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Microreview

Nitric oxide signalling functions in plant–pathogen interactions

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Summary

Nitric oxide (NO) is a highly reactive molecule that rapidly diffuses and permeates cell membranes. During the last few years NO has been detected in several plant species, and the increasing number of reports on its function in plants have implicated NO as a key molecular signal that participates in the regulation of several physiological processes; in particular, it has a significant role in plant resistance to pathogens by triggering resistance-associated cell death and by contributing to the local and systemic induction of defence genes. NO stimulates signal transduction pathways through protein kinases, cytosolic Ca^{2+} mobilization and protein modification (i.e. nitrosylation and nitration). In this review we will examine the synthesis of NO, its effects, functions and signalling giving rise to the hypersensitive response and systemic acquired resistance during plant–pathogen interactions.

Introduction

Nitric oxide (NO) is a bioactive molecule that exerts a number of diverse signal functions in phylogenetically distant species (Beligni and Lamattina, 2001). It is a free radical that can either gain or lose an electron to energetically more favourable structures, namely the nitrosonium cation (NO^+) and the nitroxyl radical (NO^-). Because of its unique chemistry, which permits both its stability and reactivity, NO and its exchangeable redox-activated forms are now recognized as intra- and intercellular signalling molecules (Durner *et al*., 1998). The free radical of NO has a half-life of a few seconds and rapidly reacts with O_2 to form nitrogen dioxide (NO_2) that degrades to nitrite and nitrate in aqueous solutions (Neill *et al*., 2003). However, this gaseous free radical rapidly diffuses across biological membranes and can play a part in cell-to-cell signalling in brief periods of time (Beligni and Lamattina, 2001). In addition, NO can react with the free radical superoxide (O_2^-) to form the reactive molecule peroxynitrite ($ONOO^-$). Moreover, NO also reacts rapidly with proteins, especially with reactive amino acids such as cysteine and tyrosine, as well as with various receptors and transcription factors (Stamler *et al*., 2001).

Both cytotoxic and cyto-protecting/stimulating properties of NO have been described in plants (Beligni and Lamattina, 2001). High levels of NO are associated with cell death and DNA fragmentation in *Taxus* cultures (Pedroso *et al*., 2000). Concentrations of NO greater than 10 μM inhibit the expansion of leaves, change thylakoid viscosity and impair photosynthetic electron transport in pea (Leshem *et al*., 1998). Exposure to NO has also been shown to reduce photosynthesis in oat and alfalfa leaves (Hill and Bennet, 1970) and to inhibit respiration in carrot cell suspensions (Zottini *et al*., 2002). NO stimulates seed germination in different species and determines increases in chlorophyll levels of *Arabidopsis* and lettuce seedlings grown in the dark. Hypocotyl and internode elongations, which are inhibited in processes mediated by light, are also affected by NO (see Beligni and Lamattina, 2001). NO mediates the abscisic acid-induced stomatal closure in varoious species (Desikan *et al*., 2002; Garcia-Mata and Lamattina, 2002), the auxin response leading to root organogenesis in cucumber (Pagnussat *et al*., 2003) and plays a central role in determining lateral root development in tomato (Correa-Aragunde *et al*., 2004). Moreover, low NO concentrations increase the rate of leaf expansion in pea (Leshem and Haramaty, 1996). A decrease in NO levels has also been associated with fruit maturation and flower senescence, suggesting its involvement in the modulation of these

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physiological processes as well (Beligni and Lamattina, 2001).

Exposures to low levels of NO improve the response of plants under diverse types of stresses. Indeed, pretreatment with an NO donor enhanced tolerance to drought in wheat seedlings (Garcia-Mata and Lamattina, 2002) and induced salt resistance by increasing the K^+ to Na^+ ratio in calluses of reed plants (Zhao *et al.*, 2004). Additionally, NO can strongly protect tomato plants from methylviologen damage by scavenging reactive oxygen species (ROS) (Beligni and Lamattina, 2001) confirming its anti-oxidant function. Similar effects have been observed in barley aleurone cells where the ROS-dependent giberellin-induced programmed cell death (PCD) in the presence of NO donors is delayed (Beligni *et al.*, 2002). Furthermore, NO production has been observed in response to several abiotic stressors such as high temperature, osmotic stress or UV-B (Beligni and Lamattina, 2001; Gould *et al.*, 2003). In spite of its involvement in these diverse processes, NO could not be considered as a universal plant stress response as its production has not been observed following mechanical or light stress up to 400 μmol m^{-2} s^{-1} (Gould *et al.*, 2003).

