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Programmed Macromolecule Degradation during Apoptotic-Cell Death in Oats

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ABSTRACT

The execution of programmed cell death (PCD) involves the controlled degradation of cellular macromolecules such as proteins and nucleic acids. Compared with animal systems, very little is known about the molecular mechanisms regulating macromolecule degradation during plant PCD. Victorin, a host-selective toxin produced by the fungus *Cochliobolus victoriae*, induces PCD in oat cultivars harboring the *Vb* gene. Victorin-induced PCD displays typical morphological and biochemical features of apoptosis, including nuclear DNA laddering, chromatin condensation, cell shrinkage, and a mitochondrial permeability transition. In the oat-victorin system, it has been demonstrated for the first time that certain cellular macromolecules are specifically degraded during plant PCD. One example is the specific proteolytic cleavage of the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Two subtilisin-like serine proteases that exhibit caspase-like activity have been identified as associated with Rubisco proteolysis. Another example involves the degradation of RNA molecules. Ribosomal RNA species from the cytosol, mitochondria and chloroplasts are all degraded via specific degradation intermediates during victorin-induced PCD. Concurrently with rRNA degradation, mRNAs of housekeeping genes such as *actin* and *ubiquitin* but, interestingly, not those of stress-responding genes such as *PR-1* and *PR-10*, are also targeted for specific degradation. The oat-victorin system, therefore, serves as a model for elucidating the molecular mechanisms regulating macromolecule degradation in the execution phase of plant PCD.

Keywords: apoptosis, DNA laddering, protein cleavage, rRNA/mRNA degradation, victorin

CONTENTS

INTRODUCTION

Programmed cell death (PCD) is a physiological and pathological process of cell deletion that plays important roles in normal tissue homeostasis, stress responses and immune system development (Greenberg 1996; Wertz and Hanley 1996; Jacobson *et al*. 1997; Danial and Korsmeyer 2004). Multi-cellular organisms use the mechanisms of PCD to regulate developmental morphogenesis, to remove infected or damaged cells from healthy tissues (Jacobson *et al*. 1997; Vaux and Korsmeyer 1999; Nagata 2000), and to control cell numbers (Jacobson *et al*. 1997). Intensive study of the mechanisms of PCD in animals have identified typical morphological and biochemical features of PCD (apoptosis) (Martin *et al*. 1994), including condensation and shrinkage of the cell, re-organization of the nucleus, membrane blebbing, formation of apoptotic bodies (Kerr *et al*. 1972), chromatin condensation (Earnshaw 1995; O'Brien *et al*. 1998), and nuclear DNA laddering (Wyllie 1980; Earnshaw 1995). In plants, PCD has also been recognized as an integral part of development and survival programs (Greenberg 1996). PCD is essential for plant-specific development, such as the formation of tracheary elements (Obara *et al*. 2001) and cereal aleurone cells (Swanson *et al*. 1998). The sequence of the events leading to animal PCD is also detectable in plants during development and in response to different biotic or abiotic stimuli (Baillieul *et al*. 1995). However, the mechanisms of PCD are gradually becoming clearer (Drury and Gallois 2006; Bouranis *et al*. 2007), although many reports have attempted to classify PCD in plants as a form of apoptosis as seen in animals (Dangl *et al*. 1996; Pennell and Lamb 1997).

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Invited Review

During both animal and plant PCD, a broad spectrum of changes is induced in various cellular components. One of the typical changes is the degradation of specific cellular macromolecules such as proteins and nucleic acids. In animal apoptosis, various proteins, such as alpha-actinin, hnRNP K, lamin B1, PARP-1 (Kaufmann *et al*. 1993; Lazebnik *et al*. 1994) and Rho GDI 2, have been shown to be targeted for proteolytic cleavage. Through a proteomics approach, even more proteins have been identified as cleaved during apoptosis (Thiede *et al*. 2005). In most cases, activation of certain proteases, which are cysteine proteases that are activated by different pro-apoptotic stimuli (Earnshaw *et al*. 1999), precedes the proteolytic events; although it is not clear whether the activated protease is directly responsible for substrate digestion or not. Nucleic acids such as nuclear DNA, rRNA and certain mRNA species are also specifically degraded during animal apoptosis. At least in some cases, macromolecule degradation is thought to be essential for the regulation and execution of apoptosis.

In this review, we focus on macromolecule degradation during plant PCD, using oat (*Avena sativa* L.) as a model. The host-selective toxin victorin, which is produced by the phytopathogenic fungus *Cochliobolus victoriae*, induces apoptosis-like cell death in sensitive oat lines harboring the single dominant gene, *Vb* (Navarre and Wolpert 1999). Cell death induced by victorin exhibits characteristic features of animal apoptosis, such as a mitochondrial permeability transition (Curtis and Wolpert 2002, 2004), chromatin condensation (Yao *et al*. 2001), and nuclear DNA laddering (Navarre and Wolpert 1999; Tada *et al*. 2001; Yao *et al*. 2001). More recently, using this system, specific proteolytic cleavage of the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and specific degradation of various RNA species, including rRNA and mRNA, were demonstrated for the first time during plant PCD. Therefore, the oat-victorin system is one of the best models for elucidating the molecular mechanisms regulating programmed macromolecule degradation in the execution phase of plant PCD.

INTERNUCLEOSOMAL DNA FRAGMENTATION DURING VICTORIN-INDUCED PCD

Nuclear DNA laddering in oat

Internucleosomal DNA degradation, known as nuclear DNA laddering, is observed in many (but not all) apoptotic processes (Gavrieli *et al*. 1992). This process plays an essential role in reducing the autoimmune response (Nagata 2000) or inflammatory response (Bortner *et al*. 1995), and is considered to be a biochemical hallmark of apoptosis (Earnshaw 1995; Kerr *et al*. 1972; Wyllie 1980). The detailed molecular mechanisms leading to DNA laddering have been identified previously for animal apoptosis (for detail, see Nagata 1997, 2000). It is usually a two-step process in which nuclear DNA is first cleaved into 50- to 300-kb fragments, termed high molecular weight (HMW) DNA fragmentation (Walker *et al*. 1991); subsequently, the DNA is degraded into smaller fragments of oligonucleosomal size, known as low molecular weight (LMW) DNA degradation or DNA laddering (Lecoeur 2002). However, DNA laddering is not always associated with PCD in animal (Sakahira *et al*. 1999) or plant cells (Mittler and Lam 1995; Dangl *et al*. 1996).

In oat cells, as is not often the case with plant PCD, clear nuclear DNA laddering can be detected during PCD triggered by victorin as well as during PCD triggered by various elicitors, toxins or pathogen infection (**Fig. 1**) (Navarre and Wolpert 1999; Tada *et al*. 2001; Hoat *et al*. 2006). By EM-TUNEL and LM-TUNEL methods, serial morphological changes in the nucleus and other organelles were examined during victorin-induced PCD in oat (Yao *et al*. 2001). The results revealed that chromatin condensation was an early indicator of PCD, preceding DNA fragmentation, and that organelles remained morphologically intact at relatively later stages than reported in other apoptosis systems (Yao *et al*. 2001). Interestingly, EM-TUNEL positive signals were mostly observed in the heterochromachin (Yao *et al*. 2001), suggesting that DNA cleavage occurred in the more compressed nuclear DNA, rather than in open regions such as euchromatin.

