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

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apoptosis in biology

morphogenesis

immune system

nervous system

Apoptosis-related diseases

Inactive apoptosis

Malignant diseases
- Carcinoma
- Leukemia
- Lymphoma

Autoimmune Diseases
- Rheumatoid arthritis

Hematological diseases
- Polycythemia vera
- FHL

Overactive apoptosis

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

Infection
- AIDS

Ischemia
- Stroke
- Myocardial infarction

Autoimmune Diseases
- Type 1 diabetes

C. elegans

C. elegans and systems biology

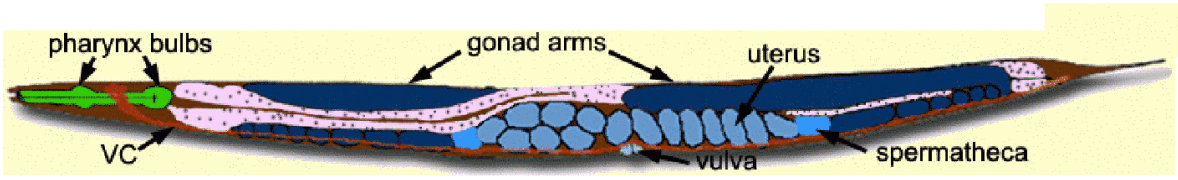
Genetics

C. elegans and systems biology

Genetics

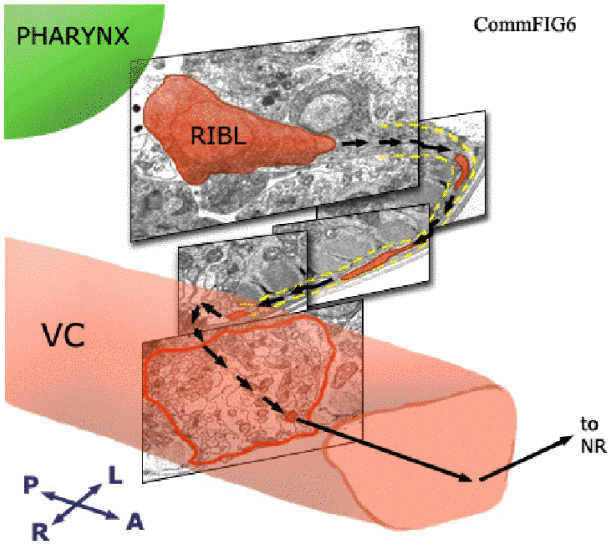
Complete cellular anatomy

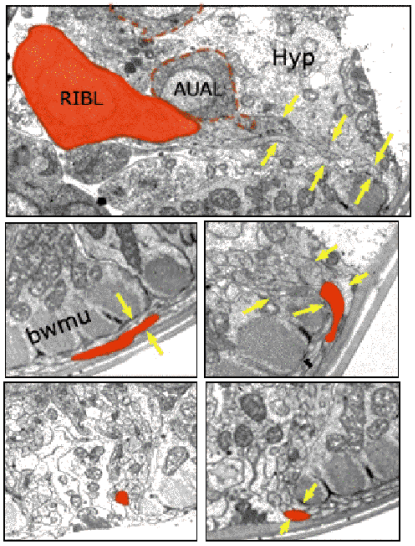
Anatomy of *C. elegans*



959 Cells
~200 cell types

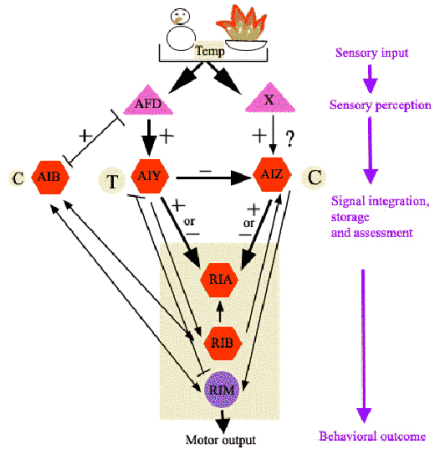
Reconstruction of nervous system



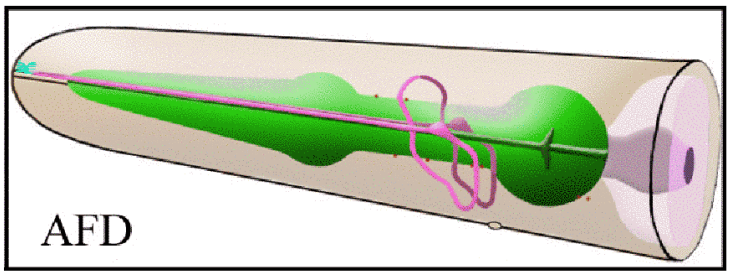


John White et al

Reconstruction of nervous system



The neural model for thermotaxis.
Chemical synapses
Gap junctions
C: Cryophilic movement; T: Thermophilic movement
Original model developed by I. Mori & Y. Ohshima (1995) and I. Mori (1999)



John White et al

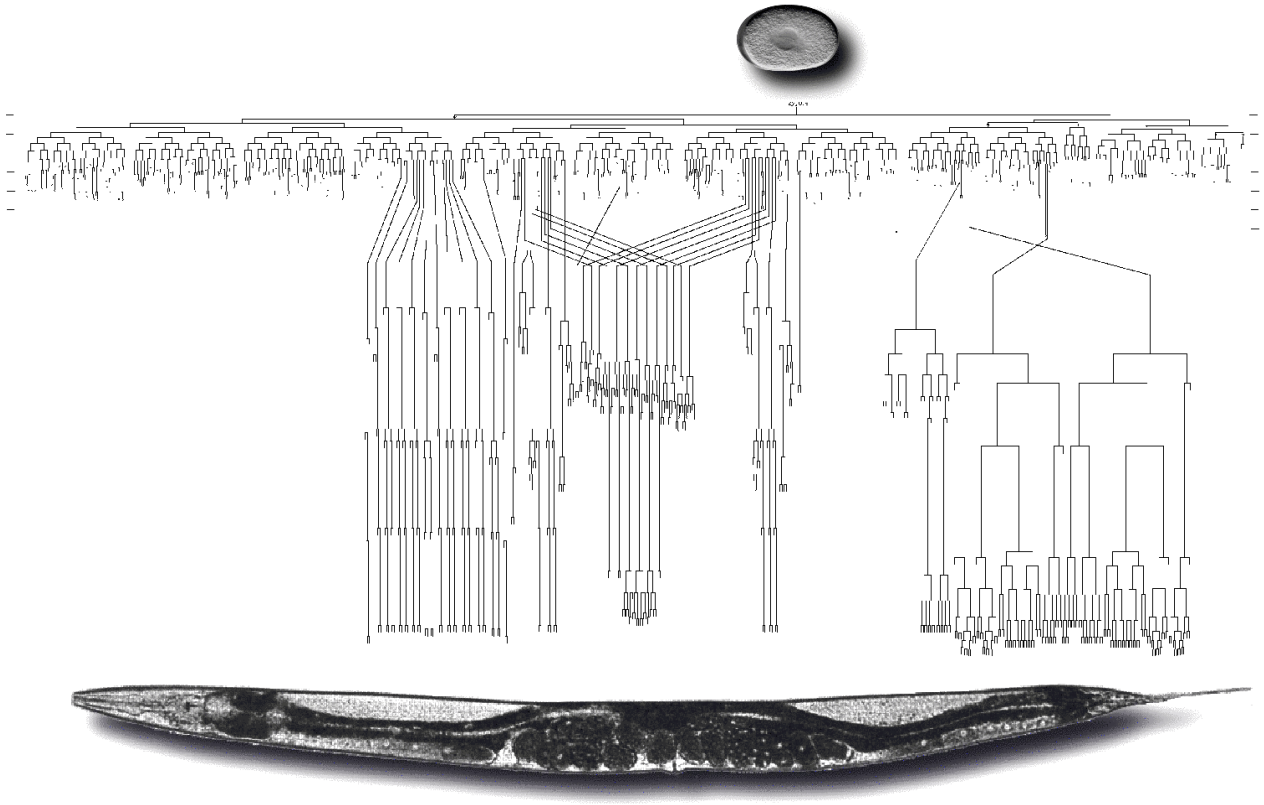
C. elegans and systems biology

Genetics

Complete cellular anatomy

Complete development

C. elegans development



C. elegans and systems biology

Genetics

Complete cellular anatomy

Complete development

Complete genome sequence
(100,258,171 bp)

C. elegans and systems biology

Genetics

Complete cellular anatomy

Complete development

Complete genome sequence
(100,258,171 bp)

Large scale functional genomics

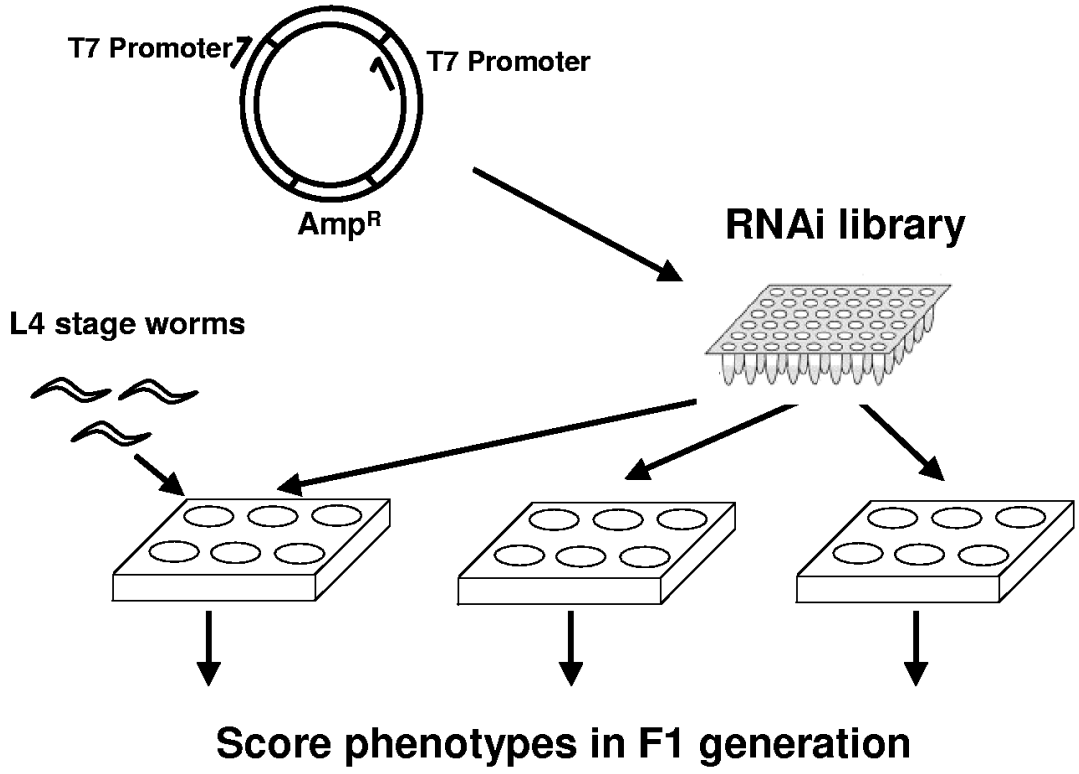
functional genomics with RNAi

dsRNA → "instant gene knockout"

feed bacteria expressing dsRNA

genome-wide screen (~19,000 genes)

