Caspase Biology and Function in Apoptosis

The ECDO Training Course

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Chia, Sardinia, Italy

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What is a caspase ?

Cysteine-dependent aspartate specific protease

OR

Cysteinyl aspartate proteinase

OR

Cysteine aspase

(Only other known aspase is Granzyme B)

Caspases make up the effector arm of the apoptosis execution machinery

The discovery of caspases

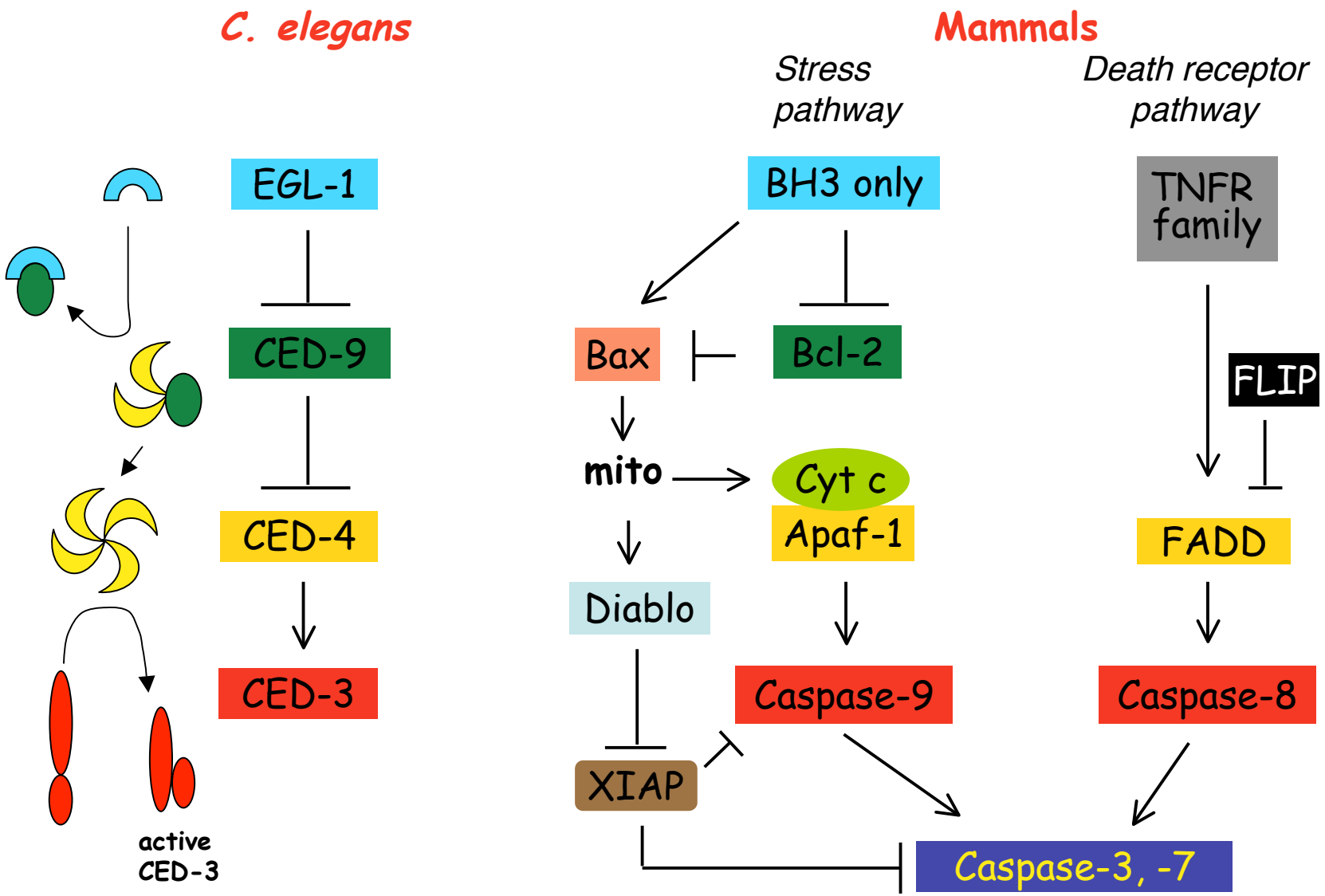
1992 Two groups reported the identification of inteleukin-1beta converting enzyme (ICE), now known as caspase-1

1993 *Caenorhabditis elegan* death gene ced-3 cloned and its product shown to be similar to mammalian ICE (caspase-1) and Nedd2 (now known as caspase-2)

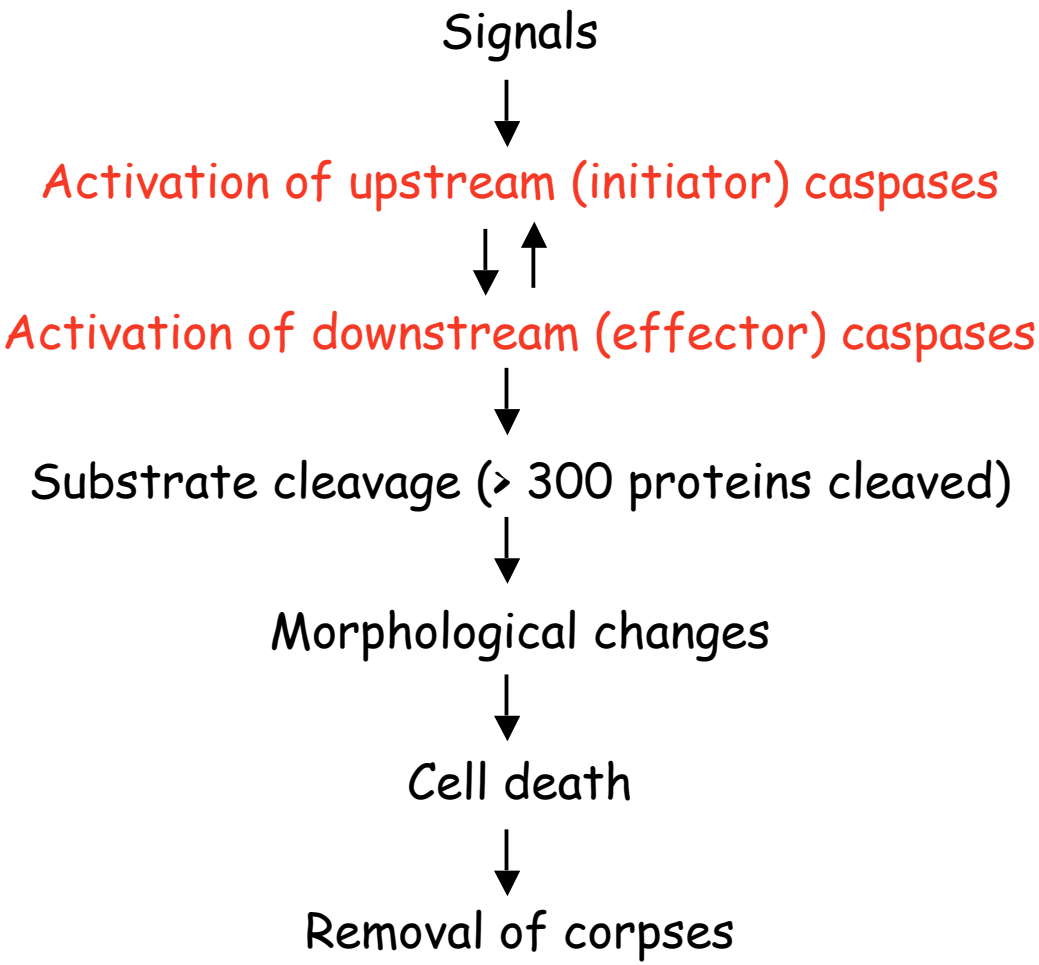
1994- Several caspases from mammals and other metazoans cloned

1996 Caspase terminology proposed to define this new group of proteases

Caspases are conserved components of the cell death machinery



Caspases are the key apoptosis mediators



However, caspases also function in some nonapoptotic pathways

Apoptotic caspases:
Caspase-3, -6, -7, -8, -9, -10
CED-3 (*C. elegans*)
DRONC, DRICE (*Drosophila*)