Nitric oxide has also been implicated in disease resistance to avirulent pathogens attack. After pathogen recognition, a complex signal transduction system triggers defence responses based on accumulation of ROS and NO (Fig. 1; Levine *et al.*, 1994; Delledonne *et al.*, 1998). In this review we will evaluate different components of NO production and functions during plant–pathogen interaction, including the binomial rate NO/ROS to induce cell death and NO targets as pieces of its signalling pathways.

Nitric oxide synthesis in plants

In animals, biosynthesis of NO is primarily catalysed by the enzyme nitric oxide synthase (NOS) that oxidizes L-arginine to L-citrulline and NO. Three isoforms of NOS have been identified: neuronal NOS (nNOS) and endothelial NOS (eNOS), which are referred to as constitutive NOSs, and inducible NOS (iNOS) that is expressed in macrophages and other cell types in response to inflammatory agents and cytokines (Wendehenne *et al.*, 2001). Non-enzymatic production of NO from nitrite in an acidic/reducing environment has also been demonstrated in humans (Weitzberg and Lundberg, 1998). In plants, NO can be synthesized either by an inorganic nitrogen pathway or by enzymatic catalysis. Slow and spontaneous liberation of NO occurs from nitrite at neutral pH (Yamasaki, 2000). Acid pH and reducing agents such as ascorbate and phenolics, which are abundant in plants, can also accelerate the rate of NO production (Yamasaki, 2000; Berthke *et al.*, 2004). Recently, Berthke and colleagues have demonstrated synthesis of NO via the non-enzymatic reduction of apoplastic nitrite in seeds (Berthke *et al.*, 2004), although it is unlikely that this route of production of NO is significant in response to pathogen attack as the apoplastic pH is likely to be too high (Bolwell *et al.*, 2002).

In plants, the first enzyme found to be implicated in NO synthesis was nitrate reductase (NR). This protein has a fundamental role in nitrogen assimilation and produces NO when photosynthetic activity is absent or inhibited and when nitrite, the substrate for NR-dependent NO synthesis, can be accumulated (Yamasaki, 2000). As NR is involved in many physiological responses (see Neill *et al.*,

