Chapter 17
The Role of Nitric Oxide in Programmed Cell Death in Higher Plants

Hu-Yi He, Ming-Hua Gu and Long-Fei He

Abstract Programmed cell death (PCD) is a genetically controlled biological process involved in defense, development, and stress response. Generally, the characters of plant PCD are similar to animal apoptosis, for instance cytoplasm shrinkage, chromatin condensation, and DNA fragmentation. An important signaling molecule, nitric oxide (NO) has been implicated in environmental-induced plant PCD, but its signaling and controlling network is still unknown. Whether NO promotes or suppresses PCD depends on NO sources and concentration in different plant species and environmental conditions. The effects of NO on developmental PCD were extensively studied. NO not only plays a crucial role in hypersensitive response (HR) during plant-pathogen interactions, but is also involved in abiotic stress-induced PCD including heat shock, salt, drought, cold, UV radiation, ozone, and heavy metals (mainly cadmium, aluminum). Previous studies showed the mitochondrion as a modulating center of PCD and also control NO level *in planta*. Vacuole processing enzyme (VPE) and caspase-like protein are involved in PCD. NO regulates the expression of PCD-associated genes via mitogen-activated protein kinase (MAPK) cascade, *S*-nitrosylation, and cGMP-dependent pathway. In addition, there are diverse interactions between NO and other signals such as hydrogen peroxide, calcium, ethylene, and salicylic acid (SA) during PCD. Based on understanding of related knowledge, NO signaling network in response to PCD in higher plants is presented in this chapter.

Keywords Higher plants · Nitric oxide · Programmed cell death · Signaling network · Stress

H.-Y. He · M.-H. Gu · L.-F. He (✉)
College of Agronomy, Guangxi University, Nanning 530004, People's Republic of China
e-mail: lfhe@gxu.edu.cn

M. N. Khan et al. (eds.), *Nitric Oxide in Plants: Metabolism and Role in Stress Physiology*, DOI: 10.1007/978-3-319-06710-0_17,

17.1 Introduction

Programmed cell death (PCD) occurs in various forms throughout the plant life cycle, probably with both common and specific aspects. PCD is a genetically controlled biological process which activates an intrinsic suicide program of cells. It not only controls the degradation of intracellular components, but facilitates removal of unwanted, incorrect, or damaged cells from multicellular organisms. It plays an important role in defense response, development, and environmental stress. Leaf senescence, hypersensitive response (HR), lysigenous aerenchyma formation, and aleurone degradation are all the forms of PCD in pants.

Generally, the characters of plant PCD are similar to animal apoptosis, such as cytoplasm shrinkage, chromatin condensation, membrane blebbing, DNA fragmentation, and selective cleavage of proteins. During HR development, plant also can form apoptotic bodies. Although individual processes differ in the triggering factors such as vacuole collapse, releasing sequestered hydrolases, may be the universal trigger of plant PCD. It is indicated that the molecular machinery underlying PCD is well conserved in eukaryotic organisms. The executive phases and typical hallmarks of PCD differ under different occasions. Cleavage of genomic DNA during apoptotic PCD is divided into two subsequent steps; an early cleavage into high molecular weight fragments, whose sizes coincidence with chromatin loop domains, and later an intense fragmentation, usually forming oligonucleosomal fragments (Brotner et al. 1995), that can be detected by DNA electrophoresis in the whole tissue or cell population, also visualized by TUNEL reaction (TdT-mediated dUTP nick-end labeling) in individual cells (Gavrieli et al. 1992; Zhan et al. 2013).

Nitric oxide (NO) a simple diatomic, diffusible, gaseous free radical, involved in many physiological processes such as PCD, seed germination, lateral root initiation, flowering, stomatal closure, and responses to stress in plants. Moreover, as an important signaling molecule, NO has been implicated in environmental-induced plant PCD, but its signaling network is still unknown. Whether NO promotes or suppresses PCD is dependent on sources and concentration of NO in different plant species.

17.2 Evolution of NO and Dual Function During Plant Programmed Cell Death

Owing to the essential function of NO in plant signaling network, its endogenous source is very important. There are two ways to generate NO in plants viz. L-arginine-dependent nitric oxide synthase (NOS) pathway and nitrite-dependent nitrate reductase (NR) pathway. Although NOS-like activity has been detected in plants, this enzyme remains enigmatic. No gene or protein with sequence homology to known mammalian type NOS has been found (Crawford 2006).

NR works as a major enzymatic source of NO production in plants. It can convert nitrite to NO in vitro and in vivo (Desikan et al. 2002). In *Arabidopsis*, NR is encoded by two genes, *NIA1* and *NIA2*, which contribute differently to the synthesis of NO in different tissues.

Another is nonenzymatic conversion of nitrite to NO in the apoplast. NO produced in plants at low concentration may rapidly eliminate lipid peroxyl radicals, alter the species and components of reactive oxygen species (ROS), and block the injuries from ROS, induce the expression of antioxidant genes and the activity of antioxidant enzymes (Lamattina et al. 2003).

17.3 Effects of NO on Developmental PCD

The effects of NO on developmental PCD have been extensively studied (Table 17.1). Gibberellin (GA)-induced PCD in barley aleurone layers is mediated by ROS, because GA greatly reduces the amount of CAT (catalase) and SOD (superoxide dismutase). NO donors, SNP (sodium nitroprusside) and SNAP (*S*-nitroso-*N*-acetylpenicillamine) delay the loss of two enzymes and PCD in barley aleurone layers treated with GA, but stimulate slightly the secretion of α-amylase. It is suggested that NO may be an endogenous modulator of PCD in barley aleurone layers (Beligni et al. 2002).