High-throughput functional genomic screening



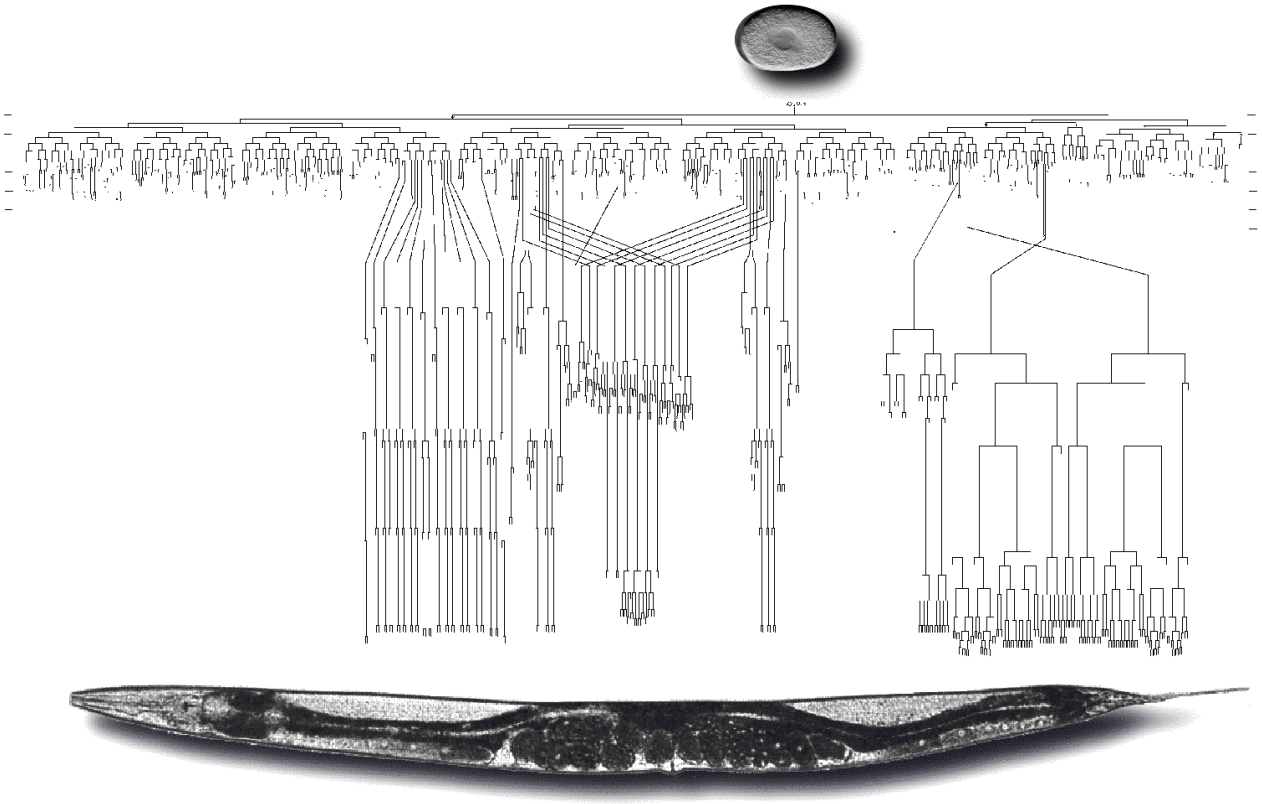
C. elegans and systems biology

- Genetics
- Complete cellular anatomy
- Complete development
- Complete genome sequence (100,258,171 bp)
- Large scale functional genomics
- Model for humans diseases

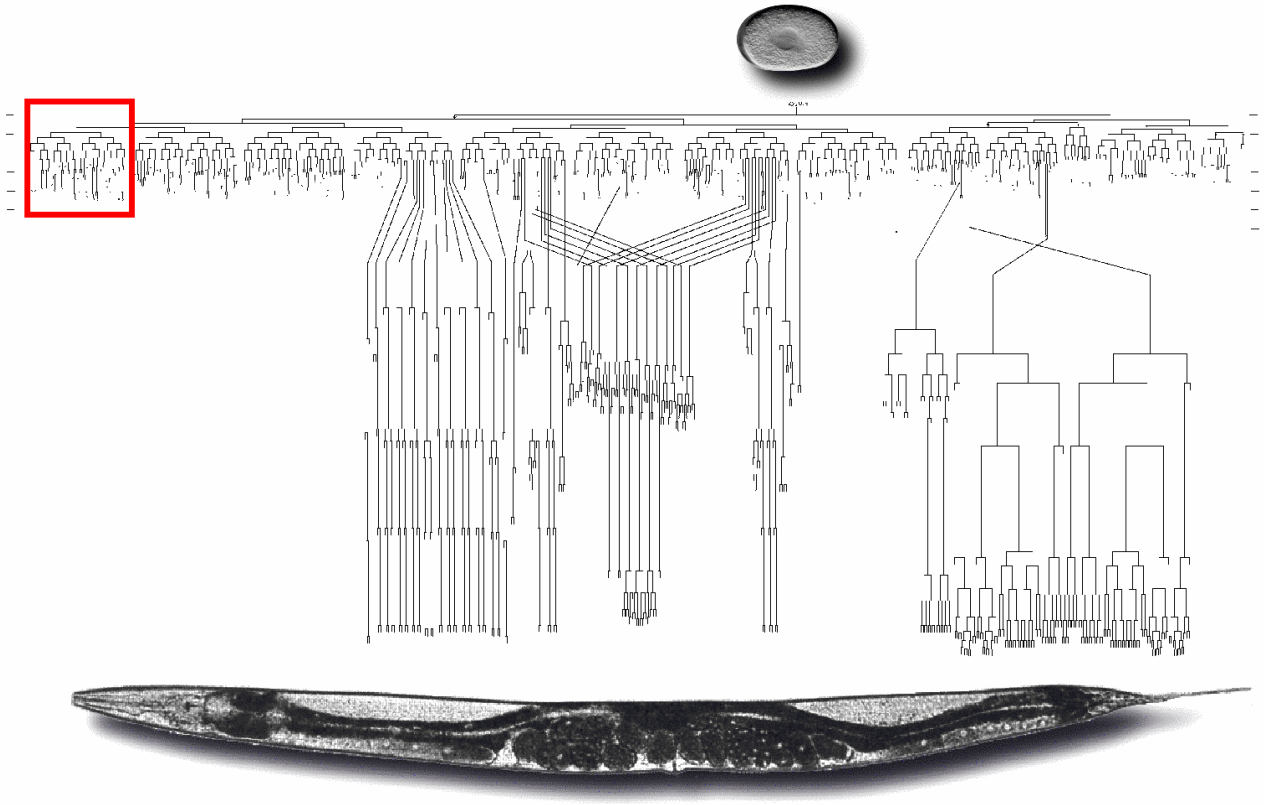
Some unique contributions of *C. elegans*

Complete lineage analysis
Laser cell ablation/fusion
Complete nervous system reconstruction
Green fluorescence protein (GFP)
RNA interference (RNAi)
miRNAs
Programmed cell death

