The 13th Symposium on Plant Biotechnology **13:** 69-73, 1999.
1999 Korea-Japan Join Symposium.

Programmed cell death in plants

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Key words: apoptosis, cysteine protease, Dnase, necrosis, programmed cell death, tracheary element differentiation, vacuole, *Zinnia elegans*

Abstract

In plants as well as in other multicellular organisms, programmed cell death plays essential roles in the abortion or formation of specific cells and tissues during development to organize the plant body [11, 15, 18]. A typical example of developmentally programmed cell death in plants is the death during differentiation of tracheary elements which are components of vessels and tracheids, a water-conducting system. The programming of cell death during tracheary element differentiation has been revealed to be unique to plant cells by using the *in vitro Zinnia* mesophyll cell culture system. In particular, new biosynthesis of autolysis-related enzymes such as cysteine proteases and nucleases, their accumulation of the vacuole and the programmed collapse of the vacuole are essential to the death of tracheary elements and differ greatly from the process of the apoptotic cell death in animals.

Programmed cell death in development

During the development of reproductive organs, the cell death process functions by both aborting specific tissues or cells and forming new types of cells as summarized in Figure 1. For example, in maize, unisexual male flowers are formed through the abortion of the primordia of female organs by programmed cell death [5]. This sex determination is under genetic control. Lesions in the TASSELSEED (TS) genes cause feminization of male flowers. Among them the TS2 gene has been cloned and found to encode a short-chain alcohol dehydrogenase which may be involved in steroid metabolism [6]. Maturation of pollens requires the death of surrounding cells called tapetum cells to form sophisticated walls of pollens with substrates that have been accumulated in these cells [12]. Root cap cells derived from the root meristem are continuously displaced to the root periphery by new cells and die at the periphery. This cell death is similar to apoptosis in animals, as hallmarked by nuclear condensation and fragmentation, DNA ladder, and TUNEL positive staining [20]. The death of the suspensor of embryos and of the aleurone layer is also under a strict program. Another conspicuous example of programmed cell death in vegetative tissues is cell death of tracheary elements [8, 9, 10]. Tracheary elements are emptied by the loss of all cell contents including the nucleus to form hollow tubes as a pathway for fluids.

Cytological aspects of cell death during tracheary-element differentiation

Mesophyll cells isolated from the first leaves of *Zinnia* seedlings differentiate into tracheary elements. Epifluorescent microscopic observations revealed that differentiating tracheary elements of *Zinnia* are very active and have all organelles such as the nucleus, vacuole, and many active mitochondria and chloroplasts, but that several hours after the formation of visible secondary wall thickenings such cell contents are lost abruptly. The sudden degradation of the organelles results from the collapse of the vacuole [8, 10, 13]. Single membrane-organelles, ER and Golgi bodies, after vacuole collapse, first swell at the ends and then over their entire length, become balloon-like structures, and disappear (Figure 2). A little later than the sign of degeneration of single membrane-organelles, the degeneration of double membrane-organelles such as chloroplasts and mitochondria becomes visible. The condensation and fragmentation of the nucleus, which are typical features of apoptotic cell death in animals, do not occur in association with the cell death of tracheary elements. Instead, the first sign of nuclear degradation is swelling of the nuclear membrane after the vacuole col-