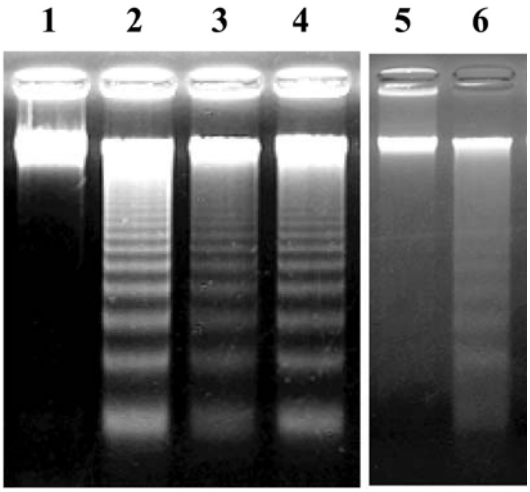

Fig. 1 Nuclear DNA laddering in oat cells. Primary leaf segments of the victorin-sensitive oat line cv. Iowa X 469 were treated with water (lane 1); 5 ng ml^{-1} victorin (lane 2); 5 mM $CuSO_4$ (lane 3); NaN3 (lane 4) for 6 h. Another oat cultivar Shokan 1 was inoculated with an incompatible race of the crown rust fungus (lane 6). Lane 5 represents genomic DNA from uninoculated control plants. Nuclear DNA was separated in a 2% agarose gel and photographed after staining with 0.5 ug ml^{-1} ethidium bromdide.

Signaling molecules and enzymes associated with nuclear DNA laddering in oat

Pharmacological studies have indicated that various types of Ca^{2+} inhibitor strongly suppress victorin-induced DNA laddering (Tada *et al*. 2001). Most Ca^{2+} inhibitors also completely prevent victorin-induced cell death as well as other cell death-related phenomena such as chromatin condensation, RNA degradation and Rubisco cleavage (Navarre and Wolpert 1999; Yao *et al*. 2001; Hoat *et al*. 2006). Therefore, an influx of Ca^{2+} seems to be an upstream and crucial signal during victorin-induced cell death. Indeed, in oats, the administration of the calcium ionophore A23187 alone causes cell death exhibiting nuclear DNA laddering (Tada *et al*. 2001). The protein kinase inhibitor K-252a was shown to have no effect on DNA laddering, but it did block chromatin condensation induced by victorin (Tada *et al*. 2001; Yao *et al*. 2001). Interestingly, A23187-induced DNA laddering was significantly suppressed by K-252a. Therefore, protein phosphorylation appears to be involved in upstream cell death signaling triggered by A23187, but not by victorin. N-acetyl-L-cysteine (NAC), a ROS scavenger was reported to inhibit the mitochondrial oxidative burst and delay victorin-induced chromatin condensation and DNA degradation (Yao *et al*. 2002b). We also found that NO stimulates the accumulation of ROS in the hypersensitive reaction (HR) lesion (Tada *et al*. 2004) and functions as an essential mediator in the modulation of H_2O_2 accumulation during the defense response (Tada *et al*. 2004).

Protease inhibitors such as E-64 (a cysteine protease inhibitor) and aprotinin (a serine protease inhibitor) blocked both victorin- and A23187-induced DNA laddering (Tada *et al*. 2001). Interestingly, cell extracts derived from oat tissues undergoing victorin-induced PCD caused nuclear collapse and internucleosomal DNA fragmentation in isolated nuclei (Kusaka *et al*. 2004). In a cell-free apoptosis system with isolated nuclei, E-64 but not aprotinin still strongly suppressed victorin-induced DNA laddering and nuclear collapse (Kusaka *et al*. 2004), indicating that at least one of the steps involving an E-64 sensitive cysteine protease is

likely to occur in nuclei. Interestingly, in the cell-free system, E-64 effectively prevented DNA laddering but none of the specific inhibitors of caspase-1, 2, 3, 4, 5, 6, 8, 9 or granzyme B was effective for suppressing DNA laddering or nuclear collapse (Kusaka *et al*. 2004). Therefore, the E-64-sensitive cysteine protease acting in or with oat nuclei to achieve DNA laddering appears to be a protease with different catalytic features from caspases or granzyme B.

In animals, certain endonucleases, including DNase I (Peitsch *et al*. 1993), DNase II (Barry and Eastman 1993), NUC18 (Hughes and Cidlowski 1994), CAD (Enari *et al*. 1998), and endonuclease G (Li *et al*. 2001; Parrish *et al*. 2001), have been implicated in the degradation of chromatin into multiples of 180-bp nucleosomal units. These nucleases differ in their cation requirement and location within the cell (Peitsch *et al*. 1994). In some cases, such as with CAD, caspase activation precedes the activation of endonuclease for DNA laddering (Enari *et al*. 1995a, 1995b; Martin *et al*. 1995; Enari *et al*. 1996, 1998; Nagata 2000). It was reported that CAD is responsible not only for DNA fragmentation but also for the morphological changes in nuclei (Nagata 2003), and that this process is sufficient to kill cells (Nagata 2000).

In plant cells undergoing PCD, activation of several specific endonucleases has been reported (for detail see Mittler and Lam 1995; Dominguez and Cejudo 2006). One of these endonuceases, ZEN1, has been cloned and shown to degrade nuclear DNA in tracheary elements without characteristic ladder formation (Ito and Fukuda 2002). During apoptotic cell death induced by victorin, activation of a specific endonuclease of 28 kDa (p28) was detected (Tada *et al*. 2001; Kusaka *et al*. 2004). The activation of p28 preceded the occurrence of chromosomal DNA degradation, and mostly paralleled DNA laddering regardless of cell death triggers (Tada *et al*. 2001). Pharmacological studies using a cell-free system revealed that nucleases and the cysteine proteases were essential components for nuclear DNA fragmentation, and both types of enzymes acted cooperatively to induce DNA laddering and nuclear collapse (Kusaka *et al*. 2004).

Is PCD a general response to pathogen attack in plants?

Nuclear DNA laddering was observed in oat leaves infected with a wide range of plant pathogens including an obligate parasite, *P. coronata f. sp. avenae* (Tada *et al*. 2001; Yao *et al*. 2002a; Tada *et al*. 2004); a facultative biotroph parasite, *M. grisea*; pathogenic bacteria, *P. syringae* pv. *atropurpurea* and *P. syringae* pv. *Coronafaciens*; and ryegrass mottle virus. All of these pathogens induced most of the apoptotic features, such as chromatin condensation, in and around the infection sites (Yao *et al*. 2002a). Intriguingly, apoptotic responses occurred in both incompatible and compatible interactions. In the case of the crown rust fungus, DNA laddering was observed at a later stage of infection in the compatible interaction compared with the incompatible one. In contrast, when oat was inoculated with the blast fungus, chromatin condensation and DNA laddering were detected earlier in oat cells infected with a compatible strain than an incompatible one (Yao *et al*. 2002a). Previous investigations in other plant species also demonstrated that, in some cases, DNA laddering is detected in both incompatible and compatible plant-pathogen interactions (Ryerson and Heath 1996; Dickman *et al*. 2001; Kiba *et al*. 2006), whereas in other cases, no nuclear DNA laddering was observed in either compatible or incompatible interactions (Mittler and Lam 1995; Ryerson and Heath 1996; Del Pozo and Lam 1998). Because PCD can be triggered by initiation of a hypersensitive response and by some toxins, it can occur in both compatible and incompatible interactions. At least in oats, PCD seems to be a common and general response to pathogen attack (Yao *et al*. 2002a).