Leaf senescence is a highly coordinated process that involves PCD. Early stages of leaf senescence occurring during normal leaf ontogenesis, but not triggered by stress factors, are poorly known. Kolodziejek et al. (2007) found that both nDNA fragmentation and chromatin condensation occurred quite early during barley leaf senescence and always in the same order. NO was localized in vivo and in situ within the cytoplasm, mainly in mitochondria, in leaves at the same stage as those in which chromatin condensation was observed. The highest concentration of NO was found in the cytoplasm of mesophyll cells in the earliest stage of senescence, and lower concentrations were found during later stages that might suggest that NO plays an inductive role in PCD in leaf senescence.

During the seed development, the cells of the nucleus suffer a degenerative process early after fertilization as the cellular endosperm expands and accumulates reserves. Nuclear cell degeneration has been characterized as a form of developmental PCD. Lombardi et al. (2010) showed that nucleus PCD is accompanied by a considerable production of both NO and hydrogen peroxide (H_2O_2), and each of the two molecules is able to induce the production of the other and to cause PCD when applied to a living nucleus. Xylem cells have to be killed so as to facilitate the formation of rigid hollow tubes specialized for water transport. NO is also a key factor regulating PCD and lignification during xylem formation (Neill et al. 2005).

Table 17.1 Reports of nitric oxide-mediated PCD in plants

PCD types	Inducing factors	Species	NO alteration	Effect	Reference
Growth and development	Gibberellin	Barley aleurone layers	Decrease	Protect against oxidative damage	Beligni et al. (2002)
	Xylogenesis	*Zinnia elegans*			Neill et al. (2005)
	Early senescence	Barley leaves	Increase		Kolodziejek et al. (2007)
	Seed development	*Sechium edule nucellus*	Increase		Lombardi et al. (2010)
	Self-incompatibility	Papaver	Increase	Actin reorganization	Wilkins et al. (2011)
HR	*Pseudomonas syringae*	Tobacco suspension cells	Increase	cGMP pathway	Durner et al. (1998)
		Soybean suspension cells	Increase	NO/H_2O_2 cooperation	Delledonne et al. (2001)
		Arabidopsis	Increase	Intercellular signal	Zhang et al. (2003) Guo et al. (2004)
	Yeast elicitor	*Cupressus lusitanica*	Increase	Production of peroxynitrite	Zhao et al. (2007)
		Arabidopsis thaliana suspension cultures	Increase		Clarke et al. (2000)
		sycamore (*Acer pseudoplatanus*) cultured cells		Actin depolymerization	Malerba et al. (2008)
Abiotic stress	Cadmium	*Arabidopsis* suspension cells	Increase	Induce *SAG12* expression	De Michele et al. (2009)
		Tobacco BY-2 cells	Increase	Promote Cd^{2+} accumulation	Ma et al. (2010)
		Arabidopsis	Increase	MPK6-mediated caspase-3-like activation	Ye et al. (2012)
		Yellow lupine	Increase		Arasimowicz-Jelonek et al. (2012)

(continued)

Table 17.1 (continued)

PCD types	Inducing factors	Species	NO alteration	Effect	Reference
	High light	Tobacco leaf	Increase	Cross-talk between NO and H_2O_2	
		Rice leaf	Increase	Protein *S*-nitrosylation	Zago et al. (2006) Lin et al. (2011)
	Ozone	Tobacco BY-2 cells	Increase	Antioxidant systems	De Pinto et al. (2002)
		Arabidopsis leaf	Increase	SA signaling genes	Ahlfors et al. (2009a, b)
	NO donor	*Arabidopsis thaliana* and *Nicotiana tabacum* cells	Increase	Suppression of ROS-scavenging systems	Murgia et al. (2004)
		Tobacco protoplasts	Increase	Mitochondrial pathway regulated by Ca^{2+}	Wang et al. (2010a, b)

17.4 Role of NO in Hypersensitive Response

NO plays a crucial role in HR during plant-pathogen interactions. NO and H_2O_2 function in combination with each other all along HR cell death (Table 17.1).

Administration of NO donors or recombinant mammalian NOS to tobacco plants or tobacco suspension cells triggered expression of the defense-related genes encoding pathogenesis-related 1 protein and phenylalanine ammonia lyase (PAL). These genes were also induced by cyclic guanosine monophosphate (cGMP) and cyclic ADP-ribose, two molecules that can serve as secondary messengers for NO signaling in mammals. Consistent with cGMP acting as a secondary messenger in tobacco, NO treatment induced dramatic and transient increases in endogenous cGMP levels. Unregulated NO levels drive a diffusion limited reaction with O_2^- to generate peroxynitrite ($ONOO^-$), which is a mediator of cellular injury in many biological systems but not a mediator of HR. The HR is triggered only by balanced production of NO and reactive oxygen intermediates. Increasing the level of O_2^- reduces NO-mediated toxicity. HR is activated after interaction of NO not with O_2^- but with H_2O_2. During the HR, SOD accelerates O_2^- dismutation to H_2O_2 to minimize the loss of NO by reaction with O_2^- and to trigger HR through NO/H_2O_2 cooperation. The rates of production and dismutation of O_2^- generated during the oxidative burst play a crucial role in the modulation and integration of NO/H_2O_2 signaling in the HR (Delledonne et al. 2001). The researches on the kinetics of NO production and hypersensitive cell death showed that NO accumulation contributed to HR. NO was first seen as punctate foci at the cell surface. Subsequent NO accumulation patterns were consistent with NO being an intercellular signal that functions in cell-to-cell spread of the HR (Zhang et al. 2003).

