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Chapter

Receptor Kinases and Signal Pathway in the Arbuscular Mycorrhizal Symbiosis

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Abstract

Most terrestrial plants establish symbiotic interactions with arbuscular mycorrhizal fungi (AMF) to acquire phosphorus and nitrogen nutrients. The current understanding regarding how plants recognize symbiotic signals has now been updated. Plant Lysin-Motif receptor kinases, that is, rice OsCERK1 and OsMYR1 or orthologs from other plants, perceive Myc factor, a lipochitooligosaccharide from AMF, to initiate symbiotic signaling pathway. The Myc factor receptor model is quite similar to the known Nod factor receptors required for rhizobial symbiosis and chitin receptors for chitin-triggered immunity. Thus, the open question is how plants use similar receptor complexes to recognize structurally similar molecules to induce different signaling pathways. Upon recognition of Myc/Nod factors signaling, LysM receptors could activate the symbiosis receptor kinase (SymRK), which is an essential component of common symbiotic signaling pathway (CSSP) for both mycorrhizal symbiosis and rhizobial symbiosis. Downstream of SymRK, a clear module in the CSSP by CCaMK-CYCLOPS-DELLA was identified to promote both mycorrhizal symbiosis by activating the expression of *RAM1*, and rhizobial symbiosis by forming a complex with NSP1/NSP2 to regulate the expression of *NIN*. In this chapter, we discussed the roles of receptor kinases and CSSP in mycorrhizal symbiosis, as well as in rhizobial symbiosis.

Keywords: symbiosis, LysM-receptor kinases, root nodule symbiosis, SymRK, transcription factor, common symbiosis signal pathway

1. Introduction

Arbuscular mycorrhizal symbiosis (AMS) is a mutualistic interaction formed between more than 80% of terrestrial plants and members of the Glomeromycotina fungi, referred to as the arbuscular mycorrhizal fungi (AMF) [1]. It was proposed that AMS evolved approximately 400−450 million years ago, while root nodule symbiosis (RNS) originated about 60 million years ago [2, 3]. Thus, it was consistent with the generally accepted theory that RNS might be a result of a gradual attenuation of AMS, and both of them might evolve from the ancient plant-pathogens interaction [4, 5]. In the AMS, AMF could help plants to absorb more phosphorus and nitrogen nutrients from environment, and in return, plants provide carbohydrates mainly in the form of lipids for AMF [6–9]. As one of the pivotal nutrients for host plants' growth,

phosphate is known negatively correlate with AMS [10–12]. Meanwhile, AMF could help host plants adapt to stressful environmental conditions [13, 14]. The development of AMS is a highly dynamic process, including presymbiotic communication between both symbionts, colonization of AMF in the plant root cortex and highly branched structures called arbuscules formation [15, 16], vesicle and spore maturation. Before making physical contact, phytohormones strigolactones (SLs) secreted by the roots of plants into the rhizosphere under Pi-deficient conditions [17], promote the branching of mycorrhizal hyphae. Simultaneously, the secreted cutin monomer promotes the colonization of AMF in the host roots. But the molecular basis of SL perception by AMF spores has not yet been elucidated.

Similar to the process of rhizobial symbiosis, branched hyphae of AMF secrete mycorrhizal (Myc) factors, a mixture of short-chain chitooligosaccharides (CO4/CO5) and lipochitooligosaccharides (LCOs), both of which are similar structures with rhizobial Nod factors [18, 19], to activate the common symbiosis signaling pathway (CSSP), required for both AMS and RNS [2, 20]. Over the past two decades, advances have been made in the areas of symbiotic signals perception, including the identification of LysM receptor-like kinases in non-legumes, especially in rice, and symbiotic signaling transduction in plants. Downstream of LysM receptors, several key common components were identified as shared components for both AMS and RNS, that is, Symbiosis receptor kinase (SymRK), also called Nodulation Receptor Kinase (NORK) or Does not Make Infection 2 (DMI2) in other plant species [21, 22], calcium and calmodulin-dependent kinase (CCaMK) [23], CYCLOPS [24]. GRAS transcription factor (TF) family proteins such as DELLAs [25], Nodulation Signaling Pathway 1 (NSP1), NSP2 [26], Reduced Arbuscular Mycorrhiza 1 (RAM1) [27, 28] are also involved in AMS and/or RNS. In addition, some downstream components, such as TFs like Nodulation Inception (NIN) [29, 30], Phosphate Starvation Responses (PHRs) [31], SYG1/Pho81/XPR1 (SPX) [32], etc., participate in AMS and/or RNS. Overall, the current study has provided a rough linear pathway of AMS/RNS in plants.

2. Roles of LysM-RLKs in perceiving symbiotic signals in AMS and RNS

In nature, only a few species of microbes can establish compatible interactions with host plants to cause either pathogenic or mutualistic symbiosis. More and more data suggest that plant innate immunity plays a key role in distinguishing invading microbes to establish different interactions. Hence, how plants recognize and distinguish signals from different microbes could be precisely regulated. The existing data indicate that LysM-RLKs play such roles in distinguishing different microbes and initiating different physiological responses in plants. N-Acetylglucosamine (GlcNAc)-containing molecules are conserved components of cell walls for different microbes. For example, chitin, the major component of fungal cell wall, and bacterial peptidoglycan (PGN), function as microbe-associated molecular patterns (MAMPs) perceived by LysM-containing proteins to trigger plant immunity against invading pathogens.