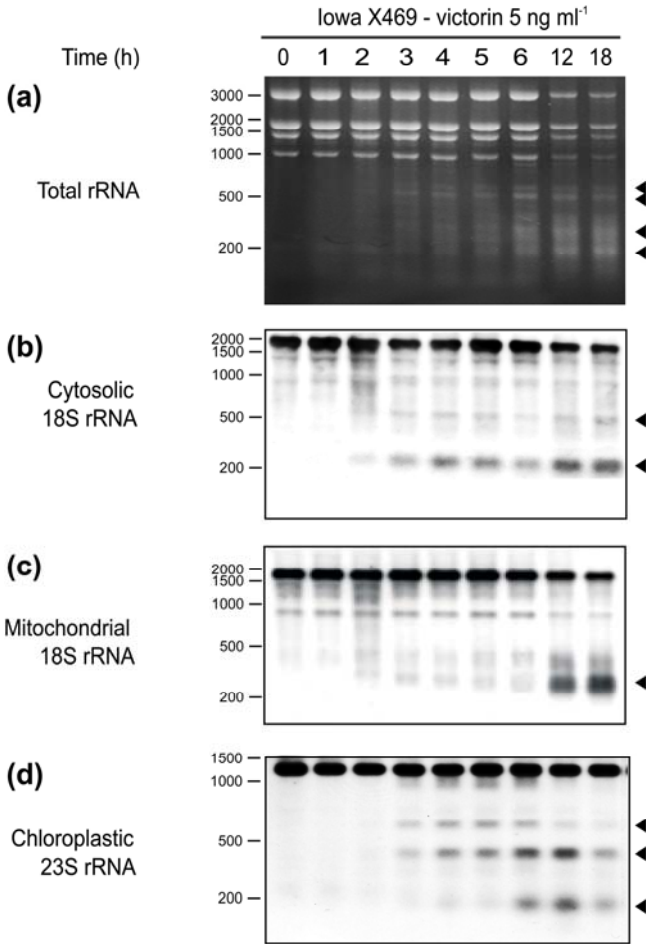

Fig. 2 Degradation of rRNA is induced in different organelles during victorin-induced apoptosic cell death. Primary leaf segments of the victorin-sensitive oat line cv. 'Iowa X469' were treated with 5 ng ml^{-1} victorin for the time periods indicated in the figure. Total RNA was extracted and analyzed by ethidium bromide staining (**a**) and by northern analysis with probes for cytosolic 18S rRNA (**b**), mitochondrial 18S rRNA (**c**) and chloroplastic 23S rRNA (**d**). The arrowheads indicate the major degradation products of the rRNA species. On the left the positions of RNA molecular length markers are indicated. (from Hoat *et al*. (2006) *The Plant Journal* **46**, 922-933, with kind permission, Blackwell Publishing.

PROGRAMMED RNA DEGRADATION DURING VICTORIN-INDUCED PCD

Specific RNA degradation in oat

Specific rRNA degradation is known to occur during animal apoptosis in some cell lines (Houge *et al*. 1995; Lafarga *et al*. 1997; Nadano and Sato 2000; King *et al*. 2000; Kulka *et al*. 2003). Originally, rRNA degradation was reported to occur in cAMP-induced apoptosis of a rat myeloid leukemia cell line (Houge *et al*. 1993) and in X-ray-induced apoptosis of human lymphocytes (Delic *et al*. 1993). Later, cleavage of rRNA during apoptosis was observed in several other combinations of cell types and triggers, but not in every cell type or in response to every trigger (Crawford *et al*. 1997; Samali *et al*. 1997; Kulka *et al*. 2003). Interestingly, 28S and 18S rRNA molecules were sometimes differently targeted for degradation (Houge *et al*. 1993; Banerjee *et al*. 2000; King *et al*. 2000).

In the oat-victorin system, various RNA species including cytosolic 28S and 18S rRNA, mitochondrial 18S RNA, and chloroplastic 23S rRNA, in addition to the mRNAs of housekeeping genes, were shown to be cleaved via specific intermediates during apoptotic cell death (**Fig. 2**) (Hoat *et al*. 2006). In contrast, the same RNA molecules were rather randomly degraded without specific intermediates during necrotic cell death induced by a high concentration (30

mM) of $CuSO_4$ or heat shock (Hoat *et al*. 2006). Interestingly, only constitutive, but not stress-inducible, mRNAs were degraded during apoptotic death of oat cells induced by victorin or other apoptotic inducers (Hoat *et al*. 2006). The degradation of housekeeping mRNAs was detectable as early as DNA laddering, but later than rRNA degradation (Hoat *et al*. 2006), suggesting that the degradation of mRNA is not an early event preceding other apoptosis hallmarks (Hoat *et al*. 2006). These observations suggest that subsets of mRNAs might be selectively targeted for, or protected from, degradation during PCD in oat. Selective degradation of mRNAs during PCD appears to be a biologically relevant phenomenon because dying cells no longer require housekeeping genes, but may need the products of stress-responsive genes such as anti-microbial proteins to prevent proliferation of an invading pathogen. In oat cells, there may be some mechanism to protect newly synthesized mRNAs; alternatively, selective mRNA degradation could just be due to the subcellular localization of an mRNA species in the dying cells. Although housekeeping mRNAs are distributed in the cytoplasm, newly synthesized mRNAs could be mostly located in the nucleus where they would be relatively safe from attack by nucleases.

The degradation of cytosolic 18S/28S rRNA and mitochondrial 18S rRNA was shown to precede the cleavage of chloroplastic 23S rRNA and DNA fragmentation during victorin-induced PCD (Hoat *et al*. 2006), suggesting that the effect of victorin on the chloroplasts occurred later than its effect on mitochondria (Navarre and Wolpert 1995). In animal systems, it was also reported that the timing of RNA degradation differed among RNA species in some cases. In HA-1 fibroblastic cells, for example, there was much less degradation of 28S/18S rRNA and this occurred much later than degradation of mitochondrial 16S rRNA, during H_2O_2-induced apoptosis (Crawford *et al*. 1997). Mitochondria play an essential role in the regulation of PCD in both animal and plant cells (Susin *et al*. 1999). A reduction in the mitochondrial membrane potential occurs in the early stages of apoptosis and coincides with a decrease in mitochondrial translation, and often with ROS production on the mitochondrial membrane (Crawford *et al*. 1997; Yao *et al*. 2002b). The victorin-induced collapse of mitochondrial membrane potential sufficiently affects mitochondrial function to influence the coordinated pathways that regulate Rubisco cleavage, DNA laddering and other morphological changes (Curtis and Wolpert 2004). Navarre and Wolpert (1995) showed binding of victorin to the glycine decarboxylase complex (GDC) in mitochondria, and inhibition of GDC activity. Nevertheless, it is still not clear whether the GCD is a biological significant target of victorin (Curtis and Wolpert 2002, 2004; Tada *et al*. 2005), dysfunction of mitochondria caused by victorin may lead to degradation of mitochondrial 16S rRNA at a relatively early stage of victorin-induced PCD.

Is RNA degradation a cause or effect of PCD?

