacco transient increases in the concentration of cytosolic Ca^{2+} in the aerial parts of the plant have been reported to occur within 5 min of localized cold shock to the root system [18]. Similarly, in bean wounding of the tip of a leaf led to an

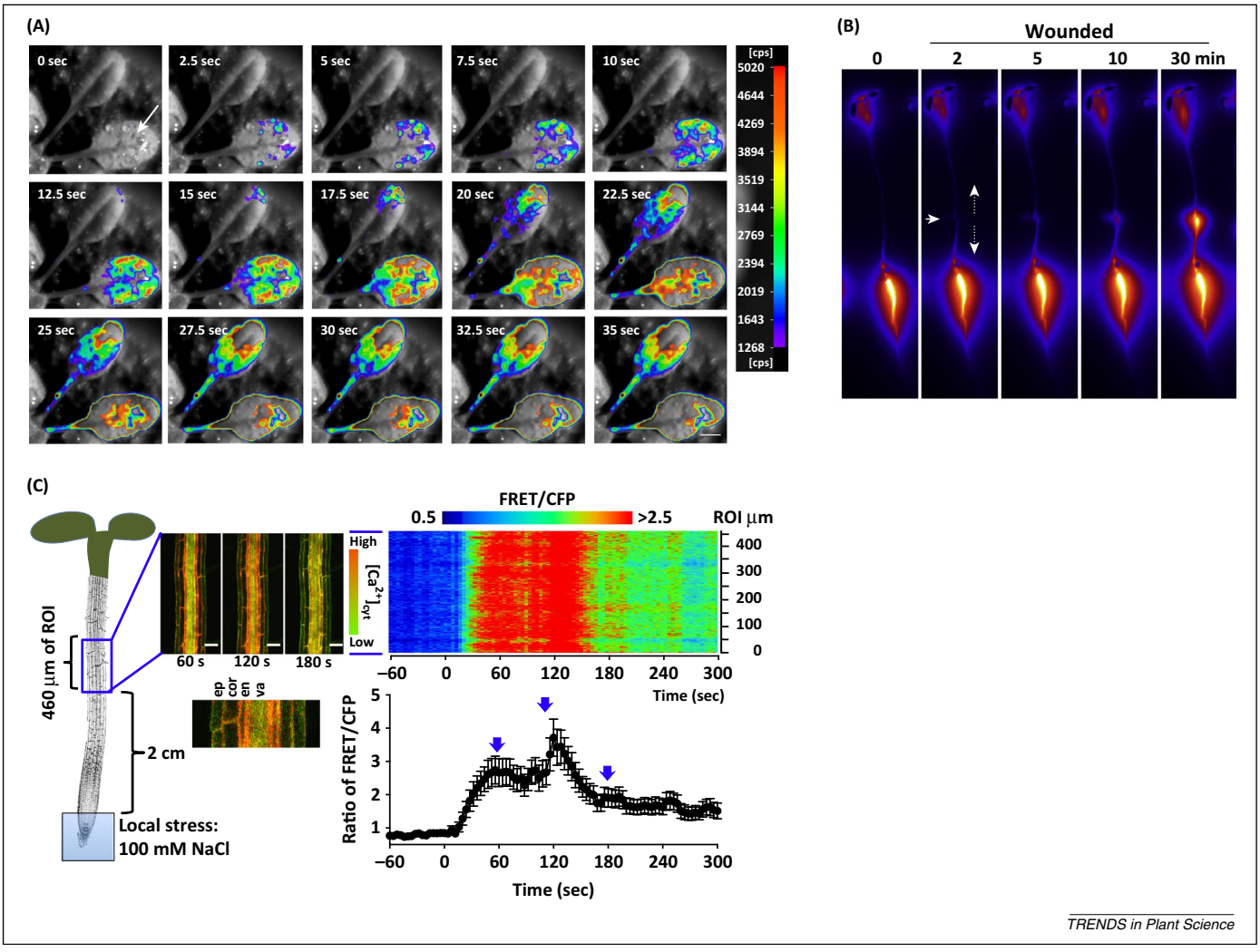

Figure 1. The reactive oxygen species (ROS) and calcium (Ca^{2+}) waves. **(A)** Time-lapse video imaging of the ROS wave using a Zat12:luc reporter gene showing the spread of the ROS wave from an injured *Arabidopsis thaliana* leaf (arrow) to an adjacent systemic leaf. Experiment was performed as described in [5]. **(B)** Wound-induced ROS wave in an *Arabidopsis* seedling stained with the H_2O_2-specific dye Amplex-red© showing the local and systemic development of the ROS signal following injury (solid arrow). Experiment was performed as described in [5] using a 10-day-old *Arabidopsis* seedling germinated in the dark for 5 days to produce a long hypocotyl. **(C)** Transmission of the Ca^{2+} wave in response to local salt stress. Experiment performed as described in [8]. Propagation of a tissue-specific Ca^{2+} wave through cortical and endodermal cells generated in response to 100 mM NaCl applied to ~50 µm of the root tip. Representative images are shown for 60 s, 120 s, and 180 s (blue arrows in the graph) following local salt stress in the root tip. Ca^{2+} was monitored using plants expressing the green fluorescent protein (GFP)-based biosensor YCNano65. For this sensor, an increase in the ratio of fluorescence resonance energy transfer (FRET) signal:cyan fluorescent protein (CFP) signal reflects an increase in Ca^{2+} levels. Scale bars = 50 µm. Data represent mean ± S.D., $n = 18$. Abbreviations: cor, cortex; en, endodermis; ep, epidermis; ROI, region of interest; va, vasculature.

increase in the cytosolic Ca^{2+} concentration in the vasculature of the same leaf but centimeters distant from the site of damage [19]. In this case, the increase in Ca^{2+} concentration was correlated with the localization of Ca^{2+} channels, leading to a model where a propagating signal elicited by the wound led to the gating of Ca^{2+} channels and so to the distant Ca^{2+} signal.