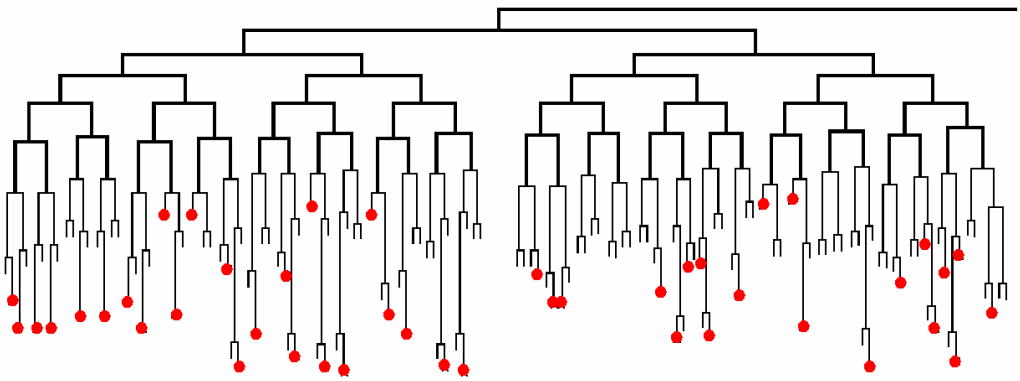
C. elegans development

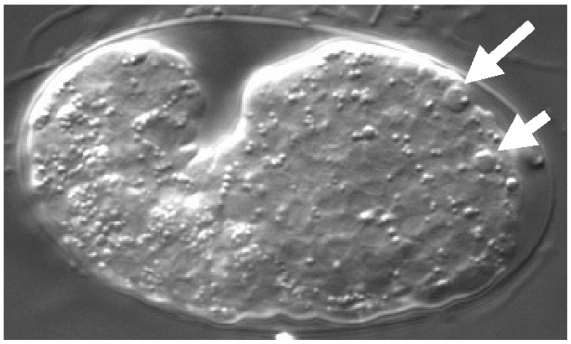


C. elegans development



invariant pattern of somatic cell death

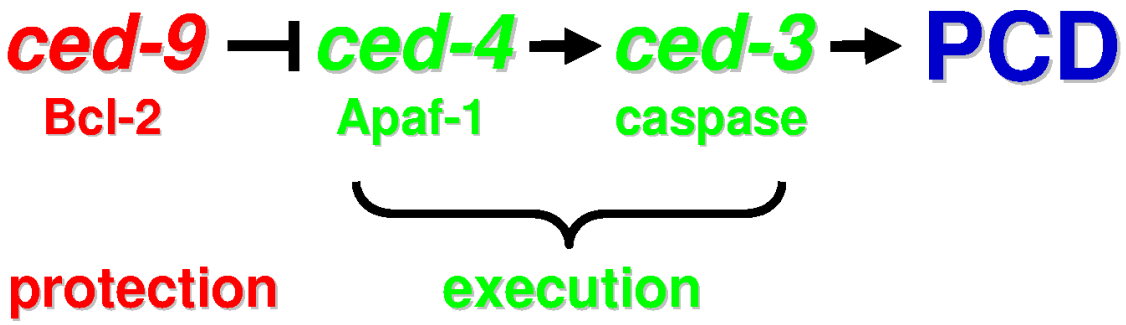




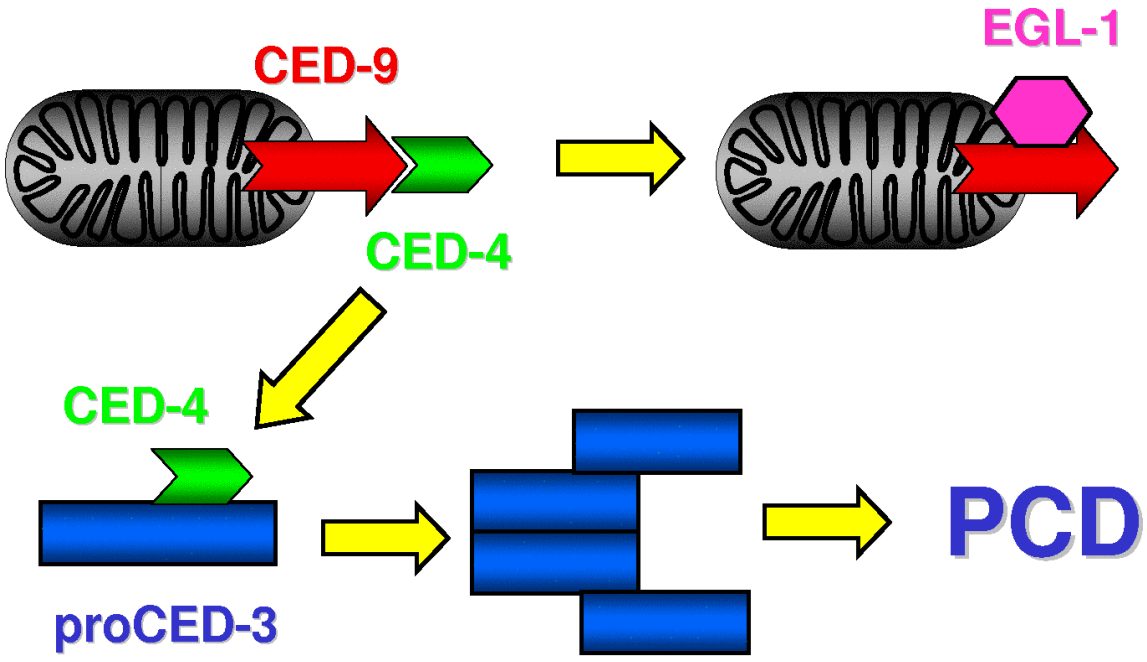
959 nuclei survive

131 nuclei die

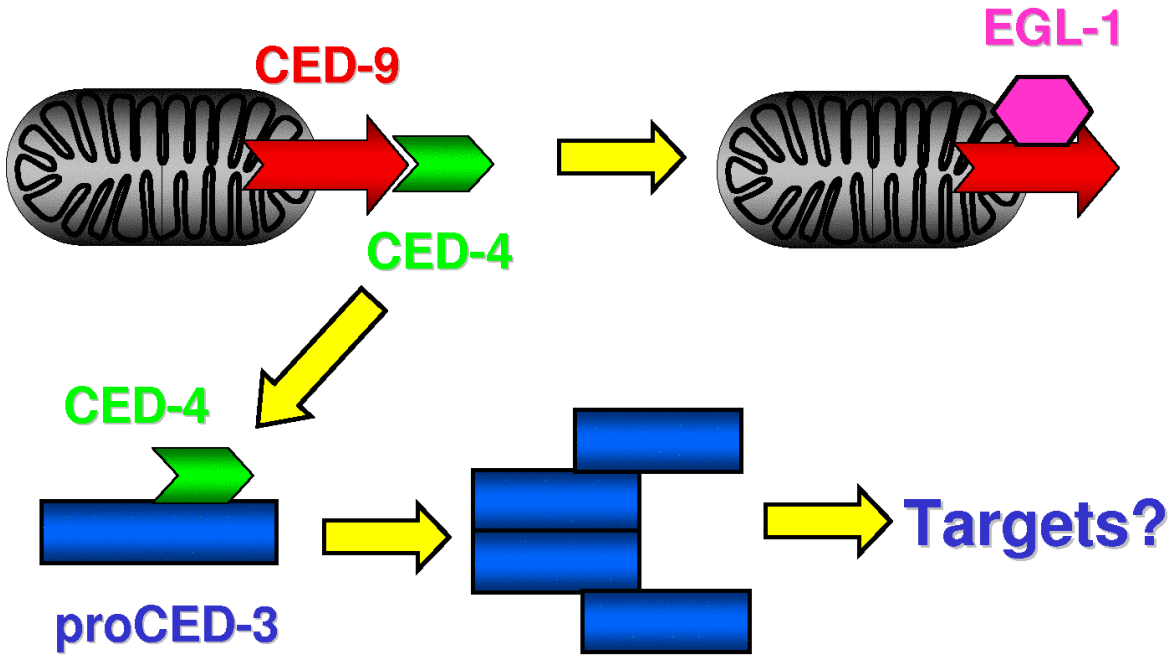
core apoptotic regulatory pathway



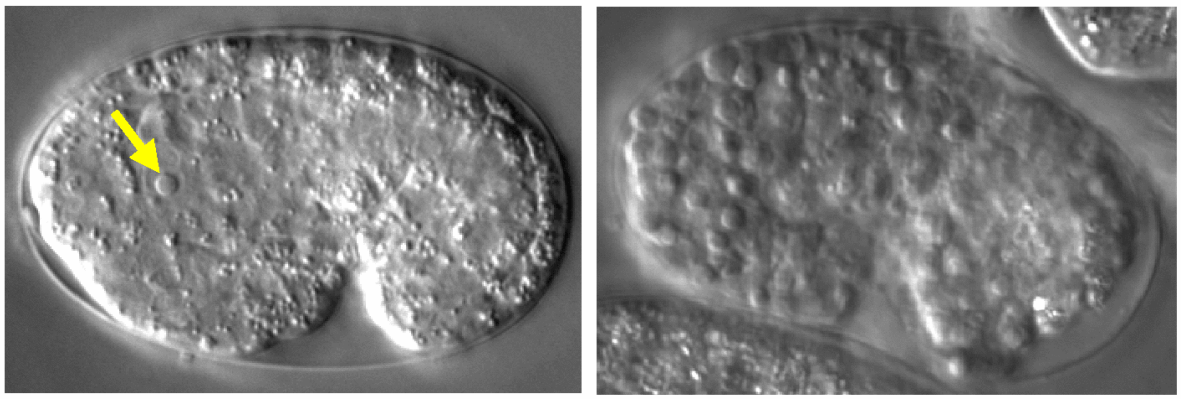
core apoptotic pathway



core apoptotic pathway



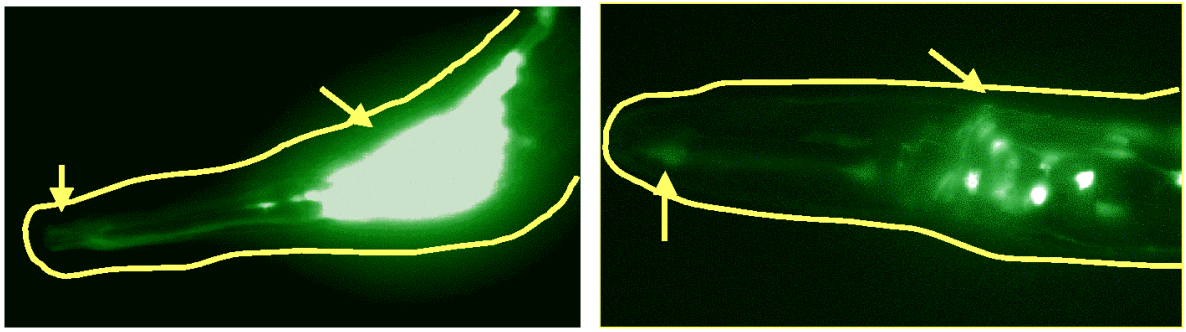
icd-1 (inhibitor of cell death-1) phenotype



wildtype

icd-1(RNAi)

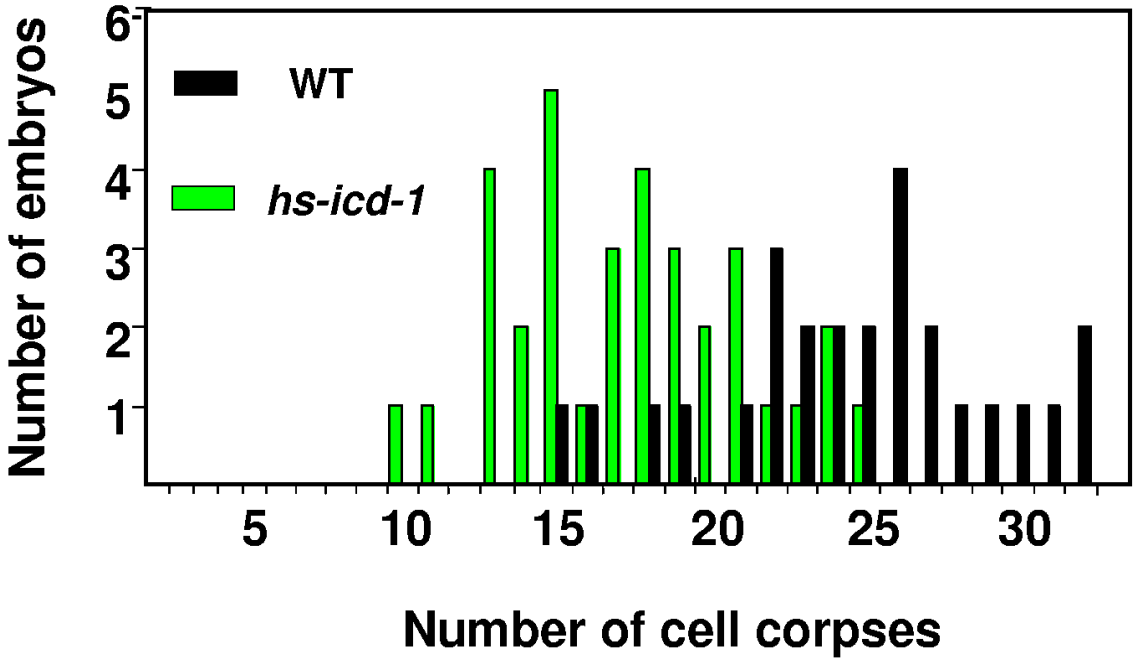
icd-1(RNAi) results in widespread loss of neurons



wild-type

icd-1(-)