Total protein synthesis is dramatically decreased in apoptotic cells (Deckwerth and Johnson 1993). The apoptosis-associated cleavage of cytoplasmic mRNAs (Del Prete *et al*. 2002) and rRNA (Houge *et al*. 1995) might cause the down-regulation of protein synthesis during apoptosis (Del Prete *et al*. 2002). In addition to RNA degradation, the apoptosis-related specific cleavage of translation initiation factors (eIF4GI, eIF4GII) (Clemens *et al*. 1998; Marissen *et al*. 2000), which act as a bridge between eIF4E and eIF3 and allow an mRNA molecule to associate with the 40S ribosomal subunit during the translation process, was suggested to be a mechanism of translational control during apoptosis (Marissen and Lloyd 1998; Bushell *et al*. 2004). The degradation of rRNA occurred coincidentally with the cessation of cellular protein synthesis in most cases (Nadano and Sato 2000). In addition, protein synthesis inhibitors such as cycloheximide or anisomycin are widely used to induce apoptosis in certain mammal cell lines, such as human promyelocytic leukemia HL-60 cells (Martin *et al*. 1990), rat myeloid leukemia cells (Houge *et al*. 1993), and S49 Neo cells (King *et al*. 2000). These results support the idea that inhibition of protein synthesis by rRNA or mRNA degradation could be a cause of apoptosis. However, there are several reports that argue against this idea. First, administration of cycloheximide does not induce apoptosis in every cell type, but rather causes significant decreases in the extent of apoptosis induced by other triggers in various cell types (for detail see Wertz and Hanley 1996; King *et al*. 2000). In addition, it was reported that the protein synthesizing ability of ribosomes remained intact after 28S rRNA degradation (Houge and Døskeland 1996; Kulka *et al*. 2003). Therefore, it is still a matter of debate whether changes in protein synthesis can be a trigger of apoptosis (Nadano and Sato 2000). In oat cells, cycloheximide did not induce DNA laddering or RNA degradation when administered alone (data not shown), but suppressed DNA laddering in victorin-induced PCD (Tada *et al*. 2001). Therefore, in oat cells, inhibition of protein synthesis itself might not be sufficient to induce apoptotic cell death even though rRNA degradation might contribute to the progression of apoptotic cell death by inhibiting unnecessary protein synthesis and/or facilitating the recycling of ribonucleotides (Hoat *et al*. 2006).

In oat, RNA degradation was mostly concomitant with nuclear DNA laddering, even though the two processes are mediated by different signaling pathways with some overlap (Hoat *et al*. 2006). In some other systems, however, it has been shown that there is no clear correlation between DNA cleavage and RNA degradation. In HL-60 cells, for example, apoptotic cell death with massive DNA cleavage was induced without any degradation of rRNA; in okadaic acid-treated Molt-4 cells, rRNA fragmentation was observed without DNA laddering (Samali *et al*. 1997). It was also indicated that rRNA degradation in virus-infected cells occurred prior to the execution of apoptosis (Goswami *et al*. 2004) and was independent of caspase-induced DNA degradation (Nadano and Sato 2000).

Enzymes associated with RNA degradation during apoptosis

Programmed RNA degradation must require activation of specific ribonucleases (King *et al*. 2000), since RNA is randomly degraded when exposed to cell lysates containing various RNase activities (Houge *et al*. 1995). In animal systems, the RNase L enzyme is thought to be a member of a multi-component system for RNA degradation during apoptosis (Goswami *et al*. 2004). RNase L activation was concomitant with the degradation of rRNA and mRNA, and caspase activation, during apoptosis (Houge *et al*. 1995; Banerjee *et al*. 2000; Del Prete *et al*. 2002). The degradation of 28S rRNA is reduced by inhibition of caspases, suggesting that caspases play an important role in RNA cleavage during apoptotic cell death (King *et al*. 2000). The caspase-3 specific inhibitor suppresses rRNA degradation as well as inhibition of protein synthesis in Jurkat cells. This may indicate that the degradation of rRNA could be a physiologically important cellular signal for the malfunction of the protein synthesis machinery during apoptosis in Jurkat cells (Nadano and Sato 2000).

In the oat-victorin system, the degradation of rRNAs occurs via the same specific intermediates regardless of the cell death trigger, indicating that cleavage of RNA molecules is catalyzed by the same or a similar ribonuclease. Pharmacological studies indicated that an E-64-sensitive cysteine protease and an aprotinin-sensitive serine protease were involved in particular steps of mRNA degradation during victorin-induced PCD (Hoat *et al*. 2006). These proteases may activate a specific RNase by proteolytic cleavage, or may make the conformation of an mRNA-containing ribo-protein complex accessible to the RNase. Administration of the protein synthesis inhibitor cycloheximide did

not significantly affect rRNA/mRNA degradation, indicating that, like other apoptotic effector molecules, the enzymes responsible for RNA degradation are already present in cells and become active when apoptosis is induced (King *et al*. 2000).

SPECIFIC PROTEIN DEGRADATION DURING VICTORIN-INDUCED PCD

A variety of proteins are targeted for proteolysis during animal apoptosis

Proteins are basic structural components of the cell and organelles, and also key players in various signaling pathways. Destruction of key substrates in cellular homeostatic pathways is an essential biochemical mechanism underlying the apoptotic process (Yuan *et al*. 1993; Casciola-Rosen *et al*. 1994). In animal systems, a variety of proteins have been shown to be specifically cleaved during apoptosis. Early in apoptosis, poly-(ADP-ribose)-polymerase (PARP) is cleaved into distinct 89- and 24-kDa fragments by the action of caspase-3 (Kaufmann *et al*. 1993; Lazebnik *et al*. 1994). The specific cleavage of PARP has been used as a hallmark of apoptotic cell death in different types of cell responding to a wide range of apoptotic triggers (Kaufmann *et al*. 1993; Lazebnik *et al*. 1994; Tewari *et al*. 1995; Hercer and Wang 1999; Whitacre *et al*. 1999). PARP synthesizes and transfers ADP-ribose polymers onto the glutamic acid residues of acceptor proteins (Schreiber *et al*. 2006); functionally, it is involved in DNA damage repair (Wang *et al*. 1997; Trucco *et al*. 1999). DNA-dependent protein kinase (DNA-PK), another protein involved in the DNA damage response and cell cycle control (Hari *et al*. 1995), was also reported to be degraded into specific fragments in several cell types undergoing apoptosis (Song *et al*. 1996). Therefore, the cleavage of PARP and/or DNA-PK may facilitate DNA fragmentation in apoptosis (Shiokawa *et al*. 1994) by inactivating the DNA repair system (Wang *et al*. 1997). This process occurs concomitantly with DNA laddering, or precedes it in some cases (Kimura *et al*. 1998). However, apoptosis can occur even when either PARP cleavage or DNA fragmentation is prevented (Herceg and Wang 1999), suggesting that these events can be dissociated and, therefore, may not be in a cause-effect relationship with each other (Li and Drazynkiewicz 2000).

Degradation of structural proteins is also an early feature of apoptosis. The degradation of lamin B1 leads to the collapse of the cell nucleus during apoptosis (Rao *et al*. 1996). Chromatin condensation and breakdown of the nuclear envelope may occur as a result of disruption of nuclear lamina architecture (Weaver *et al*. 1996). Cytoskeleton proteins such as actin (Kayalar *et al*. 1996), the cytoplasmic actin-severing protein gelsolin (Kothakota *et al*. 1997), a major component of the cortical cytoskeleton, fodrin (Martin *et al*. 1995), and a protein component of the microfilament system, Gas2 (Brancolini *et al*. 1992), are also known to be targets for proteolytic degradation during apoptosis.

An interesting class of proteins targeted for degradation during apoptosis is those involved in the splicing of mRNA (Casciola-Rosen *et al*. 1996). The 70-kDa protein component of the U1 small nuclear ribonucleoprotein, which is essential for the splicing of precursor mRNA and for the recognition of the 5′ splice site (Sharp 1994), is observed as a 40-kDa fragment during apoptosis, and the kinetics of its cleavage coincide with the appearance of cells with an apoptotic morphology (Casciola-Rosen *et al*. 1994). In addition, it has recently been shown that noncoding RNA in the spliceosomal U1 snRNP complex can be a target for degradation. The U1 snRNP complex contains the U1 snRNA molecule and the U1 snRNP specific proteins U1-70K, U1A, and U1C, plus a common set of eight proteins called the Sm proteins (Hoet *et al*. 1995). Recent studies have indicated that U1 snRNA is specifically cleaved during apoptotic cell death in many systems (Degen *et al*. 2000).

