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Nitric oxide-sphingolipid interplays in plant signaling: a new enigma from the Sphinx?

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INTRODUCTION

Nitric oxide (NO) emerged as one of the major signaling molecules operating during plant development and plant responses to its environment. Beyond the identification of the direct molecular targets of NO, a series of studies considered its interplay with other actors of signal transduction and the integration of NO into complex signaling networks. Beside the close relationships between NO and calcium or phosphatidic acid signaling pathways that are now well-established, recent reports paved the way for interplays between NO and sphingolipids (SLs). This mini-review summarizes our current knowledge of the influence of NO and SLs might exert on each other in plant physiology. Based on comparisons with examples from the animal field, it further indicates that, although SL-NO interplays are common features in signaling networks of eukaryotic cells, the underlying mechanisms and molecular targets significantly differ.

Keywords: sphingolipids, ceramides, long chain bases, nitric oxide, plant, signaling, abiotic and biotic stresses

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SPHINGOLIPID SYNTHESIS AND SIGNALING IN PLANTS

The generic term SLs designate both membrane-located complex SLs (glycosphingolipids, nystatin- and sphingosine-1-phosphorylceramides in plants) and their metabolic precursors, i.e., long chain bases (LCB) and ceramides (Cer; Patas et al., 2010). They therefore constitute a diverse family of hundreds of molecular entities (Cacas et al., 2012). Adding to this complexity, a subset of LCB and Cer can get phosphorylated by specific LCB and Cer kinases, respectively. Finally, the relative amount of the different SL species is not steady, but might undergo fluctuations due to the regulation of SL synthesis, degradation,
and/or phosphorylation/dephosphorylation leading to an over-
representation of specific SL (Kihara et al., 2007). Therefore, far
away from the early picture of SL being static constitutive entities,
the sphingolipidome now emerges as dynamic, possibly modified
in response to inside and outside signals and thereafter prompting
a range of physiological responses (Markham et al., 2013).

Parallel to the decoding of sphingolipidome, studies con-
ducted during the last decade brought tangible evidences for SL
function in signaling networks operating during plant develop-
ment and responses to environmental cues (Berkey and Xiao,
2012; Markham et al., 2013). Best documented are signaling func-
tions for the precursors of complex SL, i.e., LCB and Cer. For
instance LCB and Cer participate in the induction and/or control
of plant cell death as illustrated by several studies in which
LCB/Cer content was modified by exogenous treatments or the
disruption of key genes of SL metabolism (Liang et al., 2003;
Peer et al., 2003; Perre et al., 2010). Noteworthy complex membrane-located SL also participate
in pathogen-triggered cell death (Wang et al., 2008). Further-
more, whereas LCB/Cer promote cell death, phosphorylated LCB
content was modified by exogenous treatments or the
disruption of key genes of SL metabolism (Liang et al., 2003;
Brodersen et al., 2002; Liang et al., 2003; Peer et al., 2010).

Less documented in plants are the signaling functions
of SL related to their particular location within membrane
microdomains (rafts). Rafts are not only enriched in SL and
sterols, but also present a particular protein composition (Simon-
Plas et al., 2011; Cacas et al., 2012). Indeed, plant membrane rafts
are rich in signaling-related proteins (Morel et al., 2006; Lefeb-
vre et al., 2007). Such signaling proteins might not be permanent
raft residents but rather temporarily recruited following stimulus
perception (Minami et al., 2009; Li et al., 2011). Therefore, rafts
emerged as potent signaling platforms and the dynamic modifi-
cation of membrane structure/composition is probably involved
in signal transduction during plant development and response
to environmental cues. For instance alterations of membrane
integrity via defects in SL composition led to strong developmen-
tal phenotypes due to auxin carrier mislocalization (Brodersen et al.,
2010; Markham et al., 2011). Moreover analysis of the SL and raft
abundance and the raft lipid/protein composition during plant
acclimation to cold evidenced a close correlation between SL and
raft dynamics (Minami et al., 2010). Although the mechanisms
underlying the remodeling of rafts is far from being solved, SL
have been demonstrated as key components for raft formation in
animal membranes (Filippov et al., 2006). As proposed by Cacas
et al. (2012), this function of membrane SL is likely conserved
in plants, therefore outlining a possible link between SL-based regula-
ration of raft formation and/or structure and SL-triggered signaling
events.

