INTRODUCTION

Nitric oxide (NO) is a free-radical product of cell metabolism, being nitrate reductase the best characterized enzymatic pathway for NO production in plants. However, other reductive and oxidative routes have been also described (Lamattina and Polacco, 2007). It functions as a ubiquitous signal involved in diverse physiological processes and it is frequently implicated in multiple cell signaling events under the control of phytohormones including growth, development, and stress responses. Nevertheless, in most cases the molecular mechanisms underlying NO action in the plant cell are still undeciphered. The overlapping roles between plant hormones and NO raise the question of how both molecules may act in coordination. In general, regulatory effects of NO are mediated through protein modifications, including tyrosine nitration, metal nitrosylation, and S-nitrosylation of cysteines. Thus, the identification of NO primary targets has provided new opportunities to link NO reactivity and biological processes. In this review, we highlight the progress brought by the identification of S-nitrosylated targets proteins related to stress and growth-promoting plant hormones. Our focus is the broad role of this post-translational modification that allows NO to modulate plant hormone homeostasis as well as signaling pathways. However, the participation of NO beyond its action through S-nitrosylation in hormone-regulated processes is out of the scope of this work and it is widely covered in recent reviews by Simontacchi et al. (2013) and Astier and Lindermayr (2012).

S-NITROSYLATION AS AN EMERGING POST-TRANSLATIONAL MODIFICATION OF PLANT PROTEINS

S-nitrosylation is the reversible binding of an NO moiety to a reactive cysteine residue of a target protein to form an S-nitrosothiol (SNO; Stansler et al., 2003). It is recognized as a reversible and ubiquitous regulatory reaction. Thus, like in animals, this redox-based post-translational mechanism is also crucial for the transduction of NO bioactivity in many plant cellular responses (Hess et al., 2005). At first, protein S-nitrosylation was thought to be controlled mainly through the regulation of NO biosynthesis. However, in mammals it has been postulated as a short-range NO post-translational mechanism limited to proximity of NO sources (Martínez-Ruiz et al., 2013). In addition to the enzymatic NO-producing enzymes, it is important to consider that both, favorable environment to NO-producing enzymes, it is important to consider that both, favorable environment to S-nitrosylation agent formation as well as transnitrosylating reactions could promote the expansion of the S-nitrosylation range of action (Martínez-Ruiz et al., 2013). The SNO turnover could also provide an alternative mechanism to control protein S-nitrosylation in the cell. Given the labile nature of this post-translational modification, it was conceived initially as a spontaneous and non-regulated process. However, different denitrosylases enzymes have been described, which directly mediate denitrosylation or govern the cellular equilibrium between protein and low-molecular weight SNOs. Two main enzymatic systems have emerged as physiologically relevant denitrosylases: the glutathione/S-nitrosoglutathione reductase (GS/GSNOR) and the thioredoxin/thioredoxin reductase (Trx/TrxR; Benhar et al., 2009). S-nitrosylation of the major intracellular antioxidant tripeptide GSH forms S-nitrosoglutathione (GSNO) that functions as a mobile reservoir of NO. Consequently, the enzyme GSNOR or GSNOR1 in Arabidopsis does not display a direct denitrosylase activity but controls intracellular levels of both, GSNO and SNO affecting the global level of S-nitrosylation (Feechan et al., 2005; Malik et al., 2011). In the other side, the mechanism described in animals for Trx denitrosylation involves direct interaction between NO and Trx, with a requirement of thioredoxin reductase.
S-nitrosylation of target proteins linked to stress phytohormones

Salicylic acid (SA) and ethylene (ET) are key signaling molecules for plants in the resistance to biotic stress (Fujita et al., 2006; Loake and Grant, 2007). NO has an essential role in restriction of pathogen attack by induction of the defense response and programmed host cell death (reviewed by Mur et al., 2013). Thus, NO bioactivity may exert a role on SA and ET hormone signaling pathways.

In Arabidopsis, one of the first comprehensive proteomic studies allowed the identification of more than 100 S-nitrosylated proteins (Lindermayr et al., 2005). Interestingly, one of the identified S-nitrosylated proteins corresponded to a methionine adenosyltransferase (MAT) which catalyzes the synthesis of S-adenosylmethionine (SAM), a substrate for ET biosynthesis. Later on, Lindermayr et al. (2006) provided the first detailed molecular characterization of an S-nitrosylation regulatory mechanism in plants. This study describes the S-nitrosylation of Cys-114 residue of the MAT1 isoform and the consequent inhibition of its activity. The enzymes S-adenosylhomocysteinase and cobalamin-independent methionine synthase are also part of the methionylmethionine cycle and both enzymes have been found to be S-nitrosylated in proteomic analysis in Arabidopsis and Kulanscaceae pinunata plants (Lindermayr et al., 2010; Abat et al., 2008). Activation/inactivation of these enzymes controls the SAM pool impacting in ET biosynthesis. All these evidences point out a multi-step control of ET biosynthesis by S-nitrosylation and opened the possibility to elucidate new mechanisms of NO and ET cross-talk (Figure 1A).