Specific proteolytic degradation in oat

Various researchers have reported that caspase-specific inhibitors can suppress different forms of plant PCD induced by pathogen attack (Del Pozo and Lam 1998) or treatment with elicitors (Elbaz *et al*. 2002). Activation of a caspase-3–like protease was reported to occur concomitantly with PARP cleavage during heat shock–induced PCD in *N. tabacum* suspension cells (Tian *et al*. 2000). However, no plant proteases exhibiting significant sequence homology to caspases have been identified, even in *Arabidopsis* or rice, whose genomes have been fully decoded.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39), (Rubisco), the most abundant protein in leaves, is a bifunctional enzyme that catalyzes two competing reactions, photosynthetic CO_2 fixation and photorespiratory carbon oxidation, in the stroma of chloroplasts (Evans and Seemann 1989; Ishida *et al*. 1998). Rubisco is subjected to several forms of post-translational modification, including the removal of two N terminal amino acid residues and acetylation of Pro-3 of the large subunit of Rubisco (LSU) (Mulligan *et al*. 1988); e-methylation of Lys-14 of the LSU (Houtz *et al*. 1989); proteolytic cleavage of the small subunit (SSU) by the stromal processing protease (VanderVere *et al*. 1995); and a-methylation of Met-1 of the processed form of the SSU (Grimm *et al*. 1997). Navarre and Wolpert (1999) first reported that treatment of oat leaf slices with victorin in the dark led to the accumulation of a truncated form of the LSU missing the first 14 amino acids. The effect of victorin on the LSU is markedly different between light and dark conditions. Under light conditions, no detectable truncated LSU was observed, indicating that the cleaved product was degraded too quickly, or that there may be a different mechanism of LSU breakdown in the light (Navarre and Wolpert 1999). The degradation of Rubisco, which can occur under various stressful conditions (Ferreira and Teixeira 1992), may affect photosynthesis and nitrogen economy in plants during senescence (Mehta *et al*. 1992; Ishida *et al*. 1998). It was reported that reactive oxygen species (ROS) can trigger the degradation of Rubisco (Mehta *et al*. 1992; Mitsuhashi *et al*. 1992) or directly digest Rubisco (Ishida *et al*. 1998) during ROS-induced cell death (Casano and Trippi 1992).

The victorin-induced cleavage of Rubisco was prevented by E-64 (a Cys protease inhibitor) and leupeptin (a Cys and Ser protease inhibitor) (Navarre and Wolpert 1999; Coffeen and Wolpert 2004) as well as by caspase-specific inhibitors (Coffeen and Wolpert 2004). The initial step of Rubisco fragmentation may occur within chloroplasts, possibly due to the action of a Rubisco specific protease(s) or the specific modification of Rubisco, followed by general proteolysis (Huffaker 1990). Similar to DNA laddering and RNA degradation, inhibitors of calcium signaling were mostly effective in blocking LSU cleavage induced by victorin (Navarre and Wolpert 1999). Interestingly, naphthyl acid phosphate (NAP), a phosphatase inhibitor, caused LSU cleavage in leaf slices in the absence of victorin (Navarre and Wolpert 1999). Pretreatment of leaf slices with cycloheximide or kanamycin did not suppress Rubisco cleavage, indicating that the protease involved is post-translationally activated (Navarre and Wolpert 1999). Recently, two proteases that are apparently involved in the Rubisco proteolytic cascade were purified and characterized (Coffeen and Wolpert 2004). These proteases, designated as saspase-1 and saspase-2, were shown to be sensitive to caspase-specific inhibitors that were effective in suppressing LSU cleavage (Coffeen and Wolpert 2004). Surprisingly, the saspases displayed amino acid sequences homologous to plant subtilisin-like Ser proteases, indicating that, in plants, Ser proteases may exhibit biological functions similar to those of animal caspases belonging to the family of Cys proteases. Unlike caspases, saspases were constitutively present in an active form in the cell, but were likely relocalized to the

extracellular fluid after the induction of PCD by victorin (Coffeen and Wolpert 2004).

CONCLUDING REMARKS

Because PCD is a form of cell death, it involves a dismantling of cellular structures through degradation of biomacromolecules in the cell. In fact, the great majority of molecular events during PCD consist of degradation of various cellular macromolecules. The macromolecular degradation appears to function either as a key process for facilitating PCD or just as a cleaning process of dead cells for recycling cellular materials. In either case, most of the processes seem to be dictated by an innate program. Elucidating the molecular mechanisms of macromolecule degradation, therefore, will help decipher the innate program of PCD in plants as well as in animals.

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REFERENCES

Baillieul F, Genetet I, Kopp M, Saindrenan P, Fritig B, Kauffmann S (1995) A new elicitor of the hypersensitive response in tobacco: a fungal glycoprotein elicits ceil death, expression of defence genes, production of Salicylic acid, and induction of systemic acquired resistance. *The Plant Journal* **8**, 551-560

Banerjee S, An S, Zhou A, Silverman RH, Makino S (2000) RNase L-independent specific 28S rRNA cleavage in murine coronavirus-infected cells. *Journal of Virology* **74**, 8793-8802

Barry MA, Eastman A (1993) Identification of deoxyribonuclease II as an endonuclease involved in apoptosis. *Archives of Biochemistry and Biophysics* **300**, 440-450

Bortner CD, Oldenberg NBE, Cidlowski A (1995) The role of DNA fragmentation in apoptosis. *Trends in Cell Biology* **5**, 21-26

Bouranis DL, Chorianopoulou SN, Siyiannis VF, Protonotarios VE, Hawkesford MJ (2007) Lysigenous aerenchyma development in roots – triggers and cross-talks for a cell elimination program. *International Journal of Plant Developmental Biology* **1**, 127-140

Brancolini C, Bottega S, Schneider C (1992) Gas2, a growth arrest-specific protein, is a component of the microfilament network system. *The Journal of Cell Biology* **117**, 1251-1261

Bushell M, Stoneley M, Sarnow P, Willis AE (2004) Translation inhibition during the induction of apoptosis: RNA and protein degradation? *Biochemical Society Transaction* **32**, 606-610

Casano LM, Trippi VS (1992) The effect of oxygen radicals on proteolysis in isolated oat chloroplasts. *Plant and Cell Physiology* **33**, 329-332

Casciola-Rosen LA, Miller DK, Anhalt GJ, Rosen A (1994) Specific cleavage of the 70-kDa protein component of the U1 small nuclear ribonucleoprotein is a characteristic biochemical feature of apoptotic cell death. *The Journal of Biological Chemistry* **269**, 30757-30760

Casciola-Rosen L, Nicholson DW, Chong T, Rowan KR, Thornberry NA, Miller DK, Rosen A (1996) Apopain/CPP32 cleaves proteins that are essential for cellular repair: a fundamental principle of apoptotic death. *Journal of Experimental Medicine* **183**, 1947-1951

Clemens MJ, Bushell M, Morley SJ (1998) Degradation of eukaryotic polypeptide chain initiation factor (eIF) 4G in responses to induction of apoptosis in human lymphoma cell lines. *Oncogene* **17**, 2921-2931

Coffeen W, Wolpert TJ (2004) Purification and characterization of serine proteases that exhibit caspase-like activity and are associated with programmed cell death in *Avena sativa*. *The Plant Cell* **16**, 857-873

Crawford DR, Lauzon RJ, Wang Y, Mazurkiewicz JE, Schools GP, Davies KJA (1997) 16S mitochondrial ribosomal RNA degradation is associated with apoptosis. *Free Radical Biology Medicine* **22**, 1295-1300

Curtis MJ, Wolpert TJ (2002) The oat mitochondrial permeability transition and its implication in victorin binding and induced cell death. *The Plant Journal* **29**, 295-312