ICD-1 overexpression suppresses PCD



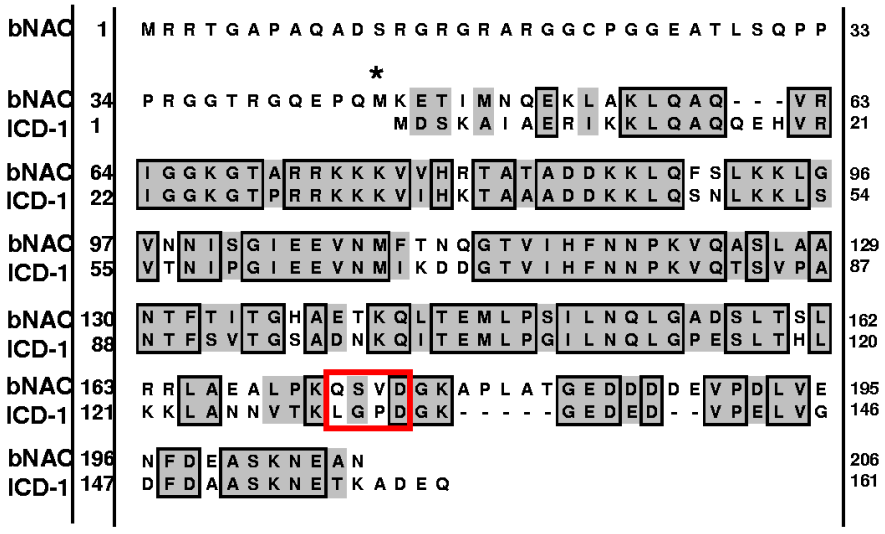
ICD-1: nascent chain associated complex (NAC)

α / β complex implicated in protein targeting

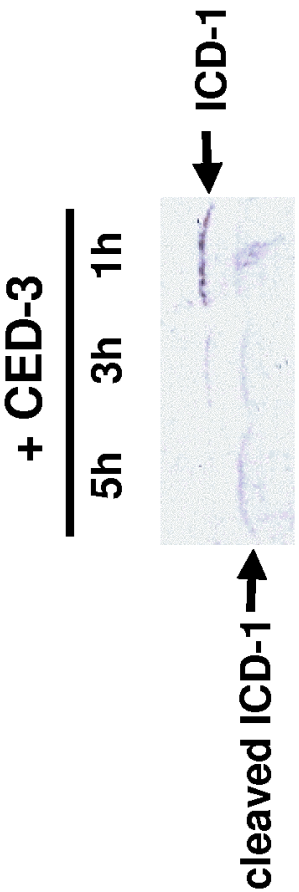
β NAC eliminated early in apoptosis of Jurkat and Burkitt's lymphoma cells

β NAC cleaved by caspase-3

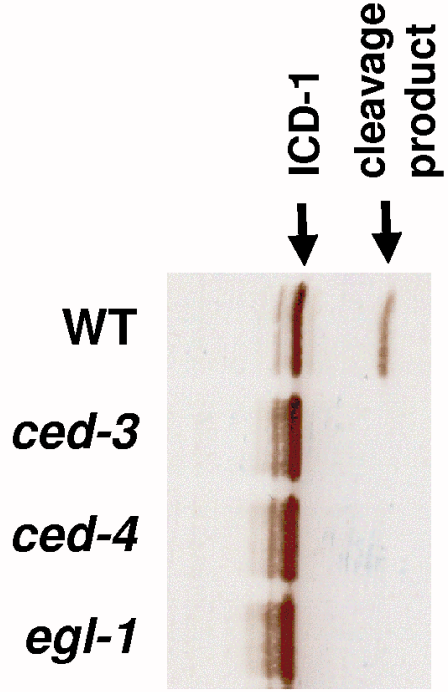
ICD-1 contains a putative caspase cleavage site



ICD-1 is cleaved by CED-3 *in vitro*

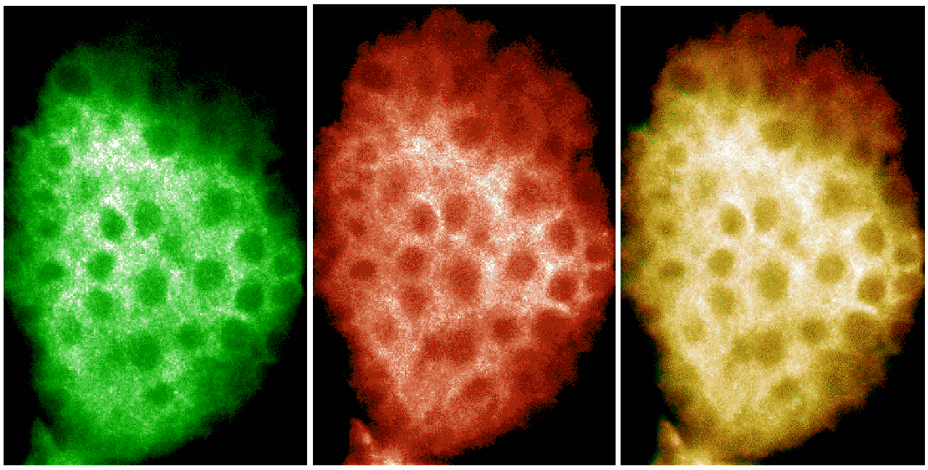


ICD-1 is cleaved *in vivo*



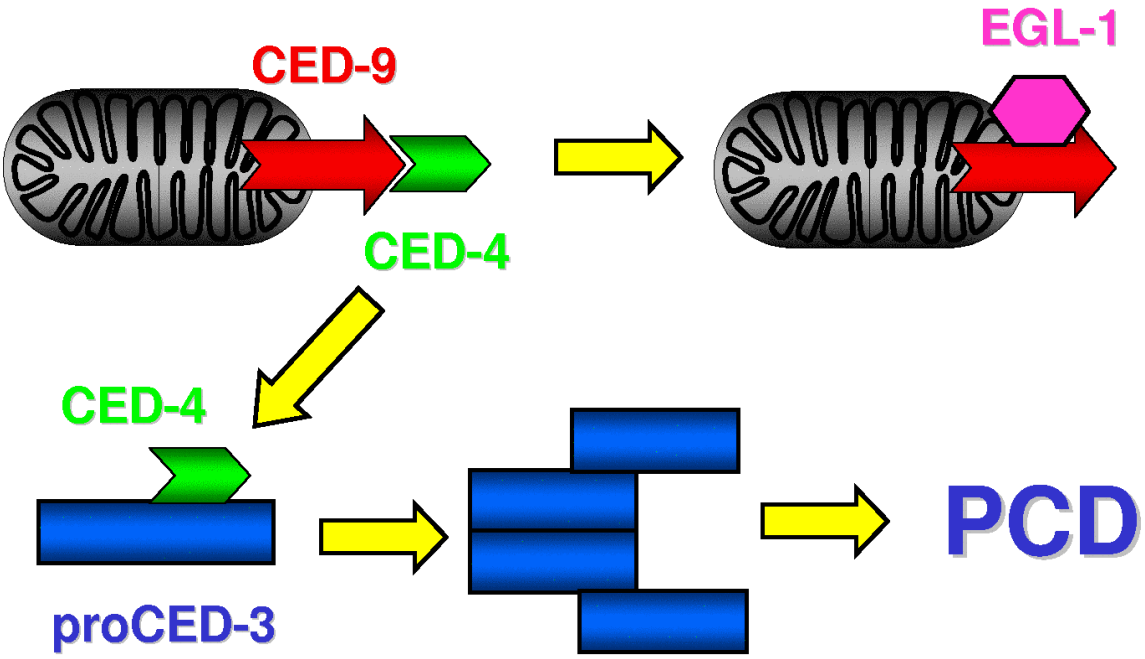
ICD-1 contains a putative caspase recruitment domain (CARD)