Whereas lipo-chitooligosaccharides (LCOs), for example, rhizobial NFs and mycorrhizal Myc factors are key signals recognized by two LysM-RLKs to induce symbiotic signaling transduction in plants [33]. Rhizobial NF, a short-chain of chitin with different modifications at the terminal residues, plays an important role in specific recognition between rhizobial and legumes [34]. In AMS, Myc factors that contain Myc-LCOs, and Myc-COs can activate the CSSP with resultant calcium oscillations

in root epidermal cells [35–37]. Myc factors were proposed to be mixtures of CO4/5 and LCO, while the function of CO4 appears to be the predominant molecule activating symbiotic responses in rice. Thus, symbiotic signaling pathways induced by Myc-LCOs and COs seem to be a little bit different since AMF produces a mixture of molecules during the symbiotic interaction with hosts [18, 37, 38]. In this process, a class of LysM receptor kinases (LYKs) participate in the discrimination of these GlcNAc molecules and determine the outcomes of the downstream signaling pathway to immunity or symbiosis [39–41].

In the establishment of symbiosis between rhizobial and legume host plants, LjNFR1 (Nod Factor Receptor 1) and LjNFR5 in *Lotus japonicus*, also named MtLYK3 (LysM containing receptor Kinase 3) and MtNFP (Nod Factor Perception) in *Medicago truncatula* are two essential LYKs regulating NFs specific perception [42–44]. In non-legume species, for example, rice OsCERK1 (Chitin-elicitor receptor kinase 1), a LysM-RLK, is a necessary receptor involved in COs-induced immunity and mycorrhizal symbiotic responses. CERK1 was originally identified as an essential receptor for chitin elicitor signaling in *Arabidopsis thaliana*, and the KO mutant for *AtCERK1* completely lost the ability to respond to chitin [45]. Subsequent studies have demonstrated that OsCERK1 and a LysM protein OsCEBiP (Chitin Elicitor binding protein) could cooperatively regulate chitin elicitor signaling in rice [39, 46]. But, interestingly, OsCERK1 plays a dual role in mediating both AMS and immunity [47, 48]. The AMS in the rice *Oscerk1* mutant plants was severely diminished but was normal in the *Oscebip* mutant, the function of OsCERK1 in mediating symbiosis or immune responses seems to be dependent on specific interaction with different receptors in response to either symbiotic signals or pathogenic signals [19]. Recent breakthroughs have revealed OsCERK1[DY] from Dongxiang wild rice with two amino acids substitutions in the second LysM domain exhibited stronger colonization with AMF than the rice cultivar Zhongzao 35 (ZZ35), as well as promoting phosphorus acquisition [49].

CO4/CO5 are necessary signals for symbiotic interactions between AMF and host plants, however, rice OsCERK1 does not seem to bind to CO4 directly [39, 50]. It was implied that another component was needed to perceive these signals, just like the sandwich models of OsCERK1/OsCEBiP in mediating chitin-triggered immunity and LjNFR1/LjNFR5 complex for rhizobial symbiosis in *L. japonicus* [44, 46]. OsMYR1/OsLYK2/OsNFR5/OsRLK2, grouped in the same clade as LjNFR5/MtNFP/SlLYK10, is the co-receptor of OsCERK1 required for AMS. OsMYR1 directly binds to CO4 but not Nod factors or lipopolysaccharides (LPS) [20]. Significant reduction of AMF colonization, as well as transcription levels of AM-specific marker genes and calcium spiking, were observed in the *Osmyr1-1/Oslyk2-1* mutant compared to wide type (WT) inoculated with *Rhizophagus irregularis* spores [20]. However, the AM colonization in a high dosage did not show too many differences between *Osmyr1-1/Osnfr5* and WT plants, but a significant decrease in transcript levels of AM-responsive gene was detected in the *Osmyr1-1/Osnfr5* mutant plants [51]. Whether OsMYR1/OsNFR5 responds differentially to different dosages of *R. irregularis* is unclear. But all these data indicated that OsMYR1 seems to be a binding receptor for sensing CO4, and the subsequent dimerization and phosphorylation between OsMYR1 and OsCERK1 activate symbiotic signaling pathway [20].

It was identified that only the long chain of COs with polymer of degree between 6 and 8 (CO6, CO7, and CO8) but not CO4 or CO5 could trigger a plant immunity [52, 53]. A recent study has shown that CO8 has a similar function as CO4 to induce symbiotic nuclear calcium oscillations and activates some of the symbiosis-related

