Update on Nuclear Calcium Signaling

Nuclear Calcium Signaling in Plants[1]

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An astonishing variety of plant and animal cellular functions are coordinated by intracellular calcium (Ca^{2+}; Berridge et al., 2000; White and Broadley, 2003). Although Ca^{2+} is a common second messenger, it is translated into specific developmental processes, implying specificity of recognition of individual Ca^{2+} responses (Dolmetsch et al., 1997, 1998). Such specificity in Ca^{2+} signaling is a function of its mechanism of activation, the spatial nature of its release, and the developmental context within which the Ca^{2+} response occurs (Berridge et al., 2000; Evans et al., 2001; Ng and McAinsh, 2003; Di Capite et al., 2009). Together, the frequency, amplitude, and spatial location of the Ca^{2+} release can differ, and these lead to variations in Ca^{2+} responses, commonly known as the Ca^{2+} signature (McAinsh and Pittman, 2009). The spatial differences across Ca^{2+} responses derive from Ca^{2+} release from diverse stores, with differential activation of Ca^{2+} channels that occur in restricted locations within the cell (Berridge et al., 2000; Di Capite et al., 2009; McAinsh and Pittman, 2009).

It has been hotly debated whether the nucleus of animal cells can act as an independent Ca^{2+} compartment from the rest of the cell. In animal cells, the generation of nucleoplasmic Ca^{2+} signals has been shown to be essential to regulate specific processes, including transcription, cell growth, and proliferation (Bootman et al., 2009), but the origin of nucleoplasmic Ca^{2+} increase in animal cells remains contentious. The Ca^{2+} channels inositol-1,4,5-trisphosphate and ryanodine receptors, as well as Ca^{2+}-ATPases, are all present on the inner nuclear envelope (Bootman et al., 2009), highlighting the potential of the nucleus to independently generate Ca^{2+} signals. In contrast to the animal field, it is widely accepted that the nuclei of plant cells can produce an autonomous Ca^{2+} response (Pauly et al., 2000, 2001; Mazars et al., 2009), and these nuclear Ca^{2+} events have distinct biological roles from those regulated by cytosolic Ca^{2+} release. For example, transient nuclear Ca^{2+} events are required for wind-induced calmodulin expression (van Der Luit et al., 1999) and for sphingolipid-induced programmed cell death (Lachaud et al., 2010). Nuclear Ca^{2+}, whether autonomous or of cytoplasmic origin, functions as a second messenger to stimulate numerous Ca^{2+}-sensitive processes, notably transcriptional regulation (Kaplan et al., 2006; Whalley et al., 2011), by binding to Ca^{2+}-sensing proteins such as calmodulin, transcription factors, kinases, or phosphatases (Galon et al., 2010; Reddy et al., 2011). In this review, we discuss the latest knowledge on nuclear Ca^{2+} signaling in plants. Among of the best plant models for nuclear Ca^{2+} signaling are the Ca^{2+} oscillations that occur during symbiotic signaling (Ehrhardt et al., 1996; Miwa et al., 2006; Sieberer et al., 2009; Chabaud et al., 2011), and while not exclusive, this review will focus on this signaling process. Over the past decade, tremendous progress has been made in understanding the mechanisms of encoding and decoding Ca^{2+} oscillations during symbiotic signaling, and this provides a platform for understanding nuclear Ca^{2+} signaling more broadly in plants.