INTERPLAYS BETWEEN SL AND NO SIGNALING: SOME
LESSONS FROM MAMMAL CELLS

Studies in the animal field initiated in the late 1990s brought to
light interconnections between SL and NO signaling in (patho-
)physiological situations (reviewed in Huwiler and Pfeilschifter,
2001; Igarashi and Michel, 2008; Perrotta and Clementi, 2010).
The models hypothesized from these studies principally implicate
Cer and sphingosine 1-P (S1P), the major LCB signal in animal
cells (Figures 1A,B). First, NO can regulate sphingomyelinasers
(SMase) that generate the formation of Cer from sphingomyelin
(SM), a major membrane SL in mammalian cells (Figure 1A; Perre-
otta et al., 2008). Interestingly NO might regulate SMase activities
in a different way, depending on the intracellular NO level. On the
one hand low physiological concentrations of NO lead to the inhi-
bition of SMases, thereby preventing cell death in a large range
of (patho)physiological models by reducing the intracellular Cer
concentration (Falcone et al., 2004; Perrotta et al., 2007). On the
other hand high levels of NO lead to the increase of Cer concentra-
tion, thereby driving cells to apoptosis (Takeda et al., 1999; Pilane
and Lalleb, 2004). In this last case (Figure 1A), NO promotes
(i) the activation of SMases that generate Cer and (ii) represses Cer
degradation via the inhibition of ceramidase activities (Huwiler
et al., 1999; Franken et al., 2002). How NO up-regulates SMases
and down-regulates ceramidas under such conditions is cur-
rently unknown. A SMase isoform has been recently identified as
S-nitrosylated in mouse, thus providing a possible mechanism for
SMase regulation by high NO concentration (Kobr et al., 2011).

Under low NO concentrations SMase inhibition is likely indi-
rect and involves a GMP/PKG-dependent pathway, possibly in
relation with the regulation of SMase intracellular localization
(Falcone et al., 2004; Perrotta and Clementi, 2010).

A second model is illustrated by the regulation of NO forma-
tion by the endothelial NO synthase (eNOS) mediated by S1P
(Figure 1B; Igarashi and Michel, 2008). At least two mechanisms
are at play in this process. Firstly the association of eNOS with
and its inhibition by caveolin, a transmembrane protein located in
the raft-related caveolae microdomains, is reverted by a Ca2+
dependent mechanism mediated by S1P (Igarashi and Michel,
2008). Secondly S1P via its binding to S1P receptors activates a
signaling cascade involving AMP-activated protein kinase, Rac1
G protein, PI3 kinase, and Akt kinase, ending up at eNOS phos-
phorylation and activation (Levine et al., 2007). Strikingly most

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NO/sphingolipid interplays in plants
FIGURE 1 | Examples of interplays between SL and NO signaling in animal (A,B) and plant cells (C,D). (A) NO regulates Cer formation from sphingomyelin in a dose-dependent process. Low NO concentrations inhibit sphingomyelinase activity (SMase) leading to low Cer levels that are further degraded to sphingosine (Sph) by ceramidases. High NO concentrations stimulate SMase activities while inhibiting ceramidases, therefore leading to high Cer levels. This differential control of SMases by NO participates in Cer-dependent cell survival or death. (B) Cer formation indirectly triggers endothelial NOS (eNOS) activation and NO formation. Sphingosine-1P (S1P) is formed from Cer degradation and subsequent phosphorylation of Sph. S1P is externalized and perceived on the outer cell surface by specific S1P receptors (S1PR). Activated S1PR trigger eNOS activation via an increase of cytosolic Ca2+ concentration and/or via the regulation of the PI3K/Akt signaling pathway. eNOS-evoked NO eventually down-regulates Cer-activated signaling pathways. (C) Complexes SL (fungal cerebrosides), SL precursors (LCB), or SL analogs (AAL fungal toxin) act as potent inducers of NO formation. Such NO production might participate in specific aspects of SL-triggered cell death or defense responses. (D) Plant exposure to cold triggers the formation of NO that down-regulates the synthesis of phospho-SL (i.e., Cer-P and LCB-P). In addition, NO participates in the modification of membrane SL content resulting from low temperature exposure.