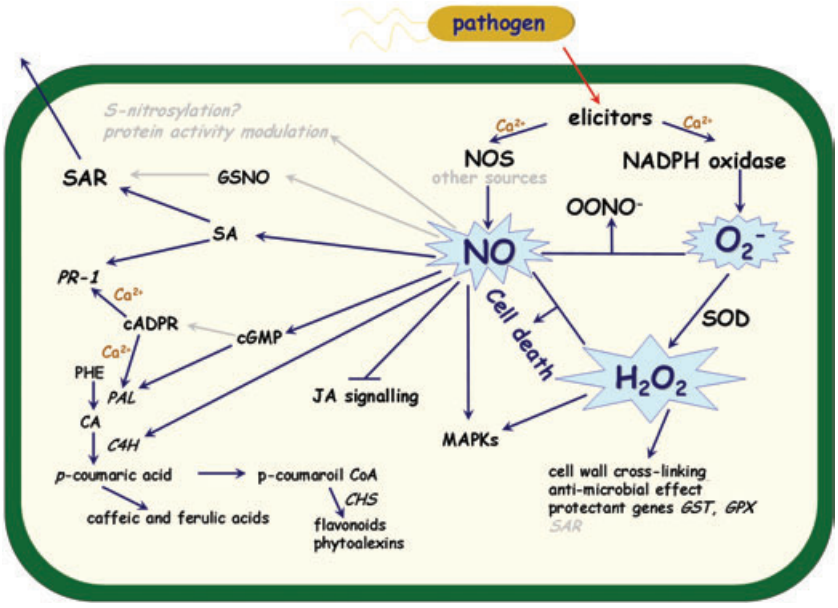

Fig. 1. Representation of NO signalling functions during the HR. Grey arrows represent potential NO functions and synthesis; blue arrows represent experimental supported results.
CHS, chalcone synthase; C4H, cinnamic acid-4-hydroxylase; CA, cinnamic acid; Ca^{2+}, calcium influx; cADPR, cyclic ADP ribose; cGMP, cyclic GMP; GPX, glutathione peroxidase; GSNO, S-nitroso-L-glutathione; GST, glutathione S-transferase; H_2O_2, hydrogen peroxide; HR, hypersensitive response; JA, jasmonic acid; MAPK, mitogen-activated protein kinase; NO, nitric oxide; NOS, nitric oxide synthase; $ONOO^-$, peroxynitrite; PAL, phenylalanine ammonia lyase; PHE, phenylalanine; PR, pathogenesis-related proteins; SA, salicylic acid; SAR, systemic acquired resistance; SOD, superoxide dismutase.

2003), it has been viewed as a candidate for NO production during plant–pathogen interactions. Yamamoto *et al.* (2003) reported that fungal infection of potato tubers causes a transient increase of the NR transcript. However, no significant differences in NO accumulation have been observed in response to infection with avirulent pathogens between wild-type *Arabidopsis* plants and the double mutant *nia1/nia2* that has no NR activity (Zhang *et al.*, 2003).

During the last few years, several groups have provided evidence for the existence of NOS-like activity in plants. NOS-like activities, detected by the oxidation of arginine to citrulline or by electron paramagnetic resonance, have been identified in several plant species such as *Mucuna hassjoo*, *Lupinus albus*, tobacco, pea, maize and soybean (see Neill *et al.*, 2003). In addition, inhibition of NO production by mammalian NOS inhibitors has been observed in pea, soybean, tobacco and *Taxus brevifolia* (see Wendehenne *et al.*, 2001). Furthermore, confirmatory results have been obtained from immunological and immunocytochemical analysis using antibodies raised against mammalian NOS isoforms (Modolo *et al.*, 2002). Nonetheless, the existence of a plant NOS has been object of discussion because mammalian NOS antibodies may recognize many plant proteins that are unrelated to NOS (Butt *et al.*, 2003) and because no gene with high sequence similarity to known mammalian NOS was found in the genome of *Arabidopsis* (Neill *et al.*, 2003).

A pathogen-inducible NOS (iNOS) has been recently identified in tobacco and *Arabidopsis* (Chandok *et al.*, 2003). Purification of NOS-like activity from tobacco demonstrated that the protein is ≈120 kDa and is a variant of the P protein (varP) of the glycine decarboxylase complex (Chandok *et al.*, 2003). In fact, in addition to mammalian inhibitors, the P protein inhibitors carboxymethoxylamine (CM) and aminoacetonitrile (AA) suppresse the ability of tobacco iNOS to synthesize NO (Chandok *et al.*, 2003). Although plant and mammalian iNOS have almost no homology, sequence analysis identified several motifs that may have similar functions. Plant iNOS has similar kinetic properties to its animal counterpart and also requires tetrahydrobiopterin (H_4B), FAD, NADPH, Ca^{2+}, calmodulin (CaM) for activity *in vitro* (Chandok *et al.*, 2003). Moreover, it also contains a pyridoxal phosphate (PP) binding domain that probably participates in NO synthesis as CM inhibits the function of P protein by reacting with PP (Chandok *et al.*, 2003). The activity of iNOS is strongly induced both in resistant tobacco after infiltration of leaves with tobacco mosaic virus (TMV) and in *Arabidopsis*-resistant plants infected with turnip crinkle virus (TCV) (Chandok *et al.*, 2003). Preliminary studies have indicated a dramatic rise in varP mRNA levels in TCV-infected resistant *Arabidopsis* plants (Chandok *et al.*, 2003). The rapid NO production measured in epidermal cells after treatment with the elicitor cryptogein (Foissner *et al.*, 2000) as well as the requirement of Ca^{2+} influx and protein phosphorylation during NOS-dependent NO accumulation in ginseng cells treated with oligogalacturonic acid (Hu *et al.*, 2003) suggest that plant iNOS could also be post-translationally regulated or that another, possibly constitutive, plant NOS could be post-translationally regulated in order to produce NO in a rapid manner.