MEASURING Ca^{2+} IN PLANT NUCLEI

A number of approaches have been used to measure Ca^{2+} responses in plants and, in particular, in the nucleus. Initially, microinjection with Ca^{2+}-responsive dyes was used, and this revealed nuclear Ca^{2+} responses, such as the Ca^{2+} oscillations induced in legume root hair cells in response to the rhizobial signaling molecule Nod factor (Ehrhardt et al., 1996). Such dyes are not restricted to the nucleus; thus, the nuclear changes could only be surmised by measuring fluorescence changes within the nuclear region. Concurrent with the use of Ca^{2+}-responsive dyes has been the development of Ca^{2+}-responsive proteins, whether naturally occurring such as aequorin or synthetic Ca^{2+} reporters such as cameleon (Allen et al., 1999; Knight et al., 1991; van Der Luit et al., 1999). The advantage of such protein reporters is the ability to target these proteins to different cellular compartments, including the nucleus (Mithöfer and Mazars, 2002; Krebs et al., 2012; Mehlmer et al., 2012). This has provided conclusive proof of Ca^{2+} changes within the nucleoplasm of plant cells as well as providing the ease of measurement of nuclear Ca^{2+} events in response to a variety of different stimuli, such as sphingolipids (Lachaud et al., 2010), wind or cold stresses (van Der Luit et al., 1999), mastoparan (Pauly et al., 2001), osmotic shocks (Pauly et al., 2000), pathogen elicitors (Lecourieux et al., 2005), jasmonic acid (Walter et al., 2007), and

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symbiotic signals (Sieberer et al., 2009; Chabaud et al., 2011).

LINKING SIGNAL PERCEPTION TO THE ACTIVATION OF NUCLEAR Ca^{2+} RELEASE

The best-studied inducers of nuclear Ca^{2+} responses are the symbiotic signals produced by rhizobial bacteria and arbuscular mycorrhizal (AM) fungi. Such Nod factors and Myc factors are lipochitooligosaccharides (LCOs) with a variety of modifications, dependent on the producing organism (Lerouge et al., 1990; Maillet et al., 2011; Genre et al., 2013). Recognition of these LCO signals involves plasma membrane LysM receptor-like kinases (Fig. 1; Amor et al., 2003; Madsen et al., 2003, 2011; Radutoiu et al., 2003; Smit et al., 2007; Antolín-Llovera et al., 2012) that, at least for the Nod factor receptors, have been shown to bind directly to the appropriate LCO signal (Broghammer et al., 2012). Recognition of rhizobia, AM fungi, and their LCO signals leads to the induction of Ca^{2+} oscillations in the nucleus (Sieberer et al., 2009; Genre et al., 2013) and, considering their recognition by plasma membrane-localized receptors, implies the production of diffusible secondary messengers that can link recognition at the plasma membrane to the Ca^{2+} changes in the nucleus. While the precise nature of these secondary messengers remains elusive, a number of clues provide indications of their potential structure.

The G-protein agonist mastoparan and its synthetic analog Mas7 were shown to activate Ca^{2+} oscillations in a manner analogous to Nod factor-induced responses (Charron et al., 2004; Sun et al., 2007). Furthermore, inhibitors of phospholipase D and phospholipase C block Nod factor-induced Ca^{2+} oscillations (Engstrom et al., 2002; Charron et al., 2004). Together, these data indicate that G-proteins may induce nuclear Ca^{2+} oscillations through their regulation of phospholipases. Inositol phosphates, which are products of phospholipase C, can coordinate Ca^{2+} responses in plant cells (Gilroy et al., 1990), and they are major activators of Ca^{2+} channels in animal cells (Bootman et al., 2009). However, there is currently no direct link demonstrated between the Nod factor receptors and the phospholipases or G-proteins; furthermore, there is little evidence for inositol phosphates functioning in symbiosis signaling. At the plasma membrane, the symbiosis receptor-like kinase, SYMRK, is hypothesized to complex with the Nod and Myc factor receptors (Antolín-Llovera et al., 2012) and to be associated with the production of the secondary messenger that activates nucleoplasmic Ca^{2+} oscillations. Proteins that interact with SYMRK include a plant mitogen-activated protein kinase kinase (Chen et al., 2012), and a 3-hydroxy-3-methylglutaryl-CoA reductase, HMGR1 (Kevei et al., 2007), and both of these were found to positively regulate the rhizobial association (Kevei et al., 2007; Chen et al., 2012). 3-Hydroxy-3-methylglutaryl-CoA reductase, an enzyme involved in lipid signaling via mevalonate production, and mitogen-activated protein kinase kinase could both be involved in downstream signaling through the generation of secondary messengers and phosphorylation cascades, respectively (Stermer et al., 1994; Taj et al., 2010). Both could be associated directly or indirectly in the activation or modulation of the symbiotic Ca^{2+} channel.