ICD-1 is mitochondrial

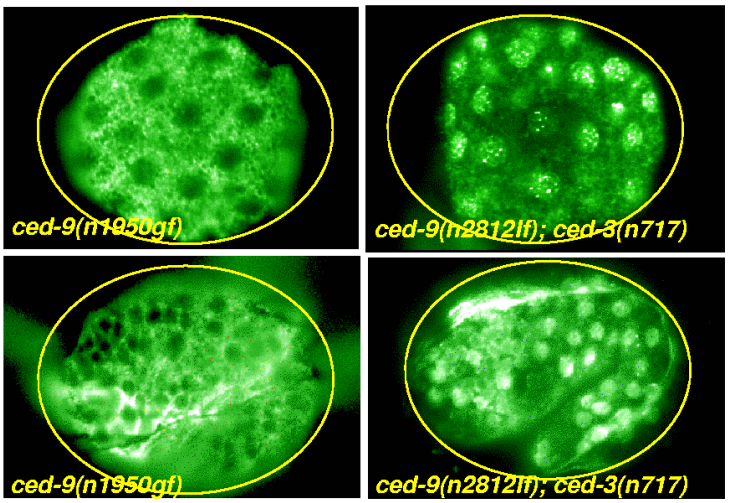


ICD-1 mito merge

core apoptotic pathway

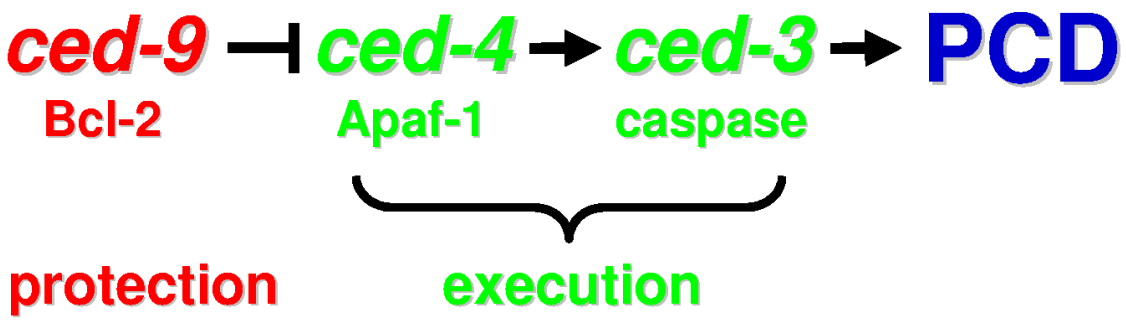


CED-9 is required for ICD-1 localization to mitochondria

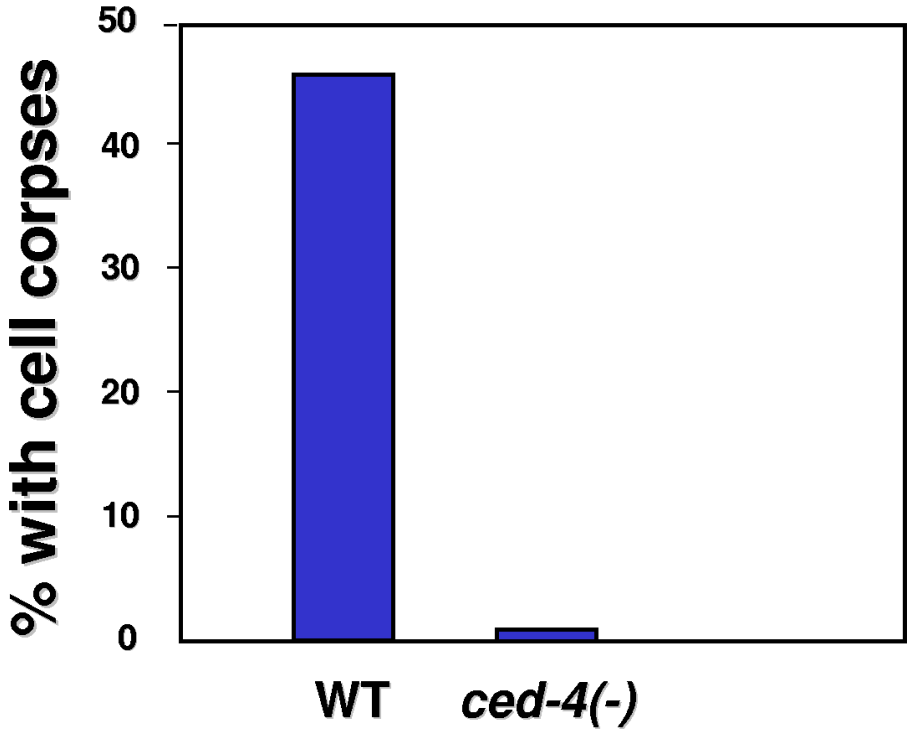


Genotype	Early embryo	Late embryo
WT	nuclear	mito.
ced-9(lf)	nuclear	nuclear
ced-9(gf)	mito.	mito.

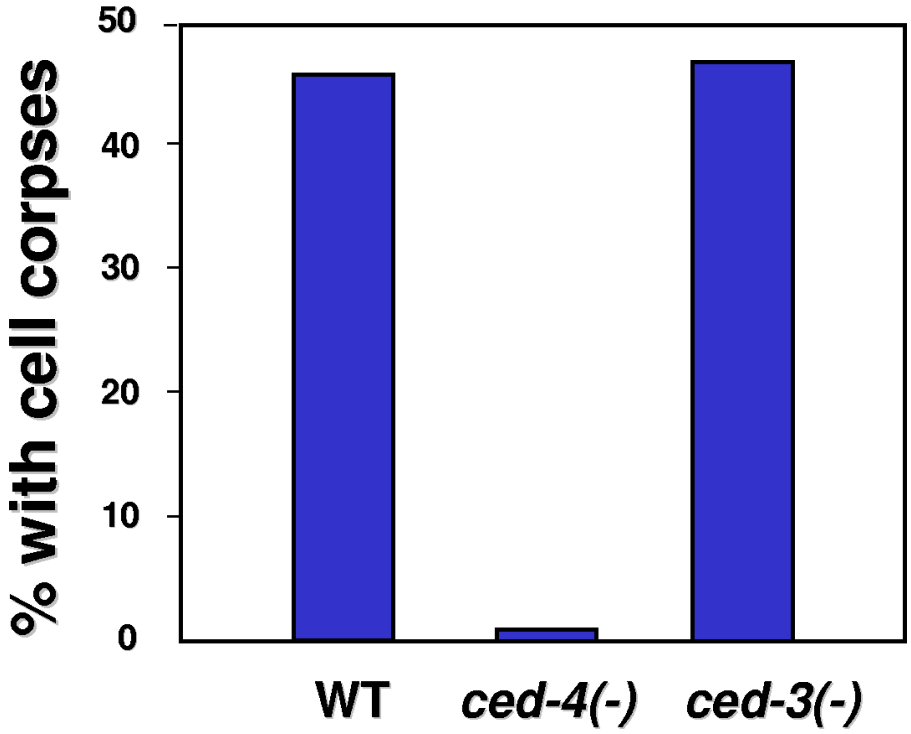
core apoptotic regulatory pathway



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



cell death in *icd-1(-)* is *CED-3-independent*!

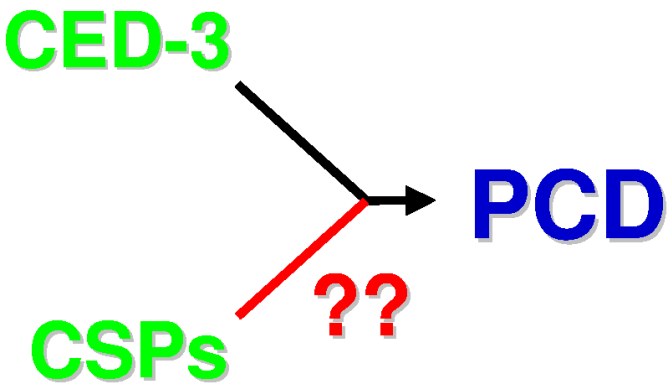


αNAC (ICD-2) is associated with apoptosis-related disease

- downregulated in Alzheimer's neurons
- upregulated in malignant brain tumors
- *icd-2(RNAi)* → CED-3-independent PCD in *C. elegans*

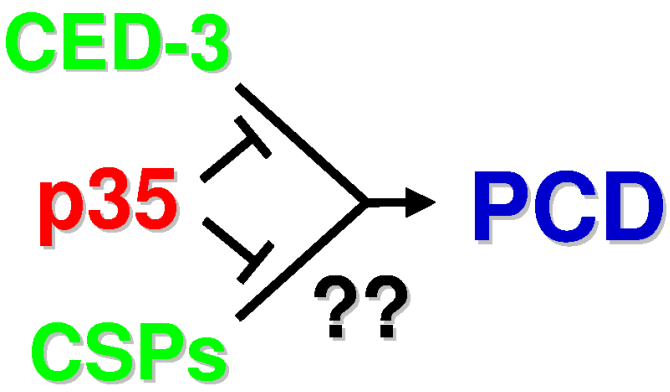
Why is PCD in *icd-1/2(-)* mutants CED-3-independent?

caspase redundancy?