Curtis MJ, Wolpert TJ (2004) The victorin-induced mitochondrial permeability transition precedes cell shrinkage and biochemical markers of cell death, and shrinkage occurs without loss of membrane integrity. *The Plant Journal* **38**, 244-259

Dangl JL, Dietrich RA, Richberg MH (1996) Death don't have no mercy: cell death programs in plant-microbe interactions. *The Plant Cell* **8**, 1793-1807

Danial NN, Korsmeyer SJ (2004) Cell death: critical control points cell death. *Cell* **116**, 205-219

Deckwerth TL, Johnson EM Jr. (1993) Temporal analysis of events associated with programmed cell death (apoptosis) of sympathetic neurons deprived of nerve growth factor. *Journal of Cell Biology* **123**, 1207-1222

Degen WG, Aarssen Y, Pruijn GJ, Utz PJ, van Venrooij WJ (2000) The fate of U1 snRNP during anti-Fas induced apoptosis: specific cleavage of the U1 snRNA molecule. *Cell Death and Differentiation* **7**, 70-79

Delic J, Coppey-Moisan M, Magdelenat H (1993) X-Ray-induced transcription and apoptosis-associated loss of 28S rRNA in interphase human lymphocytes. *International Journal of Radiation Biology* **64**, 39-46

Del Pozo O, Lam E (1998) Caspases and programmed cell death in the hypersensitive response of plants to pathogens. *Current Biology* **8**, 1129-1132

Del Prete MJ, Robles MS, Guío A, Martínez AC, Izqueirdo M, Garcia-Sanz JA (2002) Degradation of cellular mRNA is a general early apoptosis-induced event. *FASEB Journal* **16**, 2003-2005

Dickman MB, Park YK, Oltersdorf T, Li W, Clemente T, French R (2001) Abrogation of disease development in plants expressing animal antiapoptotic genes. *Proceedings of the National Academy of Sciences USA* **98**, 6957-6962

Dominguez F, Cejudo FJ (2006) Identification of a nuclear-localized nuclease from wheat cells undergoing programmed cell death that is able to trigger DNA fragmentation and apoptotic morphology on nuclei from human cells. *The Biochemical Journal* **397**, 529-536

Drury GE, Gallois P (2006) Programmed Cell Death in plants and flowers. In: Teixeira da Silva JA (Ed) *Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues* (1st Edn, Vol I), Global Science Books, London, pp 141-156

Earnshaw WC (1995) Nuclear changes in apoptosis. *Current Opinion in Cell Biology* **7**, 337-343

Earnshaw WC, Martins LM, Kaufmann SH (1999) Mammalian caspases: structure, activation, substrates, and functions during apoptosis. *Annual Review of Biochemistry* **68**, 383-424

Elbaz M, Avni A, Weil M (2002) Constitutive caspase-like machinery executes programmed cell death in plant cells. *Cell Death and Differentiation* **9**, 726-733

Enari M, Hase A, Nagata S (1995a) Apoptosis by a cytosolic extract from Fas-activated cells. *The EMBO Journal* **14**, 5201-5208

Enari M, Hug H, Nagata S (1995b) Involvement of an ICE-like protease in Fas-mediated apoptosis. *Nature* **375**, 78-78

Enari M, Sahahira H, Yokoyama H, Okawa K, Iwamatsu A, Nagata S (1998) A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature* **391**, 43-50

Enari M, Talanian RV, Wong WW, Nagata S (1996) Sequential activation of ICE-like and CPP32-like proteases during Fas-mediated apoptosis. *Nature* **380**, 723-726

Evans JR, Seemann JR (1989) The allocation of protein nitrogen in the photosynthetic apparatus: cost, consequence and control. In: Briggs W (Ed) *Photosynthesis*, Alan R Liss Inc, New York, pp 183-205

Ferreira RB, Davies DD (1987) Protein degradation in *Lemna* with particular reference to ribulose bisphosphate carboxylase: II the effect of nutrient starvation. *Plant Physiology* **83**, 878-883

Ferreira RM, Teixeira AR (1992) Sulfur starvation in *Lemna* leads to degradation of ribulose-bisphosphate carboxylase without plant death. *The Journal of Biological Chemistry* **267**, 7253-7257

Gavrieli Y, Sherman Y, Ben-Sasson SA (1992) Identification of programmed cell death *in situ* via specific labeling of nuclear DNA fragmentation. *The Journal of Cell Biology* **119**, 493-501

Goswami BB, Kulka M, Ngo D, Cebula TA (2004) Apoptosis induced by a cytopathic hepatitis A virus is dependent on caspase activation following ribosomal RNA degradation but occurs in the absence of 2'-5' oligoadenylate synthetase. *Antiviral Research* **63**, 153-166

Greenberg JT (1996) Programmed cell death: A way of life for plants. *Proceedings of the National Academy of Sciences USA* **93**, 12094-12097

Grimm R, Grimm M, Eckerskorn C, Pohlmeyer K, Rohl T, Soll J (1997) Postimport methylation of the small subunit of ribulose-1,5-bisphosphate carboxylase in chloroplasts. *FEBS Letter* **408**, 350-354

Hari KL, Santerre A, Sekelsky JJ, McKim KS, Boyd JB, Hawley RS (1995) The mei-41 gene of *Drosophila melanogaster* is a structural and functional homolog of the human ataxia telangiectasia gene. *Cell* **82**, 815-821

Herceg Z, Wang ZQ (1999) Failure of poly(ADP-ribose) polymerase cleavage by caspases leads to induction of necrosis and enhanced apoptosis. *Molecular and Cellular Biology* **19**, 5124-5133

Hoat TX, Nakayashiki H, Tosa Y, Mayama S (2006) Specific cleavage of ribosomal RNA and mRNA during victorin-induced apoptotic cell death in oat. *The Plant Journal* **46**, 922-933

Hoet RMA, Kastner B, Luhrmann R, van Venrooij WJ (1993) Purification and characterization of human autoantibodies directed to specific regions on U1RNA; recognition of native U1RNP complexes. *Nucleic Acids Research* **21**, 5130-5136

Houge G, Døskeland SO (1996) Divergence towards a dead end? Cleavage of the divergent domains of ribosomal RNA in apoptosis. *Experientia* **52**, 963-967

Houge G, Døskeland SO, Bøe R, Lanotte M (1993) Selective cleavage of 28S rRNA variable regions V3 and V13 in myeloid leukemia cell apoptosis. *FEBS Letters* **315**, 16-20

Houge G, Robaye B, Eikhom TS, Golstein J, Mellgren G, Gjertsen BT, Lanotte M, Døskeland SO (1995) Fine mapping of 28S rRNA sites specifically

cleaved in cells undergoing apoptosis. *Molecular and Cellular Biology* **15**, 2051-2062

Houtz RL, Stults JT, Mulligan RM, Tolbert NE (1989) Post-translational modifications in the large subunit of ribulose bisphosphate carboxylase/oxygenase. *Proceedings of the National Academy of Sciences USA* **86**, 1855-1859

Huffaker RC (1990) Proteolytic activity during senescence of plants. *New Phytologist* **116**, 199-231

Hughes FM Jr, Cidlowski JA (1994) Regulation of apoptosis in S49 cells. *The Journal of Steroid Biochemistry and Molecular Biology* **49**, 303-310

Ishida H, Shimizu S, Makino A, Mae T (1998) Light-dependent fragmentation of the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase in chloroplasts isolated from wheat leaves. *Planta* **204**, 305-309

Ito J, Fukuda H (2002) ZEN1 is a key enzyme in the degradation of nuclear DNA during programmed cell death of tracheary elements. *Plant Cell* **14**, 3201-3211

Jacobson MD, Weil M, Raff MC (1997) Programmed cell death in animal development. *Cell* **88**, 347-354