The existence of different isoforms of NOS in animals suggests that plants may also possess more than one isoform. This hypothesis was recently confirmed by the identification of a gene in *Arabidopsis* that encodes a protein with sequence similarity to another protein that has been implicated in NO synthesis in snail *Helix pomatia* (Guo *et al.*, 2003). The protein shows NOS activity that is dependent on NADPH, CaM, Ca^{2+} and independent on BH_4, FAD, FMN and haem. This gene plays a vital role in plant growth, fertility, stomatal movements and hormone signalling (Guo *et al.*, 2003), while its possible role in pathogen response has not been investigated.

Nitric oxide detection during plant–pathogen interaction

Most of the experimental data available on NO detection during plant–pathogen interactions come from studies of infections by biotrophic pathogens (Table 1). Rapid accumulation of NO in response to avirulent bacteria has been observed in soybean and *Arabidopsis* suspension-cultured cells (Delledonne *et al.*, 1998; Clarke *et al.*, 2000) as well as in *Arabidopsis* plants (Zhang *et al.*, 2003). Similarly, a rapid accumulation of NO has been observed in tobacco leaves treated with the fungal elicitor cryptogein (Foissner *et al.*, 2000) and in potato tubers treated with an elicitor from *Phytophthora infestans* (Yamamoto *et al.*, 2003). Also, direct contact of avirulent crown rust fungus with oat plants induces the production of NO at an early stage in the defence response (Tada *et al.*, 2004). Additionally, an increase in NOS activity correlated with the pathogen resistance response has been observed in resistant tobacco during TMV infection (Durner *et al.*, 1998; Chandok *et al.*, 2003) and in soybean cotyledons challenged with a fungal elicitor (Modolo *et al.*, 2002). Oligogalacturonic acid, an elicitor produced from plant cell wall degradation, stimulates NO accumulation and induces increased NOS activity in ginseng cells (Hu *et al.*, 2003). In contrast, virulent bacteria cause only an extremely modest accumulation of NO in soybean or *Arabidopsis* cell suspensions (Delledonne *et al.*, 1998; Clarke *et al.*, 2000). These results suggest that after challenge with avirulent pathogen or elicitor NO accumulates in resistant plants, and establish a direct correlation between disease resistance responses against biotophic pathogens and NO.

Table 1. Evidence of NO production during plant–pathogen interaction in various systems.

Plant	Pathogen/elicitor	Assay	Reference
Arabidopsis leaves	*P. s. m. avrRpm1*	NOS inhibitors, indirect method	Delledonne *et al.* (1998)
Arabidopsis cell suspension	*P. s. m.* m6	Haemoglobin assay	Clarke *et al.* (2000)
Arabidopsis leaves	TCV	NOS activity	Chandok *et al.* (2003)
Arabidopsis leaves	*P. s. t. avrB* or *avrRpt2*	DAF detection	Zhang et al. (2003)
Ginseng cells	OGA	NOS activity	Hu *et al.* (2003)
Oat leaves	Rust fungus	DAF detection	Tada *et al.* (2004)
Potato tuber disks	HWC	DAF detection in protein extract	Yamamoto *et al.* (2003)
Soybean cell suspension	*P. s. g.*	Haemoglobin assay	Delledonne *et al.* (1998)
Soybean cell suspension	*P. s. g. avr*A	Haemoglobin assay	Delledonne *et al.* (1998)
Soybean cotyledons	Dpm	NOS activity	Modolo *et al.* (2002)
Tobacco leaves	TMV	NOS activity	Durner *et al.* (1998)
Tobacco epidermal section	Crypt	DAF detection	Foissner *et al.* (2000)
Tobacco leaves	TMV	NOS activity	Chandok *et al.* (2003)

