The Role of Protease Cathepsin B during non-host HR

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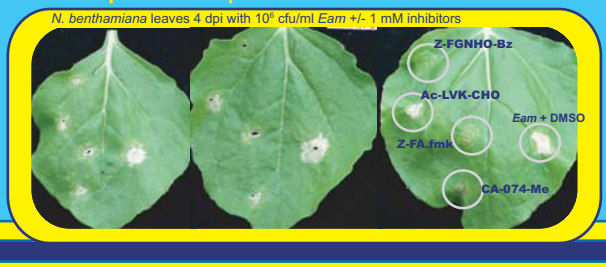
Introduction

Programmed cell death (PCD) is a fundamental process in development and in responses to biotic and abiotic stresses. Caspase activities are involved in PCD in both animals and plants, although the genes encoding them are highly dissimilar. In addition, the cysteine protease cathepsin B has been demonstrated to act as both an effector and regulator in animal PCD, where it is implicated in relocalisation of cytochrome c from mitochondria to the cytosol. We demonstrate that cathepsin B is also required for PCD in a plant disease resistance hypersensitive response (HR). The bacterium *Erwinia amylovora* (*Eam*) induced a rapid non-host HR in *Nicotiana benthamiana*. The HR was preceded by a transient increase in cathepsin B activity and coincident early relocalisation of cytochrome C. These events and the subsequent HR were prevented by cathepsin B-specific inhibitors. Virus-induced gene silencing (VIGS) was used to confirm that cathepsin B was essential for *Eam*-mediated HR and showed that this HR was dependent on the ubiquitin ligase-associated protein SGT1. The requirement for cathepsin B in the HR reveals a structurally conserved cysteine protease in an ancient PCD mechanism shared by plants and animals.

Cathepsin B-specific Inhibitors delay the HR

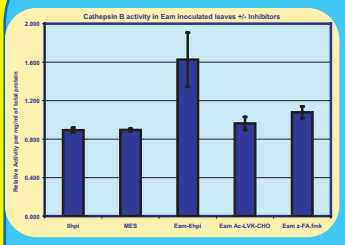
To investigate involvement of cathepsin B in the *Eam*-mediated HR, cathepsin B-specific inhibitors I (Z-FA.fmk), II (Ac-LVK-CHO), IV (CA-074 Me) and the broad cathepsin B, S, L and papain inhibitor Z-FGNHO-Bz were each infiltrated with 10^6 cfu ml^{-1} of *Eam* into *N. benthamiana* leaves. In the absence of inhibitor, *Eam* elicited a visible HR by 24 hours post-infiltration (hpi). In contrast, infiltration of *Eam* in the presence of 1 mM of inhibitors consistently abolished or considerably delayed and reduced the HR.

>Co-infiltration of *Eam* and mammalian cathepsin B-specific inhibitors can perturb the plant HR .



Inhibitors Reduce CathB Activity and Increase *Eam* Recovery

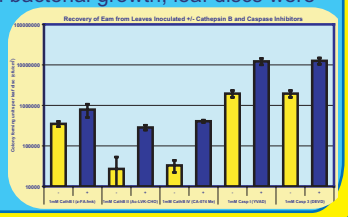
Cathepsin B activity in uninfiltrated *N. benthamiana* leaves (0), and 6 hpi with *Eam*, with the buffer in which *Eam* is prepared (MES) or with *Eam* containing 1 mM Ac-LVK-CHO or CA-074-Me.



>Cathepsin B activity at 6 hpi is absent when co-inoculated with inhibitors. This correlates with observed suppression of the HR.

In order to measure differences in bacterial growth, leaf discs were cut from *Eam* (10^6 cfu/ml) inoculated with and without 1 mM CathepsinB and Caspase inhibitors at 4 dpi.