genes expression [18], raising a question that nuclear calcium oscillation might not be a specific signal representing symbiosis. In *Medicago truncatula*, MtCERK1 and MtLYR4 can bind both CO4 and CO8, in which MtCERK1 is required in both COs induced immune and symbiotic signaling pathways [18]. Interestingly, the plant defense triggered by CO8 could be suppressed by additional LCOs in both legumes and non-legumes, and CO4 could reduce CO8-triggered ROS generation via OsMYR1 in rice or AtLYK3 in Arabidopsis [18, 19, 54], suggesting that non-legumes could still respond to LCOs. Hence, a Single Pole Double Throw (SPDT) switch model in symbiosis and defense signaling pathways was proposed [19]. In this model, OsMYR1 can recognize CO4 from symbiotic fungi and then associates with OsCERK1, which suppresses the formation of chitin-induced OsCEBiP/OsCERK1 complex and trans-phosphorylation of immune-associated substrates, then activate mycorrhizal colonization. When OsCEBiP senses CO8, it competes with OsMYR1 to bind OsCERK1, which induces immunity and negatively regulates AMS [19].
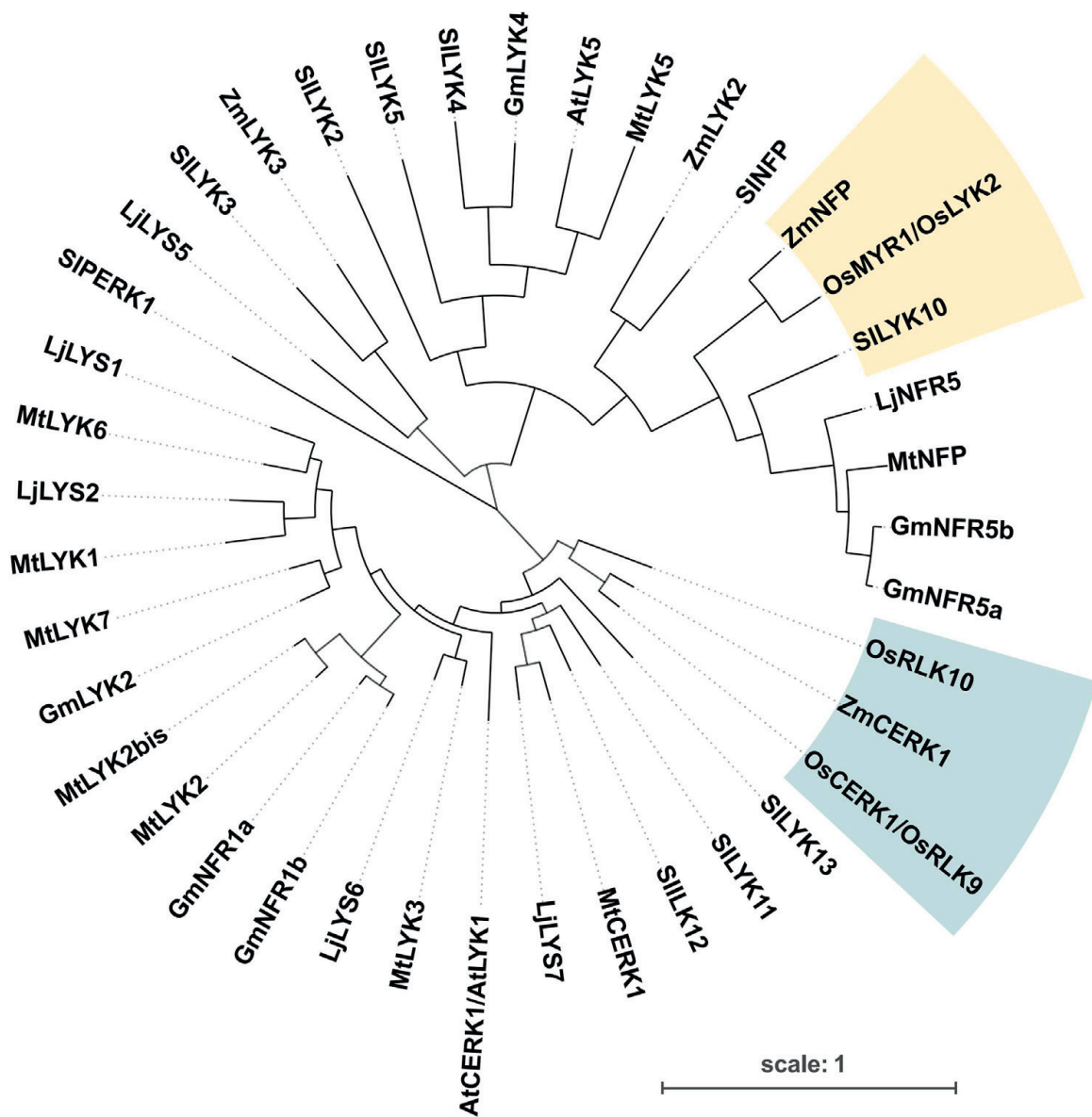

Figure 1.
Phylogenetic tree of LysM-containing receptor-like kinases. Molecular phylogeny of OsCERK1 and OsMYR1 with homologs from Oryza sativa (Os), Medicago truncatula (Mt), Glycine max (Gm), Lotus Japonicus (Lj), Arabidopsis thaliana (At), Zea mays (Zm), Solanum lycopersicum (Sl) was constructed using a maximum-likelihood algorithm in MEGA-X with 1000 bootstraps value.

Rice is a very-well studied model species used for AMS study. The dual function of OsCERK1 homologs in both symbiosis and immunity was also studied in other plant species. Similar to rice OsCERK1 in symbiosis and immunity, OsCERK1 homologs in leguminous plants also play a dual role in both symbiosis and immunity (**Figure 1**). For example, MtLYK9 in *M. truncatula* and PsLYK9, a close ortholog of CERK1 in *Pisum sativum* regulate both plant immunity and AMS [55, 56]. Likewise, in *Parasponia andersonii*, the only known non-legume plant with an ability of RNS, PanLYK1, and PanLYK3 were essential for intracellular arbuscule formation, while PanLYK3 also acted as a chitin receptor for innate immunity signaling [57]. Nonetheless, LYKs in some species, such as the CERK1 homologs in tomato (SlLYK1, SlLYK11, SlLYK12, and SlLYK13) are also functional in AMS and chitin-triggered defense. It has been found that knockdown of *SlLYK12* would significantly reduce AMF colonization, but the chitin-induced defense response was unaffected, whereas SlLYK1 and SlLYK13 only participate in immune signaling but not AMS [58]. Hence, plant CERK1 is such a receptor that works as a shared kinase mediating both chitin-triggered immunity and AMF symbiotic signals. The LYKs receptor model might at least support the hypothesis that symbiotic interaction might be a result of a graduate attenuation from plant-pathogens interaction until a condition that both symbionts and plants could benefit.