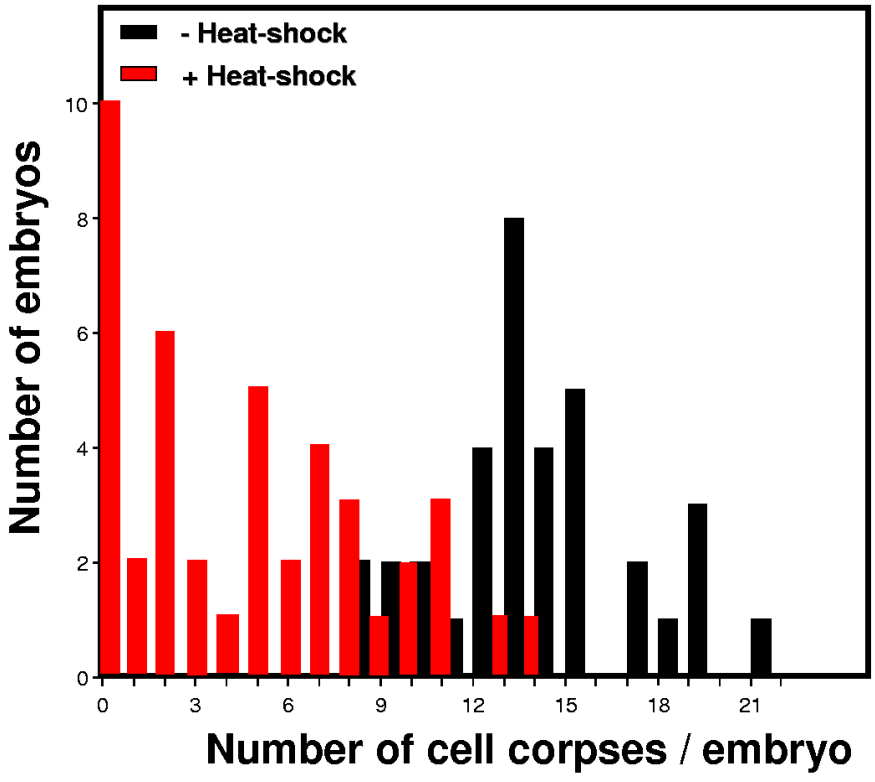


3 CSP genes → 6 caspases

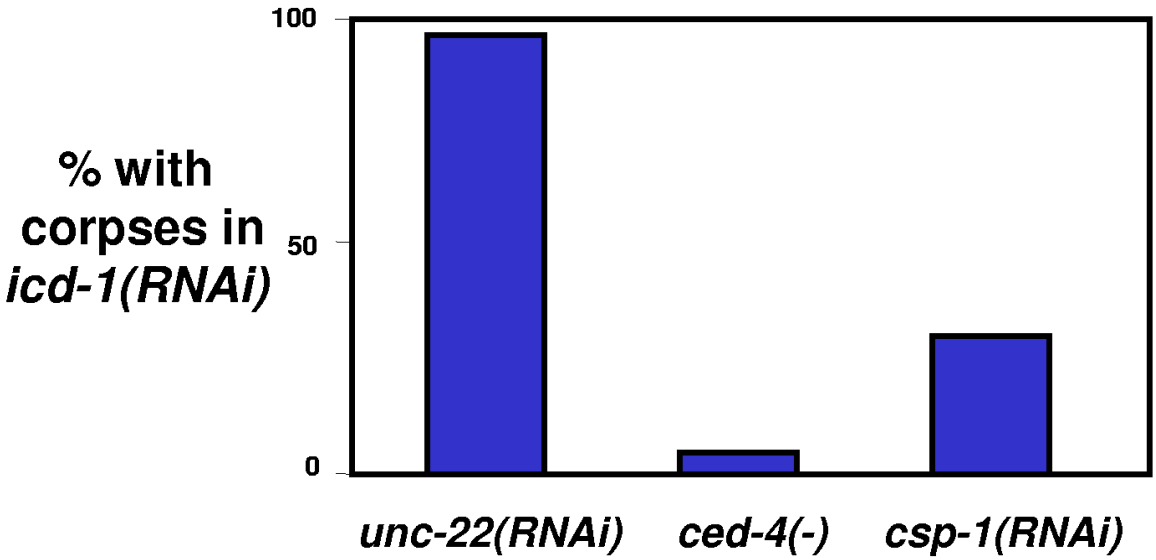
caspase inhibition by baculovirus p35



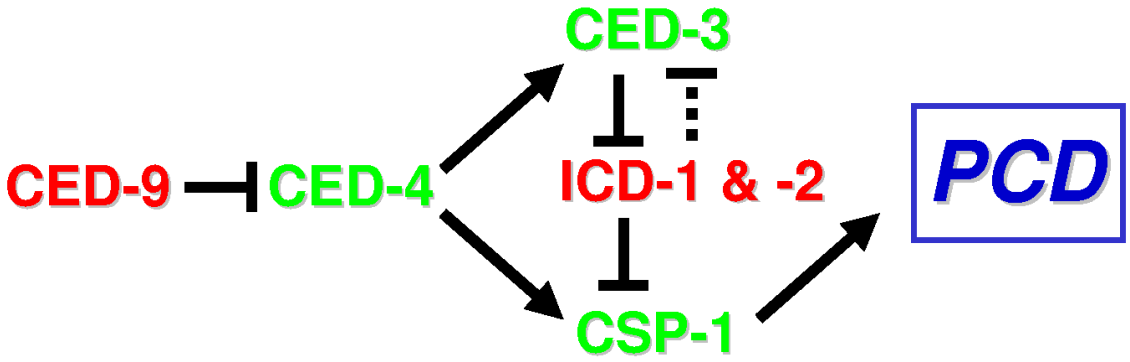
p35 suppresses inappropriate apoptosis



CSP-1 caspase is required for PCD in *icd-1(-)*



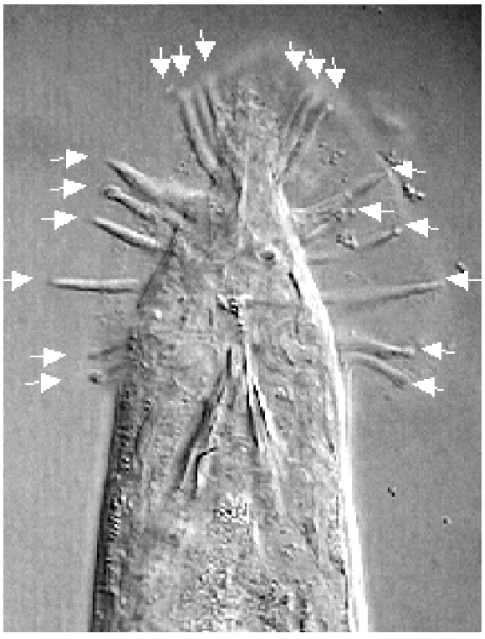
model for ICD-1-mediated repression



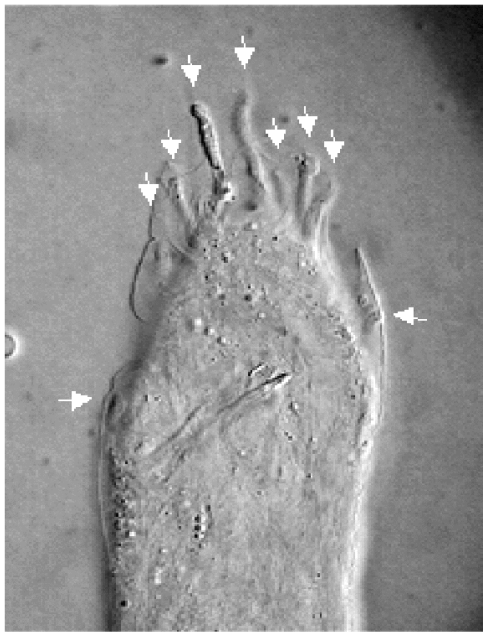
Positive feedback loop: all or none switch

Is CED-3-independent PCD relevant to normal development?

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

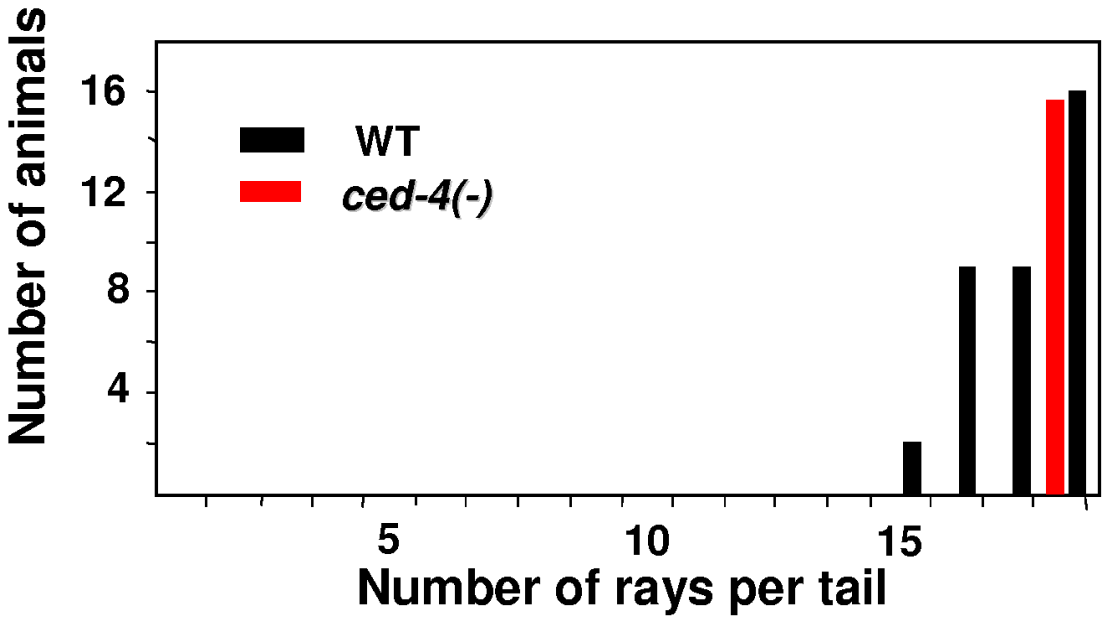


WT



icd-1(RNAi)

male tail rays in *ced-4(-)* mutants



***ced-4* mutants make more "perfect" male tails than wildtype!**

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

	% with 18 rays
Wildtype	50
ced-3(-)	87
ced-4(-)	98

Identifying the comprehensive set of PCD regulators

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

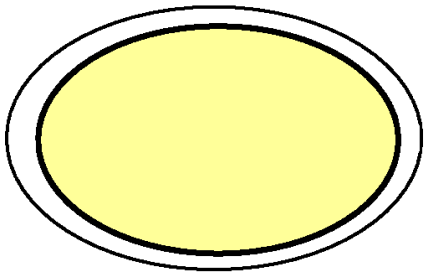
	PCD
ced-9(-)	+++
ced-9(-); ced-3(-)	-
icd-1(-)	+++
icd-1(-); ced-3(-)	+++

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

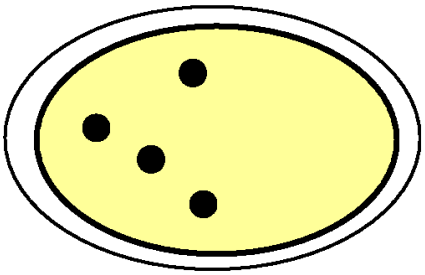
	PCD
ced-9(-)	+++
ced-9(-); ced-3(-)	-
icd-1(-)	+++
icd-1(-); ced-3(-)	+++

