C. elegans apoptosis pathway

EGL-1 — CED-9 — CED-4 — CED-3 — Effector caspases

BH3  BCL2  APAF-1  CASPASE-9

Substrates cleavage
CELL DEATH

Phagocytosis

Ced-1  Ced-2  Ced-5  Ced-6  Ced-7  Ced-10  Ced-12  PSR
Lrp  CrkII  Dock180  Gulp  ABC-1  Rac  Elmo  PSR
Phagocytosis of apoptotic cells in C. elegans

Apoptotic corpse

CED-7
(ABC-1)

CED-12
(Elmo)

CED-1
(SR-EC/LRR)

CED-7
(ABC-1)

CED-2
(CrkII)

CED-5
(Dock180)

CED-10
(Rac)

PSR1

CED-6
(GULP)

Neighboring cell
Model systems for studying Development

- Plants (A. thaliana)
- Worms (C. elegans)
- Fruit flies (D. melanogaster)
- Zebrafish (D. rerio)
Drosophila as a Model System

1. Short generation time  ~10 days

- Adults mating
- Embryo 1 day
- Embryogenesis 1 day
- Pupae 3.5-4.5 days
- Pupariation 2.5-3 days
- First Instar Larvae 3x1 day
- Second Instar Larvae 3x1 day
- Third Instar Larvae 3x1 day
Drosophila as a Model System

2. Complex body plan

- mouthpart
- frontal plate and upper lip
- antenna
- eye
- leg
- wing
- haltere
- genitalia

adult

larva
Drosophila as a Model System

3. Only 4 chromosomes

- The first physical and genetic map
  Centimorgan : 1% recombination
  (Sturtevant and Morgan)
  TH Morgan Nobel lecture 1933

- Polytene chromosomes
  In situ hybridization

- Positional cloning, cDNA and chromosomal libraries (Hogness)

- Genome project completed
Drosophila as a Model System

4. Genetic approach:
   - **Loss of function genetics:**
     Chemically generated mutants (EMS, ENU)
     Chromosomal deficiencies (~ 85% of the genome, X-rays, γ-rays, P-elements)
     Transposable elements
     Gene knockout by homologous recombination
     Mosaic analysis: making clones of mutant cells in a normal tissue (FLP/FRT)
   - **Gain of function genetics:**
     Dominant mutants
     Chromosome duplications
     Tissue specific expression systems: UAS/Gal-4
   - **Biochemical**
     HPLC purification from whole organism extracts
Drosophila as a Model System

5. Molecular approach:

- A physical map that corresponds to the genetic map: Fully sequenced, genetically marked genome.
- Excellent cytogenetics: Genome is arrayed on overlapping Bacterial Artificial Chromosome (BAC) clones, SNPs mapping.
- ESTs (Expressed Sequence Tags) characterized for all stages of the life cycle,
- Genome-wide microarrays,
- Antibodies and transposable elements to mark gene products, cell morphology and subcellular structure,
- Stable single copy transformation,
- Targeted, tissue specific expression systems (UAS/Gal-4),
- Genome-wide RNAi library allow double stranded RNA bathing/ transfection of cultured cells to address partial or complete LOF phenotypes,
- First genome-wide two hybrid screen (protein-protein interaction)
- And the fly community will not stop there!
Normal Cell

- Cell shrinkage away from neighbouring cells
  - Plasma membrane blebbing
  - Cytoplasmic and nuclear condensation
    - Margination of condensed chromatin
    - Nuclear and cellular fragmentation
      - Apoptotic Bodies
      - Phagocytosis
The importance of PCD in development and homeostasis
Consequences of deregulation of PCD

**Excessive cell death:**
- Degenerative neurological disorders
- Stroke, cardiac ischemia,
- Immune suppression associated with AIDS

**Suppression of cell death:**
- Autoimmune diseases
- Cancer
Programmed Cell Death (PCD)

1. Cell Death Signal
2. Execution of Death
3. Engulfment of remains
Programmed cell death during *Drosophila* embryogenesis

John M. Abrams1, Kristin White1, Liselotte I. Fessler2 and Hermann Steller1

**Development 117, 29-43 (1993)**

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[Image: Acridine orange staining](image)

Allowed for the initiation of a deficiency screen for PCD mutants
A deficiency kit that covers over 85% of the genome!!!

