The PHYTOGLOBIN-NO cycle regulates plant mycorrhizal symbiosis

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Spotlight Article

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Abstract

The production of the redox-active, signalling molecule, NO has long been associated with interactions between microbes and their host plants. The emerging evidence now suggests that specific NO signatures and cognate patterns of *PHYTOGLOBIN1* (*PHYTOGB1*) expression, a key regulator of cellular NO homeostasis, may help determine either symbiosis or pathogenicity.

Main Text

Mycorrhizal fungi spend a minor portion of their life cycle as free living organisms and a majority of their life cycle associated with their respective host plant. It has been estimated that over 90% of land plants are associated with Mycorrhizal fungi [1]. Among various mycorrhizal associations the arbuscular mycorrhizal (AM) association with plants is one of the most important, as they play a major role in shaping both agricultural and natural ecosystems and their associated productivity. AM fungi establish themselves in root cortical cells facilitating the uptake of key molecules, especially phosphorous, to their host plant, thus providing a unique source of essential micronutrients under limiting conditions, promoting plant growth. In turn, AM fungi receive photosynthate from their host plants [1]. AM fungi also convey additional advantages to their host plants such as increasing disease resistance due to presence of 'elicitor' molecules on their surface which trigger microbial associated molecular pattern (MAMP) immunity [2] and further, activation of the symbiotic regulatory (SYM) pathway, which partially supresses the host immune response facilitating colonization [3].

The accumulating evidence suggests that the free radical signalling molecule, nitric oxide (NO) plays a key role in plant symbiotic interactions [3]. AM fungi have also been reported to induce disease resistance in soybean against *Phytophthora sojae*, an economically significant pathogen of this plant. Further, NO is thought to be a key component in the signalling network establishing this resistance [4]. In the association between leguminous plants and rhizobium bacteria both partners contribute to NO production [5]. Significantly, NO plays a key role from the initial stages of the interaction through the development of mature root nodules and their subsequent senescence [5]. In this context, nitrate reductase (NR), mitochondrial electron transport chain mediated nitrite NO reduction and nitric oxide synthase-like (NOS-like) activity have all been proposed to generate the observed NO production. Further, an important function for NO turnover has also been studied [5].Thus, a delicate balance between NO production and removal is thought to determine key signalling outputs associated with plant-microbe symbiosis [5].

The major NO scavenging pathways are thought to be mediated by phytoglobin (Pgb) and S-nitrosoglutatione reductase (GSNOR). Phytoglobins are a group of non-symbiotic hemoglobins. These hexacoordinate hemoglobins are functionally and genetically distinct from symbiotic hemoglobins and possess high affinity for both oxygen and NO under certain conditions such as hypoxia, thereby functioning as effective molecular scavengers for these molecules [5]. The generated nitrate via

oxygenation of NO via Pgb can subsequently become a substrate for NR to produce nitrite and concomitantly, NO. This cycling of NO mediated by Pgb is termed the "Pgb-NO cycle" [5]. Although NO is known to play a key signal in the establishment of AM fungal-plant interactions the underpinning molecular details have remained enigmatic.

Excitingly, Martinez-Medina et al.,[6] now demonstrate that NO-dependent regulation of *PHYTOGB1* (class 1 hemoglobin) transcription plays a key role in these mycorrhizal-plant interactions. Significantly, they also identify specific NO-based signatures that precede colonisation by *Rhizophagus irregularis*, employed as a soil inoculant in agriculture and horticulture, that regulate *PHYTOGB1* expression. Using transgenic tomato hairy roots, these authors demonstrated that PHYTOGB1 controls the levels of NO in tomato roots during colonization of the AM fungus, *R. irregularis*. Further, PHYTOGB1 also modulated NO accrual during the interaction of tomato plants with the necrotrophic fungal pathogen, *Fusarium oxysporum*. In the case of *R. irregularis,* initial contact with the fungus or exudates derived from its germinating spores, generated rapid and subtle oscillations of NO accumulation, specifically in tomato epidermal and root hair cells, followed subsequently by transcriptional up-regulation of *PHYTOGB1* at the later stages of this response. Remarkably, cell wall extracts from *R. irregularis* failed to induce these NO oscillations, suggesting recognition of microbial associated molecular patterns (MAMPS) [7] by their cognate pattern recognition receptors (PRRs) [7] were not integral to this response. Rather, the observed signature of NO accumulation appeared to be an early plant response to diffusible factors released from germinating spores of AM fungi. In addition, this *R. irregularis* induced NO signature also triggered activation and subsequent analogous oscillations in the expression pattern of NO-inducible *PHYTOGB1*, presumably reducing NO levels (Fig 1A). Overexpression of *PHYTOGB1* in tomato hairy roots, further decreasing NO levels, promoted increased *R. irregularis* colonisation but did not alter the abundance of arbuscules in the colonized areas, supporting a role of *PHYTOGB1* in the early events of root colonization and its extension, but not in arbuscule formation. Counter-intuitively, RNAi silencing of *PHYTOGB1* also resulted in enhanced colonization, suggesting perturbing NO oscillations *per se* is sufficient to enhance AM fungal colonization, possibly by disrupting plant immune responses that might be activated by this NO signature.