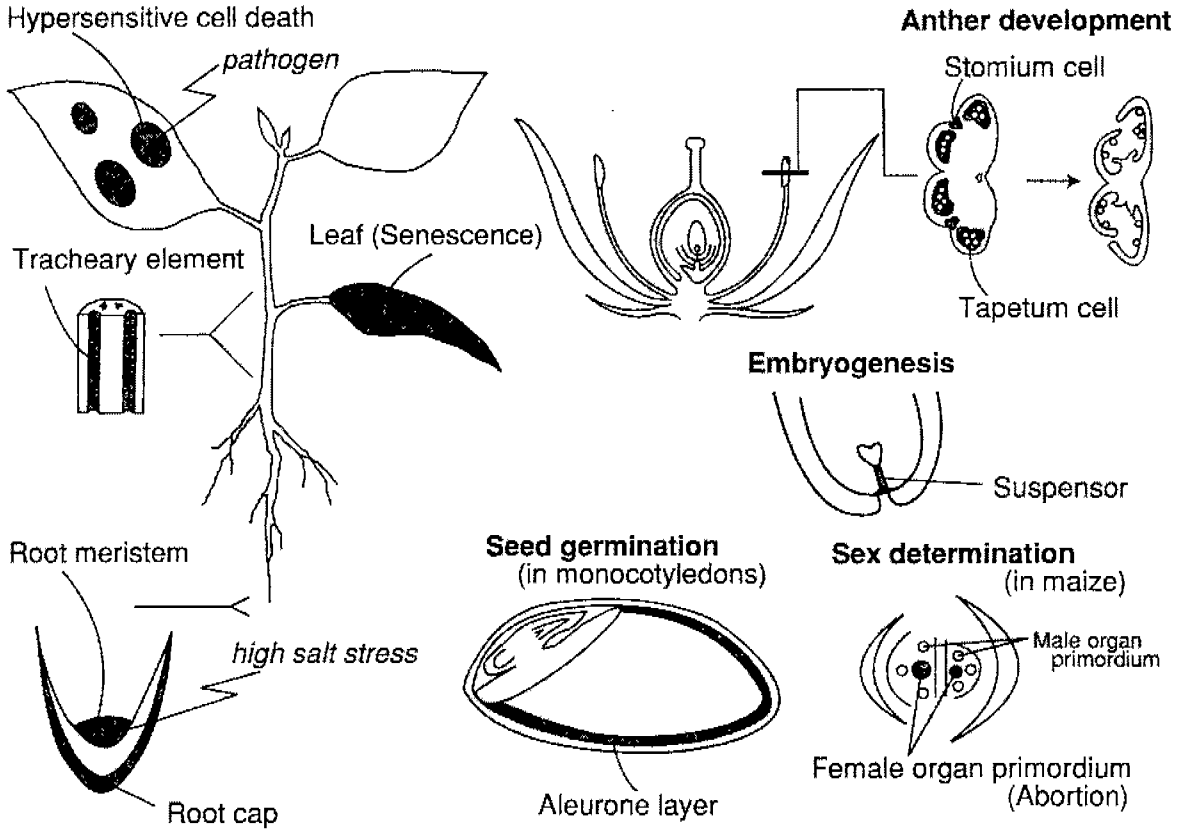

Figure 1. Typical examples of programmed cell death in plants. The death of specific cells is developmentally programmed in the processes of sex determination, anther development, embryogenesis, seed germination, root formation, xylem formation and leaf senescence. (after Fukuda, 1998)

lapse, and then nuclear matrix is degraded. Therefore, one of the most critical irreversible points of death of tracheary elements is the tonoplast collapse. Mittler and Lam [17] and Groove *et al.* [14] reported the presence of fragmented nuclear DNA in differentiating tracheary elements by the TUNEL method. However, their result is explainable in terms of DNA fragmentation by DNase that is accumulated in the vacuole, rather than by apoptosis-specific endonucleases. Indeed, DNA ladder has not been detected in differentiating tracheary elements. Thus, although death of tracheary elements is a developmentally programmed and active process, its cytological feature is closer to necrosis than to apoptosis.

Initiation of cell death during tracheary-element differentiation

The process of differentiation from *Zinnia* mesophyll cells to tracheary elements is divided into 3 stages, Stage I to Stage III (Figure 3) [7, 9]. In the final stage, Stage III, cells are engaged in cell death program. The transition from Stage II to Stage III seems to be an irreversible checkpoint toward cell death. We revealed that uniconazole specifically suppresses the accumulation of transcripts for genes that were induced in Stage III but not the accumulation of transcripts that appear in Stages I and II [21]. This suppression was overcome by the addition of brassinolide. Indeed, we found that differentiating cells produce active brassinosteroids. It is possible, therefore, to assume that endogenous brassinosteroids induce transition from Stage II into Stage III toward cell death.

Involvement of proteases in cell death

In-gel protease assay with embedded single tracheary elements revealed that a differentiating tracheary element itself has high protease activity, suggesting that cell lysis occurs autonomously [2]. We found that the Z-phe-Arg-MCA hydrolytic activity rapidly increased in a differentiation-specific manner just before the start of autolysis in *Zinnia*. [16]. This activity was derived from a cysteine protease(s) with about a 30 kD molecular mass and a pH optimum of

