Life or death decisions: the network regulating programmed cell death in C. elegans

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UC Santa Barbara

apoptosis in biology
morphogenesis
immune system
nervous system
Apoptosis-related diseases

Malignant diseases
- Carcinoma
- Leukemia
- Lymphoma

Autoimmune Diseases
- Rheumatoid arthritis
- Polycythemia vera

Infectious diseases
- Infection
- AIDS

Neurodegenerative diseases
- Alzheimer's Disease
- Huntington's Disease

Ischemia
- Stroke

Cardiovascular Diseases
- Myocardial infarction

Autoimmune Diseases
- Type 1 diabetes

C. elegans
Anatomy of *C. elegans*

959 Cells
~200 cell types

Reconstruction of nervous system

John White et al
Reconstruction of nervous system

The neural model for the nervous system:
- Chemical synapses
- Gap junctions
C: Cytokinesis movement; T: Thermopile movement
Original model developed by I. Mert & Y. Ohshima (1995) and I. Mert (1999)

John White et al

C. elegans and systems biology

Genetics
Complete cellular anatomy
Complete development
Complete genome sequence
Complete development
Complete cellular anatomy
Genetics

Biology
C. elegans and systems

C. elegans development
C. elegans and systems biology

Genetics
Complete cellular anatomy
Complete development
Complete genome sequence
(100,258,171 bp)
Large scale functional genomics

Functional genomics with RNAi

dsRNA $\rightarrow$ “instant gene knockout”

feed bacteria expressing dsRNA

geno-mer-wide screen (≈19,000 genes)
High-throughput functional genomic screening

C. elegans and systems biology

Genetics
Complete cellular anatomy
Complete development
Complete genome sequence
(100,258,171 bp)
Large scale functional genomics
Model for humans diseases
Some unique contributions of *C. elegans*

- Complete lineage analysis
- Laser cell ablation/fusion
- Green fluorescence protein (GFP)
- RNA interference (RNAi)
- miRNAs
- Programmed cell death
C. elegans development

Invariant pattern of somatic cell death

959 nuclei survive
131 nuclei die
**core apoptotic pathway**

CED-9 → CED-4 → EGL-1

CED-4 → proCED-3

active CED-3 caspase → Targets?

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**icd-1** (inhibitor of cell death-1) phenotype

wildtype

icd-1(RNAi)
ICD-1 overexpression suppresses PCD
ICD-1: nascent chain associated complex (NAC)

α/β complex implicated in protein targeting

β NAC eliminated early in apoptosis of Jurkat and Burkitt’s lymphoma cells

β NAC cleaved by caspase-3

ICD-1 contains a putative caspase cleavage site
ICD-1 is cleaved by CED-3 \textit{in vitro}.

\begin{center}
\begin{tabular}{ccc}
  + CED-3 & \\ 5h & 3h & 1h \\
\end{tabular}
\end{center}

ICD-1 is cleaved \textit{in vivo}.

\begin{center}
\begin{tabular}{cccc}
  egl-1 & ced-4 & ced-3 & WT \\
\end{tabular}
\end{center}
ICD-1 contains a putative caspase recruitment domain (CARD)

<table>
<thead>
<tr>
<th>bNAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>MRRTQTAPAQADSRGRGRARGGCPGGEATLSQP</td>
</tr>
<tr>
<td>34</td>
</tr>
<tr>
<td>PRQGTRQGEPQMKETIMNGEKLAKLQAQQEVR</td>
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<tr>
<td>64</td>
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<td>IGKQTARRKVKVMTAADDKLCGLSLKLGQ54</td>
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<tr>
<td>97</td>
</tr>
<tr>
<td>VNTISQIEEVMFTNQGTIVHFNPNKVKQALGLAA</td>
</tr>
<tr>
<td>130</td>
</tr>
<tr>
<td>NTFTITGHAETIKLGTEMLPSILNLGLADSLTS</td>
</tr>
<tr>
<td>162</td>
</tr>
<tr>
<td>RRLAEALPKQSVDGKAPELDEVEDDEVPLVE</td>
</tr>
<tr>
<td>196</td>
</tr>
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<td>FDEASKNEAN</td>
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</table>

ICD-1 is mitochondrial

ICD-1    mito    merge
core apoptotic pathway

CED-9 → CED-4 → EGL-1

CED-4 → proCED-3 → active CED-3 caspase → PCD

CED-9 is required for ICD-1 localization to mitochondria

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Early embryo</th>
<th>Late embryo</th>
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</thead>
<tbody>
<tr>
<td>WT</td>
<td>nuclear</td>
<td>mito.</td>
</tr>
<tr>
<td>ced-9(If)</td>
<td>nuclear</td>
<td><strong>nuclear</strong></td>
</tr>
<tr>
<td>ced-9(gf)</td>
<td><strong>mito.</strong></td>
<td>mito.</td>
</tr>
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</table>
core apoptotic regulatory pathway