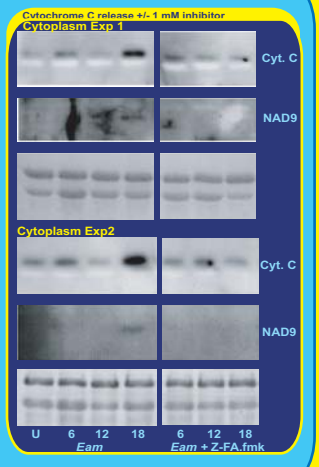
>Suggests a link between suppression of HR and the number of *Eam* recovered.



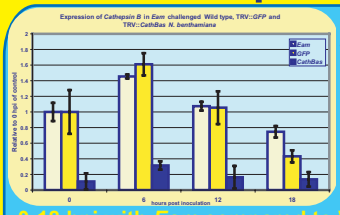
CathB Inhibitor Perturbs Cyt. C Release from Mitochondria

Western blots indicating presence of cytochrome c or NAD9 proteins in cytoplasmic fraction (repeated twice) prepared from untreated leaves (U); leaves 6, 12 or 18 hpi with *Eam*; *Eam* with 1 mM Z-FA.fmk (Cathepsin B I) inhibitor. The same result was observed using 1 mM Ac-LVK-CHO (Cathepsin B II) inhibitor. Equal loading is indicated by Ponceau staining.

>Cathepsin B-specific inhibitor prevents *Eam*-induced relocalisation of cytochrome c from the mitochondria to the cytoplasm.



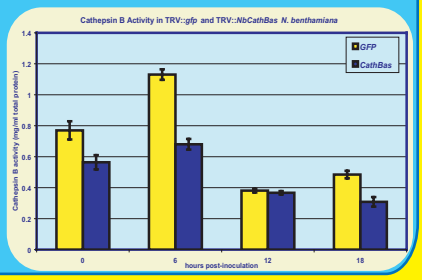
VIGS effect on Cathepsin B Expression and Protein Activity



Relative expression of *NbCathB* in wild type or infected (TRV::gfp or TRV::NbCathB) *N. benthamiana* leaves that were untreated (0), or inoculated at 6, 12 and 18 hpi with *Eam*. **>TRV::*NbCathB* plants have 80% reduced *CathB* expression 0-18 hpi with *Eam* compared to WT and TRV::*gfp*.**

Cathepsin B activity in *N. benthamiana* leaves infected with TRV::*gfp* or TRV::*NbCathB* at 0, 6, 12 or 18 hpi with *Eam*.

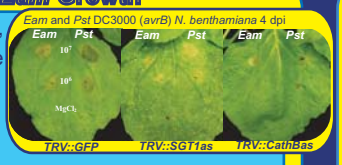
>TRV::*NbCathB* plants have reduced Cathepsin B activity at 0 and 6 hpi.

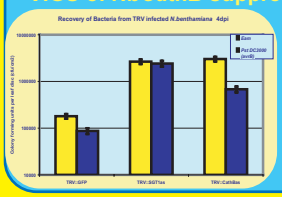


VIGS of Cathepsin B on HR and *Eam* Growth

N. benthamiana inoculated with TRV::*gfp*, TRV::*NbCathB* and TRV::*Nbsgt1* were infiltrated with 10^5 or 10^6 cfu.ml^{-1} *Eam*.

>VIGS of *NbCathB* suppresses the HR





Leaf discs were cut from *Eam* and *Pst* (10^6 cfu/cm^2) inoculated VIGSed *N. benthamiana*.

>VIGS of *NbCathB* and *Sgt1* permits 10-fold greater bacterial growth 4 dpi.

Conclusion

We propose that PCD in plants, as in animals, involves a cascade of interacting proteases, including cysteine proteases. Although caspase activities are involved in both plant and animal PCD, the genes encoding them are not similar. In contrast, the requirement for cathepsin B in the HR has revealed an ancient PCD mechanism executed or regulated by a cysteine protease that is structurally conserved in plants and animals.

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