Programmed cell death (PCD) is a fundamental cellular process observed in eukaryotic cells of different origin (Huh et al., 2002; Buss et al., 2006; Piszczek and Gutman, 2007; Lam, 2008). Being an ordered series of events, PCD facilitates the removal of redundant, misplaced, or damaged cells and is essential for cellular differentiation and tissue homeostasis (Hoeberichts and Woltering, 2003; Chen and Dickman, 2004). Phloem differentiation, root cap and aerenchyma formation, aleurone and endosperm cell death, and leaf senescence are all known examples of PCD in plants. PCD is often associated with the occurrence of specific biochemical and morphological features such as condensation of the nucleus and cytoplasm, fragmentation of genomic DNA (‘DNA laddering’) and fragmentation of the cell into membrane-contained vesicles (apoptotic bodies) (Hoeberichts and Woltering, 2003). Other typical hallmarks of PCD in plants are an increase in caspase-like proteolytic activity and cytochrome c release from mitochondria. Being essential for cell and tissue homeostasis and specialization, PCD also plays an important role in mediating plant adaptive responses to the environment. The most characterized type of PCD in plants is a hypersensitive response (HR) observed in plants in response to pathogen attack (Pennell and Lamb, 1997; Lam et al., 2001). Recently, PCD has also been proved to occur in response to various abiotic stresses such as salinity, cold stress, waterlogging, and hypoxia (Katsuura and Kawasaki, 1996; Drew et al., 2000; Kratsch and Wise, 2000; Huh et al., 2002).

In contrast to the relatively well-described cell death pathway in animals, often referred to as apoptosis, mechanisms and regulation of plant PCD are still ill-defined (Hoeberichts and Woltering, 2003). It appears that the mechanism of DNA laddering varies in different species or even in different tissues of one organism (Jiang et al., 2008). A key role for caspase-like proteases has been suggested (Piszczek and Gutman, 2007), although caspase-encoding genes are not found in plants at the nucleotide sequence level (Hatsugai et al., 2004; Chichkova et al., 2004).

In this issue, Affenzeller and colleagues report salinity-induced PCD in a freshwater green algae Micrasterias denticulata. They have shown that prolonged salt stress (24 h) led to degradation of organelles by autophagy, a special form of PCD where organelles are degenerated and enclosed by membranous structures derived from ER. This finding extends the phenomenon of salinity-induced PCD, previously reported in higher plants (Katsuura and Kawasaki, 1996; Katsuura, 1997; Katsuura and Shibasaki, 2000; Lin et al., 2005; Li et al., 2007a, b) and yeasts (Huh et al., 2002), to algal species. Importantly, DNA laddering, one of the hallmarks of PCD, was visible as soon as 1 h after the onset of NaCl stress (Affenzeller et al., 2009) while previous reports suggested that at least 4 h of salinity treatment was needed (Li et al., 2007a).

Another important aspect of Affenzeller and his colleagues’ work was the finding that the observed DNA laddering occurred in NaCl but not in sorbitol-stressed cells. This indicates that the ionic rather than the osmotic component of salt stress led to the activation of the endonuclease resulting in PCD. To the best of my knowledge, the only previous report on such ionic specificity of PCD was by Huh et al. (2002). Although the exact mechanisms beyond this specificity remain elusive, several lines of evidence suggest that changes in the cytosolic K+/Na+ ratio may be crucial for triggering PCD in living cells.

Under saline conditions, strong membrane depolarization caused by Na+ uptake favours K+ efflux via depolarization-activated outward-rectifying K+ channels (Shabala et al., 2006). By contrast, isotonic mannitol or sorbitol solution causes significant membrane hyperpolarization, resulting in increased K+ uptake (Shabala et al., 2000; Shabala and Lew, 2002). This will result in a dramatic difference in cytosolic K+ level between these two types of stresses (Shabala and Cuin, 2008). In animal tissues, caspase activity is significantly increased by a low cytosolic K+ content (Hughes and Cidlowski, 1999). Assuming plant caspase-like proteases are regulated in a similar way, a decrease in the cytosolic K+ pool will activate caspase-like proteases leading to PCD after NaCl but not sorbitol treatment.