Arabidopsis suspension cultures generate elevated levels of NO in response to challenge by avirulent bacteria, and NO are sufficient to induce cell death in *Arabidopsis* cells independently of ROS. NO-induced cell death is a form of PCD, requiring gene expression, and has a number of characteristics of PCD such as chromatin condensation and caspase-like activity in *Arabidopsis cells* (Clarke et al. 2000). Phytotoxin fusicoccin induces another form of cell death in sycamore (*Acer pseudoplatanus* L.) cultured cells, likely mediated by NO and independent of cytochrome c release, and they make it tempting to speculate that changes in actin cytoskeleton are involved in this form of PCD (Malerba et al. 2008).

17.5 Involvement of NO in Abiotic Stress-Induced PCD

NO is also involved in PCD induced by abiotic stress including heat shock, salt, drought, cold, UV radiation, ozone, and heavy metals (mainly cadmium, aluminum) (Table 17.1).

Arabidopsis thaliana cell suspension cultures underwent a PCD process when exposed to 100 and 150 mM $CdCl_2$. As suggested by the expression of the marker senescence-associated gene12 (*SAG12*), this process resembled an accelerated

senescence. $CdCl_2$ treatment was accompanied by a rapid increase in NO and phytochelatin (PC) synthesis, which continued to be high as long as cells remained viable. NO is actually required for Cd^{2+}-induced cell death, because the inhibition of NO synthesis by NG-monomethylarginine monoacetate (L-NMMA) resulted in partial prevention of H_2O_2 increase, *SAG12* expression, and mortality. NO also modulated the extent of PC content and their function by *S*-nitrosylation (De Michele et al. 2009). Tobacco BY-2 cells exposed to 150 μM $CdCl_2$ underwent PCD with TUNEL-positive nuclei, significant chromatin condensation and the increasing expression of a PCD-related gene *Hsr203J*. Accompanied with the PCD, the production of NO increased significantly. NO played a positive role in $CdCl_2$-induced PCD by modulating Cd^{2+} uptake and thus promoting Cd^{2+} accumulation in BY-2 cells (Ma et al. 2010). The roots of 3-day-old yellow lupine seedlings exposed to 89 mM $CdCl_2$ resulted in PCD starting from 24 h of stress duration. Cd-induced PCD was preceded by a relatively early burst of NO localized mainly in the root tips. Above changes were accompanied by the NADPH-oxidase-dependent superoxide anion ($O_2^{\cdot-}$) production. NADPH-oxidase inhibitor and NO-scavenger significantly reduced $O_2^{\cdot-}$ and NO production, respectively, as well as diminished the pool of cells undergoing PCD (Arasimowicz-Jelonek et al. 2012).

Tobacco leaves, exposed to moderate high light, dramatically potentiated NO-mediated cell death in catalase-deficient (CAT1AS) but not in wild-type plants. The results consolidate significant crosstalk between NO and H_2O_2, and provide new insight into the early transcriptional response of plants to increased NO and H_2O_2 levels, and identify target genes of the combined action of NO and H_2O_2 during the induction of plant cell death (Zago et al. 2006). Lin et al. (2011) identified an NO accumulation mutant *noe1* (*nitric oxide excess 1*) in rice and analyzed its role in NO-mediated leaf cell death. The *NOE1*, encoded a rice catalase *OsCATC*, that increased the H_2O_2 in the leaves, which consequently promoted NO production via activation of NR. Removal of excess NO reduced cell death in both leaves and suspension cultures derived from *noe1* plants, implicating NO as an important endogenous mediator of H_2O_2-induced leaf cell death.

Ozone (O_3) induced a rapid accumulation of NO, which started from guard cells, spread to adjacent epidermal cells and eventually moved to mesophyll cells. NO production coincided with the formation of HR-like lesions. SNP and O_3 individually induced a large set of defense-related genes; however, in a combined treatment SNP attenuated the O_3 induction of salicylic acid (SA) biosynthesis and other defense-related genes. SNP treatment decreased O_3-induced SA accumulation. The O_3-sensitive mutant *rcd1* was found to be an NO overproducer; in contrast, *Atnoa1/rif1* (*Arabidopsis* nitric oxide associated 1/resistant to inhibition by FSM1), a mutant with decreased production of NO, was also O_3 sensitive. NO can modify signaling, hormone biosynthesis and gene expression in plants during O_3 exposure. NO is an important signaling molecule, which production is needed for a proper O_3 response (Ahlfors et al. 2009a, b).

The involvement of cellular antioxidant metabolism in the signal transduction triggered by these bioactive molecules has been investigated. NO and ROS levels were singularly or simultaneously increased in tobacco (*Nicotiana tabacum* cv