RNAi screen for PCD suppressors

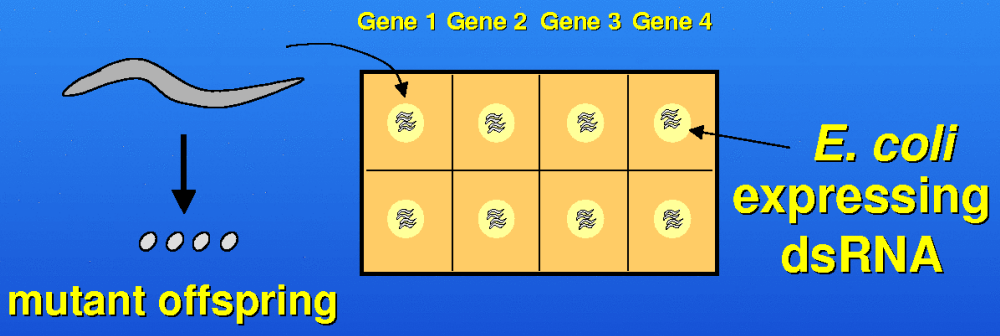
ced-3(-)
embryo



ced-3(-); gene X(RNAi)
embryo

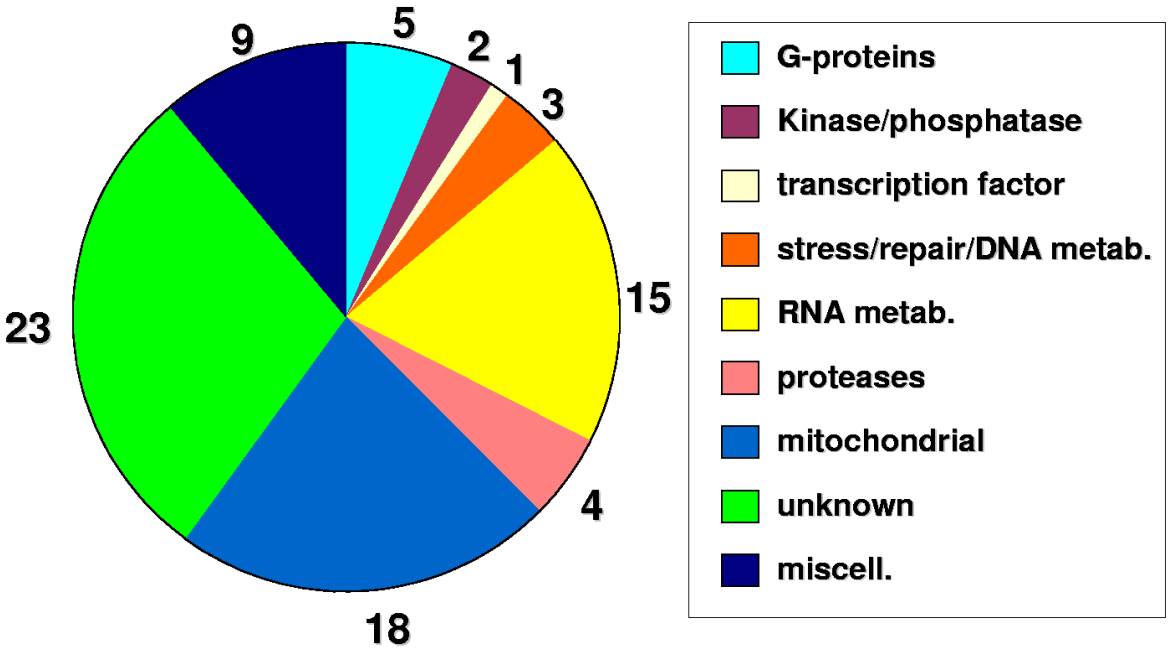


High throughput RNAi screen



Rapid functional screen of entire genome

distribution of 80 genes that repress apoptosis



screen identifies neurodegenerative disease genes

Hallervorden Spatz disease:
mitochondrial pantothenate kinase

Spastic periplegia:
AAA ATPase

identification of cancer genes

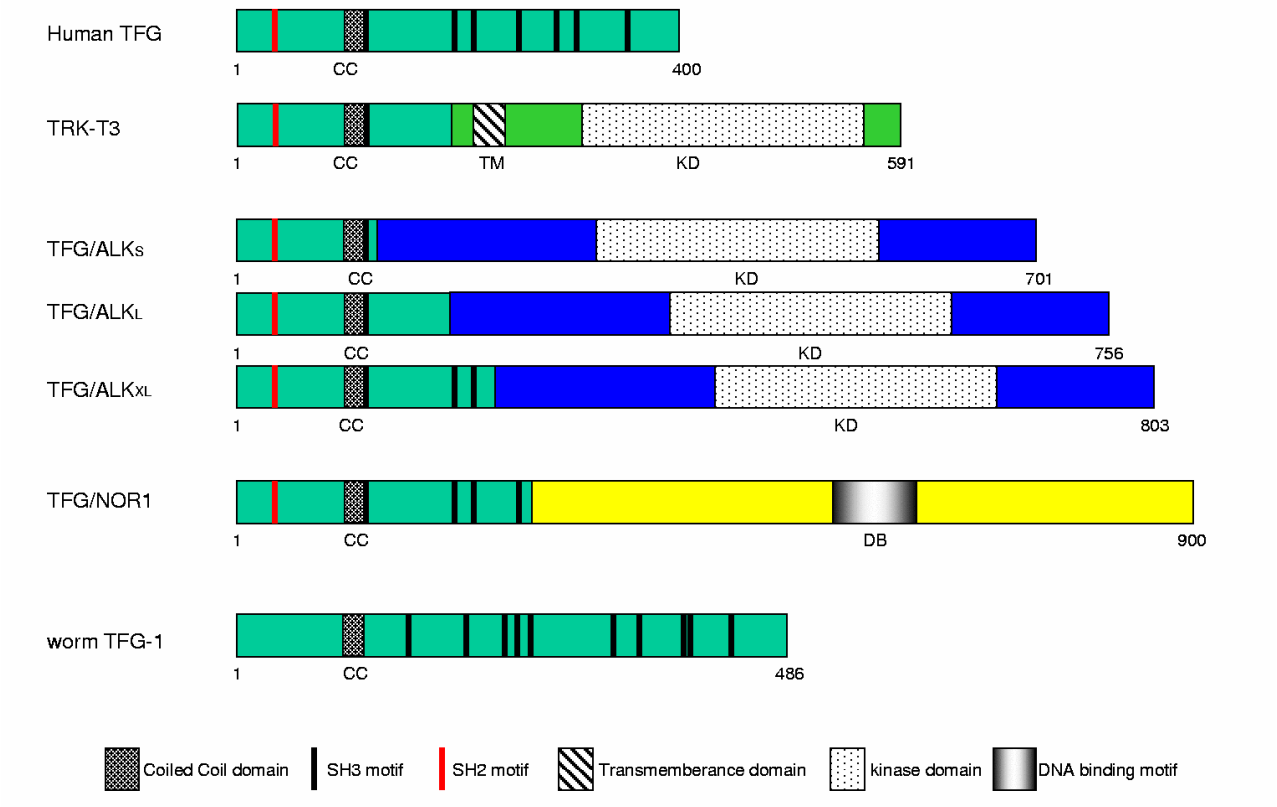
VBP-1
binds Von Hippel-Lindau tumor suppressor

TFG
lymphomas and thyroid cancers

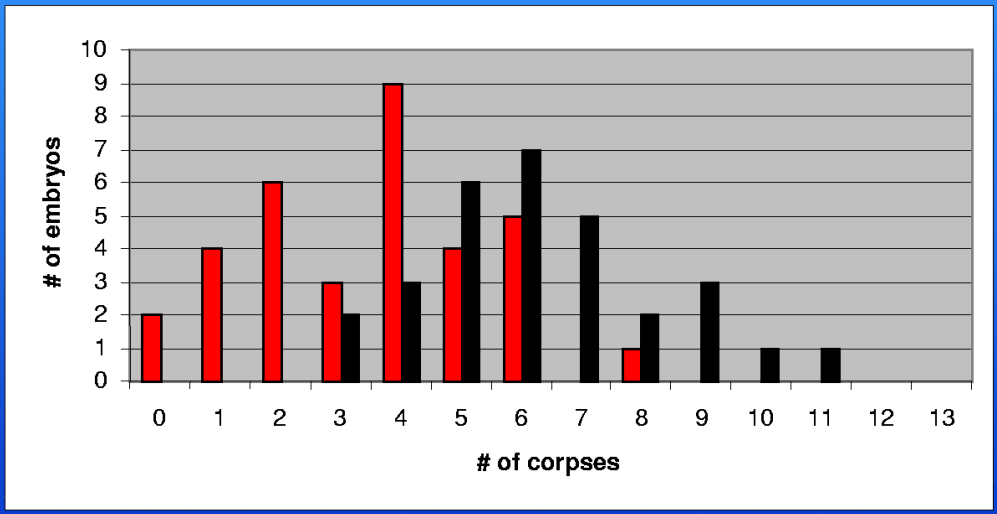
TFG

rearranged in large cell lymphomas and papillary thyroid cancers

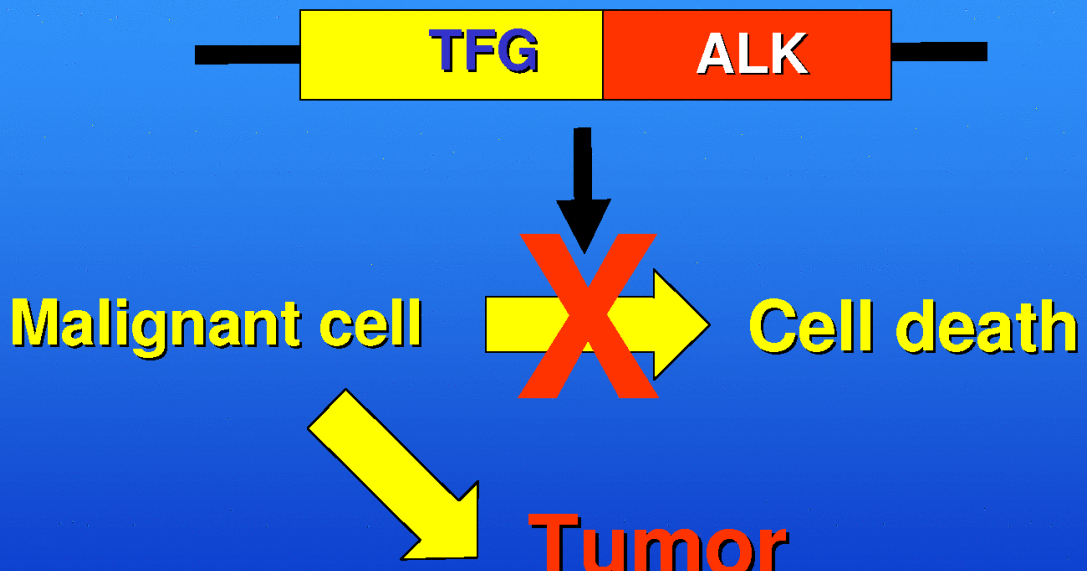
upregulated by TALL-1 receptor (B cell proliferation and autoimmunity)



TFG-1 inhibits PCD



TFG and cancer

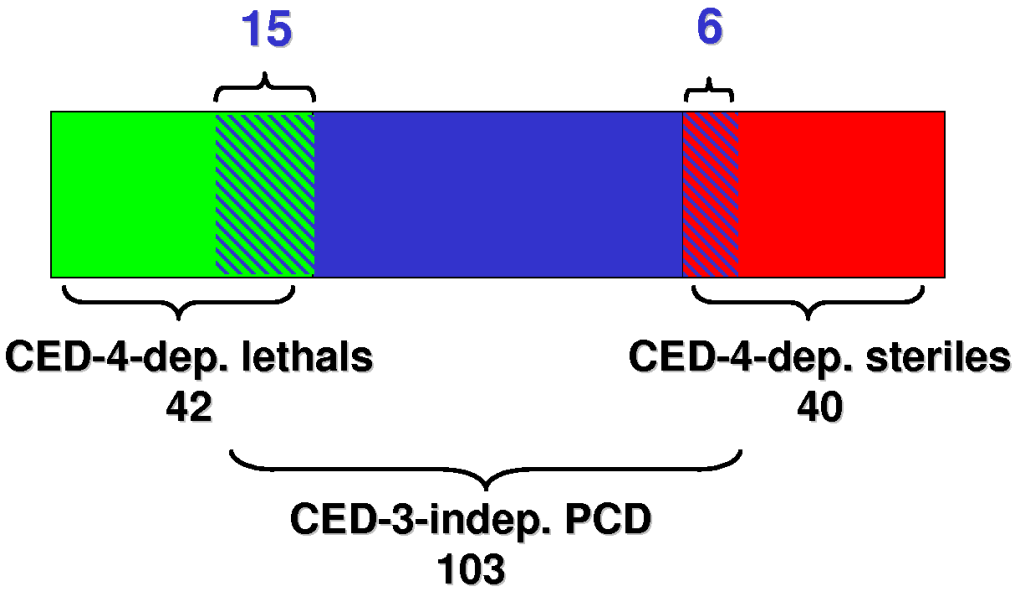


Analyzing the network of PCD regulators

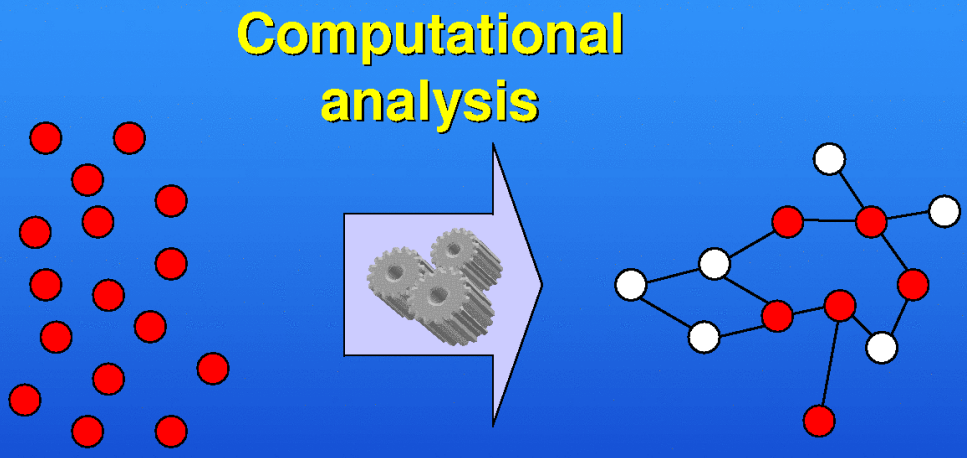
Three RNAi screens for apoptotic suppressors