Thus, there is strong evidence for stress signals triggering Ca^{2+}-dependent events in distant tissues. However, precisely how the site of perception and response are coupled and the possible relationship between the ROS wave and these Ca^{2+}-dependent events remains largely undefined.

It is thought that information about the nature of stimuli perceived by plant cells may well be encoded in the spatial and temporal dynamics of cellular Ca^{2+} changes, the so-called Ca^{2+} signature of the stimulus [15]. Decoding of these Ca^{2+} signatures would then lead to stimulus-specific cellular responses. Consistent with such ideas, the frequency of transients in cytosolic Ca^{2+} appears important for signaling in stomatal guard cells [16,20] and Nod factors elicit symbiosis-related genes in *Medicago truncatula* but only after the target cells have accumulated 36 spikes in nuclear Ca^{2+} concentration [21]. Biochemical mechanisms for responding to specific frequencies of Ca^{2+} transients have also been reported, such as the fine-tuned regulation of the kinase activity of mammalian Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) by the frequency of cellular Ca^{2+} transients [22]. At the transcriptional level of response, the authors of this study [23] applied electrical stimulation to generate Ca^{2+} changes in specific patterns in *Arabidopsis thaliana* and then used microarray analyses to follow transcriptional responses. Specific patterns of Ca^{2+} change were observed to be linked to specific and unique transcriptional changes. In addition, this analysis led to the identification of a common Ca^{2+}-response element (CARE) in several of the promoters of the upregulated genes. This CARE had already been discovered in another guise, as an ABA response element (ABRE), providing a strong

candidate for a mechanistic link between ABA and Ca^{2+} signaling. However, these kinds of cellular reactions to specific Ca^{2+} signatures are generally discussed in the context of local stimulus response. Any role these systems might have in the systemic transmission of information about specific stimuli remains undefined.