Salicylic acid is synthesized by plants in response to pathogen infection and is essential to the establishment of resistance mechanisms, including host cell death and systemic acquired resistance. Mutations in ATGSNOR1 showed a pivotal role in the GSNO turnover, influencing cellular SNO levels under both, basal conditions and attempted microbial attack (Fecher et al., 2003). Interestingly, in the absence of ATGSNOR1 both SA biosynthesis and signaling are affected, suggesting that S-nitrosylation may control at least, two nodes of the SA-signaling network. GSNO1 regulates the S-nitrosylation extent of non-expressor of pathogenesis-related gene1 (NPR1) and SA binding protein 3 (SABP3; Tada et al., 2008; Wang et al., 2009). S-nitrosylation of SABP3 is triggered during bacterial infection and suppresses SA binding capacity and carbonic anhydrase (CA) activity (Wang et al., 2009). Since, CA activity is required for the establishment of plant disease resistance, its inhibition by S-nitrosylation during late infection stages could contribute to a negative feedback loop which could be crucial for the proper modulation of SA-dependent plant defense mechanism (Figure 1B). S-nitrosylation also exerts a key redox control of systemic acquired resistance in plants through targeting NPR1/TGA1 system. The SA NPR1-dependent signaling mechanism is mediated by redox changes that lead to reduction of NPR1 cysteines. This event switches NPR1 from cytosolic, disulfide-bound oligomers, to active monomers that are subsequently translocated into the nucleus and interacts with the TGA class of basic leucine zipper transcription factors. The result is an enhanced binding activity of TGA1 to the promoter region of pathogenesis-related (PR) genes, stimulating SA-dependent immune defense (Vlot et al., 2009). Upon pathogen attack, SA induces Trx which facilitates NPR1 monomerization, nuclear translocation, and activation of PR genes (Tada et al., 2008). Additionally, Tada et al. (2008) demonstrated that NPR1 is an S-nitrosylated protein. Notably, TGA1 is regulated by S-nitrosylation and S-glutathionylation improving TGA1 binding activity to PR1 promoter region (Lindermayr et al., 2010). However, it has not been demonstrated which type of modification, S-nitrosylation, S-glutathionylation, and/or both, is responsible for such protein–DNA binding activity (Figure 1B).

Concluding, plant immunity is regulated by a precise redox balance between the opposing actions of distinct redox-signals that catalyze NPR1 oligomer–monomer switch and NPR1/TGA1 interaction through transient redox fluctuations that includes S-nitrosylation and S-glutathionylation. Moreover, in the cytosol NPR1 also contributes to the suppression of jasmonic acid (JA)-dependent responses (Speel et al., 2003), evidencing S-nitrosylation as a mediator of the integrative hormonal regulation network for guarantee immunity in plants.

Meanwhile, abscisic acid (ABA) is the major player mediating adaptation of plants to drought stress. ABA induces stomatal closure and inhibits stomatal opening by facilitating osmotic solute loss to reduce guard cell turgor. These events take place through a complex signaling network that involves multiple components including Ca2+, K+, IP3, MAPK, and H2O2 (Fan et al., 2004). NO enhances plant tolerance to drought and it contributes to stomatal closure evoked by ABA. Mechanistically, NO regulates inward-rectifying K+ channels through its action on Ca2+ release from intracellular stores. Alternative pathways have been also indicated for NO action on the outward-rectifying K+ channels, which are Ca2+ insensitive. It is probable that NO directly modifies the K+ channels.
Targets for protein S-nitrosylation in signaling pathways of growth-promoting phytohormones auxins and cytokinins