Crypt, cryptogein fungal elicitor from *Phytophthora cryptogea*; Dpm, fungal elicitor from *Diaporthe phaseolorum meridionalis*; HWC, hyphal wall component, fungal elicitor from *Phytophthora infestans*; OGA, oligogalacturonic acid, an elicitor from plant cell wall; *P. s. g.*, *Pseudomonas siringae glicinea*; *P. s. g. avr*A, *Pseudomonas siringae glicinea* carrying the *avr*A avirulence gene; *P. s. m. avrRpm1*, *Pseudomonas siringae maculicola* carrying the *avrRpm1* avirulence gene; *P. s. m.* m6, *Pseudomonas siringae maculicola* race m6, avirulent strain for *Arabidopsis*; *P. s. t. avrB* or *avrRpt2*, *Pseudomonas siringae tomato* carrying either *avrB or avrRpt2* avirulence genes; rust fungus, avirulent crown rust fungus *Puccinia coronata avenae*; TCV, turnip crinkle virus; TMV, tobacco mosaic virus.

Only a small amount of contradictory data are available regarding the production of NO during the defence response associated with necrotrophic pathogens or herbivorous insects. For example, wounding tomato leaves does not cause an increase in NO production (Orozco-Cardenas and Ryan, 2002), while significant amounts of NO are produced after wounding sweet potato or *Arabidopsis* plants (Jih *et al.*, 2003; Huang *et al.*, 2004). In addition, it has been shown that NO activates early wounding-related genes in *Arabidopsis* plants, although it does not appear to be a key player in this response as the induction of these genes after wounding is not affected by NO scavengers (Huang *et al.*, 2004). Moreover, NO can function as a negative regulator of some wound-related signals as NO donors either inhibit or delay the expression of wound-dependent genes such as proteinase inhibitors in tomato or ipomoelin (IPO) in sweet potato and reduces the production of wound-induced H_2O_2 (Orozco-Cardenas and Ryan, 2002; Jih *et al.*, 2003). Further analyses are still needed to clarify the involvement of NO in defence to necrotrophic pathogens and insect herbivory.

Nitric oxide and hypersensitive response

A widespread feature of plant disease resistance is the hypersensitive response (HR), which is characterized by the formation of necrotic lesions at the infection site that function to restrict pathogen infection and spread (Lamb and Dixon, 1997). One of the earliest events in the HR is the rapid accumulation of ROS (Keller *et al.*, 1998) and NO (Delledonne *et al.*, 1998; Durner *et al.*, 1998). A peak of NO concomitant with the oxidative burst has been detected in soybean and *Arabidopsis* suspension-cultured cells about 6 h after challenge with an avirulent pathogen (Delledonne *et al.*, 1998; Clarke *et al.*, 2000) while NO production has been detected at 3 or 5 h after infiltration of *Arabidopsis* leaves depending on the avirulence gene (Zhang *et al.*, 2003). Additionally, a peak of NOS activity has been observed between 4 and 6 h after treatment, depending on the experimental condition, in tobacco plants infected with TMV and in soybean cotyledons challenged with fungal elicitor (Durner *et al.*, 1998; Modolo *et al.*, 2002; Chandok *et al.*, 2003). Treatment with purified elicitors usually causes a faster plant response, as already observed for ROS accumulation (Kieffer *et al.*, 2000) because of the time necessary for direct or indirect release of the elicitor during whole pathogen treatment. Indeed, NO accumulation was found to occur within just 3 min in cryptogein-elicited tobacco leaves (Foissner *et al.*, 2000).