1. CED-3-independent PCD 103 genes
2. CED-4-dependent sterility 40 genes
3. CED-4-dependent lethality 42 genes

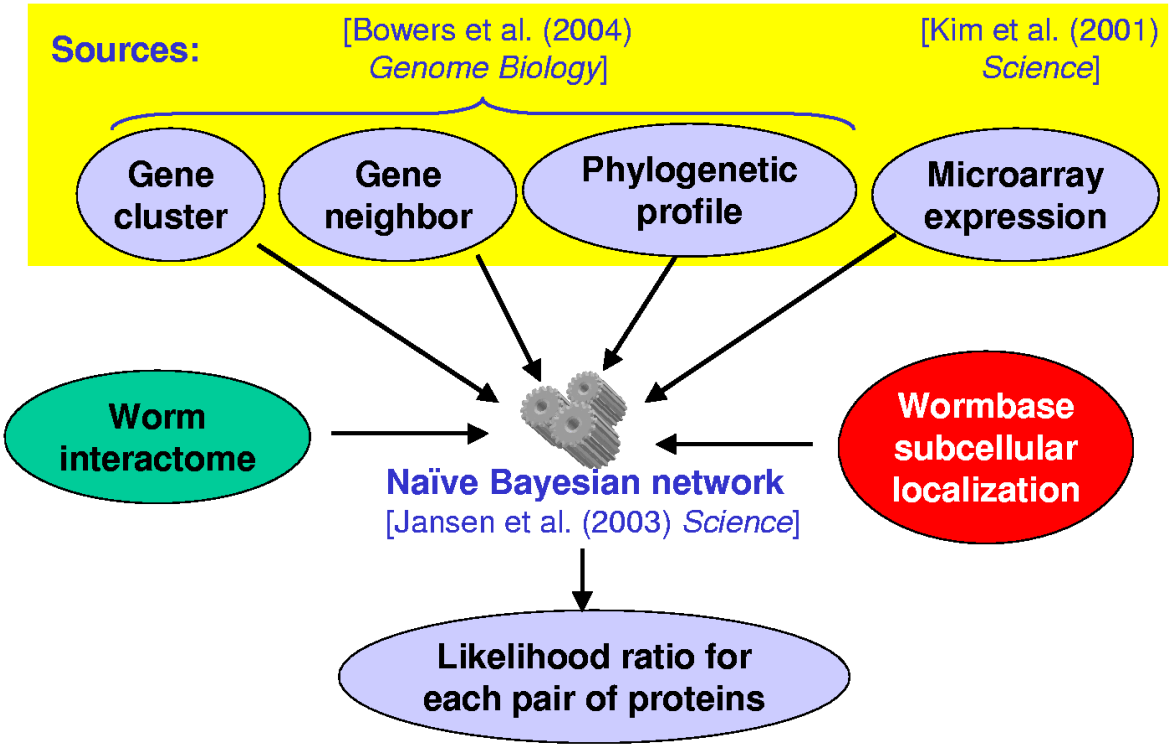
identification of overlapping gene sets



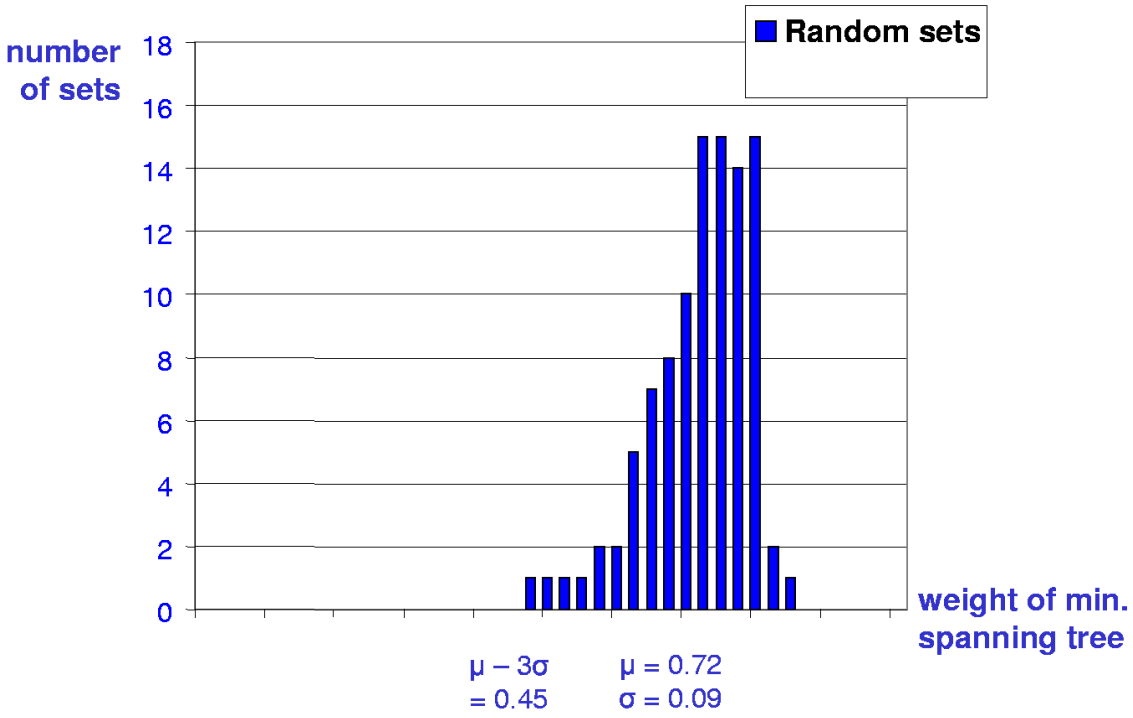
Computational prediction of networks



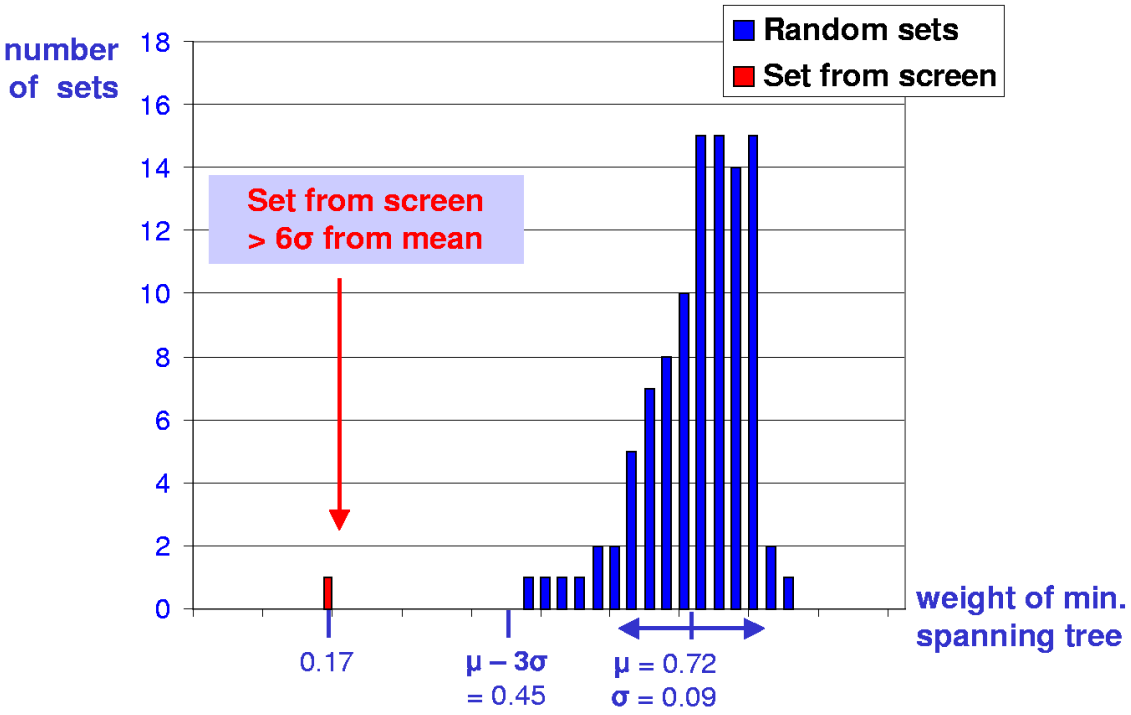
Computing Bayesian Networks



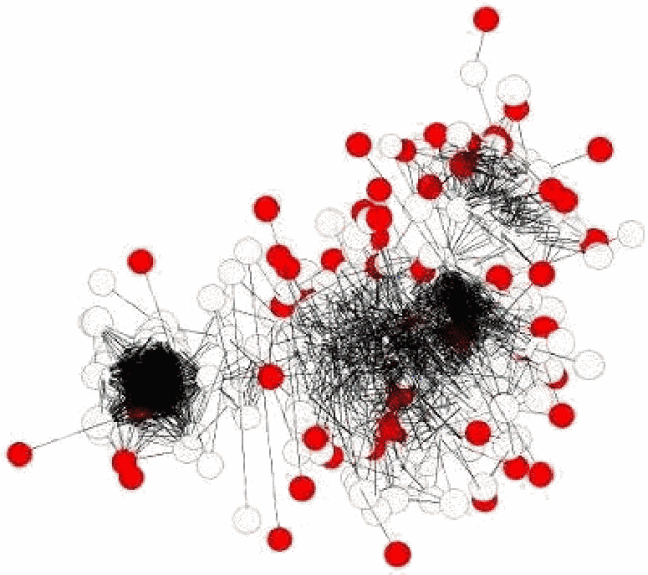
Clustering of genes



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)


Collaborators
Bayesian Network Computation:
Vebjorn Ljosa, Chao-I Chen, Ambuj K. Singh (CS)
Xiaofang Lei, Sreenivas Jammalamadaka (PSTAT)


Zoom


Researchers
Eric Witze
Niels Holten-Anderson
Tom McCloskey
Leukena Cheam
Tim Bloss
Not pictured: Jeremy Wickenheiser, Ling Chen, Chiou-Wei Tsai,
Pradeep Joshi, Ryan Magnuson, Yoko Igawa, Lingyan Chang