Auxins and cytokinins (CKs) are critical regulators of cell division, expansion, and differentiation. Relatively recent breakthroughs were found by comparing functions of NO and the well-known growth-promoting hormones (reviewed by Mur et al., 2013). There are several examples of NO and auxin overlapping effects during shoot and root organogenesis such as, NO mediation of auxin-induced adventitious and lateral roots (Pagnussat et al., 2002; Correa-Aragunde et al., 2004), root hair formation (Lombardo et al., 2006), and adventitious root formation (Pagnussat et al., 2003). NO stimulates the activation of cell division and embryogenic cell formation in leaf protoplast in the presence of auxin (Dros et al., 2005). Copper-induced morphological responses are also mediated by auxin and NO in Arabidopsis seedlings (Peto et al., 2011). All these previous evidences led to investigate the possible interplay between these two signal molecules. Briefly, in the case of auxin, its perception is mediated by the F-box protein TIR1 (transport inhibitor response1) and the related proteins, AUXIN SIGNALING F-BOX proteins (AFBs; Dharmasiri et al., 2005; Kepinski and Leyser, 2005). Auxin binding stabilizes the interaction between TIR1/AFBs and the transcriptional repressor proteins, auxin/indole-3-acetic acid (Aux/IAA) causing a rapid proteasomal degradation of them (Gray et al., 2001). Then, Aux/IAA degradation results in the activation of transcriptional responses with the concomitant impact in plant growth and development (Tan et al., 2007). In an attempt to study the possible mechanism by which NO might regulate auxin signaling, S-nitrosylation of auxin receptor was analyzed. S-nitrosylation of TIR1 was demonstrated by Terrile et al. (2012). This redox-based modification enhances the efficiency by which TIR1 interacts with Aux/IAAs facilitating their degradation and modulating auxin signaling during root growth in Arabidopsis seedlings (Figure 2). Particularly, Cys-140 is a critical residue for TIR1–Aux/IAA interaction and TIR1 function. S-nitrosylation of TIR1 represents an efficient mechanism by which NO might enhance sensitivity and/or ligand selectivity. Furthermore, NO modulation of auxin signaling is more complex...
Although several lines of evidence support the involvement of S-nitrosylation/denitrosylation in plant development, including lateral root formation and nodulation in legumes (Gonzalez-Rizzo et al., 2006; Murray et al., 2007; Tiri et al., 2007), circadian rhythms (Salome et al., 2006), and shoot and root development (Werner and Schmulling, 2009). Recently, NO-mediated CK functions have been associated to cell proliferation and meristem maintenance in Arabidopsis (Shen et al., 2013). CKs are perceived and modulated by a multi-step two-component circuit through a histidine and aspartate phosphorelay (Mulder and Sheen, 2007). CKs regulate their signals through a variety of mechanisms, such as modulating transcription, controlling phosphorelay and regulating protein localization and stability (Yu and Kieber, 2008). In a recent report, Feng et al. (2013) demonstrated that NO represses CK signaling by inhibiting the phosphorelay activity through S-nitrosylation. Interestingly, the authors showed that NO-overproducing mutants, aux/1 (NO overproducer1) and gnot/1-3 do not respond to CK-induced shoot regeneration in Arabidopsis explants. Moreover, gnot/1-3 has a substantial reduction on the expression of the primary response regulator genes (ARRs) for CK signaling. Centrally, by the use of an in vivo biotin-switch assay, it was demonstrated that the histidine phosphotransfer protein AHP1 is in planta S-nitrosylated under normal growth conditions. Cys-115 was proposed as an S-nitrosylated residue. Comprehensively, AHP1 S-nitrosylation compromises CK action revealing again, a mechanism through which CK signaling components perceive and integrate a redox signal in the regulation of plant growth and development (Figure 2).

Although several lines of evidence support the involvement of NO in CK signaling (Carimi et al., 2005; Tun et al., 2008), other works claim an opposite effect of NO in CK action (Werner et al., 2003; Furlan et al., 2006; Xiao Ping and Xi Gui, 2006). Much more recently, a direct interaction between NO and CK has been also described (Liu et al., 2013). In summary, NO roles could be of the most varied because in addition to its own action it meets specific cellular functions according to the target molecules amending within the routes of hormonal regulation in plant cells.

**CONCLUDING REMARKS AND PERSPECTIVES**

NO is a fascinating molecule with remarkable feats and properties to modulate signaling pathways in biological systems. The bioactivity of NO is high enough for it to occur in a wide variety of biochemical circumstances. S-nitrosylation/denitrosylation is currently accepted as critical redox-mediated regulation processes in plant cells. Certainly, S-nitrosylation could be a possible mechanism by which NO impacts on plant hormonal regulation by modulating hormone biosynthesis, perception, transport, and/or degradation. Clearly, multiple layers of interactions may be involved in the plant hormones and NO cross-talks, depending on complex biological and biochemical scenarios in cells.
nowadays fragmented studies on its iri live function hamper our thorough understanding on hormone–NO cross-talking. Probably, high-throughput genetic approaches in combination with a deeper understanding on the basic structure/function relationships of NO generating systems will shed light on this scientific riddle.

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