The simultaneous increase of NO and ROS activate a hypersensitive cell death in soybean and tobacco cell suspensions, while the independent increase of only one component of this binary system has little effect on induction of cell death (Delledonne *et al.*, 1998; de Pinto *et al.*, 2002). Instead, the role of NO as an intercellular signal that triggers cell death in adjacent cells has been recently proposed for *Arabidopsis* leaves infected with two different *Pseudomonas* avirulent strains as well. The kinetics of accumulation of NO and progression of the HR suggest the involvement of NO in cell-to-cell spreading of HR rather than in triggering cell death (Zhang *et al.*, 2003). Moreover, cytological observations have shown that either administration of NO donors or alteration of H_2O_2 levels has no effect on the elicitation of the HR in infected cells in oat plants, although both molecules are required for the onset of cell death in adjacent cells (Tada *et al.*, 2004).

In many biological systems, the cytotoxic effects of NO and ROS derive from the diffusion-limited reaction of NO with O_2^- to form the peroxynitrite anion $ONOO^-$, which then interacts with several cellular components (Koppenol *et al*., 1992). It has been shown that urate, an $ONOO^-$ scavenger, decreases lesion formation in *Arabidopsis* leaves challenged with avirulent *Pseudomonas*. However, urate did not exert any protective effects against damage originating from NO + H_2O_2 (Alamillo and Garcia-Olmedo, 2001). Nonetheless, this result should be interpreted with caution as in plants urate oxidase catalyses the oxidation of uric acid in the presence of O_2 to form allantoin and H_2O_2 (Fraisse *et al*., 2002). Furthermore, it has been shown that when soybean cell suspensions are exposed to a wide range of concentrations of $ONOO^-$, cellular viability remains unaltered (Delledonne *et al*., 2001). Although $ONOO^-$ does not appear to be an essential intermediate of NO-induced cell death, it is expected to have important physiological and signalling functions in plants, as reported for animal cells (Beckman *et al*., 1990).

Exactly how NO cooperates with H_2O_2 to trigger hypersensitive cell death is still the object of extensive investigation. *In vitro* studies have suggested that a reaction between gaseous NO and H_2O_2 produces either singlet oxygen or hydroxyl radicals (Noronha-Dutra *et al*., 1993). Alternatively, the toxicity of NO/H_2O_2 may result from the production of a potent oxidant formed via a trace metal, H_2O_2 and an NO-dependent process (Farias-Eisner *et al*., 1996). However, experimental evidence indicates that NO can induce cell death by triggering an active process in which proteases appear to play a crucial role. Cystatin-sensitive proteases have been found to be critical regulators for HR cell death in a soybean model system (Belenghi *et al*., 2003). A gene encoding the cysteine protease RD21 was found to be induced by NO in *Arabidopsis* (Polverari *et al*., 2003) and overexpression of a cysteine protease inhibitor was found to block cell death activated either by avirulent pathogens or by nitrosative stress in *Arabidopsis* and tobacco plants (Belenghi *et al*., 2003). Finally, caspase-specific protein fragmentation has recently been revealed during the HR in tobacco plants infected with TMV (Chichkova *et al*., 2004), and Ac-YVAD-CMK, an irreversible inhibitor of mammalian caspase-1, was shown to block NO-induced cell death (Clarke *et al*., 2000).

The observation that cell death during the HR is under control of a balanced accumulation of NO and H_2O_2 has physiological consequences. The formation of ROS is an inevitable event in normal cell metabolism and excesses of ROS accumulate during exposure to various stress (Mittler, 2002). The emission of NO from plants occurs under stress situations as well as under normal growth conditions and is linked to the accumulation of NO_2 (Klepper, 1990). Based on these observations, it may be hypothesized that $ONOO^-$ is continuously formed in healthy cells. Consequently, plant cells may have developed specific mechanisms to overcome the toxicity of $ONOO^-$, and may have adopted different, still unknown, NO/ROS signals for triggering cell death during the HR.

