The role of DMI1 in establishing \( \text{Ca}^{2+} \) oscillations in legume symbioses

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Abbreviations: SYM, common symbiosis; ER, endoplasmic reticulum; DMI1, Does Not Make Infections1; MCA8, Medicago truncatula Serca Type \( \text{Ca}^{2+} \) ATPase; MthK, \( \text{Ca}^{2+} \)-gated K\(^+\) channel from Methanobacterium autotrophicum; HEK, human embryonic kidney

Calcium (\( \text{Ca}^{2+} \)) is a key secondary messenger in many plant signaling pathways. One such pathway is the SYM pathway, required in the establishment of both arbuscular mycorrhizal and rhizobial root symbioses with legume host plants. When the host plant has perceived the diffusible signals from the microbial symbionts, one of the earliest physiological responses are \( \text{Ca}^{2+} \) oscillations in and around the nucleus. These oscillations are essential for activating downstream gene expression, but the precise mechanisms of encoding and decoding the \( \text{Ca}^{2+} \) signals are unclear and still under intense investigation. Here we put forward a hypothesis for the mechanism of the cation channel DMI1.

In a recent study we presented a mathematical model based on three key components involved in the production of the \( \text{Ca}^{2+} \) oscillations in the SYM pathway: a cation channel, a \( \text{Ca}^{2+} \) pump and an an as yet unidentified \( \text{Ca}^{2+} \) channel. The components involved in the model are located on the inner nuclear membrane and therefore the model hypothesizes that the lumen of the nuclear envelope contiguous with the ER is the \( \text{Ca}^{2+} \) store. The model also included \( \text{Ca}^{2+} \)-binding proteins, so called \( \text{Ca}^{2+} \) buffers, which can be any \( \text{Ca}^{2+} \)-binding proteins such as calmodulin, \( \text{Ca}^{2+} \)/calmodulin dependent protein kinase or \( \text{Ca}^{2+} \) reporters used to measure \( \text{Ca}^{2+} \) levels in biological systems. Our study revealed that varying the binding characteristics and concentrations of the buffers can affect both \( \text{Ca}^{2+} \) spike shapes and period lengths, induce rapid \( \text{Ca}^{2+} \) spiking and can initiate and terminate \( \text{Ca}^{2+} \) oscillations. Overall, the mathematical model strikingly reproduces the \( \text{Ca}^{2+} \) oscillations observed experimentally and suggests that buffering capacity, so far unexplored in this system, can explain changes in shape and frequency of the nuclear localized \( \text{Ca}^{2+} \) oscillations in the SYM pathway.

One of the three components used to generate the mathematical model is the cation channel, named DMI1 in Medicago truncatula, which has been genetically identified and characterized. Although DMI1 is essential for generating nuclear \( \text{Ca}^{2+} \) oscillations, its role is currently not well understood and prone to speculation. Upon activation by symbiotic secondary messengers, DMI1 could counter-balance the flow of positive charges generated by the simultaneous activation of a yet to be unidentified \( \text{Ca}^{2+} \) channel. Alternatively, the activation of DMI1 could directly trigger the opening of a voltage-gated \( \text{Ca}^{2+} \) channel by hyperpolarizing the nuclear membrane potential. These two hypotheses are experimentally unresolved, but we could use our mathematical model, that successfully recapitulates the experimental \( \text{Ca}^{2+} \) oscillations, to test the potential functions of DMI1.

As explained in our recent study, we envisage the membrane potential of the nuclear envelope to be negatively charged on the nucleoplasmic side relative to the perinuclear space. In this context our mathematical model reveals that \( \text{Ca}^{2+} \) spiking is only initiated upon the simultaneous activation of both DMI1 and the \( \text{Ca}^{2+} \) channel; we hypothesize that this may be triggered by unidentified symbiotic secondary messengers. Once both channels have opened, the generation and sustainability of the \( \text{Ca}^{2+} \) oscillation is dependent on the interplay between the key players DMI1 and the \( \text{Ca}^{2+} \) channel via their respective \( \text{K}^{+} \) and \( \text{Ca}^{2+} \) electrochemical driving force and their conductance (Fig. 1). At the start of a spike (Fig. 1A and B) the membrane potential is negative and close to the \( \text{K}^{+} \) resting potential, such that DMI1 is weakly conducting (Fig. 1C, position 1). This negative membrane potential drives a \( \text{Ca}^{2+} \) current into the nucleus (Fig. 1D, position 1). The membrane potential depolarizes until it reaches the resting potential of \( \text{Ca}^{2+} \) and consequently stops the transient
Ca\(^{2+}\) current (Fig. 1C and D, position 1). This first step has two important consequences: the localized release of Ca\(^{2+}\) increases the opening of DMI1, and the positive membrane potential increases the K\(^{+}\) electrochemical driving force.

