Proteases and Programmed Cell Death in Fungi

Submitted by Derek Wilkinson of the University of Exeter as a thesis for the degree of Doctor of Philosophy in Biological Sciences in July 2011

I certify that all the material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other university.

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Abstract

Programmed cell death in animals, plants and protists is in part regulated by a variety of proteases, including cysteine aspartyl proteases, (caspases, paracaspases and metacaspases), cathepsins, subtilisin-like serine proteases, vacuolar processing enzymes and the proteasome. The role of different proteases in the cell death responses of the fungi is however largely unknown. A greater understanding of the fungal cell death machinery may provide new insights into the mechanisms and evolution of PCD and potentially reveal novel targets for a new generation of antifungal drugs.

The role of a metacaspase encoding gene, *MCA1*, in the cell death response of the human pathogen *Candida albicans* pathogen has been investigated by functional analysis. *MCA1* deletion not only alters the sensitivity of cells to a number of cell death stimuli, it also enhances virulence in an insect model. *C. albicans* shows altered cell and colony morphology on Lee's medium. Evidence is presented to suggest that these functions appear to be dependent upon active mitochondria.

In this study it has also been shown that key caspase substrates may be conserved between humans and the yeasts *Saccharomyces cerevisiae* and *Candida albicans*. Many substrates, particularly those which are essential, have retained their caspase cleavage motifs. 14 protease mutants displayed altered activity against caspase 1, 3, 6 or 8 substrates during acetic acid-induced PCD and caspase 1-like activity appeared to be particularly associated with PCD.

Using a novel bioinformatic analysis of experimental LC-MS/MS data, changes in the degradation patterns of the proteome (destructome) following acetic acid-induced cell death have been investigated in wild-type yeast. In addition, potential native substrates of the yeast Mca1 have also been identified.

The future challenge is to characterise the destructome of different proteases under a range of cell death conditions. In this way it may be possible to identify key components of the cell death machinery and their substrates and so reveal the most promising targets for future therapeutics.

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