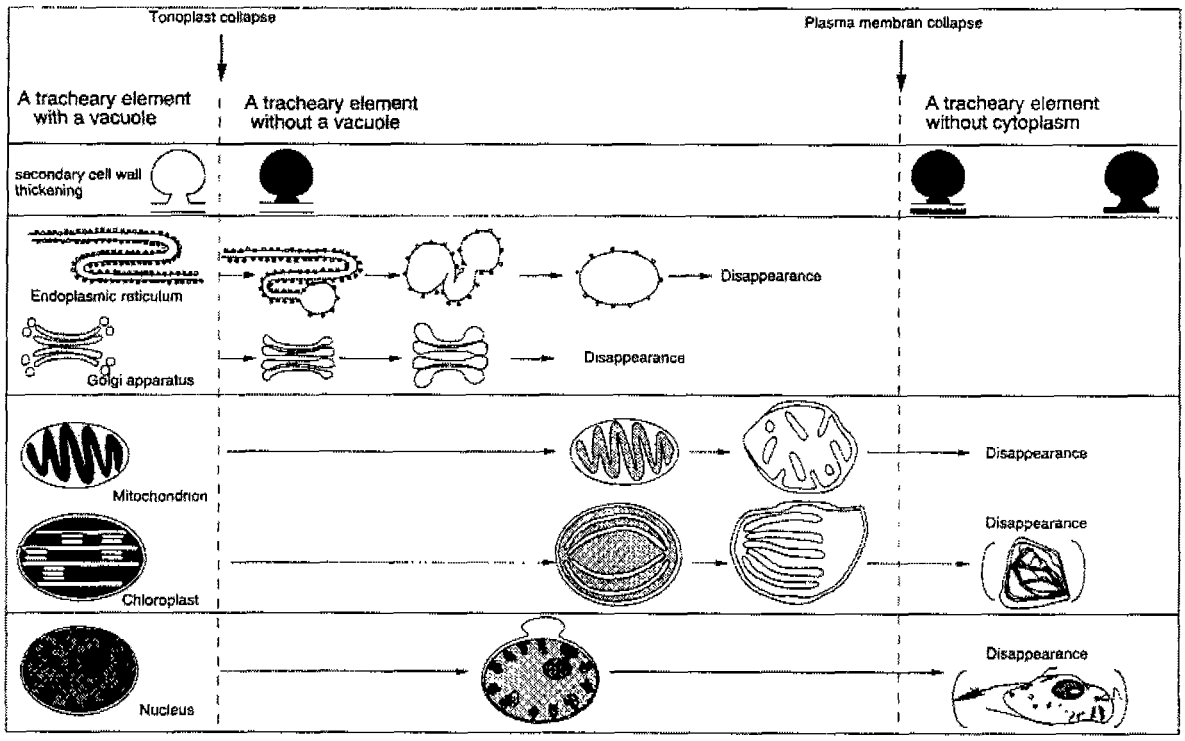

Figure 2. Structural changes in organelles during autolysis of differentiating *Zinnia* tracheary elements. All the organelles are degraded after the vacuole collapses. (after Fukuda, 1996)

around pH 5.5. Because this protease inhibited strongly by E-64, we examined the effect of a membrane permeable E-64 analogue on cell death. The addition of the protease inhibitor caused conspicuous inhibition of disruption of the nucleus, although it did not inhibit the vacuole collapse. This clearly shows that cysteine protease(s) is involved in nuclear degradation. The autolysis-specific cysteine protease activity seems to result from the new induction of two similar cysteine protease genes prior to the autolysis [21; Minami and Fukuda, unpublished]. These cysteine proteases have a putative presequence which directs the transport of the protein into some organelles or out of the cell.

Involvement of DNase in cell death

Elevation of nuclease activities is coupled with the cell death of tracheary elements [19]. Among several species of nucleases that have been shown to be expressed in association with tracheary element differentiation to date, a 43 kD nuclease is only one that can hydrolyze DNA. It has an activity hydrolyzing both single stranded DNA/RNA and double stranded in the presence of Zn^{2+}. This type of nuclease is also found to be expressed specifically in other cell death processes in higher plants, for example, in cell death in the endosperm and aleurone during germination [4], and in leaf senescence [3]. This may imply that similar types of Zn^{2+}-activated nucleases may be involved commonly in different cell death processes in higher plants. Very recently, we isolated cDNA clones, *BEN1* and *ZEN1* that correspond to the Zn^{2+}-activated nuclease from barley aleurone and from differentiating *Zinnia* tracheary elements, respectively [1]. Their predicted amino acid sequences showed that BEN1 and ZEN1 are similar to each other (45% identity) and to S1 nuclease of *Aspergillus,* indicating that the S1 type of nucleases with DNase activity are commonly involved in nuclear degradation during different programmed cell death processes in plants. Furthermore, ZEN1 nuclease possesses the putative signal peptide for targeting specific organelles or for secretion. To know the function and localization of the S1-type nucleases in plants, we tried to clone and overexpress full length of cDNAs of *ZEN1* and *BEN1* in *E. coli* and yeast. However, we failed in both their cloning and overexpression in *E. coli* and yeast, probably because of their severe toxicity. Then we devised to introduce their genome clones including introns that are fused to the DEX-inducible promoter into tobacco BY2