THE NUCLEAR MACHINERY REQUIRED FOR SYMBIOTIC Ca^{2+} RESPONSES

The genetic dissection of plant symbioses has led to the identification of a number of proteins present in the nucleus that have roles in the generation of Ca^{2+} oscillations. Three nucleoporins, NUP85, NUP133, and NENA (Kanamori et al., 2006; Saito et al., 2007; Groth et al., 2010), are all part of the nucleopore scaffold (Alber et al., 2007) and are required for symbiotic Ca^{2+} oscillations (Fig. 1). The main function of the nucleopore complex is to mediate macromolecular transport, such as mRNA export and protein import across the nuclear envelope (Alber et al., 2007). The role of these nucleoporins in the generation of Ca^{2+} oscillations could be associated with the diffusion of a symbiotic signal from the plasma membrane to the nucleus in order to activate nucleoplasmic Ca^{2+} oscillations. However, an alternative explanation is the transport of specific integral membrane proteins to the inner nuclear membrane, and in *Saccharomyces cerevisiae*, nucleoporins are required for this function (Deng and Hochstrasser, 2006; King et al., 2006). The specific yeast nucleoporins required are Nup188 and Nup170, which, like NUP85, NUP133, and NENA, locate at the nucleopore scaffold (Alber et al., 2007). These observations suggest that the nucleopore scaffold could play a role in translocating proteins to the inner nuclear membrane that are essential for the generation of Ca^{2+} oscillations. In *Medicago truncatula*, the ion channel DMI1 (*Lotus japonicus* homolog POLLUX) and the SERCA-type Ca^{2+}-ATPase MCA8 are essential for nucleoplasmic Ca^{2+} oscillations (Capoen et al., 2011; Venkateshwaran et al., 2012), and both localize to the nuclear membranes (refer to Fig. 1; Riely et al., 2007; Capoen et al., 2011). However, in contrast to MCA8, DMI1 was shown to preferentially localize to the inner nuclear membrane (Capoen et al., 2011), and the targeting of this protein may be at least one of the roles of the nuclear pore scaffold in the generation of symbiotic Ca^{2+} oscillations.

The localization of ion channels and a Ca^{2+}-ATPase at the nuclear envelope (Fig. 1; Riely et al., 2007; Charpentier et al., 2008; Capoen et al., 2011), as well as the spatiotemporal analyses showing the emergence of Ca^{2+} oscillations predominantly at the periphery of the nucleus (Sieberer et al., 2009; Capoen et al., 2011), strongly suggest that the lumen of the nuclear envelope contiguous with the endoplasmic reticulum constitutes the Ca^{2+} store for symbiotic Ca^{2+} signaling. This observation suggests that the components