There are good candidates for some of the local triggers of these kinds of stress-related Ca^{2+}-dependent responses. For example, damage to cells is thought to cause ATP to leak to the extracellular space where it triggers an increase in cytosolic Ca^{2+} concentration in adjacent cells, very likely through the recently defined Does not Respond to Nucleotides 1 (DORN1) receptor kinase perception system [24]. However, as noted below for ROS, long-distance signaling is too fast to be supported by diffusion of a chemical messenger such as eATP. In *Arabidopsis*, wounding leads to rapid (within minutes) increases in the defense hormone jasmonic acid in distant leaves, requiring a signal that moves at least at 450–700 μm/s [25,26]. The wound signal also correlates with a rapidly propagating membrane potential change that is dependent on the glutamate-receptor-like (GLR) channels: GLR3.2, GLR3.3, and GLR3.6 [27]. The involvement of such channels provides one hint as to a possible mechanism for rapid signal propagation. Knockouts in members of the GLR family of channels have been shown to attenuate amino acid induced increases in cytosolic Ca^{2+} in *Arabidopsis*, for example [28,29], and heterologous expression of the plant GLR3.4 has definitively shown that, for this particular channel at least, these genes encode *bona fide* Ca^{2+}-permeable channels [30]. Thus, Ca^{2+} fluxes themselves may well be components of the machinery of long-distance signal propagation.

Recent measurements of plant-wide dynamics of changes in cytosolic Ca^{2+} concentrations in response to localized stress application strongly support the idea of systemic Ca^{2+}-dependent signal propagation. Thus, advances in the green fluorescent protein (GFP)-based bioreporters available for the imaging of Ca^{2+} signals [31] have allowed a much higher sensitivity analysis of Ca^{2+} changes than previously available in plants. Such measurements have revealed that, in response to local stimulation such as salt stress in the root tip, a wave of increased cytosolic Ca^{2+} level does in fact move systemically through the plant, paralleling the mobile, systemic nature of the ROS wave (Figure 1C) [8]. The Ca^{2+} wave travels at ~400 μm/s and can spread throughout the root system and be transmitted to the aerial parts of the plant. Blocking the wave with the Ca^{2+} channel blocker lanthanum (La^{3+}) not only inhibited transmission of the Ca^{2+} wave but also blocked systemic induction of ROS-regulated transcriptional markers such as *ZAT12*, strongly implying that these two systemic signaling systems, the ROS- and the Ca^{2+}-wave, are closely linked. The Ca^{2+} wave is limited to rapid transmission through the cortex and endodermis in the root, indicating a cell type specific machinery specialized for the funneling of this information through the plant. In addition, knocking out two pore channel (TPC1), a vacuolar ion channel, blocks the propagation of the systemic Ca^{2+} wave. TPC1 is a cation-permeable channel (including Ca^{2+}) and has been proposed to act in plant CICR [32], although this idea remains controversial [29]. Channel activity of TPC1 is sensitive to cytosolic and vacuolar Ca^{2+} levels [33] suggesting vacuolar and cytosolic Ca^{2+} dynamics could have an important role in modulating channel activity and so sustaining the wave. Interestingly, TPC1 has been shown to be sensitive to ROS (H_2O_2) in patch-clamp experiments [34]. This observation provides one mechanism for a close mechanistic link between Ca^{2+} and ROS waves. However, as with the ROS wave, local application of H_2O_2 could not trigger a self-propagating Ca^{2+} wave, implying further components of the wave-generation machinery have yet to be defined.

Paralleling the links between ROS and systemic electric signaling, a relationship between Ca^{2+} and electric signaling is also well supported in the literature. Ca^{2+}-driven action potentials are seen in animals and plants, where Ca^{2+} can have a direct or indirect role in the electric changes. For example, Ca^{2+}-dependent gating of plasma membrane ion channels can regulate the propagation of the membrane potential changes underlying plant action potentials [35]. More rarely, the Ca^{2+} fluxes themselves can drive these potential changes, as recently reported for the touch-induced propagating action potential that is supported by the mammalian piezo-type mechanosensitive ion channel component 2 (PIEZO2) Ca^{2+} channel [36]. Systemic Ca^{2+} release has also been closely linked with propagating signals through electric phenomena such as wave potentials [18]. If similar events underlie the propagating Ca^{2+} signals described above, electric and Ca^{2+} signals would be inextricably linked.

How are Ca^{2+} and ROS signaling integrated in cells?