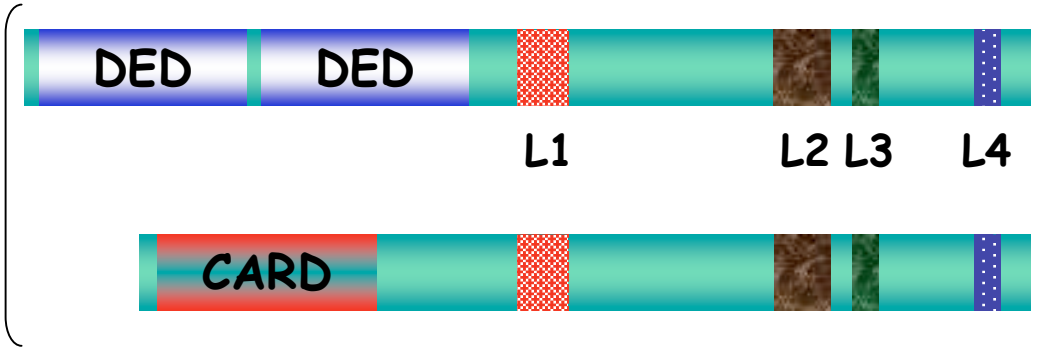
Inflammatory caspases:
Caspase-1, -4, -5 (human)
Caspase-1, -11, -12 (mouse)

Caspases implicated in proliferation & differentiation:
Caspase-3, -8, -14 (mammals)
DRONC (*Drosophila*)

Caspases implicated in innate immunity:
DREDD (*Drosophila*)

The 'initiator' and 'effector' caspases

initiator



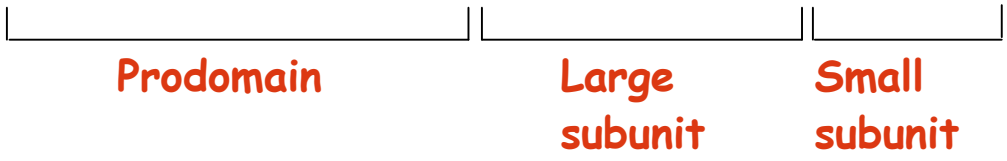
Caspases with DEDs
(e.g. DREDD, caspase-8, -10)

Caspases with CARD
(e.g. CED-3, **DRONC**, caspase-2, -9)

Caspases with no known motif (e.g. STRICA)

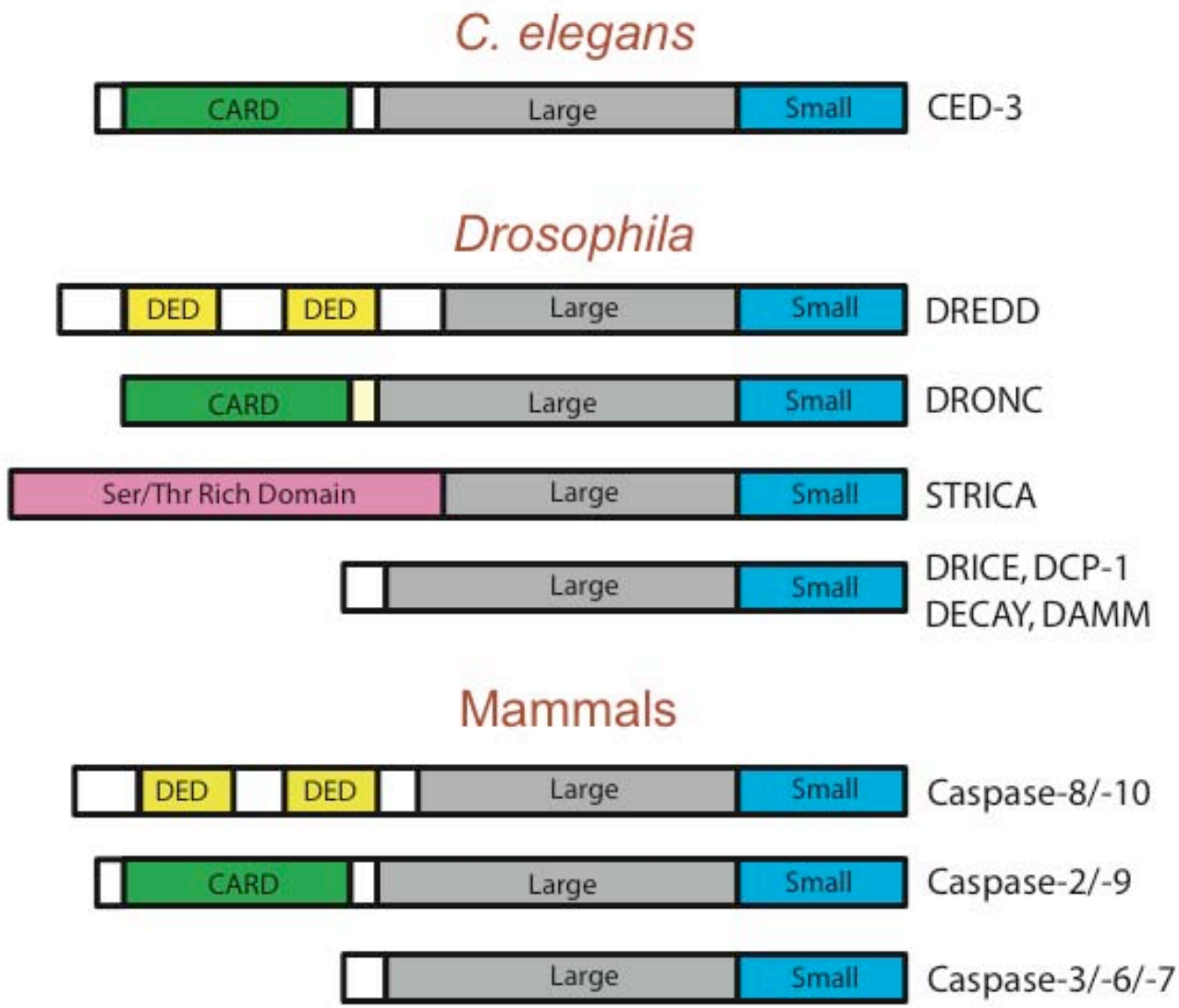
effector

Caspases with short prodomain (e.g. DCP-1, **DRICE**, caspase-3, -6, -7)



L1-L4: loops that make up the catalytic centre
L1 and L3 are highly conserved; L2 and L4 determine substrate specificity

Apoptotic caspases in the worm, fly and mammals



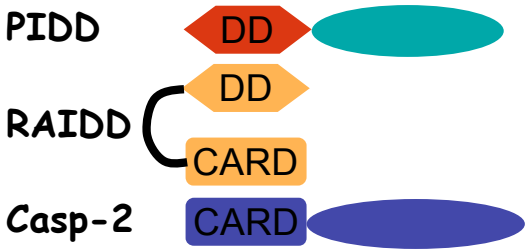
How are initiator caspases activated?

Induced proximity model

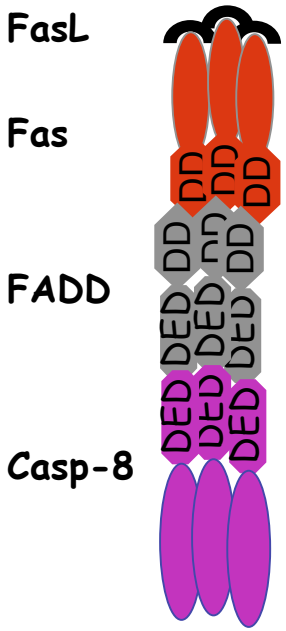
Proximity-induced dimerization and activation

Three main caspase activation complexes

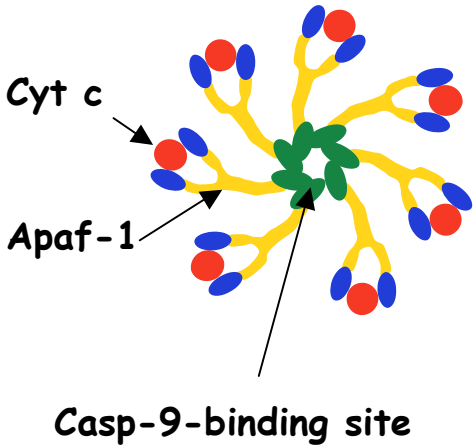
1. Piddosome (caspase-2 activation complex)
2. DISC (caspase-8, and -10 activation complex)
3. Apoptosome (CED-3, DRONC and caspase-9 activation complexes)