3. Common signaling pathway in mycorrhizal and rhizobial symbiosis

In legumes, the establishment and development of AMS and RNS require a set of common symbiosis genes [59, 60], including a conserved SymRK protein from different species and several essential TFs. When LCOs and COs from bacteria and/or fungi are recognized, SymRK is activated to associate with a set of essential proteins like HMGR1 to regulate both AMS and RNS or interact with SymRK-interacting protein 2 (SIP2) which is specifically involved in RNS. Currently, some interacting proteins of SymRK have been confirmed to participate in RNS, but whether they also take part in AMS remains unknown. As critical components of CSSP, SymRK and other receptor complexes could promote the signaling pathway downstream by triggering nuclear calcium spiking and activating CCaMK. CCaMK could interact with and phosphorylate the downstream transcription factor CYCLOPS [24, 61]. Meanwhile, DELLAs bind the CCaMK-CYCLOPS complex to promote the expression of *RAM1* and regulate AMF colonization [59]. On the other hand, DELLAs associate CCaMK-CYCLOPS with NSP1-NSP2 to enhance the expression of *NIN* which regulates RNS positively [62, 63]. Recently, several other important transcription factors, for example, PHRs and SPXs have been identified to involve in AMS under different conditions of Pi [31, 32, 64].

3.1 SymRK and its interacting proteins involved in AMS and RNS

As a typical LRR-RLK, SymRK was identified as an important membrane-localized receptor kinase required for activating a series of physiological responses in the symbiosis between AMF, rhizobial, Frankia bacteria, and their corresponding host plants [65, 66]. Studies have found only SymRK but not Nod factor receptors (NFRs) overexpression triggers the expression of AM-related genes, and the *symrk* mutant fails to form arbuscule, indicating that SymRK plays a crucial role in the exchange of signals and in the decision between the development of AMS or RNS [67]. According to polymorphisms and amino acid length of SymRK in both legumes and non-legumes, SymRK exists in at least three different structural versions. Rice

and tomato, two plant species widely used for AMS study, have a shorter form of SymRK that is sufficient for AMS but cannot fully complement *symrk* mutant in legumes for rhizobial endosymbiosis [68]. Based on the sequence comparison, the extracellular domain of SymRK homologs might play important roles in determining
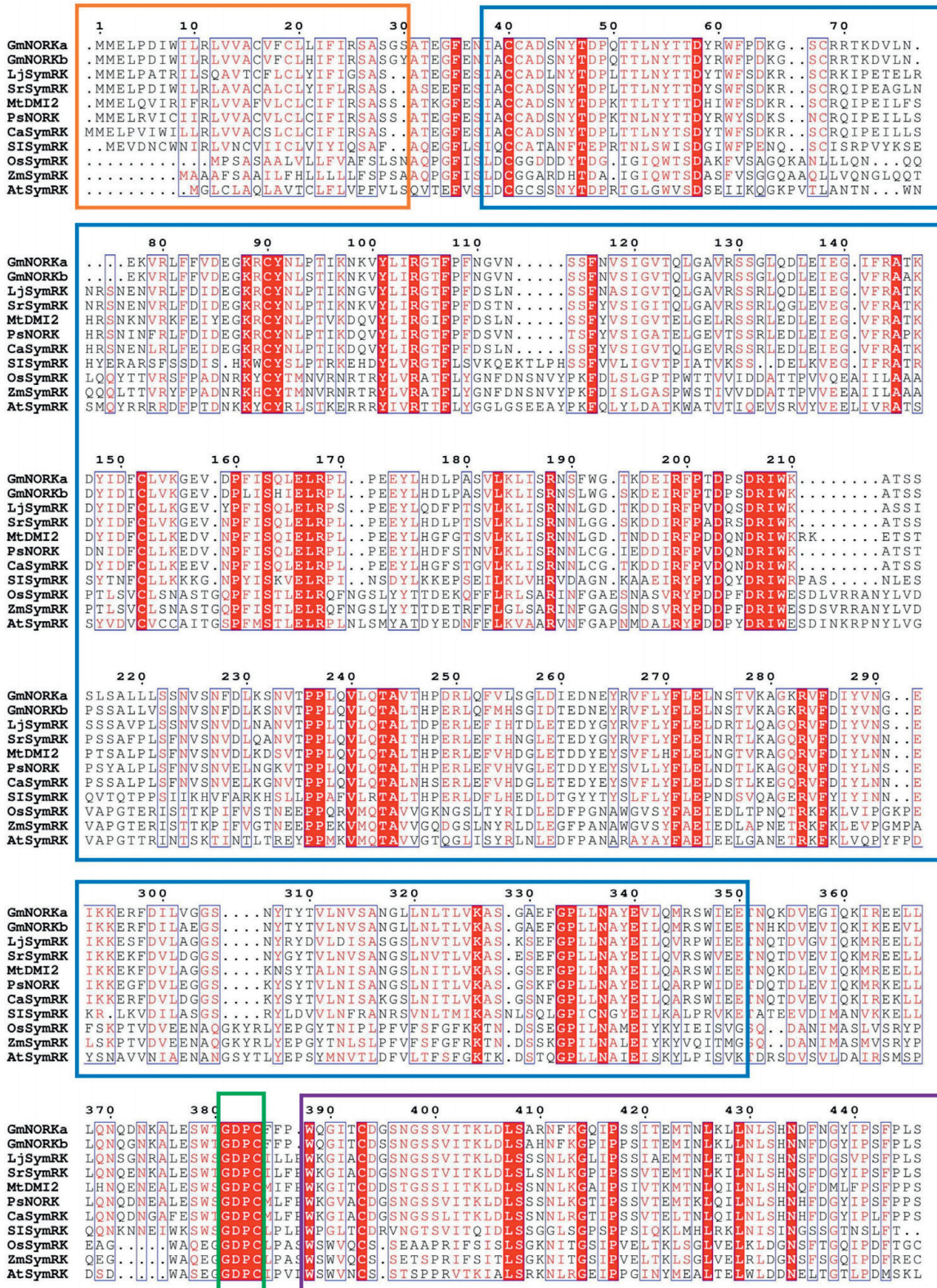