At least two different functional processes could link Ca^{2+} and ROS signaling in plant cells: Ca^{2+}-induced ROS-production (CIRP) and ROS-induced Ca^{2+}-release (RICR). CIRP could be mediated by different ROS-producing mechanisms with RBOH proteins being the most studied to date. These superoxide-generating NADPH oxidases are primarily thought to be localized on the plasma membrane (PM), but could potentially also be found on additional cellular membranes such as the mitochondria [37]. RBOH proteins were shown to be activated by Ca^{2+} via several different routes including direct binding of Ca^{2+} to the EF-hand motifs on the N terminus of RBOH proteins, Ca^{2+}-induced phosphorylation of RBOH by kinases such as CPK5, calcineurin B-interacting protein kinase 26 (CIPK26) or botrytis-induced kinase 1 (BIK1), binding of plant Rho-type (ROP)-GTPase, and Ca^{2+}-induced accumulation of phosphatidic acid (PA) that binds to different motifs at the N terminus of RBOH (Figure 2) [9,38–40].

RICR by contrast could be mediated by a direct ROS-induced activation or suppression of a Ca^{2+} channel or pump. Indeed, ROS have been shown to activate hyperpolarization-activated Ca^{2+}-permeable channels in root cells [41–43], as well as Ca^{2+}-influx channels in stomates [20,44] and *Fucus* rhizoids [45]. At a molecular or genetic level, the plant Ca^{2+}-permeable Stelar K^+ outward rectifier (SKOR) channel [46] and the Ca^{2+} conductance(s) involving annexin1 [47] have been shown to be responsive to H_2O_2. Alternatively, ROS-induced secondary messengers such as cyclic nucleotides could trigger Ca^{2+} release from different channels, for example, cyclic-nucleotide gated channels (CNGCs).