of these proteins are located within caveolae, in close vicinity with eNOS. Such common location also accounts when considering the origin of S1P. As exemplified in TNFα-stimulated HeLa cells, S1P originates from Cer released from SM by a specific SMase isoenzyme (Barsacchi et al., 2003). Cer are subsequently deacylated by ceramidases into sphingosine that gets phosphorylated to S1P. Noteworthy the SMase isoenzyme involved in this model is also located in caveolae together with eNOS (Perrotta and Clementi, 2010). Ultimately eNOS-evoked NO counteracts the apoptotic effects of Cer by inhibiting Cer signaling pathway.
These examples point out the intricate network involving NO and SL in mammalian cells. Part of this complexity resides in the diverse isoforms of SMases and ceramidases that undergo different NO-based regulations. Thereby, NO might contribute as an enhancer or a down-regulator of Cer signaling. These examples finally underline that the interplay between NO and SL signaling is not unidirectional, but can also involve bi-directional signaling according to the cellular response examined.

**INTERPLAYS BETWEEN SL AND NO SIGNALING IN PLANTS: PROMISES FROM DAWN**

At first glance, the models depicted above seem not transposable to plants as most of the molecular actors mentioned are absent from plant cells (e.g., SM, SMases, SIP receptors, eNOS). Nevertheless, several lines of evidence indicate the existence of similar interplays between SL and NO signaling in plants. First, studies have reported the capacity of SL-related molecules to trigger NO synthesis (Figure 1C). Wang et al. (2007, 2009) evidenced that treatments with cerebrosides from the fungal pathogen *Fusarium* sp IFR-121 induce NO formation in *Taxus yunnanensis* and *Artemisia annua*. Cerebrosides are complex membrane SLs widely found in soilborne fungi and are considered as pathogen-associated molecular patterns (PAMP; Umemura et al., 2004). In this context, cerebroside-evoked NO triggers the synthesis of secondary metabolites, i.e., taxol and artemisin (Wang et al., 2007, 2009). In addition to SL-related elicitors, some pathogenic fungi produce toxins structurally analogous to LCBs such as AAL toxin from *Alternaria alternata* or fumonisin B1 (FB1) from *Fusarium moniliforme*. Although the formation of NO in response to these toxins has not been directly evidenced, AAL-triggered cell death was blocked by an inhibitor of mammalian NOS suggesting that NO was required for AAL response (Goechev et al., 2004). Being LCB analogs, AAL and FB1 toxins block Cер synthesis and provoke free LCB accumulation (Abbas et al., 1994). Interestingly, Da Silva et al. (2011) recently showed that exogenous treatments with LCB triggering NO formation in tobacco cells. Nevertheless the biological outcome of LCB-stimulated NO production remains obscure as NO was not required for LCB-induced cell death. Although seminal, these studies require further investigations to establish the biological relevance of SL-triggered NO formation. For instance, one has to establish if specific SL structural features are required to trigger NO production, as reported for H₂O₂ synthesis (Shi et al., 2007). As plants lack bona fide NOS, the source of SL-evoked NO should be hunted, together with the mechanisms underlying its activation by SL. Finally it is noteworthy that the data available rely on exogenous treatment of plant material with SL-related molecules. One has therefore to examine if and how endogenous modifications of SL homeostasis might induce NO production.

Conversely to the regulation of NO formation by SL, recent data indicate that NO regulates specific aspects of SL metabolism in plants (Figure 1D). In particular it may participate in the fine-tuning of the equilibrium between LCB/Cer and LCB/P/Cer-P. This was evidenced for *Arabidopsis* response to cold where two phosphorylated SL species (i.e., the LCB phosphotyrosine-phosphate and a putative Cer-P) are rapidly and transiently formed (Cantrel et al., 2011; Duttilleul et al., 2012). In this context cold-evoked NO functions as a negative regulator of phospho-SL formation (Cantrel et al., 2011). How NO regulates phospho-SL formation during cold-stress response remains unclear. First NO could impact the activity of kinases or phosphatases metabolizing LCB-P and Cer-P. For instance sphingosine kinase 1 (Sphk1) is regulated by NO in human endothelial cells (Schwalin et al., 2010). So far only SIP lyase, which catalyses the degradation of LCB-P has been identified as a target for NO-based PTM and the biological significance of this modification remains to be established (Zhan and Desiderio, 2009). The regulation of LCB-metabolizing enzymes has been poorly studied in plants and further investigations are therefore required to decipher if NO can directly regulate these enzymes. Secondly NO might modify the availability of LCB/Cer kinase substrates. Supporting this possibility, Guillas et al. (2013) evidenced that an *Arabidopsis* mutant line over-expressing a non-symbiotic hemoglobin, and thereby exhibiting low NO levels, over-accumulates phytosphingosine. The levels of phytosphingosine were further increased after cold exposure and might afford for the highest rate of phytosphingosine-P formation observed in this mutant (Cantrel et al., 2011). Interestingly, the analysis revealed another facet of the SL response affected in this mutant. Indeed, whereas the overall amount of LCB was strongly lowered by cold exposure in WT plants, it was drastically increased in the mutant line (Guillas et al., 2013). These data therefore suggest that NO might participate in the regulation of more complex sphingolipid modifications associated with cold response.