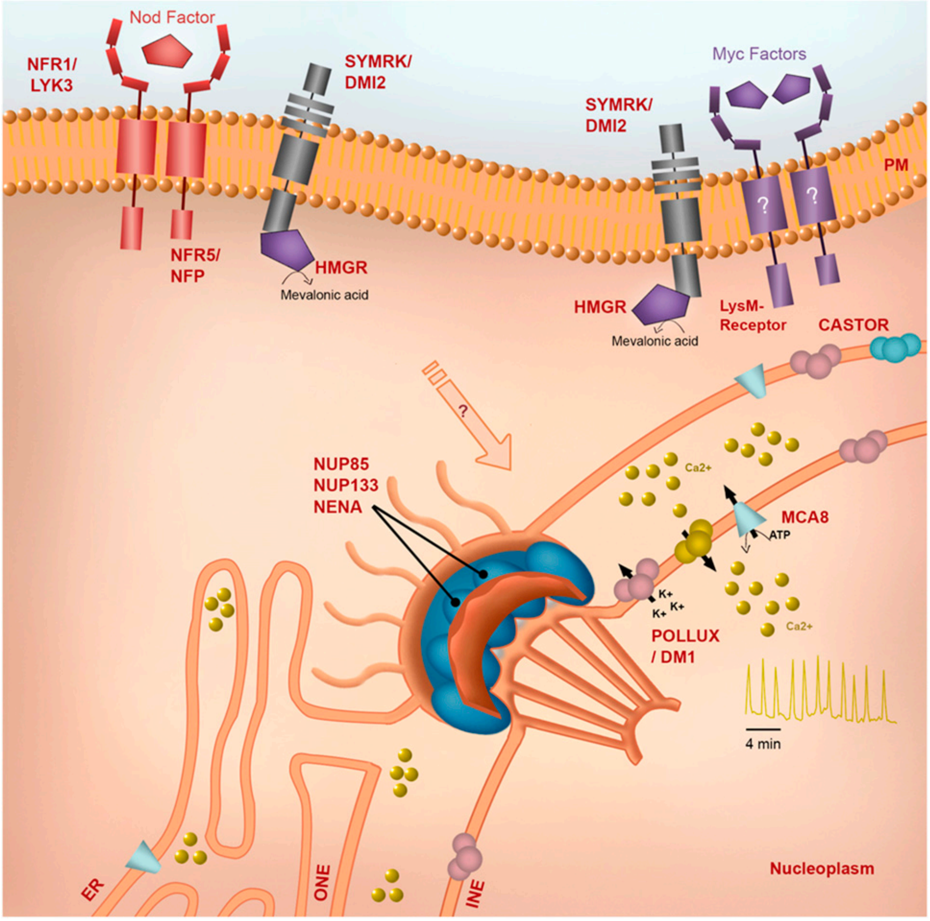

Figure 1. The symbiotic machinery required to induce nuclear-localized Ca^{2+} oscillations. In *L. japonicus* (Lj) and *M. truncatula* (Mt), the Nod factor receptors LjNFR1/MtLYK3 and LjNFR5/MtNFP are associated with the recognition of Nod factor (Amor et al., 2003; Madsen et al., 2003; Radutoiu et al., 2003; Smit et al., 2007; Broghammer et al., 2012). SYMRK is positioned at the cross point between Nod factor and AM perception (Endre et al., 2002; Stracke et al., 2002) and is proposed to form a complex with the Nod factor receptors and the yet to be identified AM receptors (Antolín-Llovera et al., 2012). SYRMK interacts with 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), which is involved in the production of mevalonic acid, a precursor for many potential second signals (Stermer et al., 1994; Kevei et al., 2007). Downstream of SYMRK are the nucleoporins NUP85, NUP133, and NENA, the ion channels LjPOLLUX (MtDMI1) and LjCASTOR, as well as the Ca^{2+} pump MCA8 (Kanamori et al., 2006; Saito et al., 2007; Charpentier et al., 2008; Groth et al., 2010; Capoen et al., 2011). In *L. japonicus*, the function of DMI1 is divided between both POLLUX and CASTOR. The selectivity filter of DMI1 differs from CASTOR and POLLUX by one amino acid, which seems sufficient to fulfill both functions (Venkateshwaran et al., 2012), making MtCASTOR redundant. ER, Endoplasmic reticulum; PM, plasma membrane.