Piddosome



DISC

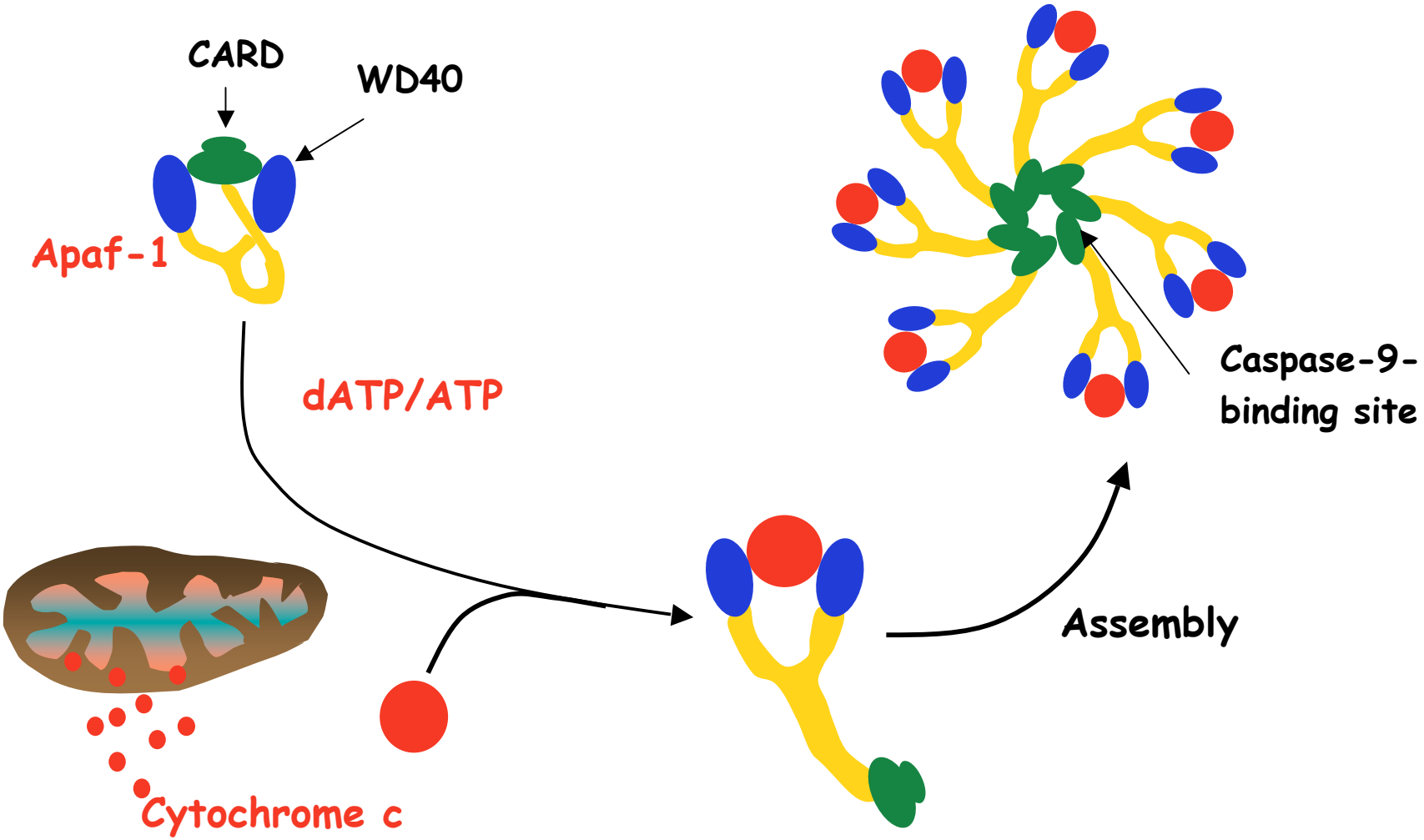


Apoptosome

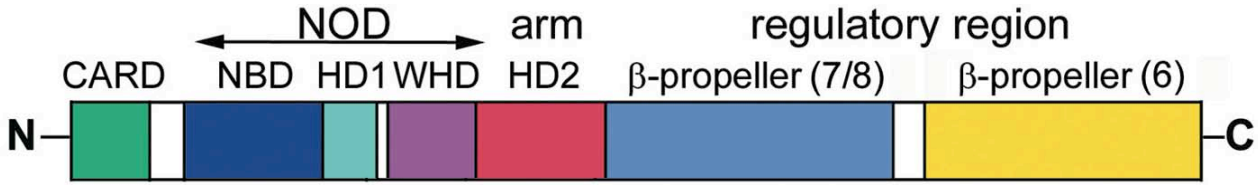
Adaptors for initiator caspase activation and the domains that mediate adaptor-caspase interactions

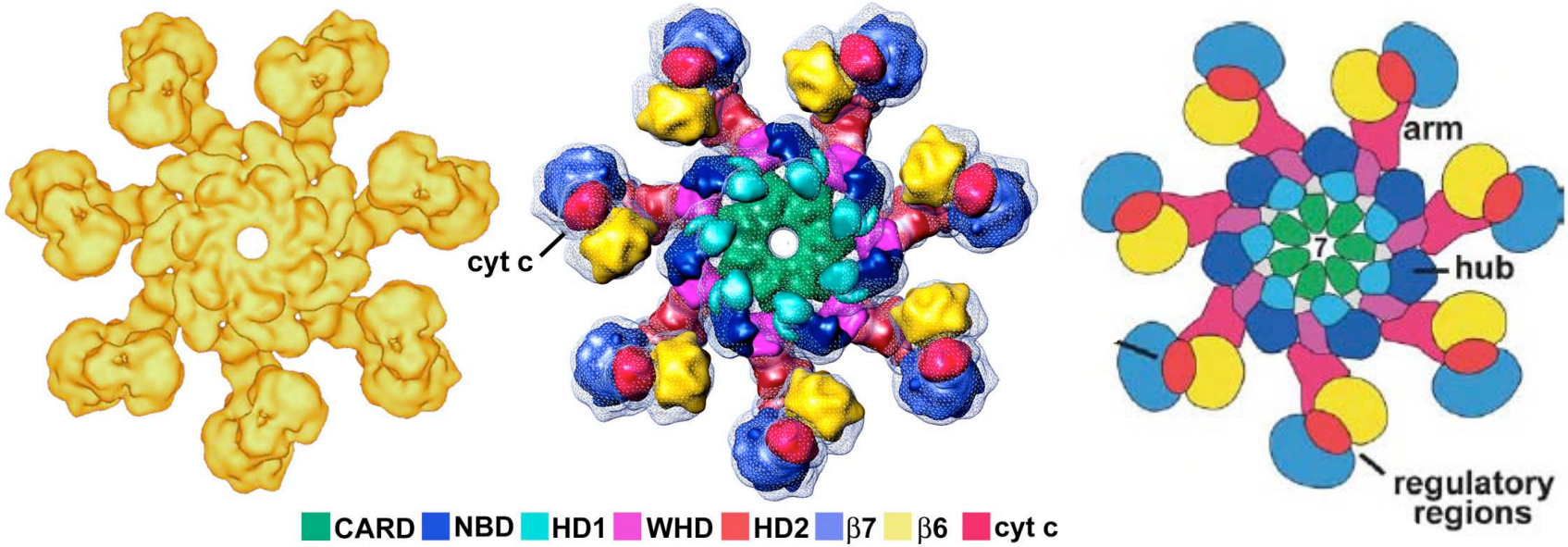
Caspase	Adaptor	Domain mediating interaction
CED-3	CED-4	CARD
DRONC	ARK	CARD
DREDD	dFADD	DED
Caspase-2	RAIDD	CARD
Caspase-8	FADD	DED
Caspase-9	Apaf-1	CARD
Caspase-10	FADD	DED