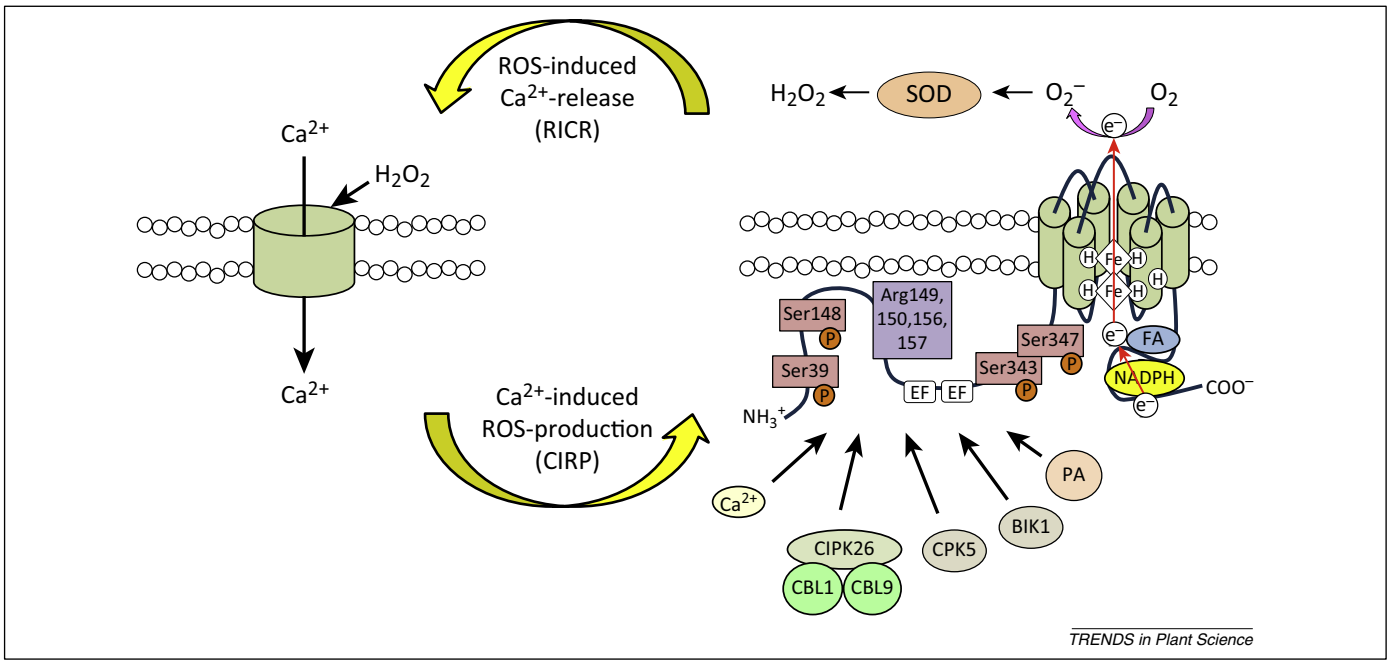

Figure 2. Integration of calcium (Ca^{2+}) and reactive oxygen species (ROS) signaling in plant cells. (Left) ROS can activate or suppress calcium channels resulting in the formation of ROS-induced calcium signatures in a process termed ROS-induced calcium release (RICR). (Right) Ca^{2+} can directly or indirectly regulate the production of ROS by respiratory burst homolog (RBOH) proteins resulting in the generation of superoxide radicals that are dismutated to H_2O_2 spontaneously or via superoxide dismutase (SOD). This process is termed calcium-induced ROS production (CIRP). The two processes depicted on the left and right of the figure (RICR and CIRP) generate different regulatory circuits with feedback function in cells. These are involved in controlling many different processes including development, response to biotic and abiotic stimuli, and the Ca^{2+} and ROS waves. Abbreviations: CBL1, calcineurin B-like protein 1; CBL9, calcineurin B-like protine 9; CIPK26, calcineurin B-like interacting protein 26; CPK5, calmodulin domain protein kinase 5; BIK1, botrytis-induced kinase 1; FAD, flavin adenine dinucleotide; NAD, nicotinamide adenine dinucleotide; PA, phosphatidic acid.

What is the relationship between the Ca^{2+} and the ROS waves and how are they integrated?

Plant tissues present a number of barriers for the transport of signals. The apoplast for example contains high levels of Ca^{2+} and is not a suitable media for the mediation of Ca^{2+} signals. The apoplast environment also contains a number of different redox and ROS production and removal mechanisms including superoxide dismutases, peroxidases, different oxidases, glutathione, ascorbic acid, and other enzymes of the ROS gene network [48]. ROS signals can accumulate in the apoplast but to keep them from being attenuated by ROS-scavenging mechanisms, their production needs to be constantly turned on. The plasma membrane itself is a very suitable media for membrane-potential-related electrical signals but, due to its sensitivity to lipid peroxidation, it is not the best media for the transport of ROS signals, and of course Ca^{2+} that is hydrophilic cannot travel long distances within membranes. In contrast to the apoplast, the cytosol is a great media for Ca^{2+} signals because Ca^{2+} levels in the cytosol are kept to a minimum. Manipulating ROS levels in the cytosol is however a different story because of the sensitivity of many cytosolic and nuclear systems for oxidative damage. Travel of long-distance systemic signals through plant tissues could therefore be mediated at least in part in the apoplast for ROS, the PM for electric signals, and the cytosol for Ca^{2+}, as well as for short distances for ROS (Figure 3A). But how do these three signals interact with each other?

A model that could integrate the ROS and Ca^{2+} waves is outlined in Figure 3B. Local sensing of abiotic stress triggers Ca^{2+} accumulation within the initiating cells at the local tissue. The enhanced levels of Ca^{2+} in these cells triggers enhanced ROS production by RBOH proteins via CPK5, CBL1/9-CPK26, or other Ca^{2+}–RBOH interactions (Figure 2). Additional CIRP mechanisms may also be involved in this process. Once RBOH proteins are activated in the initial cell(s), they cause the accumulation of ROS in the apoplast, which is in turn transported into neighboring cells via aquaporins or other channels. Two possible mechanism may then ensue: (i) apoplastic ROS entering adjacent cells may trigger RICR via TPC1, annexins, or other mechanisms, such as ROS-activated PM Ca^{2+} channels; and/or (ii) elevated cytosolic Ca^{2+} levels from the initiating cells transported via plasmodesmata to adjacent cell(s) may trigger CICR by TPC1 or other Ca^{2+} channels. The resulting enhanced cytosolic Ca^{2+} levels in the adjacent cells would then trigger the RBOH proteins of these cells. This process of Ca^{2+} inducing ROS production that induces Ca^{2+} release would then continue in a cell-to-cell autopropagation manner. The model presented in Figure 3B integrates the ROS and Ca^{2+} waves into one long-distance systemic signaling mechanism. The finding that the initiation of the ROS or Ca^{2+} waves can be blocked by inhibitors of Ca^{2+} signaling such as La^{3+} and that the propagation of the Ca^{2+} wave, as well as the induction of SAA in systemic tissues, requires the function of RBOH proteins supports the validity of the model proposed in Figure 3B.

