Regulation of Apoptosis: Lessons from *Drosophila* and Their Implications for Human Health and Disease

Prof. Hermann Steller

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Regulation of Apoptosis: Lessons from Drosophila and Their Implications for Human Health and Disease
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Reaper, Hid and Grim Are Required for All Apoptotic Cell Death


Reaper, Hid and Grim Are Key Killer Genes
- Reaper, Hid and Grim are necessary for apoptosis in Drosophila
- Active forms of Reaper, Hid and Grim are only made in cells that are doomed to die
- The activity of Reaper/Hid/Grim is controlled by many different signaling pathways
- Reaper/Hid/Grim are "messengers" of death that link many different signaling pathways with the cell death program
- The introduction of Reaper, Hid and Grim into cells that normally live is sufficient for cell-killing

A Genetic Screen for Cell Death Genes
(Julie Agapite, Andreas Bergmann, Kim McCall)

Mutagenize
YW, pGMRpr x YW, pGMRpr

Enhancers have smaller, rougher eyes
Most flies have the original eye phenotype
Suppressors have larger, smoother eyes
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**Reaper-Family Proteins**

Kill by Blocking the Anti-Apoptotic Activity of IAPs in Both Insects and Mammals

```
Reaper  ➔ IAP ➔ Caspase ➔ Apoptosis
Hid
Grim
```

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**DIAP1 Is the Target for Cell Killing by RHG Proteins**

```
BIR1 ➔ RING Domain ➔ C
N

binds Reaper, Hid, Grim & caspases

binds E2 ubiquitin-conjugating enzymes
```

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**The N-terminus of Reaper, Hid and Grim Contains a Conserved Motif**

```
Reaper (aa 1-11) MAVAFYIPDQA
Hid (aa 1-11) MAVPFYLPEGG
Grim (aa 1-11) MAVAYFIPDQA

Chen et al., Genes & Development 10, 1773-1782
```
Reaper-Family Proteins Contain a Small, Conserved N-Terminal RHG Motif

<table>
<thead>
<tr>
<th>Protein</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ppr</td>
<td>M AV A F Y I -7</td>
</tr>
<tr>
<td>Grim</td>
<td>M A I A Y F I -7</td>
</tr>
<tr>
<td>Hid</td>
<td>M A V V F Y L -7</td>
</tr>
<tr>
<td>Skl</td>
<td>M A I F F I -7</td>
</tr>
<tr>
<td>Smac/Diablo</td>
<td>C A V I A Q -61</td>
</tr>
<tr>
<td>Omi/Itta2</td>
<td>A A V S S P -149</td>
</tr>
</tbody>
</table>

White et al., (1995), Genes & Development 9, 1694-1708
Chen et al., (1998), Genes Dev. 10, 1728-1732
Dv et al., Cell 92, 33-42
Yamaguchi et al., Cell 98, 42-53
Sasaki et al., (2002), J. Biol. Chem. 277, 461-484
Verhagen et al., J. Biol. Chem. 277, 493-504
Chirol et al., (2002), Cell 106, 137-146

RHG Binding Is Conserved Between Drosophila and Mammals

Wu et al., 2001, Nat. Cell Biol 3, 104

Reaper Mimetics Can Disrupt BIR-Caspase Binding

RING Domain

BIR

IBM

Caspase
IAPs and Cancer

- IAPs are frequently over-expressed in human cancer and appear to promote tumor cell survival.
- Knock-out mice for several IAPs are overall normal and live, indicating that most individual IAPs are dispensable in normal cells.
- If tumor cells are “addicted” to an over-expressed IAP, elimination or reduction of high-level IAP expression in cancer cells may lead to selective tumor killing.
- Reaper-like molecules can antagonize and eliminate IAPs; therefore, “Reaper-mimetics” may selectively stimulate apoptosis of certain tumor cells.

Ubiquitination and Apoptosis

Hyung Don Ryoo
Andreas Bergmann (MD Anderson)
Hedva Gonen, Aaron Ciechanover
(Technion, Haifa, Israel)

Expression of Reaper Dramatically Reduces the Half-Life of IAPs

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**Drosophila IAP1 (DIAP1) is a Ubiquitin-Ligase and Reaper Stimulates DIAP1 Auto-Ubiquitination**

(Ryoo et al., 2002: Nature Cell Biol. 4, 432-438)

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**Dual Role of Reaper for Caspase-Liberation and IAP-Degradation**

Anti-apoptotic (live cells):

- Ub
- Caspase
- Cell survival

- UbcD1 (E2)
- BR
- DIAP1

Pro-apoptotic (doomed cells):

- Ub
- Reaper
- Cell death

- UbcD1 (E2)
- BF
- DIAP1

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**What Is the Role of Diap1-Mediated Ubiquitination in the Regulation of Apoptosis?**

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**diap1<sup>22-8s</sup> <s/- Cells Do Not Survive**

- Mitotic recombination (FRT/flipase)
- diap1<sup>22-8s</sup> W T
- Eliminated by apoptosis

**diap1<sup>22-8s/22-8s</sup> Cells Undergo Apoptosis**

- hs-flp; diap1<sup>22-8s</sup> FRT / GFP, FRT
- Active caspase-3 Ab marks apoptotic cells
- diap1<sup>22-8s/22-8s</sup> cells are marked by the absence of GFP

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**The RING Domain of DIAP1 Is Required to Restrict Pre-Dronc Protein Levels**