Caspase-9 activation:
Assembly of the Apaf-1 apoptosome



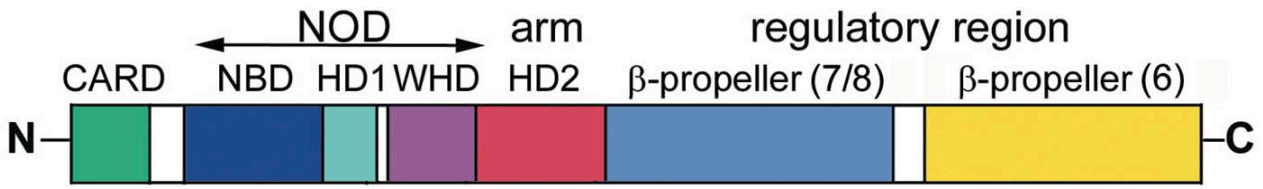
The Apaf-1 apoptosome

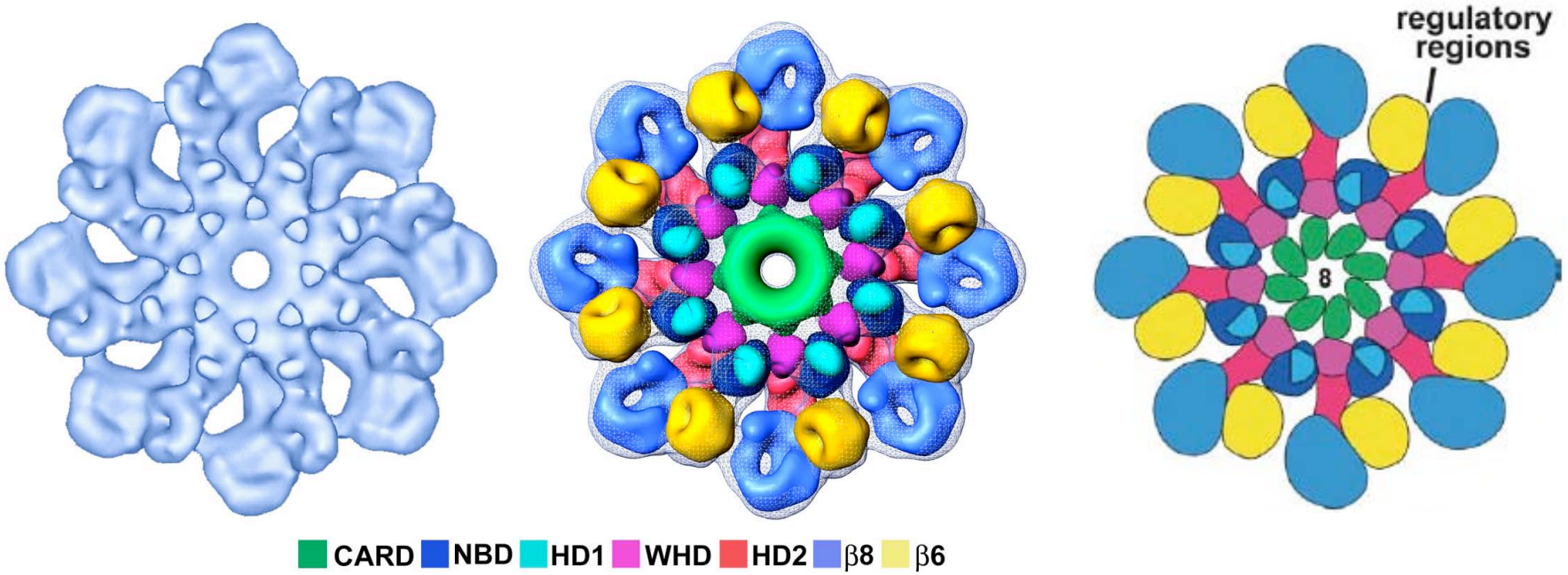




Models provided by C. Akey

The *Drosophila* ARK apoptosome



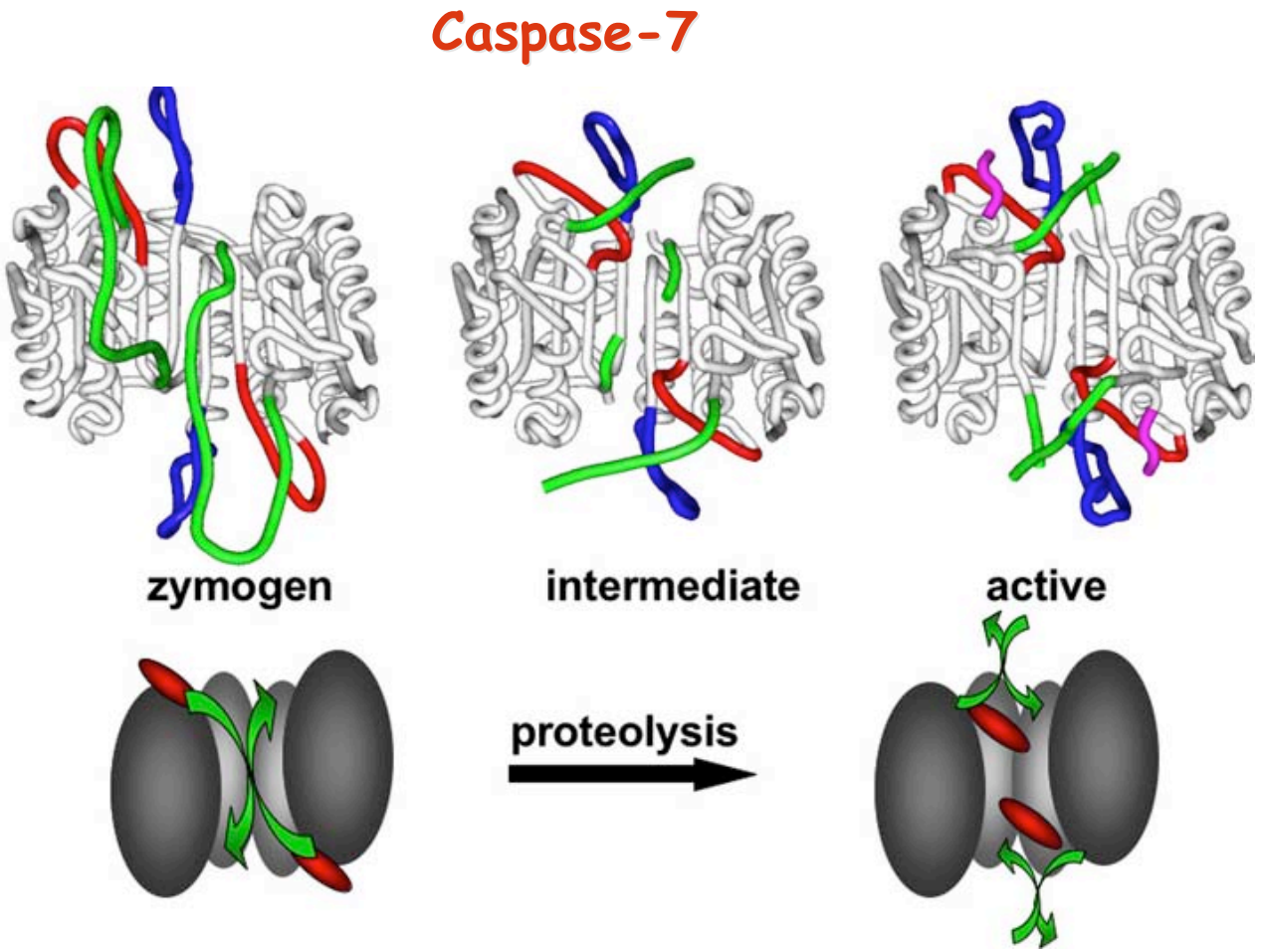


Models provided by C. Akey

Structure and activation of an effector caspase

Homodimer

Each monomer eontains 6-antiparallel β-strands



Critical loop transition following cleavage by an initiator caspase

Adapted from: Fuentes-Prior & Salvesen, Biochem. J. (2004) 384: 201–232

Substrate specificities of caspases

Group 1 caspases: Caspase-1, -4, -5 prefer a large P4 residue in the target sequence

P4-P3-P2-P1

caspase-1/-4/-5: (W/L)-E-H-D↓

Group 2 caspases: Caspase-6, -8, -9 prefer an intermediate P4 residue in the target sequence

P4-P3-P2-P1

caspase-6: V-E-H-D↓

caspase-8: L-E-T-D

caspase-9: L-E-H-D

Group 3 caspases: Caspase-2, -3, -7 prefer a small charged P4 residue in the target sequence

P5-P4-P3-P2-P1

caspase-3/-7: D-E-V-D↓

caspase-2: V-D-V-A-D (caspase-2 prefers a pentapeptide)

How to determine caspase activation and activity?