A possible failing of the model presented in Figure 3B is the rate at which the systemic ROS and Ca^{2+} waves propagate. Rates of >400 μm/s cannot be explained by simple diffusion of ROS or Ca^{2+} over long distances (Figure 4A), especially given the active buffering and

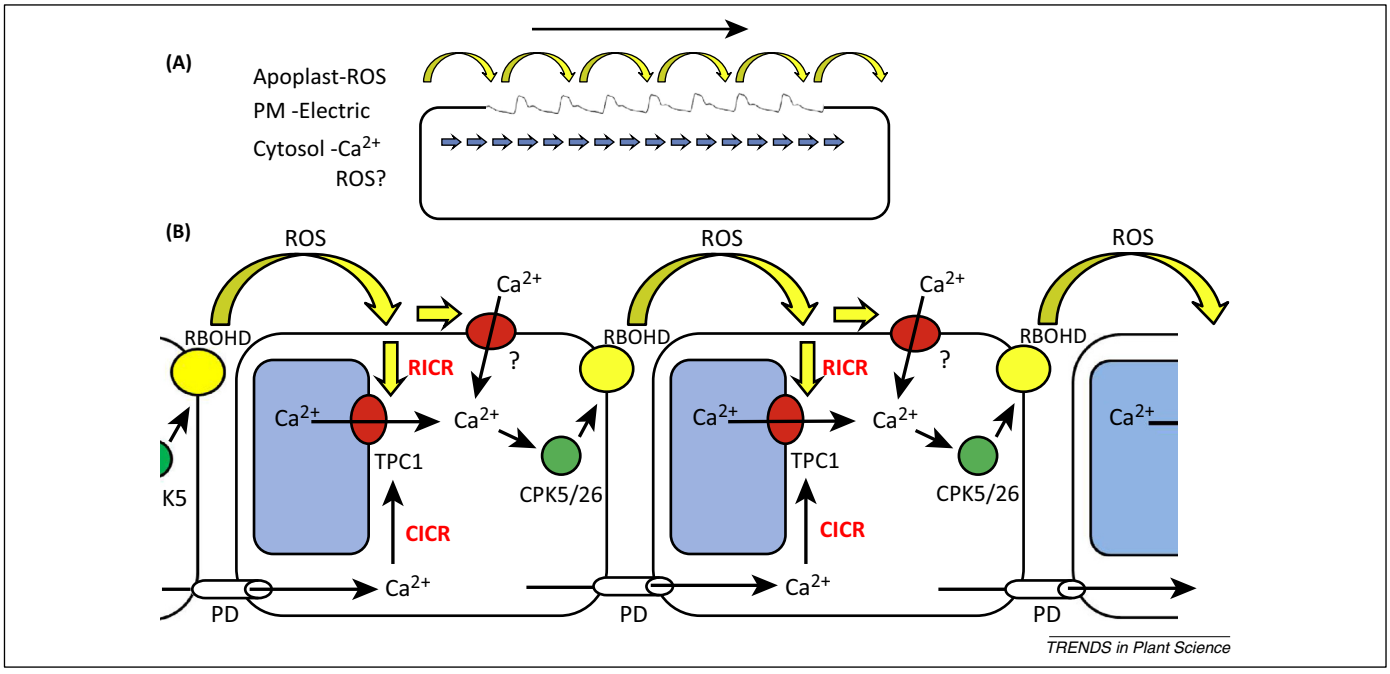

Figure 3. Cellular pathways and a model for the integration of the reactive oxygen species (ROS) and calcium (Ca^{2+}) waves. **(A)** Possible cellular routs for mediating electric signals and the ROS/Ca^{2+} waves in plant cells. **(B)** Integration of the ROS and Ca^{2+} waves in cells via the function of respiratory burst homolog (RBOH) proteins (yellow), Ca^{2+}-dependent protein kinases such as calcium-dependent protein kinase (CPK)5/26 (green), and calcium channels such as two pore channel (TPC)1 (red). The vacuole is depicted in light blue. Abbreviations: PD, plasmodesmata; PM, plasma membrane; CICR, calcium-induced calcium release; RICR, ROS-induced calcium release.