localized at the nuclear envelope/endoplasmic reticulum are primarily involved in controlling the release of Ca^{2+}. The nuclear-localized ion channel DMI1 (*L. japonicus* POLLUX), which permeates potassium, seems unlikely to be directly responsible for the Ca^{2+} release (Charpentier et al., 2008; Venkateshwaran et al., 2012). Indeed, pharmacological and yeast expression analyses highlight that DMI1 might be a tight regulator of the yet unidentified symbiotic Ca^{2+} channel (Peiter et al., 2007). In agreement with this observation, mathematical modeling reveals that the association of three components (DMI1, a putative voltage/ligand-activated Ca^{2+} channel, and a Ca^{2+} pump) is sufficient to produce the symbiotic Ca^{2+} oscillations (Granqvist et al., 2012). This mathematical modeling suggests that DMI1 functions to regulate the Ca^{2+} channel as a counter ion channel and a modulator of membrane potential in two steps (Charpentier et al., 2013). First, activation of DMI1 generates a potassium current that facilitates an initial, limited Ca^{2+} release via a partially activated Ca^{2+} channel. This Ca^{2+} release provides a positive feedback, via a predicated Ca^{2+}-binding pocket in DMI1 (Edwards et al., 2007), that fully activates DMI1, whose potassium influx hyperpolarizes the membrane to open a putative voltage-gated Ca^{2+} channel. The Ca^{2+} released is then pumped back into the store via the Ca^{2+}-ATPase. In this mathematical model, the positive Ca^{2+} feedback and the voltage fluctuation of the nuclear envelope play a major role in sustaining the Ca^{2+} oscillations. Recent studies that have demonstrated the Ca^{2+} modulation of the nuclear envelope potential to induce Ca^{2+} bursts in neurons (Yamashita, 2011) and that have shown the expression of DMI1 in human embryonic kidney cells sufficient to activate Ca^{2+} oscillations upon Ca^{2+} stimulation (Venkateshwaran et al., 2012) provide support for the mathematical modeling.

IS INFORMATION ENCODED IN THE NUCLEAR Ca^{2+} SIGNATURE?

Intrinsic to the Ca^{2+} signature hypothesis is the idea that information is encoded in the structure of the Ca^{2+} response (McAinsh and Pittman, 2009). In mammalian cells, it is well established that the amplitude and frequency of the Ca^{2+} oscillations can encode the specificity of the response (Dolmetsch et al., 1998). Thus, in T lymphocyte cells, rapid and irregular Ca^{2+} oscillations activate different Ca^{2+}-sensitive transcription factors, leading to specific gene expression patterns (Dolmetsch et al., 1998). In plants, evidence for information encoding came from studies in guard cells, where enforced Ca^{2+} oscillations of different structures gave different long-term effects for stomatal closure (Allen et al., 1999).

The nucleoplasmic Ca^{2+} oscillations induced by symbionts are cell autonomous, as nonsynchronous Ca^{2+} oscillations occur between adjacent cells (Sieberer et al., 2009; Chabaud et al., 2011). Furthermore, the structures of the oscillations differ between cells (Ehrhardt et al., 1996; Sieberer et al., 2009; Chabaud et al., 2011), providing the basis for hypothesizing information encoding within the Ca^{2+} response. Rhizobia and AM fungi utilize the same symbiosis signaling pathway for the activation of and response to the Ca^{2+} oscillations (Parniske, 2008). Despite these similarities in signaling, specificity must be maintained to ensure an appropriate response to each symbiont. Such specificity of signaling could be encoded within the Ca^{2+} oscillations, could be a function of as yet unknown parallel signaling, or could be associated with the differential cell types that the symbionts use for initial colonization: Rhizobia colonize through root hair cells, while AM fungi colonize atrichoblasts (refer to Fig. 2).

In the root hair cell, nucleoplasmic Ca^{2+} oscillations are induced after a lag of 6 to 20 min following Nod factor addition and reach a period of regular frequency (with approximately 100 s between each spike) after an initial short burst of high-frequency oscillations comprising three to six spikes (Ehrhardt et al., 1996; Miwa et al., 2006; Sieberer et al., 2009). Each spike produced is characterized by an asymmetric shape, resulting from an initial rapid release of Ca^{2+} followed by a much slower Ca^{2+} reuptake (Sieberer et al., 2009; Fig. 1). Although mathematical modeling suggests that the short burst of oscillations can be generated by a different buffering capacity of the cell (Granqvist et al., 2012), the biological relevance of this initial rapid burst of Ca^{2+} spikes remains to be determined.