In complete contrast, challenge with the pathogenic fungus, *F. oxysporum*, failed to induce subtle NO oscillations restricted to root epidermal cells. Rather, this pathogen triggered a stronger and temporally sustained production of NO throughout the root. Despite these high NO levels, *PHYTOGB1* was repressed after 24 hours post *F. oxysporum* challenge, implying this response might be driven by *F. oxysporum* to aid pathogenesis. In this context, high chronic NO levels are thought to promote pathogen susceptibility by negatively regulating key plant immune responses and promoting cell death (Fig 1B) [8], both favouring *F. oxysporum* infection. Thus, a distinct NO signature generated in tomato root epidermal cells reports the subsequent establishment of a symbiotic interaction with *R. irregularis*. Significantly, a highly dissimilar NO signature was produced by tomato roots in response to challenge with *F. oxysporum*. Further, the transcriptional profile of *PHYTOGB1*

expression was also markedly different during these two distinct types of plant-microbe interactions. Thus, Martinez-Medina and co-authors [6] provide new insights into the associations of microbes with their host plants, by the discovery that specific NO signatures and cognate patterns of *PHYTOGB1* expression may help determine either symbiosis or pathogenicity.

This interesting study supports and extends the findings of Gupta *et al.,* [9]**,** which showed the mutualistic endophyte, *Trichoderma aspelloides*, generates a specific NO signature during the early stages of an interaction with *Arabidopsis thaliana* roots. Further, it has also recently been demonstrated that *Trichoderma harzianum* triggers a rapid and transient burst of NO in roots of tomato. This NO accumulation correlated with expression of *PHYTOGB1* suggesting the possible importance of the Pgb-NO cycle in establishment of beneficial *Trichoderma* plant interactions [10]. Tight NO regulation can also be important under abiotic stress. For instance, flooding stress leads to an NO increase in specialised cortical cells promoting lysegenous aerenchyma formation and the PHYTOGB1-NO cycle is thought to be integral to this process [11]. Importantly, the enzyme responsible for NO production in plant-AM fungal interactions still remains to be established. Reductive pathways including NR and PM NI-NOR (Plama membrane Nitrite:NO reductase) may be responsible but these pathways produce NO at low oxygen conditions. However, plant roots often experience hypoxia and hence it is plausible that low root oxygen tensions might trigger mycorrihization via NO signalling. Martinez-Medina and co-authors [6] found increased AM fungal colonization in Pgb over expression lines. Previously it was demonstrated that overexpression of Pgb in barley roots leads to low internal oxygen which can trigger limited NO production, resulting in further activation of Pgb [12]. This mechanism might also explain the observed increased mycorrhization of tomato lines overexpressing Pgb [6].

Selection of plant genotypes with increased Pgb activity and by extension enhanced symbiotic ability could be the target of future breeding programs to both convey resistance against pathogens and promote colonization of beneficial microbes. Taken together, the accumulated evidence suggests that tight regulation of the NO-Pgb cycle plays an important role in NO homeostasis facilitating discrimination between microbial pathogens and symbionts.

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Figure Legend

Fig 1

(A) Scheme summarizing the role of nitric oxide (NO) in arbuscular mycorrhizal (AM) in symbiosis with plants. At pre-symbiotic stages several myc factors released by fungal spores trigger NO production via oxidative or reductive NO biosynthetic pathways. The produced NO eventually activates symbiotic regulatory pathway DMI1, DMI2, DMI3 (does not make infection genes). Activation of this pathway provides further room for fungus to grow and reach cortical cells. NO in turn induces phytoglobin1 (*PHYTOGB1*). The induced PHYTOGB1 further scavenge NO and keep the NO levels low for active colonization.
(B) During the interaction with necrotrophs such as *Fusarium*, infection pathogen-associated molecular patterns (PAMPs) generated by fungus are recognised by plant pattern-recognition receptors (PRRs) leads to production of high levels of NO and reactive oxygen species (ROS). High levels of NO or its reaction with ROS probably may cause Tyr-nitration of PHYTOGB1 leads to its inactivation, thus in turn increase NO production and subsequently initiates cell death.