Bright-Yellow 2) cells by the addition of NO and/or ROS generators to the culture medium. The generation of NO did not cause an increase in PAL activity or induction of cellular death. It only induced minor changes in ascorbate (ASC) and glutathione (GSH) metabolisms. An increase in ROS induced oxidative stress in the cells, causing an oxidation of the ASC and GSH redox pairs; however, it had no effect on PAL activity and did not induce cell death at low concentrations. In contrast, the simultaneous increase of NO and ROS activated a process of death with the typical cytological and biochemical features of hypersensitive PCD and a remarkable rise in PAL activity. Under the simultaneous generation of NO and ROS, the cellular antioxidant capabilities were also suppressed (De Pinto et al. 2002). Treatment of tobacco protoplasts with SNP resulted in a rapid $[Ca^{2+}]_{cyt}$ accumulation and decrease in mitochondrial membrane potential (potential ($\Delta\Psi m$) before the appearance of PCD. NO-induced PCD could be largely prevented not only by cPTIO, but also by Ca^{2+} chelator, EGTA (*ethylene glycol tetraacetic acid*), Ca^{2+}-channel blocker $LaCl_3$ (Lanthanum chloride) or CsA (a specific mitochondrial permeability transition pore inhibitor, which also inhibit Ca^{2+} cycling by mitochondria). NO-induced PCD is mediated through mitochondrial pathway and regulated by Ca^{2+} (Wang et al. 2010a, b). The effects of different NO-donors releasing NO with either NO^+ (SNP) or NO^- (SNAP, GSNO, NOC-18) character have been compared in plant cells. SNP behaves differently than the other NO-donors tested; indeed, SNP induces accumulation of ferritin transcripts in *Arabidopsis*, whereas SNAP (*S*-nitroso-*N*-acetyl-penicillamine) inhibits its accumulation. Only SNP caused PCD and suppression of ROS-scavenging systems (Murgia et al. 2004). Artificial NO donors are widely used as tools to study the role of NO in plants. However, reliable and reproducible characterizations of metabolic responses induced by different NO donors are complicated by the variability of their NO release characteristics. NO release characteristics of the donors SNP, *S*-nitrosoglutathione (GSNO) and NOS, both in vitro and *in planta* (*Nicotiana tabacum* L. cv. BelW3) were evaluated and their effects on NO dependent processes such as the transcriptional regulation of the mitochondrial alternative oxidase (AOX) gene, accumulation of H_2O_2 and induction of cell death were assessed. Contrary to NOS and SNP, GSNO is not an efficient NO generator in leaf tissue. In spite of the different NO release signatures by SNP and NOS in tissue, the NO-dependent responses examined were similar, suggesting that there is a critical threshold for the NO response (Ederli et al. 2009).

17.6 Regulation of NO on PCD–Associated Genes Expression

Vacuole processing enzymes (VPEs) are a vacuole-localized cysteine protease, which exhibit caspase-1-like protein activity. It can mediate the activation of caspase-3-like protein to provoke PCD and is involved in virus-induced hypersensitive cell death in tobacco (Hatsugai et al. 2004). VPE activity is also required for aluminum (Al)-induced PCD in plants. Ced-9 inhibited both the Al-induced activity of

caspase-like VPE and Al-induced PCD in tobacco (Wang et al. 2009). Senescence-associated gene 12 (*SAG12*) is considered the best molecular marker of senescence. The expression of *SAG12* increased at 2 and 3 d after 100 μM $CdCl_2$ treatment (De Michele et al. 2009). Al-induced PCD was promoted by *AhSAG*, a senescence-associated gene in *Arachis hypoganea* (Zhan et al. 2013). As one of the few endogenous cell death inhibitors in plants, bax inhibitor-1 (BI-1) is potentially a core regulator of PCD (Huckelhoven 2004). PpBI-1 can attenuate Al-induced PCD and enhance Al tolerance in transgenic yeast (Zheng et al. 2007). The programmed cell death 5 (*PDCD5*) gene encodes a protein that shares significant homology with the corresponding proteins of species ranging from yeast to mice (Liu et al. 1999). Overexpression of *OsPDCD5* gene induces PCD in rice (Attia et al. 2005).

In tobacco, mechanical wounding induced the rapid transcript accumulation and activation of wound-induced protein kinase (WIPK) (Seo et al. 1995). Transgenic tobacco plants ectopically expressing *AhMPK3* exhibited enhanced resistance to first and second instar larvae of *Spodoptera litura* (Kumar et al. 2009). The conditional overexpression of *AhMPK6* resulted in HR-like cell death in tobacco (Kumar and Kirti 2010). MPK kinase 6-mediated activation of VPE modulates heat shock-induced PCD in *Arabidopsis* (Li et al. 2012). NO promotes *MPK6*-mediated caspase-3-like activation in cadmium-induced *Arabidopsis thaliana* PCD (Ye et al. 2012). Over-expression of *OsGSNOR* reduced intracellular SNO levels, which regulates global levels of protein *S*-nitrosylation, alleviated leaf cell death in *noe1* plants (Lin et al. 2011).

Cytochorme c gets to the cytoplasm at least via two mechanisms. One is via formation of a transient mitochondrial permeability transition pore (MPTP), which is produced by the voltage-dependent anion channel (VDAC) on the outer membrane, the adenine nucleotide transporter (ANT) from the inner membrane and cyclophilin D in the matrix (Green and Reed 1998). Another is directly via the VDAC (Shimizu et al. 1999). Because the expression of AOX, the unique respiratory terminal oxidase in plants, can scavenge excess superoxide anion so that the balance of NO and H_2O_2 is destroyed, AOX plays protective roles in Al-induced *Arabidopsis* protoplast death (Li and Xing 2011). As a molecular chaperone, mitochondrial HSP70 may be involved in PCD initiation by reducing $\Delta\psi_m$ in mitochondrial outer membrane (Chen et al. 2009). Through NO/H_2O_2 cooperation, SOD accelerates O_2^- dismutation to H_2O_2 to minimize the loss of NO by reaction with O_2^- and to trigger hypersensitive cell death (Delledonne et al. 2001). Some genes associated with PCD are listed in Table 17.2.