Initial studies of Ca^{2+} oscillations induced at early stages of AM-fungal interactions before contact with the epidermal cell indicated differences to the Ca^{2+} oscillations activated by Nod factor, providing the basis for hypothesizing that the specificity of symbiosis signaling may be encoded within the structure of the Ca^{2+} response (Kosuta et al., 2008; Chabaud et al., 2011). Interestingly, chitooligosaccharides, which are produced by AM fungi in addition to LCOs, activate similar irregular and low-frequency Ca^{2+} oscillations (Genre et al., 2013). However, upon contact with the fungal hyphopodium, the nuclear Ca^{2+} oscillations induced are similar in shape and frequency to the rhizobial bacteria-induced Ca^{2+} oscillations (Chabaud et al., 2011; Sieberer et al., 2012). Furthermore, a direct comparison of cortical cells colonized by AM fungi and rhizobial bacteria indicates that both symbionts induced similar nuclear Ca^{2+} oscillations of low frequency prior to colonization and of high frequency upon colonization (Sieberer et al., 2012). These observations suggest that the Myc and Nod factors delivered by AM fungi and rhizobial bacteria might trigger diverse Ca^{2+} signaling responses, with the periodicity and shape of the Ca^{2+} response being dependent upon the concentration of symbiotic factors and probably the mix of Myc factors.

During symbiont colonization, the path of infection through the root is predicted by a preinfection structure that predicts the route of the invading AM fungus or rhizobia-colonized infection thread (van Brussel et al., 1992; Genre et al., 2005). This predicted path of infection is always directed by the cells where nuclei exhibit high-frequency Ca^{2+} oscillations (Sieberer et al., 2012). During rhizobial infection of root cortical cells, this high-frequency Ca^{2+} oscillation is sustained for 40 to 55 min, which corresponds to 35 to 45 spikes and attenuates synchronously with the infection progression (Sieberer et al., 2012). Although it is unclear whether low- and high-frequency Ca^{2+} oscillations induce different posttranslational or transcriptional changes, previous studies suggested that a minimum of 36 spikes were required to induce a symbiotic marker in response to Nod factor (Miwa et al., 2006). While low-frequency Ca^{2+} oscillations could induce the symbiotic marker, its expression was considerably delayed (Miwa et al., 2006). We propose that although the Ca^{2+} oscillations do not encode specific information for rhizobial or AM fungal colonization, they do encode information regarding the nature and concentration of the symbiotic signals perceived by the cell: only those cells perceiving the appropriate mix of factors at the right concentration support robust and sustained Ca^{2+} oscillations. Such cells undergo the appropriate programming for symbiont colonization, and these are the cells that undergo the developmental changes associated with prepenetration. It is possible that the irregular Ca^{2+} oscillations observed prior to symbiont colonization may play a role in the initial stages of priming the cell for symbiotic associations, or they may simply reflect cellular signaling that is insufficient to sustain a symbiotic response.

DECODING THE NUCLEOPLASMIC Ca^{2+} RESPONSES

The direct sensing of Ca^{2+} requires Ca^{2+}-binding proteins (Batistič and Kudla, 2012), and a number of Ca^{2+} sensors, such as calmodulin-domain protein kinases and calmodulins, are predicted to be present in the nucleus (Biro et al., 1984; Rodríguez-Concepción et al., 1999; Reddy et al., 2011). The nuclear location of such Ca^{2+}-decoding proteins implies that the nucleus itself has the capability to independently respond to Ca^{2+} signals. Clearly, in addition to such autonomous Ca^{2+} signaling, most cellular signaling, including Ca^{2+} signaling, will transduce to the nucleus; however, for simplicity in this review, we focus only on autonomous nuclear Ca^{2+} signaling events. Again, the best model for understanding the decoding of Ca^{2+} signals in the nucleus is in symbiotic signaling that utilizes a nuclear-localized Ca^{2+}- and calmodulin-dependent Ser/Thr protein kinase (CCaMK; Lévy et al., 2004; Mitra et al., 2004; refer to Fig. 2). Gain-of-function mutations of CCaMK are sufficient to induce symbiotic processes, such as spontaneous nodulation in the absence of rhizobia (Gleason et al., 2006; Tirichine et al., 2006), and the promotion of prepenetration structures that are associated with AM colonization (Takeda et al., 2012). Moreover, in the presence of the autoactive CCaMK, the symbiotic signaling components upstream of Ca^{2+} oscillations become dispensable for nodulation and mycorrhization (Hayashi et al., 2010; Madsen et al., 2010). These observations indicate that the main role of the upstream signaling components is to generate Ca^{2+} oscillations whose predominant function is the activation of CCaMK.