1. Use chromogenic or fluorogenic peptide substrates that release the chromogen or fluorescent tag upon cleavage by a caspase.
   Common tags: 7-amino-4-trifluoromethylcoumarin (*AFC*);
   7-amino-4-methylcoumarin (*AMC*); *p*-nitroanilide (pNA)

2. Monitor procaspase processing by immunoblotting

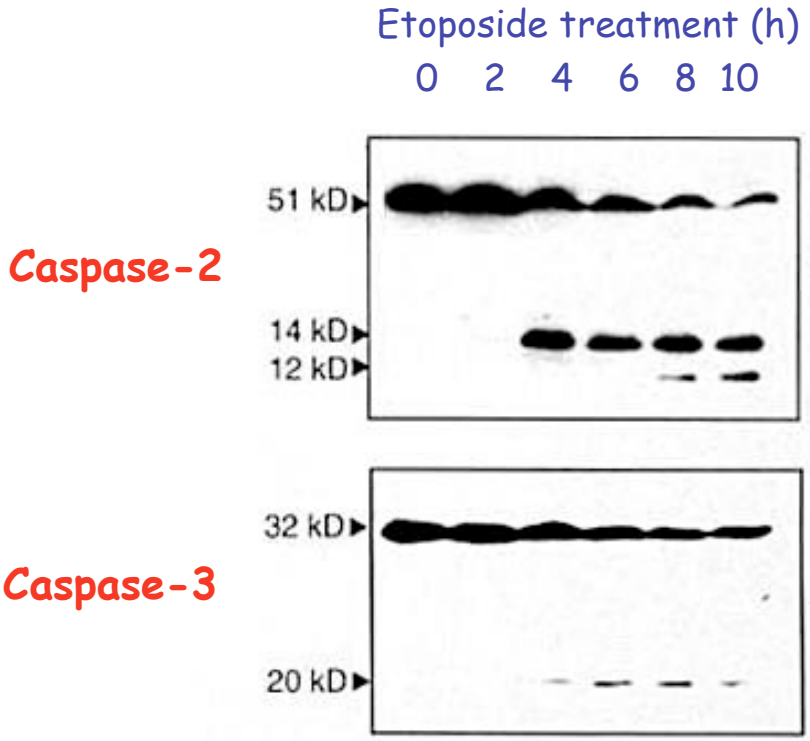
3. Monitor caspase-mediated substrate cleavage by immunoblotting

4. Immunocytochemistry using antibodies that selectively bind activated caspases

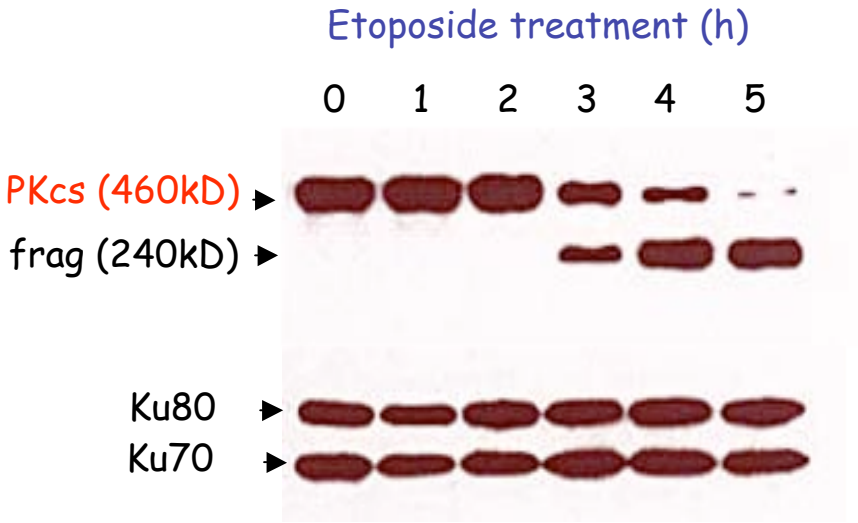
5. Monitor in situ substrate cleavage

Caspase zymogen processing and substrate cleavage in cells undergoing apoptosis

Procaspase processing



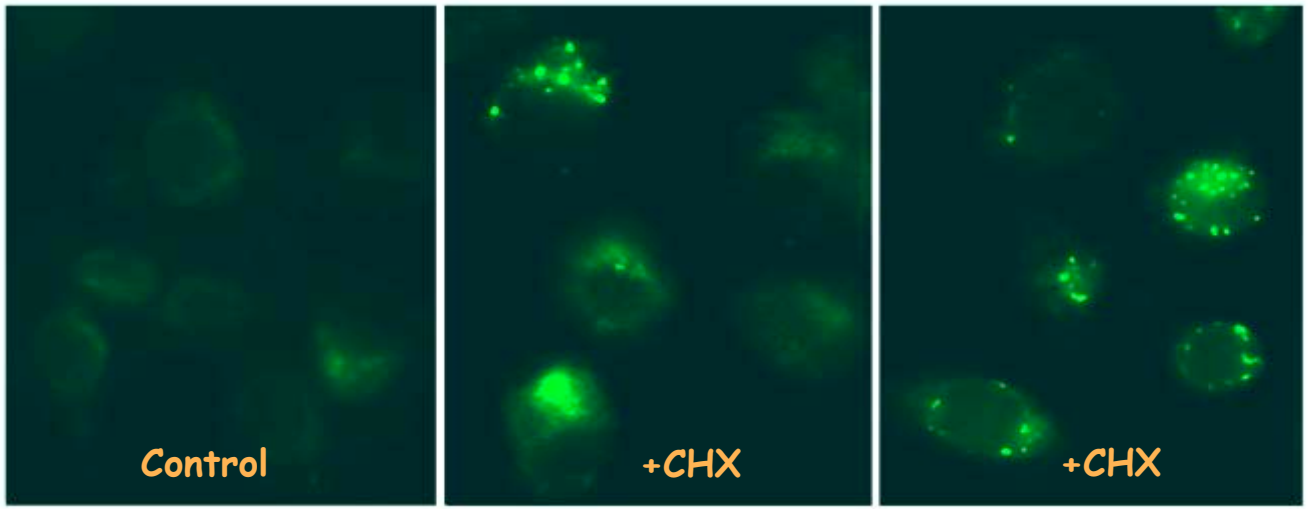
Substrate cleavage (DNA-PKcs)



Detecting caspase activation in cells and tissues

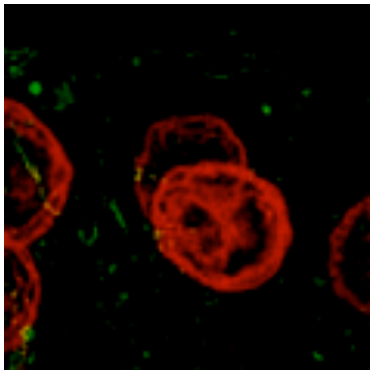
Drug-treated cells stained with an active caspase-3 ab

Green: Active caspase-3

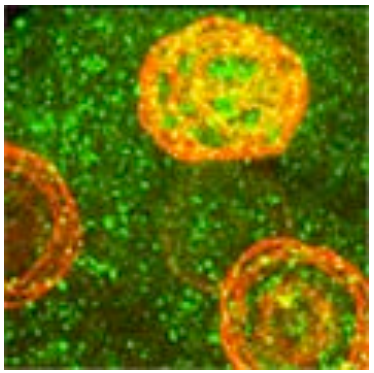


Drosophila larval and prepupal salivary glands stained with an active caspase ab

Red: Lamin
Green: Active DRICE



larval



prepupal

Caspase Substrates

Over 300 proteins are cleaved by caspases in cells undergoing apoptosis

These proteins belong to various functional classes including: apoptosis signaling proteins (eg IAPs, Bcl-2); protein kinases (eg FAK, PKC); structural/ cytoskeletal proteins (eg gelsolin, lamin); cell repair proteins (eg PARP, ATM); and cell cycle proteins (eg p21, Rb, p27)

Most proteins are inactivated following caspase cleavage, whereas some are activated

Only some caspase substrates play a direct role in mediating apoptotic changes

Some commonly mentioned examples of caspase substrates:

Procaspases (auto cleavage and cleavage of other caspases)- activated
ICAD (inhibitor of caspase-activated DNase)- inactivated
BID (cleaved by caspase-2 and -8)- activated
IAPs (cleaved by mammalian and fly effector caspases)- inactivated
PARP (first known caspase substrate)- inactivated

Intracellular localization of caspases

Almost all caspases are primarily localized in the cytosol, and this is where they are likely to be activated. However, activated caspases mediate cleavage of both nuclear and cytosolic proteins.