Figure 2.
Multiple sequence alignment of extracellular domain of SymRK from legumes and non-legumes. The orange box represents the signal peptide, the blue box represents malectin-like domain, the green box represents the conserved Gly-Asp-Pro-Cys (GDPC) sequence, and the purple box represents leucine-rich repeat (LRR) domain.

their specific functions (**Figure 2**). In legumes, such as *L. japonicus*, *M. truncatula,* and *Glycine max*, great conservation with about 75% identities is found in the extracellular domain of SymRK homologs. Such conservation might pinpoint its specific role in RNS. However, the sequence identities of SymRK homologs between legumes and non-legumes are reduced to about 40−50%. Althoug, SymRK is a central player in CSSP required for both RNS and AMS, the sequence differences at its extracellular region might give direct evidence that responds to either mycorrhizal signals or rhizobial signals. However, what determines the functional difference between legumes and non-legumes is of great interest to be studied in future, and the functional difference might be related to the evolution of AMS and RNS.

Although the *symrk* knock-out mutant completely loses the ability to allow rhizobial infection, the root hairs of *symrk* mutant plants were observed to be exaggerated after rhizobial attachments [21, 69], indicating that SymRK plays an essential role in determining rhizobial infection but not rhizobial attachment. Due to the key role of SymRK in both AMS and RNS, several SymRK-interacting proteins and protein modifications of SymRK have been studied to elucidate the precise function of SymRK in RNS and/or AMS (summarized in **Table 1**). HMGR1 (3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase1) was identified as an interacting protein of MtDMI2 (SymRK homolog in *M. truncatula*), suggesting that mevalonate biosynthesis was involved in mediating the function of SymRK to initiate calcium spiking and symbiotic gene expression in response to both rhizobia and AMF [70]. Transcriptome expression analysis characterized that SYMREM1 (Symbiotic Remorin 1) from *M. truncatula* could interact with MtDMI2 and may act as a scaffold protein for assembly of receptor complexes involved in rhizobial infection [71, 81]. In addition to interacting with DMI2/SymRK, SYMREM1 was also identified to interact with other receptor kinases in legumes, such as MtNFP/LjNFR5 and MtLYK3/LjNFR1 [81]. Thus, the function of SYMREM1 might provide corresponding structural support for the molecular network of NFR1/5-SymRK receptor complexes.

SymRK-interacting protein 1 (SIP1) is a major AT-rich sequence binding (ARID) transcription factor [72]. Two major splicing forms, SIP1 and SIP1L (a longer variant of the SIP1 transcripts) in *L. japonicus* were characterized. Interestingly, SIP1 was found to interact with SymRK, while SIP1L could not. Both SIP1L and SIP1 could specifically bind to the promoter of *NIN* to positively regulate symbiosis. Knockdown of SIP1using RNAi technology in transgenic hairy roots resulted in impairment in the nodule and arbuscular development, suggesting an important role of SIP1 in the CSSP [72, 73]. As a typical mitogen-activated protein kinase (MAPKK), SymRK-interacting protein 2 (SIP2) could specifically interact with SymRK homologs from different legumes [74]. Although SymRK and SIP2 have a strong interaction, the inter-phosphorylation between them was not detected [74, 82]. Additional studies identified that LjMPK6 is a phosphorylation target of SIP2 [83], and SymRK could inhibit the phosphorylation activity of SIP2, therefore SymRK might negatively regulate the SIP2-MPK6 signaling cascade. Like SIP1, SIP2 also functions as a positive regulator in RNS conformed by RNA interference methods. However, SIP2 is not required for AMF colonization, suggesting that SymRK-SIP2 interaction might be specific for rhizobium infection [74]. Interestingly, SymRK was also identified to directly associate with and suppress the kinase activity of LjBAK1, a homolog protein of Arabidopsis AtBAK1 that functions as a coreceptor for multiple MAMP receptors. The function of SymRK in suppressing LjBAK1-mediated immunity is required for rhizobial infection, but whether this suppression favors AMF infection is to be determined [80].