CCaMK can bind Ca^{2+} either directly via three C-terminal EF hand domains or indirectly via a Ca^{2+}/calmodulin-binding domain (Sathyanarayanan et al., 2000; Gleason et al., 2006; Tirichine et al., 2006; Swainsbury et al., 2012). This dual Ca^{2+}-binding capability of CCaMK is unique compared with

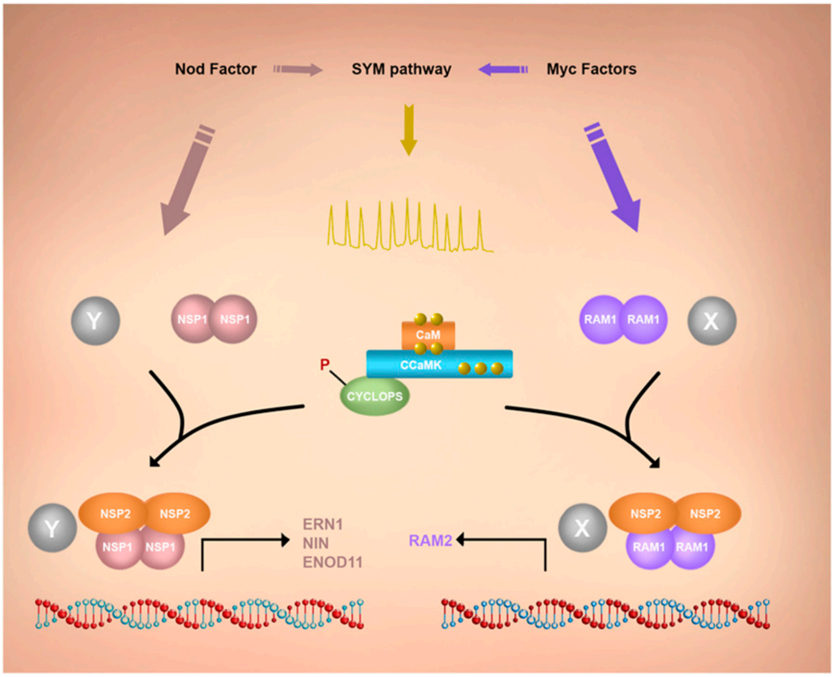

Figure 2. Symbiotic signaling downstream of Ca^{2+} oscillations. The generation of Ca^{2+} oscillations requires components essential for both rhizobia and AM symbioses, collectively referred to as the common symbiosis (SYM) signaling pathway. CCaMK is responsible for decoding the Ca^{2+} oscillations through its association with Ca^{2+} and calmodulin (CaM; Singh and Parniske, 2012). CCaMK associates with and phosphorylates CYCLOPS (Yano et al., 2008). Downstream of CCaMK and CYCLOPS, a suit of GRAS transcription factors are required to activate nodulation or AM programs (Oldroyd, 2013). NODULATION SIGNALING PATHWAY2 (NSP2) associates with both the nodulation-specific GRAS transcription factor NSP1 (Hirsch et al., 2009) and the AM-specific GRAS transcription factor REQUIRED FOR ARBUSCULAR MYCORRHIZATION1 (RAM1; Gobbato et al., 2012). The complex NSP2/NSP1 is required for the expression of nodulation genes (*ERN1*, *NIN*, and *ENOD11*), while the complex NSP2/RAM1 modulates the expression of AM-specific genes such as *RAM2* (Oldroyd, 2013). In this model, we hypothesize that upon symbiont stimulation (Myc or Nod factors), the transcription factor complexes are recruited by specific unknown AM (X) or nodulation (Y) components. The CCaMK/CYCLOPS complex is activated via Ca^{2+} oscillations and activates symbiotic gene expression, either independently or in combination with the GRAS protein complexes.