17.7 Interaction Between NO and Other Signaling Molecules During Plant PCD

There are diverse interactions between NO and other signaling molecules such as H_2O_2, calcium, ethylene, and SA during PCD. The interaction between NO and H_2O_2 can be cytotoxic or protective. NO/H_2O_2 cooperation triggers hypersensitive

Table 17.2 Genes in relation to PCD

Genes	Expression	Signal molecule	Species	PCD type	References
VPE	+	ROS	Tobacco	HR, aluminum	Hatsugai et al. (2004), Wang et al. (2010a, b)
SAG	+		*Arachis hypoganea*	Aluminum	Zhan et al. (2013)
SAG12	+	NO	*Arabidopsis*	Cadmium	De Michele et al. (2009)
BI-1	+	Ca^{2+}	*Phyllostachys prdecox*	Aluminum	Zheng et al. (2007)
PDCD5	+		Rice	Development	Attia et al. (2005)
MPK3			Tobacco	Wounding	Seo et al. (1995)
MPK6	+	ROS/Ca^{2+}, NO	*Arabidopsis*	Heat shock, cadmium	Li et al. (2012), Ye et al. (2012)
MKK4	+	H_2O_2	*Arabidopsis*	HR	Ren et al. (2002)
MEKK1			*Arabidopsis*	Innate immunity	Asai et al. (2002)
WRKY22/WRKY 29			*Arabidopsis*	Innate immunity	Asai et al. (2002)
GSNOR	+	NO	Rice	High light	Lin et al. (2011)
ANT					Green and Reed (1998)
VDAC					Shimizu et al. (1999)
AOX	+	NO, ROS	Tobacco, *Arabidopsis*	SNP, aluminum	Ederli et al. (2009), Li and Xing (2011)
HSP70	+		Rice	Salt	Chen et al. (2009)
SOD	+	NO/H_2O_2	Soybean	HR	Delledonne et al. (2001)

+ (increase)

cell death in soybean cell suspensions (Delledonne et al. 2001). Boosted NO and O_2^- production is required for Cd-induced PCD in lupine roots. Moreover, the NO-dependent Cd-induced PCD in roots of 14-day-old lupine plants was correlated with the enhanced level of the post-stress signals in leaves, including distal NO crosstalk with H_2O_2 (Arasimowicz-Jelonek et al. 2012). Using biochemical and genetic approaches in the root system, Wang et al. (2010a, b) proposed a pathway for the regulation of NO biosynthesis that involves the modulation of NIA2 by MPK6. With the increase of intracellular H_2O_2 levels, MPK6 is activated, which in turn leads to the phosphorylation of NIA2 at Ser-627. Phosphorylation of NIA2 by MPK6 dramatically.

Increases the activity of NIA2 and the production of NO and also results in morphological changes. SNP treatment resulted in a rapid $[Ca^{2+}]_{cyt}$ accumulation and the appearance of PCD in tobacco protoplasts. EGTA, $LaCl_3$ or CsA largely prevent NO-induced PCD that is mediated through mitochondrial pathway and regulated by Ca^{2+} (Wang et al. 2010a, b). Moreover, NO is involved in PCD induction via interacting with the pathways of phytohormones (Wang et al. 2010a, b). NO treatments induce ethylene production in tobacco. NO and ethylene act together to regulate O_3-induced AOX expression (Ederli et al. 2006). Transcript profiling indicated a role for NO in attenuation of certain classes of O_3 induced genes, many of which were related to SA biosynthesis or SA signaling (Ahlfors et al. 2009a, b).

17.8 NO Signaling Network in Response to PCD

Based on understanding of related knowledge, we propose NO signaling network in response to PCD in plants (Fig. 17.1). Different signals (developmental, pathogen, invasion, and abiotic stress) trigger NO production. Subsequently, NO promotes the expression of PCD-associated genes (such as VPE, AOX, HSP70, APX) via several pathways. One is cGMP-dependent pathway: NO and cGMP mediate the auxin response during adventitious root formation in cucumber (Pagnussat et al. 2003). Moreover, NO regulates the apoptotic signal cascade through protein *S*-nitrosylation (Wang et al. 2010a, b). Lin et al. (2011) suggested that *S*-nitrosylation was involved in light-dependent leaf cell death in *noe1* rice. NO targets identified only in *noe1* plants included glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and thioredoxin, which have been reported to be involved in *S*-nitrosylation regulated cell death in animals. The last one is MAPK cascade. Asai et al. (2002) identified a complete plant MAP kinase cascade (MEKK1, MKK4/MKK5, and MPK3/MPK6) and WRKY22/WRKY29 transcription factors that function downstream of the flagellin receptor FLS2. Signaling events initiated by diverse pathogens converge into a conserved MAPK cascade. An MAPK signaling cascade is activated during the adventitious rooting process induced by IAA in a NO-mediated but cGMP-independent pathway (Pagnussat et al. 2004). NO mediated caspase-3-like protease activation under Cd^{2+} stress conditions. Pretreatment with cPTIO effectively inhibited Cd^{2+}-induced MAPK activation. Cd^{2+}-induced caspase-3-like activity

was significantly suppressed in the *mpk6* mutant, suggesting that MPK6 was required for caspase-3-like protease activation (Ye et al. 2013). NO contributed caspase-3-like protease activation in Cd^{2+} induced *Arabidopsis thaliana* PCD, which was mediated by MPK6 (Ye et al. 2012). NO could also regulate the activity of Ca^{2+}-dependent protein kinase (CDPK) was addressed by Lanteri et al. (2006) who characterized a 50 kDa NO-dependent CDPK in cucumber hypocotyls. These three pathways may work synergistic or solely. In turn, gene expressions provoke some downstream events such as PCD.