Second, no DNA laddering was detected by Affenzeller and co-authors in 0.5 mM Zn2+-pretreated cells exposed to...
NaCl stress. The authors explain this finding by the fact that one of the major classes of endonucleases, the so-called Ca^{2+}-dependent endonucleases, are inhibited by elevated Zn^{2+} concentrations. However, it should also be noted that, similar to other divalent cations, Zn^{2+} is a potent blocker of both non-selective cation channels (NSCC, a major route of Na^{+} uptake in plant cells; Demidchik et al., 2002), and depolarization-activated outward rectifying K^{+} channels (Shabala et al., 2006, 2007). Thus, under saline conditions, the cytosolic K^{+}/Na^{+} ratio is expected to be much higher in Zn^{2+}-pretreated Micrasterias cells compared with untreated ones. Consistent with this idea is the finding by Li et al. (2007b) that 10 μM La^{3+} treatment was effective in preventing salt stress-induced PCD in rice roots. La^{3+} is a known NSCC channel blocker (Demidchik and Tester, 2002) and, thus, La^{3+} treatment was expected to have a beneficial effect on cytosolic K^{+}/Na^{+} ratio under saline conditions.

In animal systems, caspase-3 is instrumental to cell apoptosis. As mentioned above, no caspase orthologues have been identified in plant genomes so far. However, up to eight distinct caspase-like enzymes have been reported in plants (Bonneau et al., 2008). Increase in the caspase-3-like activity was reported in various algal cells during PCD (Zuppini et al., 2007). Interestingly, no such increase was found by Affenzeller and co-authors in NaCl-treated Micrasterias cells. This suggests that multiple biochemical pathways may lead to PCD and calls for more thorough investigation of the signal transduction pathways involved. Reactive oxygen species (ROS) have emerged as important signals in the activation of plant PCD (Hoeberichts and Woltering, 2003). In addition, several plant hormones (e.g. ET, JA, SA, ABA, GA) may exert their respective effects on plant PCD through the regulation of ROS accumulation. Recently, heterologous expression of the animal anti-apoptotic CED-9 gene was shown to increase plant salt and oxidative stress tolerance by altering K^{+} and H^{+} flux patterns across the plasma membrane of tobacco mesophyll cells (Shabala et al., 2007), pointing to ROS-regulated ion channels as an important component of the PCD machinery in plants. More direct experiments are needed to reveal the full complexity and cross-talks between multiple pathways controlling salinity-induced PCD in plant cells.

At the moment, the following model can be suggested (Fig. 1). Under saline conditions, Na^{+} enters the cell through NSCC (1), causing a significant membrane depolarization (2) and resulting in a massive K^{+} leak (3) from the cell through depolarization-activated TEA^{+}-sensitive KOR channels (Shabala et al., 2006; Shabala and Cuin, 2008). At the same time, salinity-induced elevation in cytosolic Ca^{2+} (Tracey et al., 2008) will lead to a dramatic raise in ROS level (4) resulting from \([\text{Ca}^{2+}]_{\text{cyt}}\) activation of NADPH oxidase (5) via positive feedback mechanism (6; Lecourieux et al., 2002). This will cause an additional K^{+} efflux via ROS-activated NSCC channels (7; Demidchik et al., 2003). The resultant decrease in the cytosolic K^{+} pool may activate caspase-like proteases (8) leading to PCD (Fig. 1). No decline in cytosolic K^{+} pools (hence, no PCD) will be observed in sorbitol-treated cells, or in cells where membrane depolarization is prevented by Zn^{2+} or La^{3+} pretreatment. Several predictions can also be drawn from this model. (i) NaCl-induced PCD should be substantially

![Fig. 1. The proposed model of ion specific signaling during PCD in plants. KOR, depolarization-activated outward-rectifying K^{+} channel; NSCC, non-selective cation channel; PCD, programmed cell death; ROS, reactive oxygen species. See text for the explanation.](https://academic.oup.com/jxb/article-abstract/60/3/709/453035)
attenuated (or absent) in plants lacking KOR channels (e.g. *Arabidopsis gork* mutants); (ii) PCD in salt-treated cells may also be prevented by more efficient scavenging of ROS by endogenous (e.g. antioxidant enzymes) or exogenous (e.g. diphenylene iodonium, mannitol) means; (iii) plants capable of better maintaining negative membrane potential under saline conditions (e.g. ones with intrinsically higher H+-ATPase activity) must be more prone to NaCl-induced PCD. Given the important adaptive significance of PCD for salinity tolerance (Huh *et al.*, 2002), testing these predictions and validating and exploring various components of this model may be an important priority in the future.

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**References**