Subsequently the current generated by DMI1 slowly increases and hyperpolarizes the membrane potential, as K\(^{+}\) flows into the perinuclear space (Fig. 1C and D, position 2). This hyperpolarization generates a Ca\(^{2+}\) current (Fig. 1D, position 2-3) leading to the Ca\(^{2+}\) release which shapes the upward slope of a Ca\(^{2+}\) spike (Fig. 1C and D, position 2-3). As soon as the membrane potential reaches the resting potential of K\(^{+}\), the DMI1 current and the Ca\(^{2+}\) channel current almost cease (Fig. 1D, position 3-4).
The Ca\(^{2+}\) is pumped back into the store by Ca\(^{2+}\)-ATPases such as MCA8,\(^2\) decreasing the nucleoplasmic Ca\(^{2+}\) concentration which shapes the downward slope of a Ca\(^{2+}\) spike (Fig. 1B, position 4). Although both channels are weakly conducting, the electrochemical driving force of the Ca\(^{2+}\) channel is very strong (Fig. 1E, position 4). As soon as the membrane potential returns to the initial value, the conductance of the depolarization-activated Ca\(^{2+}\) channel increases again leading to a release of Ca\(^{2+}\). The Ca\(^{2+}\) release increases DMI1 conductance and subsequently the DMI1 current hyperpolarizes the membrane; the cycle starts and repeats to generate a Ca\(^{2+}\) oscillation.

Overall, the mathematical model suggests that DMI1 acts predominantly as a counter ion channel, but importantly initial conductance from DMI1 is necessary to facilitate calcium flow. In this regard DMI1 function is neither purely a counter ion balance, nor purely an activator of the calcium channel. Rather the interplay of DMI1 and the calcium channel together derive the oscillatory calcium behavior. The model predicts that a key parameter of DMI1 action is modulation by Ca\(^{2+}\) in order to sustain an oscillatory mechanism. Indeed the increased K\(^+\) current generated by DMI1 synchronously triggers the Ca\(^{2+}\) release (Fig. 1E) and can be obtained by direct positive feedback of Ca\(^{2+}\) on DMI1 conductance. This positive feedback could potentially occur by direct binding of Ca\(^{2+}\) to DMI1. In agreement with this hypothesis, a previous study suggested the presence of Ca\(^{2+}\)-binding pockets in the C-terminal region of DMI1 based on homology modeling with its closest structural relative MthK.\(^10\) In addition, Venkateshwaran and colleagues recently demonstrated that expression of DMI1 in HEK cells is sufficient to activate Ca\(^{2+}\) induced Ca\(^{2+}\) release mechanism upon Ca\(^{2+}\) stimulation. Therefore a positive Ca\(^{2+}\) feedback could explain the oscillatory mechanism but also the sustainability of the Ca\(^{2+}\) oscillation. Indeed, the oscillation is sustained until the buffering capacity of the cell breaks the positive Ca\(^{2+}\) feedback. Ca\(^{2+}\) could be buffered such that the Ca\(^{2+}\) concentration is no longer sufficient to modulate DMI1 conductance and would therefore terminate the oscillation.\(^3\) The positive feedback mechanism of Ca\(^{2+}\) on DMI1 conductance however has yet to be experimentally validated.

A recent study suggested that the role of DMI1 is to activate a yet unidentified voltage-gated Ca\(^{2+}\) channel by hyperpolarizing the nuclear membrane potential, and that its ability to do so depends on the length of DMI1 open time.\(^5\) We challenged our model with these hypotheses and could only generate one Ca\(^{2+}\) spike but no Ca\(^{2+}\) oscillations. In this hypothetical scenario, a sustainable Ca\(^{2+}\) oscillation would only be obtained if the concentration of the secondary messenger activating DMI1 would oscillate synchronously with the Ca\(^{2+}\) transient. This speculation brings an additional level of complexity to the generation of the Ca\(^{2+}\) oscillation, which has never been reported so far in any Ca\(^{2+}\) induced Ca\(^{2+}\) release mechanism.

In summary, our model suggests that DMI1 plays a key role as a counter ion channel in the generation of Ca\(^{2+}\) oscillations. However, this counter ion channel function is intrinsic to the generation of the calcium current and initial K\(^+\) movement facilitates the first steps of calcium release. Without the K\(^+\) current no Ca\(^{2+}\) oscillation can be sustained. To validate this mathematical model, future experimental work is required, notably to identify the symbiotic secondary messengers activating the channels, define the effect of calcium on DMI1 function and to characterize the missing player, the Ca\(^{2+}\) channel.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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