17.9 Control of NO Level in Plant Mitochondrion

Previous studies showed mitochondrion has emerged as modulating center of plant PCD and also important sites in controlling NO levels in plants. Nitrite (the source of NO synthesis) inhibited the respiration of isolated *Arabidopsis* mitochondria, in competition with oxygen, an effect that was abolished or potentiated when electron flow occurred via AOX or cytochrome c oxidase (COX), respectively. Electron leakage from external NAD(P)H dehydrogenases contributed the most to NO degradation as higher rates of Amplex Red-detected H_2O_2 production and NO consumption were observed in NAD(P)H-energized mitochondria. Conversely, the NO-insensitive AOX diminished electron leakage from the respiratory chain, allowing the increase of NO half-life without interrupting oxygen consumption. The accumulation of NO derived from nitrite reduction and the superoxide-dependent mechanism of NO degradation in isolated *Arabidopsis* mitochondria are influenced by the external NAD(P)H dehydrogenases and AOX, revealing a role for these alternative proteins of the mitochondrial respiratory chain in the control of NO levels in plant cells (Wulff et al. 2009). Complex III, COX, and AOX are all involved in nitrite to NO reduction. AOX controls NO generation by directly influencing the rate of electron leakage to nitrite (Cverkovska and Vanlerberghe 2012). Robson and Vanlerberghe (2002) found that knocking down of AOX increases the susceptibility of plants to PCD. There exists a negative feedback loop where NO acts to suppress excess mitochondrial reactive nitrogen species (RNS) and presumably ROS via increased AOX expression to modulate the elicitation of PCD. Three mechanisms of AOX-mediated ROS and RNS homeostasis are suggested. First, AOX can modulate the membrane potential and reduce NO levels. Second, aconitase inhibition leads to increase in citrate which induces AOX to maintain electron flow through the electron transport chain and to lower NO concentrations (Gupta et al. 2012). Third, AOX scavenging of NO might help in decreasing ROS production by preventing over-reduction of ubiquinone pool. However, plant lead to PCD or necrotic cell death in response to stress, because NO and ROS generation from nonmitochondrial sources could swamp any AOX-mediated homeostatic mechanisms.

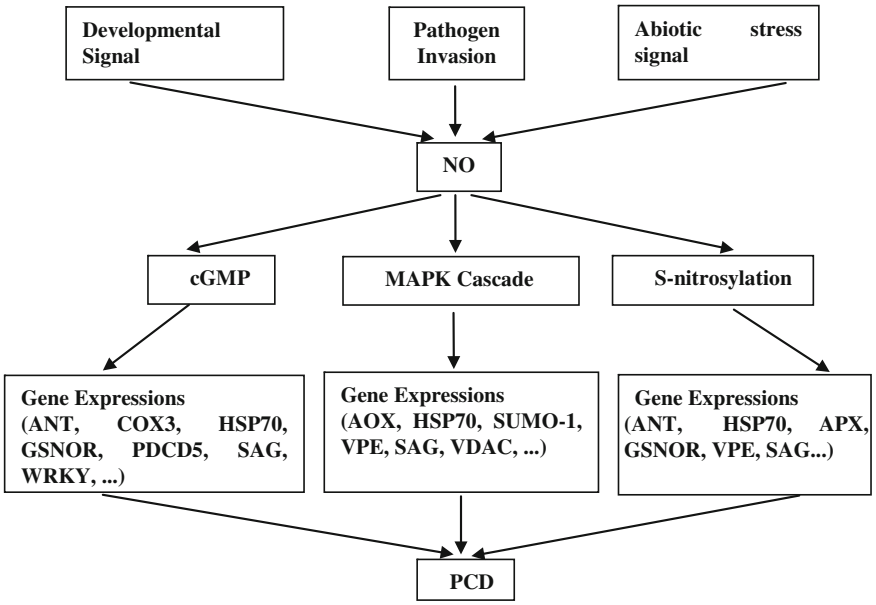

Fig. 17.1 Proposed NO signaling network in response to PCD

17.10 Conclusion and Perspectives

Adverse environmental conditions interferes NO-mediated signal transduction. By direct scavenging of ROS or activating antioxidant enzymes, exogenously applied NO might alleviate metal toxicity in plants. In contrast, NO through *S*-nitrosylation of PCs or promoting metal uptake via iron transporters contributes or even amplifies metal toxicity. The promoting and suppressing effects of NO on cell death is dependent on a variety of factors, such as cell type, cellular redox status, and the flux and dose of local NO (Wang et al. 2010a, b). Cell signaling dysregulation induced by metal not only leads to the death stimulation pathway, but might be able to activate survival signaling towards tolerance response to heavy metal. Active cell death is required for an enhanced effectiveness of protective responses in neighboring cells (Overmyer et al. 2003). In particular, the relationship between NO, ROS signaling and stress-related hormones might play a key role on the dispute on the expression of gene sets responsible for stress tolerance and in the generation of long-distance sensing from roots to shoots. NO is involved in the generation of systemic signal in systemic acquired resistance to pathogens (Vlot et al. 2008). Xiong et al. (2011) showed that tungstate is not completely a specific NR inhibitor in plant NO research. To investigate the roles of NO in plants, it is necessary to search for more NR-deficient mutants and new specific NR inhibitors. The research

on transcriptional factors and NO-regulated genes is the key to understand the mechanism of NO in PCD in higher plants. The recognition of the molecular NO targets will be an exciting challenge for future research.

Acknowledgments This work was supported by the National Science Foundation of China (31260296, 30960181, 30560070).

References

Ahlfors R, Brosche M, Kollist1 H et al (2009a) Nitric oxide modulates ozone-induced cell death, hormone biosynthesis and gene expression in *Arabidopsis thaliana*. Plant J 58: 1–12

Ahlfors R, Brosche M, Kangasjarvi J (2009b) Ozone and nitric oxide interaction in *Arabidopsis thaliana*. Plant Signaling & Behavior 4:878–879

Arasimowicz-Jelonek M, Floryszak-Wieczorek J, Deckert J et al (2012) Nitric oxide implication in cadmium-induced programmed cell death in roots and signaling response of yellow lupine plants. Plant Physiol Biochem 58:124–134

Asai T, Tena G, Plotnikova J et al (2002) MAP kinase signaling cascade in *Arabidopsis* innate immunity. Nature 415:977–983

Attia K, Li KG, Wei C et al (2005) Overexpression of *OsPDCD5* gene induces programmed cell death in rice. J Integr Plant Biol 47:1115–1122