As for SL-triggered NO formation, the example presented above questions about the ubiquity of SL–NO interplay in diverse physiological contexts and the underlying mechanisms at work. Although direct connections have not been established yet, it is likely that SL–NO crosstalks participate in ABA signaling (Zhang et al., 2009; Guo and Wang, 2012). In this framework PtdOH and signaling could be crucial to interlink SL and NO signaling. Indeed ABA activates phospholipase Dα1 (PLDα1) to synthesize PtdOH and thereby triggers NO formation (Zhang et al., 2009; Uraji et al., 2012). Strikingly PLDα1 is also a target for LCB-P that stimulate PtdOH-synthesis (Guo and Wang, 2012). This apparent simplicity turns to complexity when considering that (i) PtdOH generated by PLDα1 interacts with and further stimulates the LCB kinase Sphk1 (Guo et al., 2012) and (ii) that a ABA-triggered NO formation is also required for the activation of Phospholipase Dβ and PtdOH synthesis (Distéfano et al., 2012). In this intricate signaling network, further investigations should now examine the consequences of alterations of NO or SL signaling on each other to clearly establish possible direct NO–SL crosstalks. The interaction between *Arabidopsis* and the phytopathogenic bacteria *Pseudomonas syringae* is another context where NO–SL interactions are likely. On the one hand studies carried out on this pathosystem led to the pioneering demonstration of NO as a signal in plants (Delledonne et al., 1998). On the other hand it provided the first example of dynamic changes of LCB level triggered by biotic stress in plants (Peer et al., 2010). Although the interplay of NO and LCB has not been addressed yet in this system, it opens the possibility that NO regulates LCB metabolism as suggested above for cold stress, and/or that LCB trigger NO production as reported for ROS (Peer et al., 2011). Besides interacting within signaling
networks or interfering with each other metabolism, NO and SL might interfere at the level of protein trafficking toward membranes. In the case of auxin bioactivity, Esfandiari in either SL or NO metabolism lead to misaddressing or degradation of the auxin efflux transporter PIN1 and thereby to altered development of root system (Fernández-Marcos et al., 2011; Markham et al., 2012). Sphingolipid ER is involved in nitric oxide-induced stomatal closure. Plants 226, 1099–1907. doi: 10.1109/2412-1745-4.4.

Due to the recent involvement of NO in vesicle trafficking in roots (Lombardo and Lamattina, 2012), further analysis of its interplay with SL in this context might shed light on unexplored roles for NO in plant cell biology.

CONCLUSION

Increasing evidence plead for functional interplays between NO and lipid signaling and indirectly bring to forestage the role of biological membranes in NO biology. As exemplified in mammals and plants, SL signals generated by the catabolism or as intermediates of the synthesis of complex membrane SL, constitute new elements of the NO signaling network in a variety of physiological processes. The rising interest for SL and NO signaling in plants will undoubtedly provide new soon examples of this interplay. Future investigations should help unravel the mechanisms underlying such NO–SL signaling crosstalks. In particular a direct regulation of enzymes of the NO and SL pathway by SL and NO, respectively should be evaluated. As observed in mammals, this might include modulation of activity but also regulation of protein targeting. Finally it is likely that NO, which is liposoluble, does not only interplay with SL signaling within the cytosol, but also within the biological membranes. As it might deeply affect the activity and/or targeting of membrane-located proteins and the overall membrane structure, attention should now be paid to NO signaling within the lipid phase.

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