Interaction protein	Summary	AMS	RNS
HMGR1	As an interacting protein of MtDMI2, HMGR1 is involved in the synthesis of isoprenoid compounds and mevalonate (MVA) pathway, and positively regulates AMS and RNS [70].	🟢	🟢
SYMREM1	Symbiotic Remorin 1 (SYMREM1) has been shown to interact with SymRK and may act as a scaffold protein for assembly of signaling complexes involved in rhizobial infection [71].	🟠	🟢
SIP1	SIP1 is a major AT-rich sequence binding (ARID) transcription factor, and two major splicing forms: SIP1 and SIP1L both can specifically bind to the promoter of *NIN*, but only SIP1 can interact with SymRK [72, 73].	🟢	🟢
SIP2	SIP2 is a typical mitogen protein kinase (MPKK), which can specifically interact with legume NORK/SymRK, and SymRK can inhibit its kinase activity. It has been shown that SIP2 was positively regulating RNS but was not involved in AMS [74].	🟠	🟢
SINA4	SINA4 is induced by Nod factors and could promote the ubiquitination degradation of SymRK, which negatively regulate nodulation [75].	🟠	🟢
SIE3	As a newly discovered E3 ubiquitin ligase, SIE3 could interact with SymRK and enhance the degree of ubiquitination of SymRK in vivo. The homodimerization of SIE3 is essential for ubiquitin-related degradation of SIP1 [76–78].	🟠	🟢
PUB1	PUB1 is an E3 ubiquitin ligase and another interacting protein of DMI2, which could be phosphorylated by DMI2 and LYK3. Although PUB1 plays a negative role in RNS and AMS, DMI2 is not the ubiquitinated substrate of PUB1, only LYK3 could be degraded [79].	🟢	🟢
PUB2	MtPUB2 could be activated by MtDMI2 via phosphorylation and activated MtPUB2 directly targets MtDMI2 through ubiquitination-mediated degradation. In addition, MtDMI2-MtPUB2 negative feedback loop plays a role in symbiosis homeostasis [79].	🟠	🟢
BAK1	SymRK could directly interact with and suppress the kinase activity of LjBAK1 in *L. japonicus*, which is a well-characterized positive regulator of plant innate immunity, then suppress the plant immunity during rhizobial infection [80].	🟠	🟠

Table 1.
Summary of interacting proteins of SymRK. The green dot represents the confirmed function of SymRK in AMS or RNS, while the orange dot represents the function of SymRK in AMS/RNS that was not confirmed or studied.

SymRK-Interacting E3 ligase (SIE3) is a protein containing CTLH/CRA/RING domains, which mediates the ubiquitination of SymRK, but does not mediate the protein degradation of SymRK in an *in vitro* ubiquitination assay [76]. It is possible that ubiquitinated SymRK may allow sustained signal transduction to downstream host responses. In addition, SIE3 plays a positive role in the SymRK-mediated signaling pathway in RNS. Further study showed that SIE3 can interact with SIP1 and form a homodimer via Cys266 residue [77, 78]. SEVEN IN ABSENTIA4 (SINA4) was identified as another E3 ligase that could interact with SymRK, and coexpression of

SymRK and SINA4 caused SymRK relocalization [75]. On the contrary, SINA4 is able to mediate the degradation of SymRK and plays a negative role in RNS by inhibiting the development of infection threads [75]. It seems like SINA4 might be a key protein working in a negative feedback loop by suppressing excessive symbiotic signal responses. However, whether SIE3 and SINA4 also regulate AMS needs to be further explored.

At present, two E3 ubiquitin ligases of Plant U-Box (PUB) family, PUB1 and PUB2, have also been proved that could interact with DMI2 in *M. truncatula* [79, 84]. PUB1 could interact with two significant symbiotic receptors (LYK3 and DMI2) and modulate the establishment of both AMS and RNS [38]. In addition, PUB1 has been shown to be directly phosphorylated by LYK3 and acts as a negative regulator to inhibit rhizobial infection and nodulation [85]. Although the E3 ligase activity of PUB1 is necessary for negative regulation in RNS and AMS, DMI2 is not a ubiquitination substrate of PUB1 [79], only LYK3 could be degraded by PUB1. It is possible that the involvement of PUB1 in the early symbiosis signal pathway might be a strategy used by plants to actively suppress the excessive infection by rhizobia and AMF. The other PUB-type E3 ubiquitin ligase, MtPUB2, was identified to be a direct regulator of DMI2 in *M. truncatula* and could enhance the ligase activity of MtPUB2 via phosphorylation at Ser421, then the activated MtPUB2 directly ubiquitinates MtDMI2 for degradation in vitro. These studies demonstrated that MtDMI2-MtPUB2 forms a negative feedback loop that displays an important role in nodulation homeostasis [79]. As a key receptor kinase involved in both RNS and AMS, the direct phosphorylation target and protein modification of SymRK need to be further elucidated.

3.2 Transcription factor complexes regulate arbuscule branching

Nuclear calcium oscillations are essential components of signals leading to AMS and RNS in host plant root cells. A couple of proteins and GRAS domain TFs cooperatively mediate calcium signals and induce symbiotic process. CCaMK (as known as DMI3 in *M. truncatula*), the initiation of calcium spiking perception in nucleus, is required in both AMS and RNS [86]. A gain-of-function of CCaMK leads to exaggerated symbiosis response by forming spontaneous nodules in the absence of rhizobial in *L. japonicus*. Interestingly, the gain-of-function of CCaMK could induce rhizobial and AMF infection, as well as calcium spiking even in the mutant plants, that is, *symrk*, *castor* or *pollux*, suggesting that calcium oscillations mediated by CCaMK are a downstream response of symbiosis pathway [61, 87, 88]. The activation of CCaMK by calcium always needs two steps, direct calcium binding to three EF-hand motifs, and calmodulin (CaM) binding to the kinase domain [89]. Thus, different levels of CCaMK activity are needed in AMS and RNS. As it has been confirmed that Thr265 residue is an essential autophosphorylation site for CCaMK activation [90]. CCaMK autophosphorylated at Thr265 and disrupts the hydrogen bonds network with residues of side chains, leading to nodule organogenesis and AMF infection after two EF-hand motifs bind to calcium. CaM binds the kinase domain and stimulates the activity of CCaMK, which is only required for rhizobial infection but not for AMF colonization. Consistent with that CCaMK with kinase domain only could restore the symbiotic entry of AMF in *ccamk* mutant in *L. japonicus* [88, 91]. In addition, rice OsDMI3 is able to functionally complement the AMS in *M. truncatula*, but partially restore the nodulation phenotype in legumes [92].