Beligni MV, Fath A, Bethke PC et al (2002) Nitric oxide acts as an antioxidant and delays programmed cell death in barley aleurone layers. Plant Physiol 129:1642–1650

Brotner CD, Oldenburg NBE, Cidlowski JA (1995) The role of DNA fragmentation in apoptosis. Clinical Cancer Res 11:21–26

Chen X, Wang Y, Li JY et al (2009) Mitochondrial proteome during salt stress-induced programmed cell death in rice. Plant Physiol Biochem 47:407–415

Clarke A, Desikan R, Hurst RD et al (2000) NO way back: nitric oxide and programmed cell death in *Arabidopsis thaliana* suspension cultures. Plant J 24:667–677

Crawford NM (2006) Mechanisms for nitric oxide synthesis in plants. J Exp Bot 57:471–478

Cverkovska M, Vanlerberghe GC (2012) Alternative oxidase modulates leaf mitochondrial concentrations of superoxide and nitric oxide. New Phytol 195:32–39

Delledonne M, Zeier J, Marocco A et al (2001) Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. Proc Natl Acad Sci USA 98:13454–13459

De Michele R, Vurro E, Rigo C et al (2009) Nitric oxide is involved in cadmium-induced programmed cell death in *Arabidopsis* suspension cultures. Plant Physiol 150:217–228

De Pinto MC, Tommasi F, Gara LD (2002) Changes in the antioxidant systems as part of the signaling pathway responsible for the programmed cell death activated by nitric oxide and reactive oxygen species in tobacco Bright-Yellow 2 cells. Plant Physiol 130:698–708

Desikan R, Griffiths R, Hancock J et al (2002) A new role for an old enzyme: Nitrate reductase-mediated nitric oxide generation is required for abscisic acid-induced stomatal closure in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 99:16314–16318

Durner J, Wendehenne D, Klessig DF (1998) Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose. Proc Natl Acad Sci USA 95:10328–10333

Ederli L, Morettini R, Borgogni A et al (2006) Interaction between nitric oxide and ethylene in the induction of alternative oxidase in ozone-treated tobacco plants. Plant Physiol 142:595–608

Ederli L, Reale L, Madeo L et al (2009) NO release by nitric oxide donors in vitro and in planta. Plant Physiol Biochem 47:42–48

Gabaldon C, Ros Gomez LV, Pedrefio MA et al (2005) Nitric oxide production by the differentiating xylem of *Zinnia elegant*. New Phytol 165:121–130

Gavrieli Y, Sherman Y, Ben-Sasson SA (1992) Identification of programmed cell death *in situ* via specific labeling of nuclear DNA fragmentation. J Cell Biol 119:450–493

Green DR, Reed JC (1998) Mitochondria and apoptosis. Science 281:1309–1312

Guo Y, Cai Z, Gan S (2004) Transcriptome of Arabidopsis leaf senescence. Plant Cell Environ 27:521–549

Gupta KJ, Shah JK, Brotman Y et al (2012) Inhibition of aconitase by nitric oxide leads to induction of alternative oxidase and to a shift of metabolism towards biosynthesis of amino acids. J Exp Bot 63:1773–1784

Hatsugai N, Kuroyanagi M, Yamada K et al (2004) A plant vacuole protease, VPE, mediates virus-induced hypersensitive cell death. Science 305:855–858

Huckelhoven R (2004) BAX Inhibitor-1, an ancient cell death suppressor in animals and plants with prokaryotic relatives. Apoptosis 9:299–307

Jones A (2000) Does the plant mitochondrion integrate cellular stress and regulate programmed cell death? Trends Plant Sci 5:225–230

Kolodziejek I, Koziol-Lipinska J, Waleza M et al (2007) Aspects of programmed cell death during early senescence of barley leaves: possible role of nitric oxide. Protoplasma 232:97–108

Kumar KRR, Kirti PB (2010) A mitogen-activated protein kinase genes, *AhMPK6* from peanut localizes to the nucleus and also induces defense responses upon transient expression in tobacco. Plant Physiol Biochem 48:481–486

Kumar KRR, Srinivasan T, Kirti PB (2009) A mitogen-activated protein kinase genes, *AhMPK3* of peanut: molecular cloning, genomic organization, and heterologous expression conferring resistance against *Spodoptera litura* in tobacco. Mol Genet Genomics 282:65–81

Kuthanova A, Opatrny Z, Fischer L (2008) Is internucleosomal DNA fragmentation an indicator of programmed death in plant cells? J Exp Bot 59:2233–2240

Lamattina L, Garcia-Mata C, Graziano M et al (2003) Nitric oxide: the versatility of an extensive signal molecule. Annu Rev Plant Biol 54:109–139

Lanteri M, Pagnussat GC, Lamattina L (2006) Calcium and calcium-dependent protein kinases are involved in nitric oxide- and auxin-induced adventitious root formation in cucumber. J Exp Bot 57:1341–1351

Li Z, Xing D (2011) Mechanistic study of mitochondria-dependent programmed cell death induced by aluminum phytotoxicity using fluorescence techniques. J Exp Bot 62:331–343

Li Z, Yue HY, Xing D (2012) MAP kinase 6-mediated activation of vacuolar processing enzyme modulates heat shock-induced programmed cell death in *Arabidopsis*. New Phytol 195:85–96

Lin AH, Wang YQ, Tang JY (2011) Nitric oxide and protein *S*-nitrosylation are integral to hydrogen peroxide induced leaf cell death in rice. Plant Physiol 158:451–464

Liu HT, Wang YG, Zhang YM (1999) TFAR19, a novel apoptosis-related gene cloned from human leukemia cell line TF-1 could enhance apoptosis of some tumor cells induced by growth factor withdraw. Biochem Biophys Res Commun 245:203–210