sequestering systems that can limit the long-range movement of Ca^{2+} increases through the cytoplasm and ROS in the apoplast. To account for such speeds an active process of ROS or Ca^{2+} transport should be involved in mediating the ROS and Ca^{2+} waves across the length of the cell. Several different options are outlined in Figure 4. Structured arrays of RBOH proteins coupled with their Ca^{2+}-regulatory proteins may be arranged in rows along

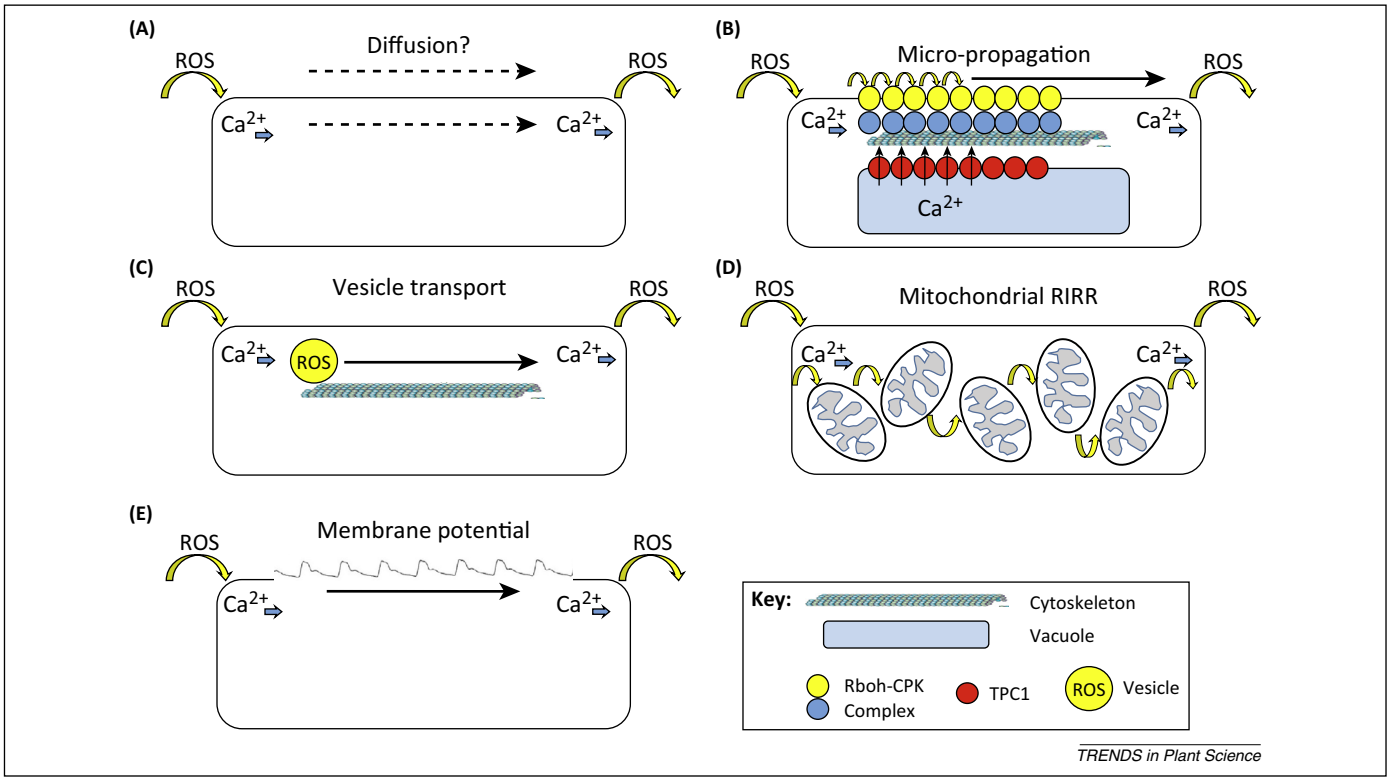

Figure 4. How can we explain the rate of the reactive oxygen species (ROS)/calcium (Ca^{2+}) waves? **(A)** The rates of the ROS/Ca^{2+} waves (>400 μm/s) cannot be explained by simple diffusion along the axis of the cell. **(B)** Structured arrays of respiratory burst homolog (RBOH) proteins together with their Ca^{2+}/phosphorylation modules may be mediating the signals via rapid micro-propagation. **(C)** ROS can be packed into vesicles that are transported along the cellular cytoskeleton routes from one end of the cell to the other. **(D)** A process of ROS-induced ROS-release may mediate the signals between different organelles in the cells. **(E)** Electric signals may mediate the signal from one polar region of the cell to the other. Abbreviations: CPK, calcium-dependent protein kinase; TPC1, two pore channel 1.

cells mediating the rapid movement of signal across the cell from complex to complex (Figure 4B). ROS and/or Ca^{2+} may be packed in vesicles that are actively transported along the cytoskeleton from one side of the cell to the other (Figure 4C). A process of RIRR may occur between organelles such as mitochondria or chloroplasts similar to the rapid RIRR mode of communication between mitochondria of human cells [11,12] (Figure 4D). In addition, perception of the ROS/Ca^{2+} wave on one side of the cell may trigger electrical signals that would propagate at a rapid rate across the PM from one side of the cell to the other (Figure 4E). The finding that systemic voltage potentials are dependent on the presence of RBOH proteins [7] supports the latter model (Figure 4E) and suggests a new and more inclusive model in which ROS, Ca^{2+}, and electric signals are integrated to mediate rapid systemic signals. In this model the processes described in Figure 3B will function at the junctions between cells to link the ROS and Ca^{2+} waves, and electric signals will connect one polar side of the cell with the other (Figure 4E). This model based around the interaction of electric signals and the ROS/Ca^{2+} networks would help address a major issue with the other models as to whether diffusion of chemical messengers within or between cells could account for the rapid movement rate of the systemic signal (Figure 4E). Of course further studies are required to elucidate the precise mechanism that mediates rapid systemic signals in plants, but at present the model shown in Figure 4E appears to provide the most sensible explanation for the rate of the ROS and Ca^{2+} waves.