Ca^{2+}-binding proteins in both animals and plants (Hrabak et al., 2003) and underlines the mechanistic complexity of this Ca^{2+}-sensing kinase. Several studies combining homology modeling with the animal Ca^{2+}/calmodulin-dependent protein kinase II and mutational analyses have highlighted the importance of the kinase domain and the autophosphorylation state of CCaMK to positively or negatively regulate its activity (Hayashi et al., 2010; Liao et al., 2012; Shimoda et al., 2012; Singh and Parniske, 2012; Takeda et al., 2012). The autophosphorylation of CCaMK is dependent on Ca^{2+} binding to the EF hand domains, while substrate phosphorylation is promoted by calmodulin binding to CCaMK (Sathyanarayanan et al., 2000; Shimoda et al., 2012). Many of the specifics of the CCaMK mode of action have been studied, and for a more detailed description of CCaMK activation, see the review by Du and Poovaiah (2013) in this edition.

CCaMK interacts with and phosphorylates the nuclear-localized CYCLOPS (Messinese et al., 2007; Yano et al., 2008; Horváth et al., 2011; refer to Fig. 2). CYCLOPS encodes a coiled-coil protein required for both AM and rhizobial infection (Yano et al., 2008; Horváth et al., 2011). Interestingly, the mutation to Asp of two of the CYCLOPS Ser residues that are phosphorylated by CCaMK creates a gain of function in CYCLOPS that leads to spontaneous nodulation when this mutant is transformed into legume roots (M. Parniske, personal communication). This observation suggests that the activation of the core complex CCaMK/CYCLOPS is sufficient to trigger downstream signaling associated with nodule organogenesis. CYCLOPS might function directly with downstream nuclear-localized GRAS family transcriptional regulators to coordinate the transcriptional events associated with rhizobial and AM invasion (Fig. 2; Kaló et al., 2005; Smit et al., 2005; Gleason et al., 2006; Hirsch et al., 2009; Gobbato et al., 2012).

CONCLUSION

The plant cell nucleus has the capability to mount an autonomous Ca^{2+} response, and such nuclear Ca^{2+} signaling is likely to be associated with a variety of processes. Because of its ease of genetic dissection, the symbiotic signaling pathway has emerged as the best model for studying nuclear Ca^{2+} signaling in plants. The perception of symbiotic signals leads to the generation of nucleoplasmic Ca^{2+} oscillations, with high-frequency Ca^{2+} oscillations associated with cellular programming that defines the pathway within the root for symbiont colonization. The establishment of nuclear Ca^{2+} oscillations involves a potassium-permeable channel, a Ca^{2+}-ATPase, and a hypothetical Ca^{2+} channel that are located on the nuclear membranes. These are predicted to function in combination to sustain Ca^{2+} oscillations, following activation by an as yet unknown secondary messenger. One of the main purposes of the Ca^{2+} oscillations appears to be the activation of CCaMK and its phosphorylation of CYCLOPS. Subsequent transcriptional reprogramming to permit either AM or rhizobial colonization is dependent on the Ca^{2+}-decoding complex defined by CCaMK/CYCLOPS and a suite of GRAS domain transcription factors (refer to Fig. 2). How the specificity of symbiosis signaling is encoded has yet to be defined, but it appears that the robustness of the Ca^{2+} oscillations is important to define the cells that will ultimately house the invading symbionts. While genetic dissection of symbiotic signaling has provided a framework to understand nuclear Ca^{2+} signaling, questions remain, particularly with regard to the nature of the secondary messenger(s) that link signal recognition at the plasma membrane to the activation of Ca^{2+} responses in the nucleus as well as the structure of the nuclear Ca^{2+} channels that coordinate this process.

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