Lombardi L, Ceccarelli N, Picciarelli P et al (2010) Nitric oxide and hydrogen peroxide involvement during programmed cell death of *Sechium edule* nucellus. Physiol Plant 140:89–102

Malerba M, Contran N, Tonelli M et al (2008) Role of nitric oxide in actin depolymerization and programmed cell death induced by fusicoccin in sycamore (*Acer pseudoplatanus*) cultured cells. Physiol Plant 133:449–457

Ma WW, Xu WZ, Xu H et al (2010) Nitric oxide modulates cadmium influx during cadmium-induced programmed cell death in tobacco BY-2 cells. Planta 232:325–335

Murgia I, de Pinto MC, Delledonne M et al (2004) Comparative effects of various nitric oxide donors on ferritin regulation, programmed cell death, and cell redox state in plant cells. J Plant Physiol 161:777–783

Neill S (2005) NO way to die – nitric oxide, programmed cell death and xylogenesis. New Phytol 165:1–2

Overmyer K, Brosche M, Kangasjarvi J (2003) Reactive oxygen species and hormonal control of cell death. Trends Plant Sci 8:335–342

Pagnussat GC, Lanteri ML, Lamattina L (2003) Nitric oxide and cyclic GMP are messengers in the IAA-induced adventitious rooting process. Plant Physiol 132:1241–1248

Pagnussat GC, Lanteri ML, Lombardo MC et al (2004) Nitric oxide mediats the indole acetic acid induction of a mitogen-activated protein kinase cascade involved in adventitious root development. Plant Physiol 135:279–286

Ren DT, Yang HP, Zhang SQ (2002) Cell death mediated by MAPK is associated with hydrogen peroxide production in *Arabidopsis*. J Biol Chem 277:559–565

Robson C, Vanlerberghe G (2002) Transgenic plant cells lacking mitochondrial alternative oxidase have increased susceptibility to mitochondria-dependent and -independent pathways of programmed cell death. Plant Physiol 129:1908–1920

Seo S, Okamoto M, Seto H et al (1995) Tobacco MAP kinase: a possible mediator in wound signal transduction pathway. Science 270:1988–1992

Shimizu S, Narita M, Tsujimoto Y (1999) Bcl-2 family proteins regulate the release of apoptogenic cytochrome c by the mitochondrial channel VDAC. Nature 399:483–487

Van Aken O, Giraud E, Clifton R et al (2009) Alternative oxidase: a target and regulator of stress responses. Physiol Plant 137:354–361

Vlot AC, Klessig DF, Park SW (2008) Systemic acquired resistance: the elusive signal(s). Curr Opin Plant Biol 11:436–442

Wang PC, Du YY, Li Y (2010a) Hydrogen peroxide-mediated activation of MAP kinase 6 modulates nitric oxide biosynthesis and signal transduction in *Arabidopsis*. Plant Cell 22:2981–2998

Wang WZ, Pan JW, Zheng K et al (2009) Ced-9 inhibits Al-induced programmed cell death and promotes Al tolerance in tobacco. Biochem Biophys Res Commun 383:141–145

Wang Y, Lin JS, Wang GX (2010b) Role of calcium in nitric oxide-induced programmed cell death in tobacco protoplasts. Biol Plant 54:471–476

Wang YQ, Chen C, Loake GL et al (2010c) Nitric oxide: promoter or suppressor of programmed cell death? Protein Cell 1:133–142

Wilkins KA, Bancroff J, Bosch M et al (2011) Reactive oxygen species and nitric oxide mediate actin reorganization and programmed cell death in the self-incompatibility response of papaver. Plant Physiol 156:404–416

Wulff A, Oliveira HC, Saviani EE et al (2009) Nitrite reduction and superoxide-dependent nitric oxide degradation by *Arabidopsis* mitochondria: Influence of external NAD(P)H dehydrogenases and alternative oxidase in the control of nitric oxide levels. Nitric Oxide 21:132–139

Xiong J, Fu G, Yang YJ et al (2011) Tungstate: is it really a specific nitrate reductase inhibitor in plant nitric oxide research? J Exp Bot 63:33–41

Ye Y, Li Z, Xing D et al (2012) Sorting out the role of nitric oxide in cadmium-induced *Arabidopsis thaliana* programmed cell death. Plant Signaling & Behavior 7:1493–1494

Ye Y, Li Z, Xing D (2013) Nitric oxide promotes MPK6-mediated caspase-3-like activation in cadmium-induced *Arabidopsis thaliana* programmed cell death. Plant, Cell Environ 36:1–15

Zago E, Morsa S, Dat JF (2006) Nitric oxide- and hydrogen peroxide- responsive gene regulation during cell death induction in tobacco. Plant Physiol 141:404–411

Zhan J, He HY, Wang TJ et al (2013) Aluminum-induced programmed cell death promoted by *AhSAG*, a senescence-associated gene in *Arachis hypoganea* L. Plant Sci 210:108–117

Zhang C, Czymmek KJ, Shapiro AD (2003) Nitric oxide does not trigger early programmed cell death events but may contribute to cell-to-cell signaling governing progression of the *Arabidopsis* hypersensitive response. MPMI 16:962–972

Zhao J, Fujita K, Sakai K (2007) Reactive oxygen species, nitric oxide, and their interactions play different roles in *Cupressus lusitanica* Cell death and phytoalexin biosynthesis. New Phytol 175:215–229

Zheng K, Pan JW, Ye L et al (2007) Programmed cell death-involved aluminum toxicity in yeast alleviated by antiapoptotic members with decreased calcium signals. Plant Physiol 143:38–49