Kaufmann SH, Desnoyers S, Ottaviano Y, Davidson NE, Poirier GG (1993) Specific proteolytic cleavage of poly(ADP-ribose) polymerase: An early marker of chemotherapyinduced apoptosis. *Cancer Research* **53**, 3976-3985

Kayalar C, Ord T, Testa MP, Zhong LT, Bredesen DE (1996) Cleavage of actin by interleukin 1 β-converting enzyme to reverse DNase I inhibition. *Proceedings of the National Academy of Sciences USA* **93**, 2234-2238

Kerr JFR, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *British Journal of Cancer* **26**, 239-257

Kiba A, Sangawa Y, Ohnishi K, Yao N, Park P, Nakayashiki H, Tosa Y, Mayama Y, Hikichi Y (2006) Induction of apoptotic cell death leads to the development of bacterial rot caused by *Pseudomonas cichorii*. *Molecular Plant-Microbe Interactions* **19**, 112-122

Kimura C, Zhao QL, Kondo T, Amatsu M, Fujiwara Y (1998) Mechanism of UV-induced apoptosis in human leukemia cells: roles for Ca21/Mg21-dependent endonuclease, caspase 3, and stress-activated protein kinases. *Experimental Cell Research* **239**, 411-422

King KL, Jewell CM, Bortner CD, Cidlowski JA (2000) 28S ribosome degradation in lymphoid cell apoptosis: evidence for caspase and Bcl-2-dependent and -independent pathways. *Cell Death and Differentiation* **7**, 994-1001

Kothakota S, Azuma T, Reinhard C, Klippel A, Tang J, Chu K, McGarry TJ, Kirschner MW, Koths K, Kwiatkowski DJ, Williams LT (1997) Caspase-3-generated fragment of gelsolin: effector of morphological change in apoptosis. *Science* **278**, 294-298

Kulka M, Chen A, Ngo D, Bhattacharya SS, Cebula TA, Goswami BB (2003) The cytopathic 18f strain of *Hepatitis A virus* induces RNA degradation in FrhK4 cells. *Archives of Virology* **148**, 1275-1300

Kusaka K, Tada Y, Shigemi T, Sakamoto M, Nakayashiki H, Tosa Y, Mayama S (2004) Coordinate involvement of cysteine protease and nuclease in the executive phase of plant apoptosis. *FEBS Letters* **578**, 363-367

Lafarga M, Lerga A, Andres MA, Polanco JI, Calle E, Berciano MT (1997) Apoptosis induced by methylazoxymethanol in developing rat cerebellum: organization of the cell nucleus and its relationship to DNA and rRNA degradation. *Cell and Tissue Research* **289**, 25-38

Lazebnik YA, Kaufmann SH, Desnoyers S, Poirier GG, Earnshaw WC (1994) Cleavage of poly(ADP-ribose) polymerase by proteinase with properties like ICE. *Nature* **371**, 346-347

Lecoeur H (2002) Nuclear apoptosis detection by flow cytometry: influence of endogenous endonucleases. *Experimental Cell Research* **277**, 1-14

Li X, Darzynkiewicz Z (2000) Cleavage of poly(ADP-ribose) polymerase measured *in situ* in individual cells: relationship to DNA fragmentation and cell cycle position during apoptosis. *Experimental Cell Research* **255**, 125-132

Li LY, Luo X, Wang X (2001) Endonuclease G is an apoptotic DNase when released from mitochondria. *Nature* **412**, 95-99

Marissen WE, Lloyd RE (1998) Eukaryotic translation initiation factor 4G is targeted for proteolytic cleavage by caspase 3 during inhibition of translation in apoptotic cells. *Molecular and Cellular Biology* **18**, 7565-7574

Marissen WE, Gradi A, Sonenberg N, Lloyd RE (2000) Cleavage of eukaryotic translation initiation factor 4GII correlates with translation inhibition during apoptosis. *Cell Death and Differentiation* **7**, 1234-1243

Martin SJ, Green DR, Cotter TG (1994) Dicing with death: dissecting the components of the apoptosis machinery. *Trends in Biochemical Sciences* **19**, 26-30

Martin SJ, Lennon SV, Bonham AM, Cotter TG (1990) Induction of apoptosis (programmed cell death) in human leukemic HL-60 cells by inhibition of RNA or protein synthesis. *Journal of Immunology* **145**, 1859-1867

Martin SJ, O'Brien GA, Nishioka WK, McGahon AJ, Mahboubi A, Saido TC, Green DR (1995) Proteolysis of fodrin (non-erythroid spectrin) during apoptosis. *The Journal of Biological Chemistry* **270**, 6425-6428

Mehta RA, Fawcette TW, Porath D, Mattoo AK (1992) Oxidative stress causes rapid membrane translocation and *in vivo* degradation of ribulose-1,5,bisphosphate carboxylase/oxygenase. *The Journal of Biological Chemistry* **267**, 2810-2816

Mitsuhashi W, Crafts-Brandner SJ, Feller U (1992) Ribulose-1,5-bisphosphate carboxylase/oxygenase degradation in isolated pea chloroplasts incubated in the light or in the dark. *Plant Physiology* **139**, 653-658

Mittler R, Lam E (1995) Identification, characterization, and purification of a tobacco *endonuclease* activity induced upon hypersensitive response cell death. *The Plant Cell* **7**, 1951-1962

Mulligan RM, Houtz RL, Tolbert NE (1988) Reaction-intermediate analogue binding by ribulose bisphosphate carboxylase/oxygenase causes specific changes in proteolytic sensitivity: the amino-terminal residue of the large subunit is acetylated proline. *Proceedings of the National Academy of Sciences USA* **85**, 1513-1517

Nadano D, Sato TA (2000) Caspase-3-dependent and -independent degradation of 28S ribosomal RNA may be involved in the inhibition of protein synthesis during apoptosis initiated by death receptor engagement. *The Journal of Biological Chemistry* **275**, 13967-13973

Nagata S (1997) Apoptosis by death factor. *Cell* **88**, 355-365

Nagata S (2000) Apoptotic DNA Fragmentation. *Experimental Cell Research* **256**, 12-18

Nagata S, Nagase H, Kawane K, Mukae N, Fukuyama H (2003) Degradation of chromosomal DNA during apoptosis. *Cell Death and Differentiation* **10**, 108-116

Navarre DA, Wolpert TJ (1995) Inhibition of the glycine decarboxylase multienzyme complex by the host-selective toxin victorin. *The Plant Cell* **7**, 463-471

Navarre DA, Wolpert TJ (1999) Victorin induction of an apoptotic/senescence-like response in oats. *The Plant Cell* **11**, 237-249

Obara K, Kuriyama H, Fukuda H (2001) Direct evidence of active and rapid nuclear degradation triggered by vacuole rupture during programmed cell death in *Zinnia*. *Plant Physiology* **125**, 615-626

O'Brien IE, Murray BG, Baguley BC, Morris BA, Ferguson IB (1998) Major changes in chromatin condensation suggest the presence of an apoptotic pathway in plant cells. *Experimental Cell Research* **241**, 46-54

Parrish JK, lotz K, Ledwich D, Wang X, Xue D (2001) Mitochondrial endonuclease G is important for apoptosis in *C. elegans*. *Nature* **412**, 90-94

Peitsch MC, Mannherz HG, Tschopp J (1994) The apoptosis endonucleases: cleaning up after cell death? *Trends in Cell Biology* **4**, 37-41

Peitsch MC, Polzar B, Stephan H, Crompton T, MacDonald HR, Mannherz HG, Tschopp J (1993) Characterization of the endogenous deoxyribonuclease involved in nuclear DNA degradation during apoptosis (programmed cell death). *The EMBO Journal* **12**, 371-377

