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Minireview

Oxidative stress and plant cell death suppressors

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Abstract Classical yeast genetic approaches have been successfully applied for identification of genes related to the suppression of cell death. Isolated genes included several reactive oxygen species (ROS)-related genes such as *SOD* (superoxide dismutase), peroxidase, and *GST* (glutathione S-transferase). The *AtBI-1* (*Arabidopsis Bax Inhibitor-1*), which is a plant homolog of mammalian antiapoptotic gene *BI-1*, was also isolated as a suppressor of Bax-mediated lethality in yeast. Overexpression of BI-1 suppresses Bax-, H_2O_2-, salicylic acid-, and elicitor- induced cell death in plant cells. These data indicate conserved overlapping pathways that regulate ROS-mediated cell death in plants and animals.

Key words: Arabidopsis, oxdative stress, programmed cell death, reactive oxygen species.

Reactive oxygen species (ROS) generated by biotic and abiotic stresses act as messenger molecules that function at the early stage in signal regulation, stress adaptation and programmed cell death (PCD). Expression of the mammalian proapoptotic protein Bax is capable of triggering apoptotic changes similar to those found in mammalian cells, in yeast (Sato et al. 1994; Hanada et al. 1995) and plants (Lacomme and Santa Cruz 1999; Kawai-Yamada et al. 2001). Oxidative stress has also reportedly been linked to the Bax phenotype (Madeo et al. 1999; Baek et al. 2004; Kawai-Yamada et al. 2004) and plant genes capable of preventing Bax-induced death in yeast and plants have been isolated. These findings were corroborated by recent molecular and biochemical evidence, and increased our understanding of oxidative stress-induced cell death in plants. The purpose of this review article is to summarize current knowledge of plant genes that have been isolated and analyzed using heterologous cell death-inducing systems, and to discuss their biological significance in the regulation of plant oxidative stress-mediated cell death.

Mammalian proapoptotic protein Bax induces lethality in plant and yeast

Relatively few endogenous plant genes that share sequence homology with the mammalian apoptotic genes have been identified to date. Nonetheless, several similarities in PCD exist in plants and animals and the expression of animal proapoptotic protein Bax in plants and yeast has been demonstrated to induce cell death (Sato et al. 1994; Hanada et al. 1995; Lacomme and Santa Cruz, 1999; Kawai-Yamada et al. 2001). Bax is thought to cause organelle dysfunction by localizing to the outer mitochondrial membrane and forming ion channel. Thus, Bax expression disrupts mitochondrial membrane potential, releases ROS and results in changes to mitochondrial morphology in plant and yeast (Madeo et al. 1999; Beak et al. 2004; Yoshinaga et al. 2005a; Yoshinaga et al. 2005b). Madeo et al. (2002) demonstrated that Yor187w, a yeast protein sharing structural homology with mammalian caspases exhibited caspase-like processing activity and regulated H_2O_2-induced yeast death. In plant, vacuolar processing enzyme (VPE) has caspase-1 activity and mediated TMV-induced HR in tobacco (Hatsugai et al. 2004; Hara-Nishimura et al. 2005). These findings suggest that some of the mechanisms associated with cell death have been conserved among the metazoa and plants.

Plant gene screening and its ability to protect yeast cells from Bax-induced lethality

The experimental advantages associated with exploiting yeast as a heterologous system for screening and

Abbreviations: AtEBP, *Arabidopsis* ethylene-responsive element binding protein; BI-1, Bax Inhibitor-1; GST, glutathione S-transferasese; HR, hypersensitive response; PCD, programmed cell death: PHGPx, phospholipid hydroperoxide glutathione peroxidase; ROS, reactive oxygen species; VAMP, vesicle associated membrane protein.

This article can be found at http://www.jspcmb.jp/

Table 1. Plant cell death-suppressor genes isolated using a yeast screening system.