Allows for the rapid screening of the entire genome for genes involved in any embryonic phenotype of interest.

Genetic definition of a deficiency:
contains at least two complementation groups,

Overlapping deficiencies provide still finer resolution,

Isogenic deletions with precise breakpoints (drosdel project)
Homozygous $Df(3L)H99$ embryos lack all PCD
The *H99* deletion allowed for the identification of three regulators of PCD, *rpr*, *hid*, and *grim*.
No homology of sequences except in their first 14 aa: RHG/IBM motif

- **Rpr** 65 aa
- **Grim** 138 aa
- **Hid** 410 aa
- **Smac** 239 aa

**Reaper** 2       AVAFYIPDQATLL 14
**Grim** 2       AIAYFIPDQAQLL 14
**Hid** 2       AVPFYL|EGGADD 14
**Sickle** 2       AIPFY|E|EHAPKS 14
**Jafrac2** -       AKPE         -
**Smac /Diablo** 54       AVPI                  66

- **Sickle** 108 aa

**Jafrac** 2       ERS  peroxidase domain 242 aa
Early embryogenesis in *Drosophila*

Nuclei cleave in a common cytoplasm (syncytium)
At cycle 10, nuclei migrate to the periphery (blastoderm)
Germ line transgenic making in the fly

**Used in:**
- Rescue experiments (replacement strategy)
- Over-expression experiments
- Homologous recombination
Genetic screens for enhancers and suppressors of PCD in the eye
Inhibitors/suppressors of apoptosis

dIAP1 blocks both reaper and hid-induced cell death
dBRUCE blocks reaper-induced cell death but not hid
dIAP2 not a very good inhibitor of PCD, cell migration role
DETERIN is most homologous to survivin as it has a single BIR

dIAP 1/thread

BIR1 ── BIR2 ── RING ── 438 aa

dIAP 2

BIR1 ── BIR2 ── BIR3 ── RING ── 498 aa

dBRUCE

BIR ── UBC ── 4876 aa

DETERIN

BIR ── 153 aa
DIAP-1: a central player

*thread* LOF mutation shows increased PCD in the embryo, and is embryonic lethal.

*thread* is epistatic to *rpr, hid* and *grim*.

GOF of *Diap1* prevents *rpr, hid* and *grim*-induced death.

GMR transgene alone has no effect, but it can inhibit Dronc and Debcl-induced death, thus it genetically interacts with *Dronc*, a caspase gene, and *Debcl*, a Death enhancer with Bcl-2 homology (pro-apoptotic Bcl-2 homologue).
DIAP1 and the N-end rule-dependent ubiquitination

Nambu’s group identified four enhancers of grim-reaper-induced death, all regulating ubiquitination: uba-1, skpA, faf, and morgue.
There are 7 *Drosophila* CASPASES

Cysteine/ASPartate proteASES related to Ced-3

**Apical**

- **DRONC**
  - CARD
  - p20
  - p14

- **DECAY**
  - large
  - small

- **DRICE**
  - p20
  - p12

**Apical**

- **DREDD/DCP2**
  - DED
  - DED
  - p20
  - p10

- **DAMM**
  - p20
  - small

- **DCP-1**
  - p20
  - p13

- **STRICA/DREAM**
  - Ser/Thr Rich
  - p20
  - small

CARD: Caspase Activation Recruitment Domain and DED: Death Effector Domain are protein-protein interactions domains
Caspases phenotypes and interactions

Dronc RNAi prevents PCD in the embryo, Dronc deficiency and Dronc dominant negative transgene genetically interact with rpr, hid and grim; Dronc over-expression induces cell death.

Dredd LOF have a defect in the immune response.

Damm dominant negative transgene genetically interacts with hid, and over-expression of Damm induces death.

DCP-1 LOF is lethal at third instar larval stage with no imaginal discs or gonads and melanotic tumors.