Downstream and phosphorylated by CCaMK [24], CYCLOPS/IPD3 is required for rhizobial infection, nodule development [93, 94], AMF infection, and arbuscule

formation [24, 95]. Acting as a member in CSSP, OsCYCLOPS could complement the AMS and RNS phenotype of *cyclops-3* mutant in *L. japonicus*, indicating functional conservation of CYCLOPS in legumes and non-legumes [24]. Recently, IPD3 and IPD3-LIKE are identified functional redundancy in AMS in *M. truncatula*, the double mutant of *IPD3* and *IPD3L* can form arbuscular but hyphal entry into epidermis cells is impaired [96, 97]. What's more, the development of AMS is remarkably reduced in *ipd3/ipd3l* under a high concentration of Pi treatment [97]. Recent research has demonstrated that other important regulators in Pi uptake, SPXs, and PHRs also take part in regulating AMF colonization under different degrees of phosphate [31, 32, 64]. The formation of arbuscule is reduced in the absence of PHRs. In low Pi conditions, PHRs could bind to P1BS cis-element in promotor of AM-associated genes and induce AM-mediated Pi uptake of host plants; however, SPXs could bind to PHRs, leading to the suppression of transcription of genes downstream in Pi-sufficient conditions. Besides, PHRs could not only regulate genes including *RAM1*, *PT11*, *and WRI5A*, which are required for arbuscule formation or nutrient exchange [31], but they also target genes like *CERK1*, *SymRK*, *NSP2*, etc., that are essential for signal perception of AMF entry in the early stage of symbiosis [64].

The CSSP plays a conserved role in regulating AMS and RNS, and plants discriminate between such processes by CCaMK-CYCLOPS complex promoting different GRAS domain TFs through DELLA proteins. Exogenous GA treatment could inhibit infection threads formation and nodule development, as well as hyphal entry and arbuscule formation in *L. japonicus* [98]. As integrators of GA signaling, DELLAs positively regulate rhizobial infection and arbuscule formation, acting as a bridge to complex CCaMK/DMI3-CYCLOPS/IPD3 with NSP1-NSP2 or RAM1 [25, 98]. In RNS, DELLAs work downstream of DMI3-IPD3, enhancing the intensity of phosphorylation of IPD3 by DMI3, then interact with NSP2 to promote the DMI3-IPD3-NSP1-NSP2 complex formation and induce the expression of symbiotic associated genes like *NIN* [99]. While in AMS, DELLAs interact with CCaMK-CYCLOPS complex, activating RAM1 via DIP1 (DELLA Interacting Protein 1) in rice, and RAD1 (Required for Arbuscule Development) in *M. truncatula* and *L. japonicus* [28, 59, 100, 101]. NSP1 specifically functions in Nod factor signaling, and NSP2 may have a minor role in AMS [35]. By contrast, RAM1 is only required in AMS to support arbuscule branching and has no role in Nod factor signaling [27]. Interestingly, the experiment in *Nicotiana benthamiana* showed that RAM1 may compete NSP2 with NSP1, indicating that RAM1 and NSP1 may be the first step downstream of CSSP to distinct Myc factors and NFs signaling [102]. Meanwhile, other GRAS-type transcription factors, maybe RAD1, are involved in Myc factors signaling, for NSP2 only has a weak function in AMS [27, 101, 102].

4. Conclusions and future perspectives

Plants establish mutualistic symbiosis with AMF and rhizobial for nutrient uptake. In AMS, mineral nutrients, especially Pi, are supplied by AMF via AM fungal hyphae, and host plants mainly concurrently transfer fatty acids to fungi as carbon resources. Although AMS facilitates the uptake of Pi, the concentration of Pi, in turn, impairs the colonization of AMF. Thus, the status of Pi is essential in AMS establishment, and some TFs involved in CSSP like PHRs play important roles in the regulation of this process. As a central regulator of Pi homeostasis, PHR2 is required for the activation of AMS-associated genes under Pi-deficient conditions. While in RNS, legumes

interact with rhizobial to fix N, and N homeostasis is closely related to NIN, a member of NIN-like protein (NLP) family.

The opening question is how plants discriminate chitin, NFs and Myc factors signals that are structurally similar and then promote different signaling pathways. Receptor kinases play critical roles in primary signal recognition in immunity and AMS and/or RNS. A part of LYKs like OsCERK1 play a dual role in both immunity and symbiosis, while some others show subfunctional in regulating these two signaling pathways. What's more, duplication of LysM genes has happened, compared with 10 LysM genes in rice, *M. truncatula* has 21 and *L. japonicus* has 26 genes that are predicted to encode LYKs. Therefore, the versatile combination of LYKs receptors plays an essential role in sensing structurally similar polysaccharides to initiate symbiosis or immunity thereby discriminating symbionts or pathogens.