Pennell RI, Lamb C (1997) Programmed cell death in plants. *The Plant Cell* **9**, 1157-1168

Rao L, Perez D, White E (1996) Lamin proteolysis facilitates nuclear events during apoptosis. *The Journal of Cell Biology* **135**, 1441-1455

Ryerson DE, Heath MC (1996) Cleavage of nuclear DNA into oligonucleosomal fragments during cell death induced by fungal infection or by abiotic treatments. *The Plant Cell* **8**, 393-402

Sakahira H, Enari M, Ohsawa Y, Uchiyama Y, Nagata S (1999) Apoptotic nuclear morphological change without DNA fragmentation. *Current Biology* **9**, 543-546

Samali A, Gilje B, Døskeland SO, Cotter TG, Houge G (1997) The ability to cleave 28S ribosomal RNA during apoptosis is a cell-type dependent trait unrelated to DNA fragmentation. *Cell Death and Differentiation* **4**, 289-293

Samejima K, Earnshaw WC (2005) Trashing the genome: the role of nucleases during apoptosis. *Nature Reviews Molecular Cell Biology* **6**, 677-688

Schreiber V, Dantzer F Amé JC, de Murcia G (2006) Poly(ADP-ribose): novel functions for an old molecule. *Nature Reviews Molecular Cell Biology* **7**, 517-528

Schwartzman RA, Cidlowski JA (1993) Apoptosis: the biochemistry and molecular biology of programmed cell death. *Endocrine Reviews* **14**, 133-151

Sharp PA (1994) Split genes and RNA splicing. *Cell* **77**, 805-815

Shiokawa D, Ohyama H, Yamada T, Takahashi K, Tanuma S (1994) Identification of an endonuclease responsible for apoptosis in rat thymocytes. *European Journal of Biochemistry* **226**, 23-30

Song Q, Lees-Miller SP, Kumar S, Zhang Z, Chan DW, Smith GC, Jackson SP, Alnemri ES, Litwack G, Khanna KK, Lavin MF (1996) DNA-dependent protein kinase catalytic subunit: a target for an ICE-like protease in apoptosis. *The EMBO Journal* **15**, 3238-3246

Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM, Mangion J, Jacotot E, Costantini P, Loeffler M, Larochette N, Goodlett DR, Aebersold R, Siderovski DP, Penninger JM, Kroemer G (1999) Molecular characterization of mitochondria apoptosis-inducing factor. *Nature* **397**, 441-446

Swanson SJ, Bethke PC, Jones RL (1998) Barley aleurone cells contain two types of vacuoles. Characterization of lytic organelles by use of fluorescent probes. *Plant Cell* **10**, 685-698

Tada Y, Hata S, Takata Y, Nakayashiki H, Tosa Y, Mayama S (2001) Induction and signaling of an apoptotic response typified by DNA laddering in the defense response of oats to infection and elicitors. *Molecular Plant-Microbe Interactions* **14**, 477-486

Tada Y, Kusaka K, Betsuyaku S, Shinogi T, Sakamoto M, Ohura Y, Hata S, Mori T, Tosa Y, Mayama S (2005) Victorin triggers programmed cell death and the defense response via interaction with a cell surface mediator. *Plant and Cell Physiology* **46**, 1787-1798

Tada Y, Mori T, Shinogi T, Yao N, Takahashi S, Betsuyaku S, Sakamoto M,

Park P, Nakayashiki H, Tosa Y, Mayama S (2004) Nitic oxide and reactive oxygen species do not elicit hypersensitive cell death but induce apoptosis in the adjacent cells during the defense response of oat. *Molecular Plant-Microbe Interactions* **17**, 245-253

Tewari M, Quan LT, O'Rourke K, Desnoyers S, Zeng Z, Beidler DR, Poirier GG, Salvesen GS, Dixit VM (1995) Yama/CPP32 β, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase. *Cell* **81**, 801-809

Thiede B, Treumann A, Kretschmer A, Sohlke J, Rudel T (2005) Shotgun proteome analysis of protein cleavage in apoptotic cells. *Proteomics* **5**, 2123-2130

Tian R, **Zhang GY**, **Yan CH**, **Dai YR** (2000) Involvement of poly(ADP-ribose) polymerase and activation of caspase-3-like protease in heat shock-induced apoptosis in tobacco suspension cells. *FEBS Letters* **474**, 11-15

Trucco C, Rolli V, Oliver FJ, Masson M, Dantzer F, Niedergang C, Dutrillaux B, Menissier-de Murcia J, de Murcia G (1999) A dual approach in the study of poly(ADPribose) polymerase: *In vitro* random mutagenesis and generation of deficient mice. *Molecular and Cellular Biochemistry* **193**, 53-60

VanderVere PS, Bennett TM, Oblong JE, Lamppa GK (1995) A chloroplast processing enzyme involved in precursor maturation shares a zinc-binding motif with a recently recognized family of metalloendopeptidases. *Proceedings of the National Academy of Sciences USA* **92**, 7177-7181

Vaux DL, Korsmeyer SJ (1999) Cell death in development. *Cell* **96**, 245-254

Walker PR, Smith C, Youdale T, Leblanc J, Whitfield JF, Sikorka M (1991) Topoisomerase II-reactive chemotherapeutic drugs induce apoptosis in thymocytes. *Cancer Research* **51**, 1078-1085

Wang ZQ, Stingl L, Morrison C, Jantch M, Schultze-Osthoff K, Wagner EF (1997) PARP is important for genomic stability but dispensable in apoptosis. *Genes and Development* **11**, 2347-2358

Weaver VM, Carson CE, Walker PR, Chaly N, Lach B, Raymond Y, Brown DL, Sikorska M (1996) Degradation of nuclear matrix and DNA cleavage in apoptotic thymocytes. *Journal of Cell Science* **109**, 45-56

Wertz IE, Hanley MR (1996) Diverse molecular provocation of programmed cell death. *Trends in Biochemical Sciences* **21**, 359-364

Whitacre CM, Zborowska E, Wilson JK, Berger NA (1999) Detection of poly(ADP-ribose) polymerase cleavage in response to treatment with topoisomerase I inihibitors: A potential surrogate to assess treatment effectiveness. *Clinical Cancer Research* **5**, 665-672

Wyllie AH (1980) Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* **284**, 555-556

Yao N, Imai S, Tada Y, Nakayashiki H, Tosa Y, Park P, Mayama S (2002a) Apoptotic cell death is a common response to pathogen attack in oats. *Molecular Plant-Microbe Interactions* **15**, 1000-1007

Yao N, Tada Y, Park P, Nakayashiki H, Tosa Y, Mayama S (2001) Novel evidence for apoptotic cell response and differential signals in chromatin condensation and DNA cleavage in victorin-treated oats. *The Plant Journal* **28**, 13-26

Yao N, Tada Y, Sakamoto M, Nakayashiki H, Park P, Tosa Y, Mayama S (2002b) Mitochondrial oxidative burst involved in apoptotic response in oats. *The Plant Journal* **30**, 567-579

Yuan J, Shaham S, Ledoux S, Ellis HM, Horvitz HR (1993) The *C. elegans* cell death gene *ced-3* encodes a protein similar to mammalian interleukin-1 β-converting enzyme. *Cell* **75**, 641-652

Yuste VJ, Sanchez-Lopez I, Sole C, Moubarak RS, Bayascas JR, Dolcet X, Encinas M, Susin SA, Comella JX (2005) The contribution of apoptosis-inducing factor caspase-activated DNase and inhibitor of caspase-activated DNase to the nuclear phenotype and DNA degradation during apoptosis. *The Journal of Biological Chemistry* **280**, 35670-35683