Nitric oxide-dependent signalling functions during the hypersensitive response

Nitric oxide functions together with ROS in triggering hypersensitive cell death, but it is also involved in other defence functions complementary to and independent of ROS. The analysis of gene expression profiles in NO-treated *Arabidopsis* leaves shows that NO can influence the transcriptional activity of a wide set of genes (Polverari *et al*., 2003). Expression levels of the defence-related genes phenylalanine ammonia lyase (PAL), the first enzyme of phenylpropanoid biosynthesis pathway, and pathogenesis-related protein PR-1, a well-defined marker of plant disease resistance whose expression during the defence response generally lags behind that of PAL, rise following administration of NO donors or recombinant mammalian NOS (Durner *et al*., 1998; de Pinto *et al*., 2002). Moreover, inhibition of NOS activity markedly reduces the accumulation of transcripts encoding PAL and chalcone synthase, the first enzyme of the branch specific for flavonoids and isoflavonoid-derived antibiotics (Delledonne *et al*., 1998). There is evidence that signal transduction pathway inducing the production of phytoalexin stimulated by NO exists in higher plant cells (Noritake *et al*., 1996). Indeed, the response of soybean cotyledons to elicitors from *Diaporthe phaseolorum* f. sp. *meridionalis* implies that NO production via an NOS-like enzyme triggers the biosynthesis of anti-microbial flavonoids (Modolo *et al*., 2002). Additionally, a transcriptional increase in cinnamate-4-hydroxylase (C4H) has been observed in *Arabidopsis* following infiltration with an NO donor in cell death-inducing conditions (Polverari *et al*., 2003). C4H is considered a key enzyme in the synthesis of phenolic compounds related to disease resistance. This result provides substantial support to the notion that defence responses related to the phenylpropanoid pathway are induced by NO in plants.

Nitric oxide signalling often operates in mammalian cells through cyclic GMP (cGMP)- and cyclic ADP ribose (cADPR)-dependent pathways (see Wendehenne *et al*., 2001), and similar mechanisms also appear to be active in plants (Klessig *et al*., 2000). The involvement of cGMP-dependent components in NO-dependent defence gene activation is suggested by accumulation of PAL and PR-1 transcripts in tobacco cell suspensions treated with a membrane permeable analogue of cGMP, and by suppression of NO-mediated induction of PAL by several inhibitors of mammalian guanylate cyclase (Durner *et al*.,

1998). cGMP synthesis is also necessary although not sufficient for NO-induced cell death in *Arabidopsis* (Clarke *et al*., 2000). The involvement of cGMP in several plant signal transduction pathways has also been demonstrated, including those that are NO-dependent (Bowler *et al*., 1994; Durner *et al*., 1998). However, the activity of guanylate cyclase from *Arabidopsis* is not affected by NO (Ludidi and Gehring, 2003).

Cyclic ADP ribose has been implicated as another second messenger for NO signalling in animals, acting in a cGMP-dependent signalling cascade to mediate calcium mobilization (Denninger and Marletta, 1999). Treatment with cADPR induces expression of PAL and PR-1 in tobacco (Durner *et al*., 1998), whereas cADPR antagonists suppress the induction of PR-1 by NO. However, this effect is incomplete, indicating that activation of defence responses by NO may occur through more than one pathway (Klessig *et al*., 2000). Other intracellular targets for NO in mammalian cells are MAP kinases (MAPKs) (Huwiler and Pfeilschifter, 1999). An MAPK has been found to be activated by NO in *Arabidopsis* (Clarke *et al*., 2000), although its function in the induction of genes involved in defence has not been clearly shown. Nevertheless, at least two MAPKs have been reported to function as regulators in the early plant defence response (Cardinale *et al*., 2000). A number of other kinases, and kinase kinases, are being identified that could represent a complex signalling network leading to resistance, which may at least partially share other responses to a number of different stresses (Nurnberger and Scheel, 2001).