SymRK functions as a vital component of the genetic basis for both plant-fungal and plant-bacterial endosymbiosis. It perceives signals dependent on extracellular malectin domain and LRR domain, whose sequences vary wildly between non-legumes and legumes, but are much more conserved in legumes. It is suggested that the diversity among them may be one of the reasons for different responses to Myc factors or NFs. SymRK activates signaling pathways downstream mediating the
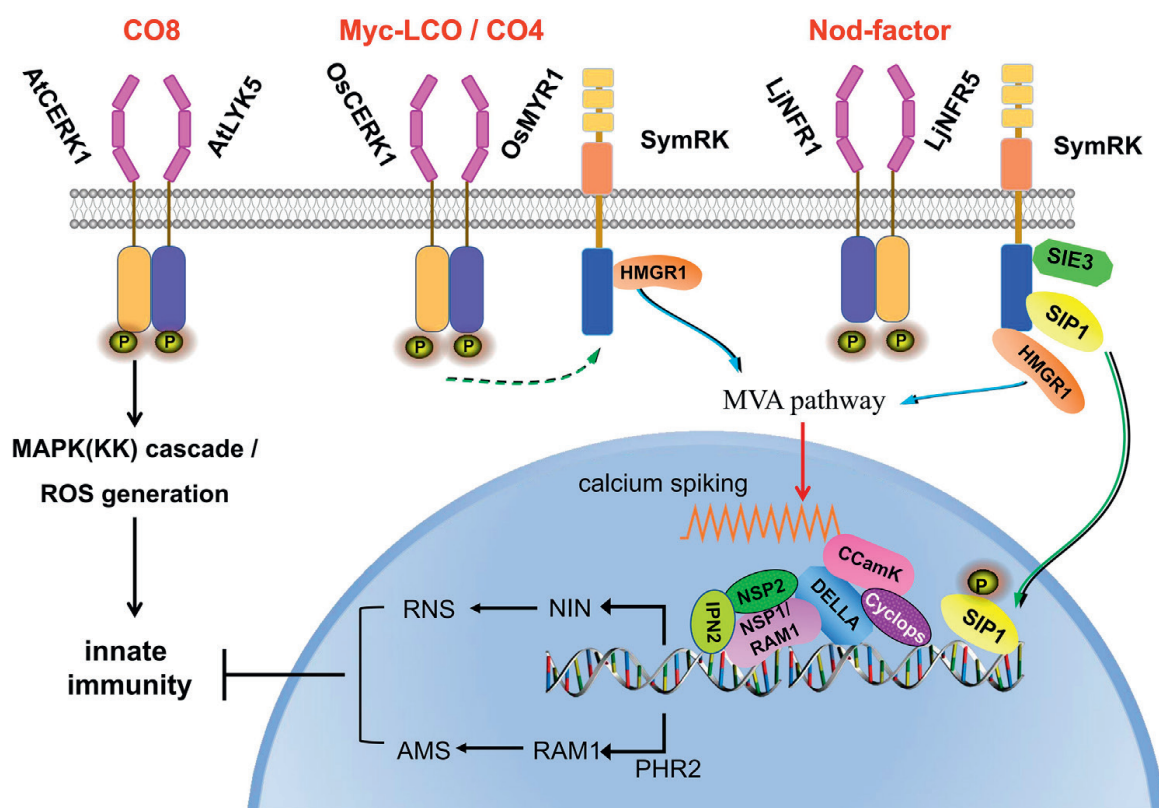

Figure 3.
The chitin-triggered immunity and common symbiotic signaling pathway. AtCERK1 and AtLYK5 form a receptor complex perceiving CO8 to induce innate immunity in A. thaliana. In AMS, the complex of OsCERK1/OsMYR1 receives Myc factors in rice. And LjNFR1/LjNFR5 receptor complex in L. japonicus perceive Nod factors (structurally similar to Myc factors) in RNS. Upon recognition of symbiotic signals of AMS/RNS, SymRK, as the coreceptor of LysM receptors, then interacts with some key proteins such as SIP1 and SIE3. Phosphorylated SIP1 could specifically bind to the promoter of NIN gene to positively regulate symbiosis. Another SymRK interacting protein, HMGR1 is a common signaling component in both AMS/RNS, which participates in MVA pathway and triggers calcium spiking in nucleus. CCaMK-CYCLOPS-DELLA complex interacts with RAM1 or NSP1/NSP2, inducing the expression of RAM1 or NIN to regulate AMS and RNS. The activation of symbiosis signaling inhibits innate immunity signaling at certain levels.

post-translational modification of the interacting proteins; however, whether these interacting proteins function in RNS also participate in AMS remains to be further investigated.

It is probable that a single pathway mediating both AMS and RNS after the recognition of signal molecules, for some homolog proteins such as CCaMK and CYCLOPS play the same role in both AMS and RNS. There may also be some parallel signaling pathways to regulate the TFs in nucleus. The complex of CCaMK-CYCLOPS directly regulates at least 3 genes in different pathways: *NIN*(RNS), *RAM1*(AMS), *CBP1*(RNS and AMS) [103]. In addition, NIN and RAM1 may be the first divergence for AMS or RNS in CSSP, and we wonder whether there are other TFs involved or not.

In summary, receptor kinases are essential in the specific recognition of signals, and OsCERK1/OsMYR1 were confirmed to be the receptor complex perceiving Myc factors in recent research, but the pivotal receptors for Myc factors in other species remain to be studied. As SymRK could receive symbiotic signals from NFRs, it is of great interest whether SymRK could receive Myc-factor signals to participate in symbiotic signal transduction between AMF and plants. Activated by the calcium spiking, CCaMK-CYCLOPS-DELLA complex could regulate TFs which promote the expression of AMS-related or RNS-related genes (**Figure 3**). Therefore, further study on the difference in signal recognition and signaling pathways between AMS and RNS may help us to apply RNS in non-legumes.

Acknowledgements

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