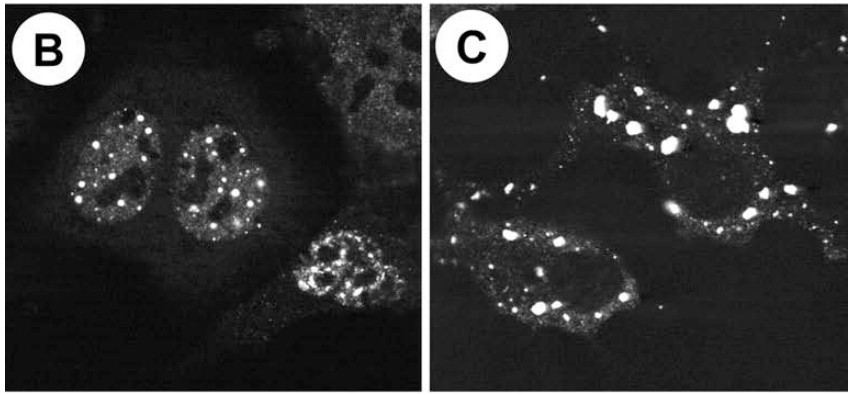
In addition to cytosol caspase-2 also localizes to nucleus and the Golgi. However, the function of caspase-2 in these compartments is not known.

Drosophila caspases DRONC and DRICE show partial localization to mitochondria. However, this localization is not necessary for their function.

Caspase-2 localization in nucleus

A

PPH**K**QLR**L**	Mouse caspase-2
PPH**K**QSR**L**	Rat caspase-2
PLY**K**KLR**L**	Human caspase-2
PAA**K**RVK**L**	Human c-Myc



A: A nuclear localization signal in caspase-2

B: Caspase-2-GFP protein in transfected COS cells

C: Caspase-2-GFP (K152A) mutant protein in transfected COS cells

Caspase inhibitors and regulators

Viral inhibitors

Crm A (Cowpox virus) inhibits caspase-1, -8, -10

P35 (Baculovirus)- broad spectrum caspase inhibitor

P49 (Baculovirus)- broad spectrum caspase inhibitor

vFLIP (γ-herpesvirus)- inhibits caspase-8 activation

Cellular inhibitors

DIAP1 (Drosophila)- strong inhibitor of DRONC and DRICE

XIAP (mammalian) inhibits caspase-3, -7 and -9

cIAP1 and cIAP2 (mammalian)- weak inhibitors of caspase-3 and -7

FLIP (mammalian) inhibits activation of caspase-8

Chemical inhibitors

Synthetic peptides (e.g. DEVD-CHO, zVAD-FMK)

Nonpeptide inhibitors (e.g. IDN-6556)

Analysis of caspase function

1. **Using cell permeable caspase inhibitors.** Lack of specificity often confounds observations and interpretation of data.

2. **Antisense and siRNA-mediated gene ablation in cultured cells.** More robust and specific but efficiency of knockdown in mammalian cells may not always be high.

3. **Gene knockout (KO) in mouse.** Powerful tool, but functional redundancy, compensation and genetic background-dependent phenotypes often lead to confusion.

4. **Use of simpler model organisms (e.g. *C. elegans* and *Drosophila*) with less redundancy.** Powerful tools, often generate useful information, but results may not always reflect the complexity of pathways in mammals.

In vivo functional analyses of worm and fly caspases

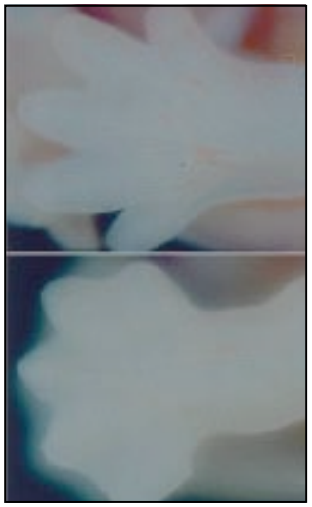
Caspase	Mutant phenotype and in vivo function
C. elegans	
CED-3	Animals have extra cells. Essential for PCD of somatic cells.
Drosophila	
DRONC	Pupal lethal. Multiple cell death defects in embryos, larvae and prepupae. Essential for most PCD and stress induced apoptosis.
DREDD	Normal development- cell death phenotype. No role in PCD.
STRICA	Mutants not characterized.
DCP-1	Mutants viable and fertile. Minor role in PCD.
DRICE	Most animals pupal lethal. Some survive to adulthood. Required for most developmental PCD and stress induced apoptosis.
DECAY	Mutants viable and fertile. Minor (if any) role in PCD.
DAMM	Mutants not available.

Functional analyses of mammalian caspases using gene knockout in mice

Caspase	Mutant phenotype and in vivo function
Caspase-2	Mice are viable and fertile. MEFs show some resistance to killing by heat shock and specific drugs. Context-dependent role in cell death.
Caspase-3	Perinatal lethal, in mixed genetic background. Hyperplasia in brain. Partly redundant role in apoptosis.
Caspase-6	Normal development. No reported cell death defects in mutants.
Caspase-7	Normal development. No apparent cell death defects in mutants. DKO with caspase-3 leads to perinatal lethality and apoptotic defects.
Caspase-8	Embryonic lethal. Defects in cardiac and T cell development. All death receptor mediated apoptosis impaired in -/- cells. Familial mutations in humans associated with immunodeficiency.
Caspase-9	Perinatal lethal, but some animals survive to adulthood. Hyperplasia in brain. Some cells resistant to stress-induced apoptosis.

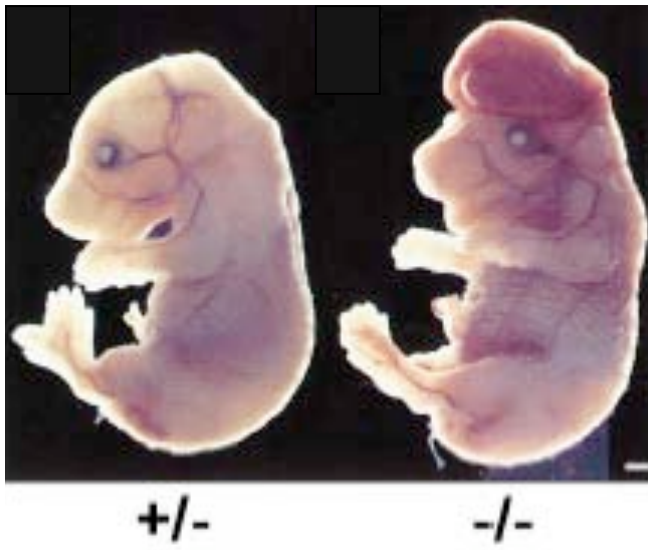
The lack of apoptosome components leads to cell death defects in mice

Apaf-1



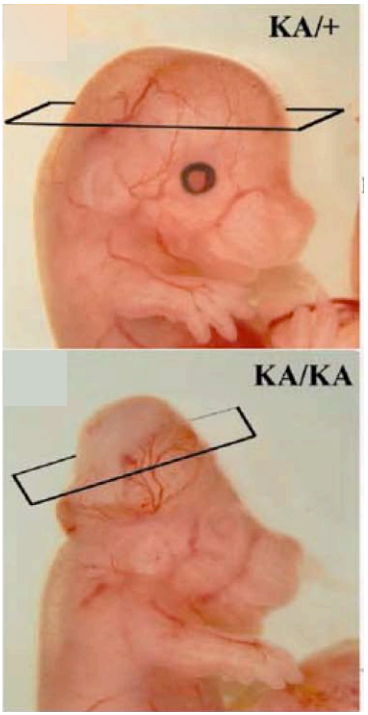
Cecconi et al., (1998)
Yoshida et al. (1998)

Caspase-9 or Apaf-1



Kuida et al. (1998)
Hakem et al., (1998)

Cyt c
KA knock-in



Hao et al. (2005)

Cyt c → Apaf-1 → Caspase-9

Stress-induced caspase activation and apoptosis in the absence of Apaf-1/caspase-9 ?