It is recognized that NO and related species can oxidize, nitrate or nitrosylate proteins. Thus, it can modify the thiol groups present in reactive amino acids such a cysteine and tyrosine as well as the transition metal centres of a wide functional spectrum of proteins (Stamler *et al*., 2001). These modifications are both reversible and specific, allowing cells to flexibly and precisely change protein function in response to environmental signals (Mannick and Schonhoff, 2002). NO-related post-translational modifications operate as a signal in mammalian cells and are used to fight invasion by microbes and tumour cells (Stamler *et al*., 2001). However, the occurrence of NO-dependent post-translational modification of proteins in plants must still be assessed. The identification of the proteins that are susceptible to this modification will undoubtedly help to understand better the functional consequences and the relevance of S-nitrosylation under both physiological and pathophysiological conditions.

Systemic acquired resistance and nitric oxide

Plants react to pathogen invasion by mounting a localized hypersensitive reaction at the site of infection and by establishing systemic acquired resistance (SAR), a long-lasting systemic immunity that protects the entire plant from subsequent invasion of a broad range of pathogens (Ryals *et al*., 1996). Establishment of SAR is associated with the systemic expression of defence gene families encoding pathogenesis-related proteins whose physiological functions have not yet been clarified, despite the fact that many possess anti-microbial activity (Van der Biezen and Jones, 1998).

Salicylic acid (SA) plays an important role during incompatible plant–pathogen interactions in both local and systemic resistance, resulting in the stimulation of the initial oxidative burst and leading to defence gene expression (Shirasu *et al*., 1997). NO treatment induces SA accumulation and its conjugates in tobacco (Durner *et al*., 1998). Activation of PR-1 by NO is mediated by SA, because it is blocked in transgenic *NahG* plants which are unable to accumulate SA (Durner *et al*., 1998). Moreover, when tobacco plants are treated with NO donors, the lesions caused by TMV on non-treated leaves are reduced in wild-type, but not in *NahG* plants (Song and Goodman, 2001), whereas treatments with NOS inhibitors or NO scavengers diminish SA-induced SAR (Song and Goodman, 2001). These results indicate that NO plays an important role in the induction of signalling pathway(s) leading to establishment of SAR in tobacco, although its activity is fully dependent on the function of SA.

Although SA is an important molecule required for defence gene induction in uninfected distal tissue, it is not the key signal that activates systemic resistance (Mauch-Mani and Métraux, 1998). Additional molecules, such as lipids and lipid derivatives, have been suggested to be short- and long-distance mobile signals (Maldonado *et al*., 2002). Recently, an endogenous peptide elicitor released by an aspartic protease has been proposed as portable signal (Xia *et al*., 2004). Yet another candidate for mobile signalling is nitroso glutathione (GSNO; Durner *et al*., 1999) that is similar to mammals wherein NO circulates as S-nitroso thiol adducts of proteins, or as low molecular weight S-nitroso thiol. GSNO is believed to act as both an intra- and intercellular NO carrier. In fact, glutathione is a major metabolite in phloem, where the SAR signal is most probably transmitted. It has been shown that GSNO induces systemic resistance against TMV infection in tobacco (Song and Goodman, 2001). In addition, GSNO is a powerful inducer of plant defence genes (Durner *et al*., 1998) and recently a GSNO-catabolizing enzyme and its encoding gene (GS-FDH) have been characterized (Sakamoto *et al*., 2002). Moreover, a mutant yeast has been characterized that shows enhanced susceptibility to nitrosative stress (Liu *et al*., 2001). A similar gene has also been identified in both pea and *Arabidopsis* (Shafqat *et al*., 1996; Sakamoto *et al*., 2002), suggesting that plants may be able to modulate the bioactivity and signalling function of this stabilized form of NO.

Concluding remarks

A number of recent publications have evidenced the broad spectrum of cellular functions modulated by NO in plants. In particular, NO has been shown to play a key role during plant–pathogen interactions by triggering resistance-associated cell death and inducing defence-related genes. This finding has contributed to increasing the interest in NO and its synthesis and functions. Although different enzymatic sources of NO cannot be excluded, after an extensive search an NOS-like enzyme responsible for NO production during pathogen resistance response has been finally identified. Notwithstanding, many aspects of NO and the downstream mechanisms of its action remain to be elucidated.

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