Isolated genes	References
Unknown function	
Ethylene-responsive element binding protein (*AtEBP*)	Pan et al. 2001; Ogawa et al. 2005
Bax inhibitor-1 (*AtBI-1*)	Kawai et al. 1999; Kawai-Yamada et al. 2001
ROS related genes	
Fe-superoxide dismutase (*Fe-SOD*)	Pan et al. 2001
Peroxidase	Pan et al. 2001
Glutathione S-transferase (*GST*)	Pan et al. 2001
Glutathione S-transferase/*peroxidase* (*GST*/*GPX*) (tomato)	Kampranis et al. 2000
Ascorbate peroxidase (*soybean*)	Moon et al. 2002
Phospholipid hydroperoxide glutathione peroxidase (*PHGPx*) (tomato)	Chen et al. 2004
Vesicle transport	
Vesicle-associated membrane protein (*VAMP*)	Levine et al. 2001

identification of candidate genes that functionally regulate plant cell death have been demonstrated by both ourselves and several other investigators. Plant genes that have been reported interfere with Bax-induced yeast lethality are listed in Table 1. The most abundant gene isolated in our screening assays was *AtEBP, Arabidopsis ethylene-responsive element binding protein* (Pan et al. 2001). It was demonstrated that the nuclear localization of AtEBP protein was essential for the suppression of cell death in yeast. Recently, Ogawa et al. (2005) demonstrated that overexpression of AtEBP in plant cells exhibited resistance to Bax-, heat- and H_2O_2 -induced plant cell death, suggesting a new position of AtEBP in ethylene signaling pathway.

Levine et al. (2001) demonstrated that vesicle associated membrane protein (VAMP) of *Arabidopsis* suppressed Bax-induced lethality in yeast. VAMPs, which are conserved from yeast to mammals, play an important role in vesicle docking through interaction with their counterpart, t-SNARE, in target membranes. These authors found that VAMP expression blocked Bax-induced lethality downstream of the oxidative burst, and also prevented H_2O_2-induced cell death in yeast and *Arabidopsis*. Reduced oxidation of lipids was detected in the AtVAMP expressing yeast, suggesting the possibility of improved membrane repair.

The ROS-related proteins, such as Fe- superoxide dismutase (Fe-SOD), peroxidase, glutathione S-transferases (GST, Pan et al. 2001), glutathione S-transferase/peroxidase (GST/GPX, Kampranis et al. 2000), ascorbate peroxidase (Moon et al. 2002) and phospholipid hydroperoxide glutathione peroxidase (PHGPx) (Chen et al. 2004), have all been reported to suppress Bax-induced cell death. This implies that Bax-induced cell death in yeast includes ROS generation and perturbation of redox homeostasis as described in Madeo et al. (1999).

Glutathione levels are known to play a regulatory role in the apoptotic process (Liu et al. 1998). Transgenic tobacco plants overexpressing tobacco-Tau class GSTs are more tolerant to chilling and osmotic stress (Roxas et al. 1997). Transgenic tobacco plants possessing antisense ascorbate peroxidase demonstrated hyperresponsiveness to *Pseudomonas syringae* (Mittler et al. 1999). Chen et al. (2004) showed that tomato PHGPx inhibited, not only oxidative stress-induced cell death in yeast, but also inhibited salt, heat, and Bax-induced cell death in tobacco plants. These data indicate the involvement of ROS in plant cell death.

BI-1 as an oxidative stress resistant protein

Bax Inhibitor-1 (BI-1) is one of most intensively researched plant cell death suppressors that is conserved in metazoans and plants (Lam et al. 2001; Chae et al. 2003; Hückelhoven et al. 2004). Xu and Reed (1998) identified a human cDNA that suppresses Bax-mediated cell death in yeast and named corresponding protein Bax Inhibitor-1. Plant *BI-1* genes have been isolated form various plant species such as rice (Kawai et al. 1999), *Arabidopsis* (Kawai et al. 1999; Sanchez et al. 2000; Yu et al. 2002), tobacco (Bolduc and Brisson 2003), *Brassica napus* (Bolduc and Brisson 2003) and barley (Hüchelhoven et al. 2001). The BI-1 protein has six or seven transmembrane domains and is thought to be localized in the endoplasmic reticulum (ER) membrane (Xu and Reed 1998; Kawai-Yamada et al. 2001; Bolduc et al. 2003). *Arabidopsis* plants express both Bax and AtBI-1, suggesting that the plant BI-1 is biologically active in suppressing mammalian Bax action in plants. We demonstrated that ROS production induced by the ectopic expression of Bax was not suppressed by coexpression of AtBI-1. Furthermore, H_2O_2- or salicylic acid-mediated cell death was also suppressed in tobacco BY-2 cells overexpressing AtBI-1 (Kawai-Yamada et al. 2004). These data suggest that BI-1 suppresses cell death downstream of ROS generation. It was recently reported that BI-1 deficient mice cells showed hypersensitivity toward agents that induce ER stress, such as tunicamycin or thapsigargin (Chae et al. 2004). However, the mechanism of BI-1-induced suppression of apoptosis or cell death is still unclear.