Canonical caspase activation pathway not essential for apoptosis in mammals?

Caspases play redundant roles in cell death?

Functional compensation in caspase KO animals?

Caspases have context (cell and signal) specific function in cell death?

Physiological cell death can occur in caspase-independent manner?

Caspases play a role only in amplifying cell death signals?

Alternative routes of caspase activation in the absence of main components?

Using simpler models to study caspase function:

Drosophila as a model system

Why use flies?

Genetics is well understood

Entire genome sequence is available

>60% human disease genes are conserved in fly

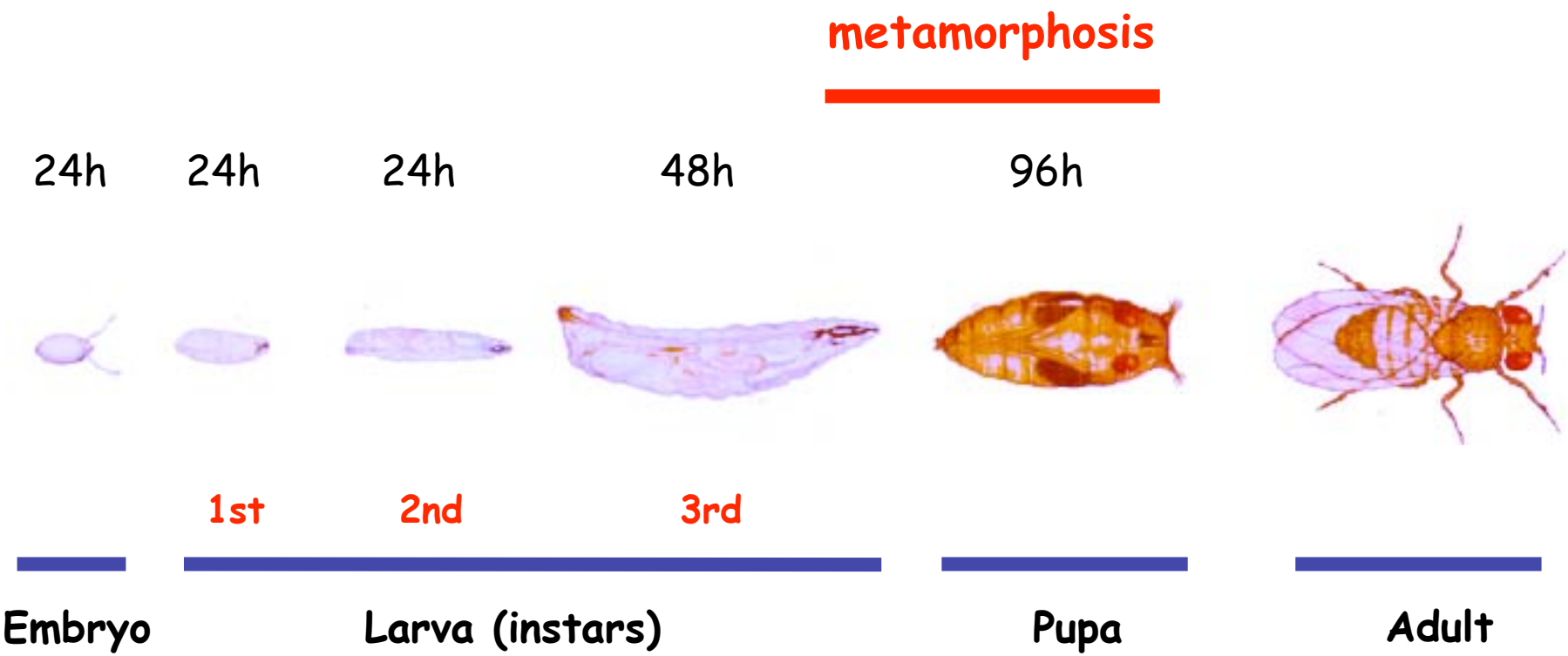
Many developmental pathways are well characterised

Core cell death machinery is conserved

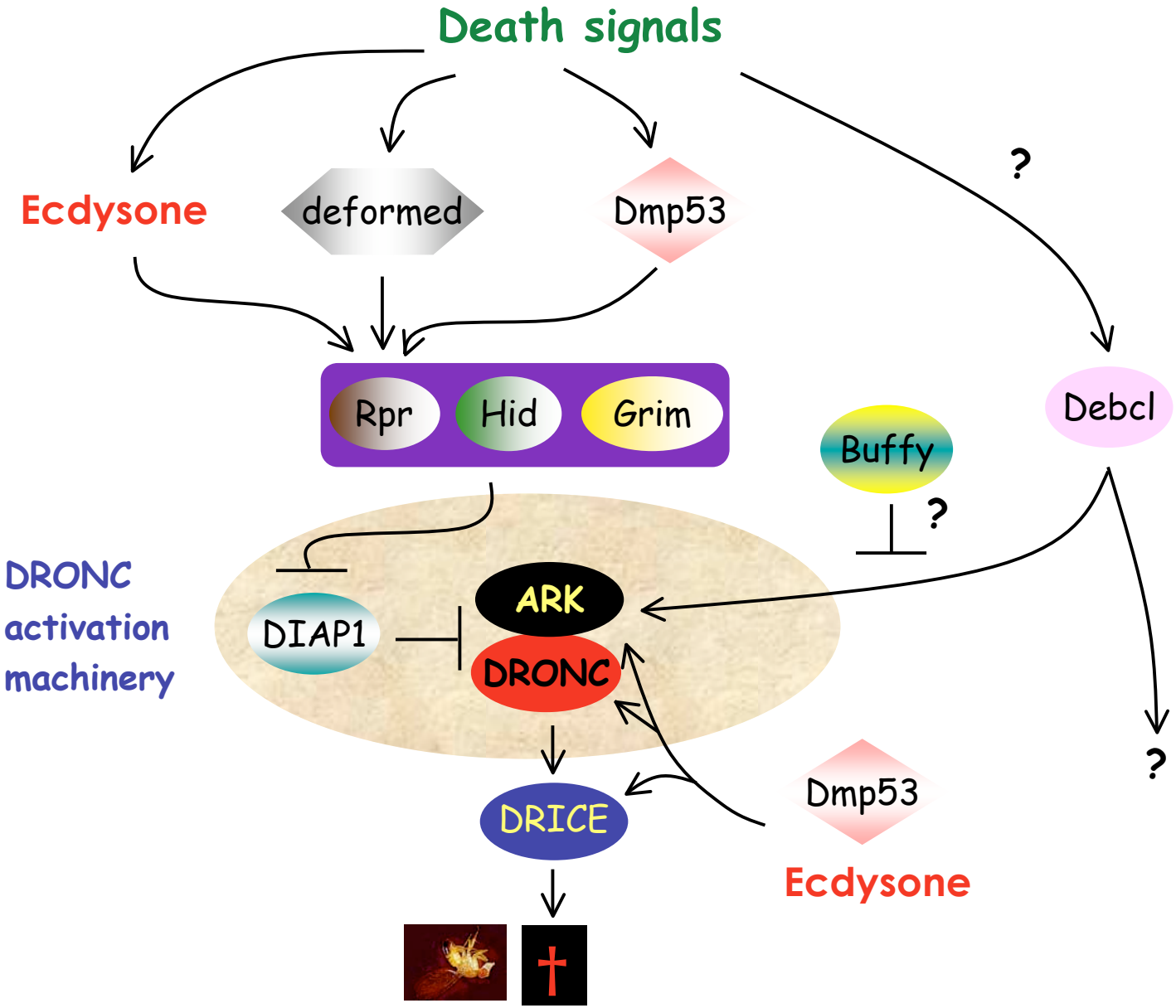
Amenable to sophisticated genetic experiments not possible in mammals