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The E3-Ubiquitin Ligase Activity of DIAP1 Is Required in Most Drosophila Cells to Prevent Inappropriate Apoptosis

Apoptotic Cells Can Express Mitogens to Induce Compensatory Cell Proliferation

Hyung Don Ryoo
Travis Gorenc


In Proliferating Tissues, Damage Induced Cell Death Is Compensated by Extra-Growth of the Neighboring Cells

Passive Model

Active Model

Compensatory growth

Cellular damage
In Proliferating Tissues, Damage Induced Cell Death Is Compensated by Extra-Growth of the Neighboring Cells

Evidence for the “Active Model” of Compensatory Proliferation

hid and p35 Expressing Undead Cells Express wg and dpp
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**Wg Signaling Is Necessary and Sufficient for Cell Proliferation in Imaginal Discs**

![Diagram showing Wg signaling](image)

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**Model for Compensatory Proliferation**

![Diagram showing model for compensatory proliferation](image)

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**Do Mitochondria Regulate Caspases in *Drosophila***?

![Diagram showing mitochondrial regulation of caspases](image)

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**Developmental Cell**

**Regulation of Caspase Activation During Drosophila Spermatogenesis**


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**Spermatid Individualization in Culture**

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**Activation of the Effector Caspase-3-Like drICE at the Onset of Individualization**

Nuclei – DAPI
Individualization Complex (IC; Actin) – Phalloidin
Active drICE – CM1 antibody
Inhibition of Caspase Activity
Blocks Individualization - *In Vivo*
Rescue Constructs for the Male Sterility of cyt-c-d Mutants

\[ \text{Rescue Constructs for the Male Sterility of cyt-c-d Mutants} \]

\[ \begin{align*}
\text{XhoI} & \quad \text{EcoRI} & \quad \text{BamHI} & \quad \text{NptI} \\
\text{hap} & \quad \text{opt-d 5'UTR} & \quad \text{opt-d 3'UTR} \\
\text{hap} & \quad \text{opt-d 5'UTR} & \quad \text{opt-d coding} & \quad \text{opt-d 3'UTR} \\
\text{hap} & \quad \text{opt-d 5'UTR} & \quad \text{opt-d coding} & \quad \text{opt-d 3'UTR} \\
\end{align*} \]

\( \text{bh}^{11} \text{ male sterility was rescued with 6 independent lines} \)

\( \text{bh}^{10} \text{ male sterility was rescued with 3 independent lines} \)

Rescue of Active Caspase-3 Staining and Spermatid Individualization in cyt-c-d Mutant Testes upon Transgenic Expression of Either cyt-c-d or cyt-c-p

\[ \text{Rescue of Active Caspase-3 Staining and Spermatid Individualization in cyt-c-d Mutant Testes upon Transgenic Expression of Either cyt-c-d or cyt-c-p} \]

\[ \text{Active caspase-3 IC / Actin} \]

Conclusion:

Cytochrome C Is Required for Effector Caspase Activation in Drosophila Spermatids

\[ \text{Conclusion:} \]

Cytochrome C Is Required for Effector Caspase Activation in Drosophila Spermatids
Cyt-c-d and Other Apoptosome Proteins Are Required for Normal Developmental Apoptosis in the Drosophila Retina

Cesar Mendes
Eli Arama
Bertrand Mollereau

cyt-c-d Promotes Normal Developmental Cell Death in the Pupal Eye of Drosophila
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“Gas” and “Brake” Model of Apoptosis Control

Generation of Mice Deficient for Sept4/ARTS, a Mitochondrial Pro-Apoptotic Protein and Tumor Suppressor
Holger Kissel, Maria Georgescu, Gary Hunnicut (Population Council), Sarit Larisch (Rambam Medical Center, Haifa, Israel)

Summary

- ARTS is a mitochondrial pro-apoptotic protein derived from the Sept4 septin locus (Larisch et al., Nature Cell Biology 2, 915, 2000)
- ARTS translocates from mitochondria to the cytosol and eventually to the nucleus in response to pro-apoptotic stimuli
- ARTS acts, at least in part, through binding to and inhibition of the caspase inhibitor XIAP (Gottfried et al., EMBO J. 23, 1627-1635)
- ARTS expression is frequently lost in lymphocytes from leukemic patients; when ARTS is transfected into leukemic cells, their sensitivity to apoptotic stimuli is restored (Elhasid et al., Oncogene May 2004)
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**Generation of sept4-Null Mice**

**Sept4-Null Mice Retain Caspase-3-Positive Cytoplasmic Droplets That Display Increased XIAP Staining**

**ARTS Antagonizes IAPs**

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Overall Similarities of Sperm Differentiation in *Drosophila* and Mammals

- Fly
- Rat

The SEM of human spermatozoon was reproduced from Hollanders and Caner-Ward, 1996.

### Mitochondrial pro-apoptotic ARTN protein is lost in the majority of acute lymphoblastic leukemia patients

- Renio Elbashir
- Doaer Sahar
- Lucile Merling
- Yildi Ezroy
- Isak Rothen

Pharmacological Department, Northern Ireland Cancer Research Trust, Belfast, UK

### Acquired Characteristics of Cancer Cells

- Growth signal independence
- Blocked apoptosis
- Loss of tumor suppression
- Angiogenesis
- Metastatic potential
- Immortalization

Hanahan & Weinberg, 2000
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Sept4-Null Mice Develop Spontaneous Malignancies, in Particular Lymphoma

“Gas” and “Brake” Model of Apoptosis Control

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