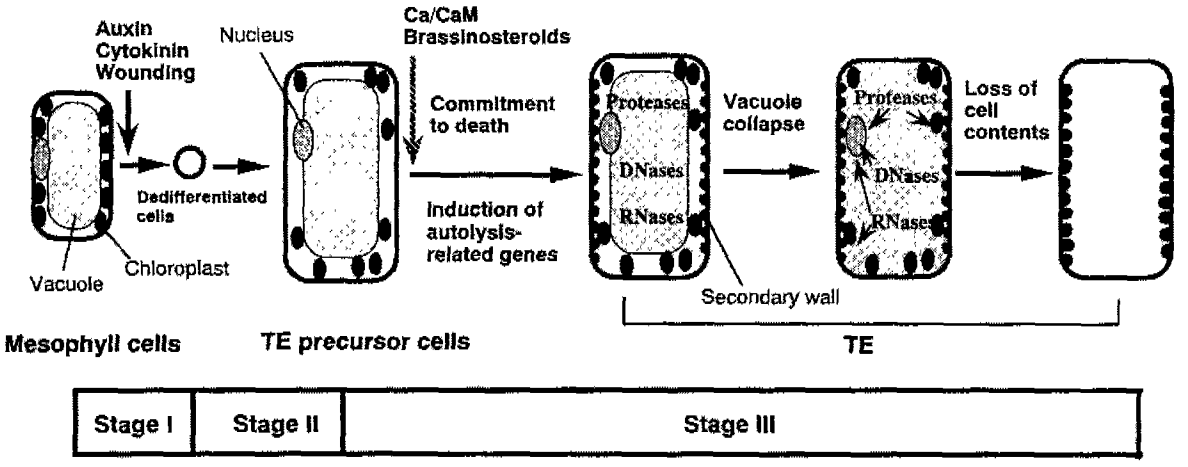

Figure 3. The process of the programmed cell death during tracheary element differentiation. The transition from Stage II into Stage III appears to be regulated by endogenous brassinosteroids. In Stage III, hydrolytic enzymes, such as DNases, RNases and proteases, accumulate in the vacuole. The tonoplast collapse causes these enzymes to invade the cytoplasm and attack various organelles, resulting in the formation of a mature tracheary element that has lost its cell contents. (after Fukuda, 1997b)

cells to make stable transformants. The experiments with the transformants demonstrated that ZEN1 nuclease is targeted into the vacuole while BEN1 nuclease is secreated out of the cell.

Cell death and the vacuole

Transcripts for *ZEN1* [1], *ZCP4* (*p48h-17*) [23, Minami and Fukuda, unpublished], and *ZRNase I* [22] accumulate transiently in a very similar pattern just before autolysis starts. Therefore, the expression of the genes encoding these enzymes may be regulated by a common mechanism. The deduced amino acid sequences of *ZEN1, ZRNase I,* and *ZCP4* (*p48h-17*) indicate the presence of putative signal peptides. These results, taken together with the above-mentioned results, allow us to speculate that all of these proteins are transported into the vacuole (Figure3). Because the pH optima of the 43 kD DNase (ZEN1 product) and the 30 kD cysteine protease (ZCP4/p48h-17 product) are both 5-5.5, which corresponds to the pH in the vacuole, these hydrolytic enzymes may function in the vacuole or, rather, in the cytoplasm after the tonoplast collapse. The tonoplast collapse could permit hydrolytic enzymes to invade the cytoplasm and attack various organelles. Thus, the tonoplast collapse is a critical event in the cell death program of tracheary elements; the mechanism of this collapse, however, is still open for investigation.

Acknowledgments

Financial support from the Ministry of Education, Science and Culture of Japan, and from the Japan Society for the Promotion of Science is gratefully acknowledged.

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