Cell death regulators are linked to pathogen defense in plants

Ectopic expression of metazoan anti-apoptosis proteins (Bcl-2, Bcl-XL, and Ced-9) in transgenic plants has been demonstrated to provide protection from pathogens (Mitsuhara et al. 1999; Dickman et al. 2001). Within

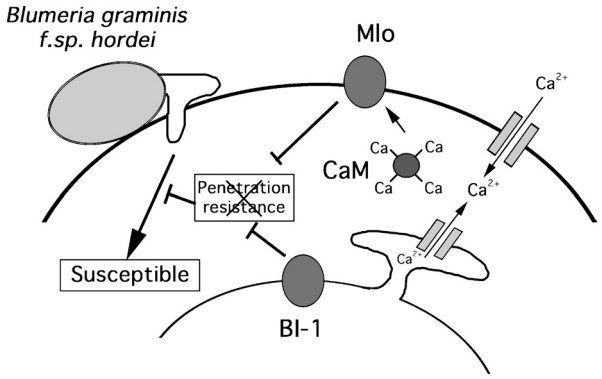

Figure 1. BI-1 and Mlo protein act as penetration resistance suppressors in barley. The Mlo protein is a plant-specific seven-transmembrane protein located in the plasma membrane and binds to calmodulin (CaM). Overexpression of barley BI-1 in *mlo5* resistant cultivar restored Mlo function in penetration by *Blumeria graminis*, suggesting that AtBI-1 and Mlo may possess similar functions in cellular defense and cell death modulation.

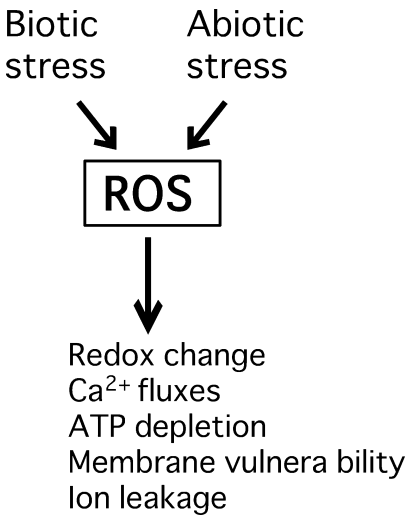

Figure 2. Biological processes leading to oxidative stress-induced cell death. Biotic and abiotic stresses can lead to ROS accumulation, which triggers various responses in plant cells.

minutes of pathogen infection, a burst in oxidative metabolism produces ROS such as H_2O_2, which subsequently trigger hypersensitive response (HR). The role of *BI-1* gene expression in the defense response has been investigated in *Arabidopsis* (Sanchez et al. 2000) and barley (Hückelhoven et al. 2001). In *Arabidopsis*, expression of the *AtBI-1* gene is rapidly upregulated during wounding or pathogen challenge (Sanchez et al. 2000). This induction was observed in both compatible and incompatible interactions between the host and pathogen. In contrast, the down regulation of rice *BI-1* mRNA was demonstrated by Matsumura et al. (2003). Treatment of suspension-cultured rice cells with cell wall extract of rice blast fungus elicited rapid H_2O_2 generation and HR. Transgenic rice cells overexpressing AtBI-1 exhibited sustained cell survival when challenged with elicitor.

Barley lines carrying recessive mutant *mlo* alleles of the *Mlo* locus, show spontaneous leaf cell death and broad-spectrum resistance to *Blumeria graminis* f.sp. hordei (*Bgh*) (Kim et al., 2002). Interestingly, Hückelhoven et al. (2003) demonstrated that overexpression of barley BI-1 induced breakdown of *mlo*-mediated penetration resistance to *Bgh* (Figure 1). Thus, BI-1 may act independently or downstream from Mlo protein.

Concluding remarks

Various biotic and abiotic stresses can cause ROS accumulation, which then lead plant cells to death (Figure 2). Despite the recent progress in our understanding of plant cellular responses, numerous uncertainties remain. The ROS cause oxidative damage to membrane lipids, proteins and nucleic acids in cells and these intracellular changes are believed to trigger a variety of responses in plant cells. The ROS signal is believed to be mediated through alterations in Ca^{2+} fluxes, redox changes, ATP depletion, membrane vulnerability, ion leakage and disruptions to cellular functioning.

Further work, such as the analysis of Ca^{2+} and redox signaling, are likely to elucidate the associated molecular mechanisms responsible for regulating cell death and survival under oxidative stress.

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