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Characterization of Bax-induced Cell-death in *Arabidopsis* Protoplasts

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Objectives

Previously, we reported that Bax, a mammalian pro-apoptotic member of the Bcl-2 family, can induce cell death when expressed in *Arabidopsis* plant. To facilitate studies of Bax-mediated cell death in plant at the cellular level, *Arabidopsis* mesophyll protoplasts that contain murine Bax cDNA under the control of a glucocorticoid-inducible promoter were used as an experimental system.

Materials and methods

- Plant material: *Arabidopsis thaliana*
- Fluorescence staining dye: Dihydrorhodamine123, DAPI
- Transformation of *Arabidopsis*
- Western blot analysis
- Fluorescent microscopy studies

Results and discussion

Transgenic protoplasts treated with dexamethasone (DEX), a potent synthetic glucocorticoid, induced Bax accumulation and this was correlated with PCD like morphological changes, namely fragmentation of DNA, increased vacuolation, loss of plasma membrane integrity. When the localized expression of Bax was examined in *in vivo* targeting experiments using a jelly fish green fluorescent protein (GFP), it was determined to be targeted to mitochondria, however, deletion of its carboxyl-terminal transmembrane domain completely abolished its mitochondrial association. Examination of reactive oxygen species (ROS) production using the fluorescence method of dihydrorhodamine123 oxidation revealed that expression of Bax specifically generates ROS. Consistent with this observation, Bax-induced cell death was inhibited by an antioxidant N-acetyl-L-cystein. It was therefore concluded that ROS generation by Bax is an evolutionarily conserved mechanism among mammalian, yeast, and plant, those in turn induces cell death. These results collectively indicate that *Arabidopsis* mesophyll protoplasts are useful experimental system to study a specific cell death program in plant induced by Bax.

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