Drice, Decay, Strica: no LOF mutation. Drice over-expression has no effect.

Drice and Dcp-1 bind to BIR1 of DIAP-1, and act downstream of Dronc
The *Drosophila* Gas and Brake model

_RPR, HID and GRIM (gas) compete with DRONC for binding to BIR2 domain of DIAP1 (brake), which inhibits DRONC.

_Upon cell death signals, RPR, HID or GRIM (gas) bind to DIAP-1 (BIR-2), competing for it with DRONC. Thus the brake (DIAP1) is released from DRONC, the caspase is activated and cell death occurs._
Ubiquitination in programmed cell death regulation

N-end-rule-dependent ubiquination

RING-dependent ubiquination

Physical interaction
Apaf-1 related DARK

IAP antagonists of the Bcl-2 family: Buffy and Debcl
The apoptosome in mammals

Closed monomer → Open dimer → Heptamer

Cyt C → WD40

procaspase ?

Inactive monomer → Inactive dimer → Active dimer
Drosophila apoptosome: how does it function?

A  Cytochrome c release involvement still a question?

C  Pro-apoptotic Debcl

B  Anti-apoptotic Buffy displaced by Debcl?
RNA interference in *Drosophila* S2 cells

**RNAi Mechanism**

- Introduction of TRIGGER dsRNA
- DICER: processes 21-23nt siRNA
- RISC: destruction of TARGET mRNA

**RNAi In S2 cells**

- dsRNA to serum-free medium
  (Clemens, et al. 2000 PNAS 97: 6499)

- Partial to complete loss of function
- Uniform penetrance

**Loss-of-function phenotype**

438 essential genes!
MicroRNAs in Drosophila apoptosis

Developmental and environmental death signals

GRIM
REAPER
SICKLE
HID

DARK

DIAP1

DRONC

DRICE

mir-14

Cell Growth and proliferation

CELL DEATH

FAT LEVELS

Transcriptional control: RNAi/transcription complex, chromatin silencing

mir-2, mir-13 family?
Drosophila intrinsic and extrinsic PCD pathways

Extrinsic death signals
- TNFR family
- dFADD
- UV

Intrinsic death signals
- Rpr, Grim, Hid
- Dmp53
- Debcl
- BH3 only

Death receptor response
- Diap-1
- Caspases

Phagocytosis
- CELL DEATH
Programmed Cell Death (PCD)

1. Cell Death Signal

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**Drosophila embryonic macrophages**

Stage 11

Stage 13

Stage 15

Stage 17

ECM production

Phagocytosis

Endocytosis

Glial cells and epithelial cells can also engulf!

Croquemort, a CD36-related macrophage receptor

Requirement for *croquemort* in phagocytosis of apoptotic cells in *Drosophila*

Peroxidasin Ab  7-AAD  Croquemort Ab

Wild type  crq-/-  crq-/-, uas-crq, hs-Gal4

C. elegans apoptosis pathway

EGL-1 → CED-9 → CED-4 → CED-3 → Effector caspases → Substrates cleavage → CELL DEATH → Phagocytosis

Ced-1 Ced-2 Ced-5 Ced-6 Ced-7 Ced-10 Ced-12 PSR Lrp CrkII Dock180 hCed-6 ABC-1 Rac Elmo PSR?
A role for \textit{Draper} in phagocytosis of apoptotic cells by \textit{l(2)mbn} and \textit{SL2} cells and embryonic macrophages

\textit{Draper} RNAi in \textit{l(2)mbn} and \textit{SL2} cells prevents engulfment of apoptotic cells

\textit{Crq} RNAi does not prevent engulfment of apoptotic cells
Although the mRNA is targeted, the endogenous protein is still present

\textit{Draper} RNAi by injection of dsRNA in embryos reduces the engulfment of apoptotic cells \textit{in vivo}.

AND THERE IS SO MUCH MORE TO LEARN!

EGFR/Ras
Eiger/TNF
MAPK/Junk pathway
Hippo/salvador/warts regulators of cell growth
PCD and cell competition
DIAP2 in innate immunity
DIAP2 in cell migration
AUTOPHAGY
Etc…