Concluding remarks and future perspectives

Additional interesting questions that arise from these new findings of wave-based systemic signaling include the following. (i) How are the two waves initiated? Is it an initial burst of ROS or a rise in cytosolic Ca^{2+} that is initially triggered in the local cells? (ii) How are the Ca^{2+} and ROS waves perceived at the target tissue? What are the decoding mechanisms that perceive the signals and trigger defense or acclimation mechanisms? At least with respect to the ROS wave it was found that ABA function is needed to induce SAA in systemic tissues upon perceiving the ROS wave and that ABA accumulation in the systemic tissue was dependent on the ROS wave [7]. Of course other plant hormones such as auxins, jasmonates, and ethylene could be involved in this process. (iii) What is the role of plasmodesmata in the propagation of the ROS and Ca^{2+} waves? Although little is known about the transfer of Ca^{2+}, ROS, or redox equivalents through these highly regulated pores, the possibility that they are involved in propagating long-distance systemic signals should be studied. (iv) Why is hydrogen peroxide application to plant tissues not enough to trigger the ROS and/or Ca^{2+} waves? It is possible that accumulation of ROS and Ca^{2+} is required, as well as crosstalk with other cellular signals. The need for specificity in rapid systemic signaling may be the answer here because only when a signal comes in its biological context are the ROS and/or Ca^{2+} waves initiated. Thus, simply applying hydrogen peroxide without a biological context cannot trigger SAA. (v) What was the evolutionary path that led to the formation of the ROS and Ca^{2+} waves? Did they co-evolve? Perhaps evolutionary studies of the RBOH, CPK, and the TPC1 gene families, as well as additional proteins involved in the Ca^{2+} and ROS networks, will answer this question. (vi) What is the relationship of the Ca^{2+} and ROS waves with electrical signals? It is known that ROS accumulation at the surface of the PM could cause membrane depolarization, but are there specific channels, such as glutamate-receptor-like channels, that regulate electric signals in response to changes in ROS and/or Ca^{2+} levels? Future studies may answer some or all of these questions.

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