Characterisation of a Novel Caspase STRICA
and the Bcl-2 Homologues BUFFY and DEBCL
in Drosophila melanogaster

by

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Abstract

Apoptosis, or Programmed Cell Death (PCD) is characterised by a number of morphological features including chromatin condensation, nuclear envelope breakdown, cytoplasmic shrinkage and plasma membrane blebbing. Cells are specified to die in order to remove excess, unwanted, or harmful cells, and to deplete cells once their function has been fulfilled. Dysfunction of the apoptotic pathway can have disastrous effects on the viability of the organism as a whole. Too little cell death can result in cancer, whereas too much apoptosis is the cause of some neurodegenerative diseases. In addition to maintaining optimal cell numbers in the adult organism, apoptosis is critical during development, where it plays a role in organ sculpting and immunity.

Apoptosis is mediated by a family of caspases (cysteiny1 aspartate-specific proteinases) that cleave their substrates following an aspartate residue. Once caspases are activated, the cell is committed to die. Cellular substrates are cleaved and the morphological features characteristic of apoptosis are observed. Caspases are present in healthy cells as inactive zymogens containing a large and small active subunit, and an N-terminal prodomain. Caspases often require proteolytic cleavage at precise residues for activation. Mature caspases are dimers comprised of two large and two small subunits. Caspases are commonly categorised into two main groups. Class I, upstream or initiator caspases have long prodomains containing protein-protein interaction domains including caspase-recruitment domains (CARDs) or death effector domains (DEDs). Recruitment of adaptor molecules brings several pro-caspase molecules together to facilitate proximity-induced activation. Class II, downstream or effector caspases have very short prodomains or lack a prodomain entirely. and are activated by initiator caspases. Activated effector caspases target many cellular proteins for degradation by recognising and cleaving specific aspartate residues.

Mechanisms are in place to regulate the activity of caspases to prevent aberrant cell death. Inhibitor of apoptosis molecules physically interact with procaspases to prevent activation by adaptor-mediated oligomerisation. Additionally, the Bcl-2 family of proteins.
containing both pro-survival and pro-apoptotic members, control initiation of the intrinsic, mitochondrial pathway of caspase activation. The core apoptotic machinery is conserved between the worm, fly and mammals. The fruitfly, *Drosophila melanogaster* is a useful model organism in the study of complex genetic pathways such as apoptosis and has homologues for most of the components of the apoptotic machinery.

At the commencement of the studies described in this thesis, six caspases were described in *Drosophila*. Two of these, DRONC and DREDD are classified as initiator caspases based on the presence of a long prodomain, while DCP-1, DRICE, DEAY and DAMM lack long prodomains and are therefore considered to be downstream caspases. Results presented in this thesis report the cloning of a novel *Drosophila* caspase, STRICA and demonstrate the ability of this molecule to function as a caspase. The *strica* gene consists of 1581 coding nucleotides and encodes a protein product of 327 amino acids. STRICA is a long-prodomain-containing caspase that has all the conserved features that characterise the caspase family but lacks a CARD or DEDs. Indeed, the prodomain of STRICA is unique and bears no significant homology to other caspase prodomains. STRICA is a cytoplasmic protein expressed throughout *Drosophila* development and is expressed in larval tissues that histotyze during fly metamorphosis. Additionally, as with most caspases identified to date, STRICA is able to induce apoptosis when overexpressed in mammalian and insect cell lines. STRICA-induced apoptosis is suppressed by DIAPI and p35 and to a lesser extent by DIAP2. The prodomain of STRICA is processed when overexpressed in cells and this processing requires the catalytic cysteine residue in the active site of STRICA. Active STRICA is also shown to cleave the key *Drosophila* survival molecule, DIAP.

Overexpression of STRICA in the fly eye results in ectopic cell death of pigment cells with a more severe small, rough eye phenotype observed in homozygous *GMR-strica* flies. This apoptotic phenotype is suppressed by DIAPI and p35, and partially suppressed by DIAP2. Physical interaction between STRICA and DIAPI and DIAP2 is demonstrated. Unlike DRONC, STRICA-induced apoptosis in the fly eye is not affected by halving the
dosage of reaper, hid and grim. A potential AKT phosphorylation site was identified in the prodomain of STRICA. The *Drosophila* AKT homologue, DAKT, inhibits STRICA-induced cell death in cultured cells and in the fly eye, to some extent.

Characterisation of the *Drosophila* Bcl-2 homologues, BUFFY and DEBCL, with particular emphasis on the differential localisation of these two proteins, is presented in this thesis. DEBCL is a proapoptotic Bcl-2 homologue that localises to mitochondria in transfected cells. BUFFY has been reported to be the prosurvival Bcl-2 homologue in *Drosophila*, and resides on the ER membrane and nuclear envelope in transfected cells.

Results demonstrate the residues that are important for intracellular targeting of BUFFY and DEBCL, and indicate that residues in the transmembrane domain (TMB) itself, in addition to TMB flanking residues, are required for appropriate localisation. The importance of the membrane anchor is demonstrated, and a functional nuclear localisation signal (NLS) in the N-terminus of BUFFY identified.

This thesis contributes to the understanding of programmed cell death in *Drosophila* by characterising a novel, *Drosophila* caspase, STRICA, and analysing the differential localisation of the *Drosophila* Bcl-2 homologues, BUFFY and DEBCL. A framework for further investigation is established. Some of the results presented have been published.
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