**ced-9** → **ced-4** → **ced-3** → PCD

Bcl-2 Apaf-1 caspase

protection execution

cell death in *icd-1(-)* is CED-4-dependent

<table>
<thead>
<tr>
<th>% with cell corpses</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>0</td>
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<tr>
<td>10</td>
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<tr>
<td>20</td>
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<tr>
<td>30</td>
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<tr>
<td>40</td>
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<tr>
<td>50</td>
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cell death in icd-1(-) is **CED-3-independent**!

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<td>50</td>
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\[ \alpha \text{NAC (ICD-2)} \text{ is associated with apoptosis-related disease} \]

- downregulated in Alzheimer’s neurons
- upregulated in malignant brain tumors
- \( icd-2(\text{RNAi}) \rightarrow \text{CED-3-independent PCD in } C. \text{ elegans} \)
Why is PCD in *icd-1/2(-)* mutants CED-3-independent?

caspase redundancy?

CED-3 → PCD

??

CSPs

3 CSP genes → 6 caspases
caspase inhibition by baculovirus p35

CED-3

p35

??

PCD

CSPs

p35 suppresses inappropriate apoptosis

![Bar chart showing the number of cell corpses per embryo with and without heat shock.](chart.png)
CSP-1 caspase is required for PCD in $icd-1(-)$

% with corpses in $icd-1(RNAi)$

unc-22(RNAi)  ced-4(-)  csp-1(RNAi)

model for ICD-1-mediated repression

CED-9 $\rightarrow$ CED-4 $\rightarrow$ CED-3

ICD-1 & -2

CSP-1

$\rightarrow$ PCD

Positive feedback loop: all or none switch
Is CED-3-independent PCD relevant to normal development?

*icd-1(RNAi)* causes dramatic loss of male rays

**WT**

**icd-1(RNAi)**
male tail rays in ced-4(-) mutants

CED-4-dependent, CED-3-independent PCD in wildtype

% with 18 rays

Wildtype  50
ced-3(-)  87
ced-4(-)  98

*ced-4* mutants make more “perfect” male tails than wildtype!
Identifying the comprehensive set of PCD regulators

**ced-9 and icd-1**: different classes of PCD suppressors

<table>
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<td>+++</td>
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**RNAi screen for PCD suppressors**

- ced-3(-) embryo
- ced-3(-); gene X(RNAi) embryo
High throughput RNAi screen

E. coli expressing dsRNA

mutant offspring

Gene 1 Gene 2 Gene 3 Gene 4

Rapid functional screen of entire genome

distribution of 80 genes that repress apoptosis

- G-proteins: 9
- Kinase/phosphatase: 5
- transcription factor: 2
- stress/repair/DNA metab.: 1
- RNA metab.: 3
- proteases: 15
- mitochonndrial: 18
- unknown: 4
- miscell.: 23
screen identifies neurodegenerative disease genes

Hallervorden Spatz disease:
mitochondrial pantothenate kinase

Spastic periplegia:
AAA ATPase

identification of cancer genes

VBP-1
binds Von Hippel-Lindau tumor suppressor

TFG
lymphomas and thyroid cancers
TFG

rearranged in large cell lymphomas and papillary thyroid cancers

upregulated by TALL-1 receptor (B cell proliferation and autoimmunity)
Analyzing the network of PCD regulators

Three RNAi screens for apoptotic suppressors

1. CED-3-independent PCD
2. CED-4-dependent sterility
3. CED-4-dependent lethality

103 genes
40 genes
42 genes
Identification of overlapping gene sets

CED-4-dep. lethals:
- 42 genes

CED-4-dep. steriles:
- 40 genes

CED-3-indep. PCD:
- 103 genes

Computational prediction of networks

Computational analysis

Apoptosis regulators from RNAi screen

Inferred relationships between genes
Clustering of genes

Predicted network

61 candidate genes cluster with 334 others
Summary

1. ICD-1 (and –2) function in PCD pathway: may establish all or none life vs. death switch

2. genome-wide RNAi screens: >100 additional cell death suppressors

3. computational methods identify network of PCD regulatory proteins

Collaborators

RNAi library:
A. Fraser, R. Kamath, J. Ahringer (Cambridge)