Regulation of complex pathways is more accessible to experimentation than in mammals

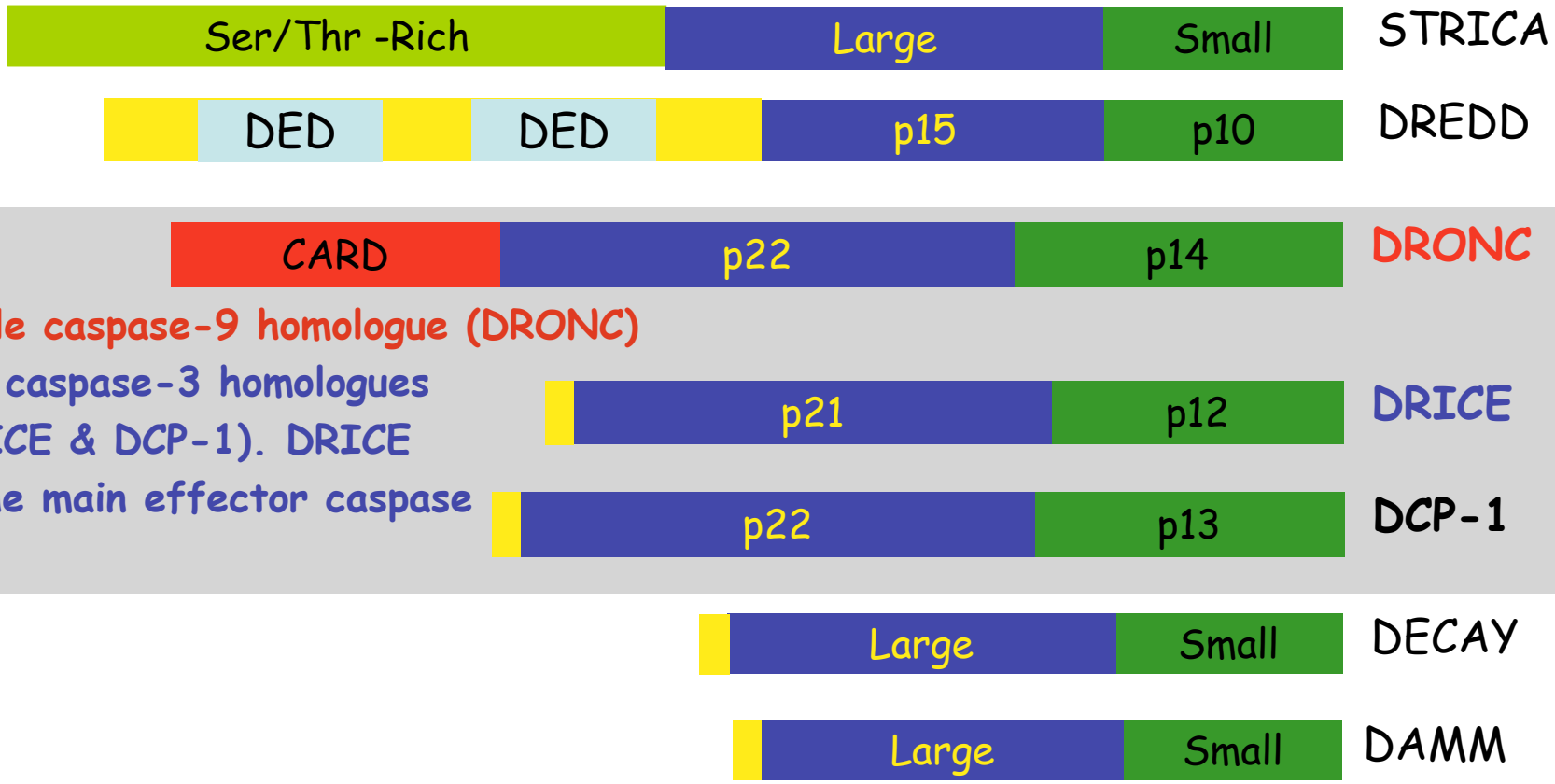
Life cycle of *Drosophila melanogaster*



Cell death machinery in *Drosophila*

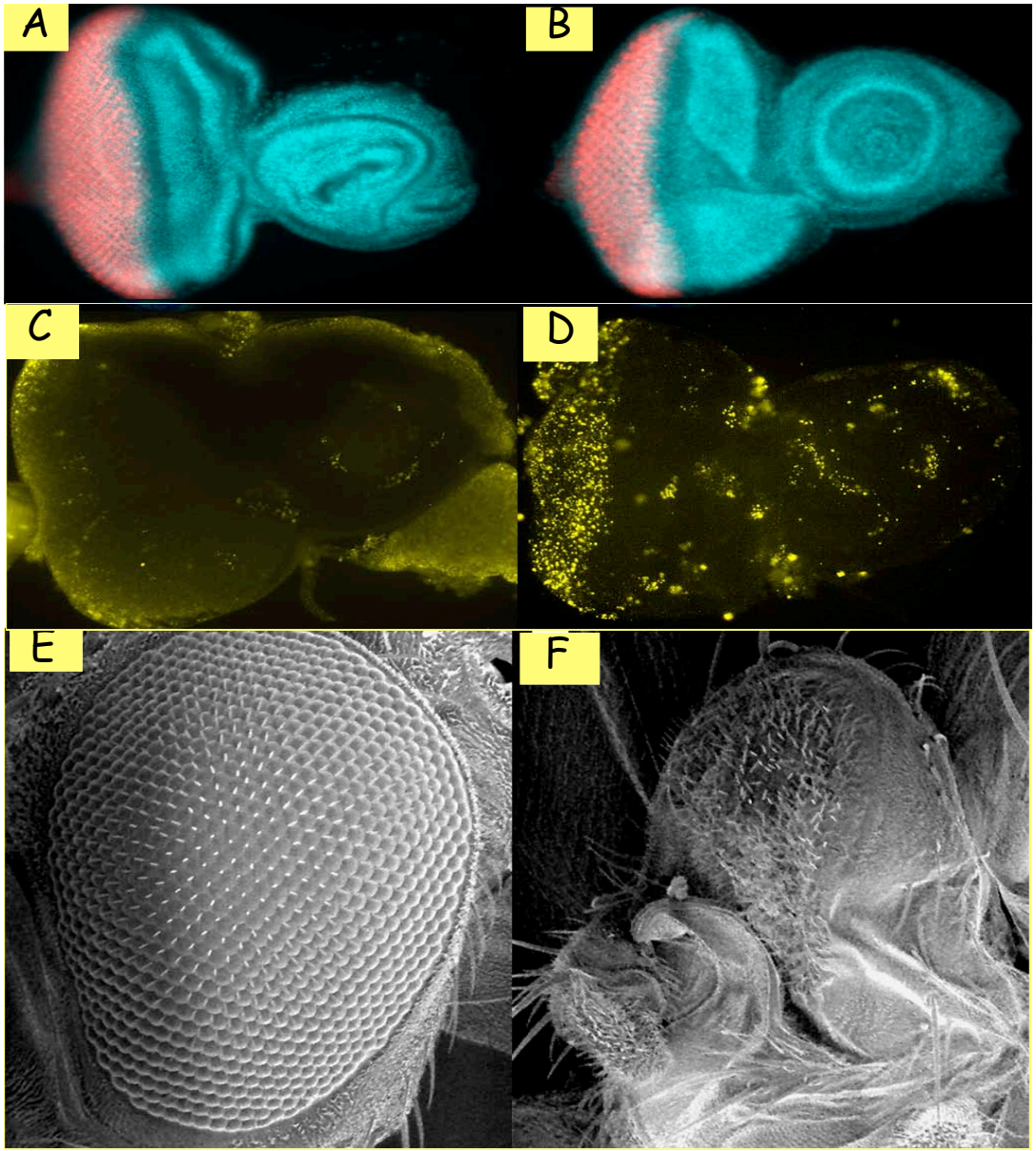


Drosophila caspases and CED-4 homologue



Single CED-4/Apaf-1 homologue (ARK/DARK)

Ectopic expression of *dronc* induces cell death



DRONC protein expression (eye disc)

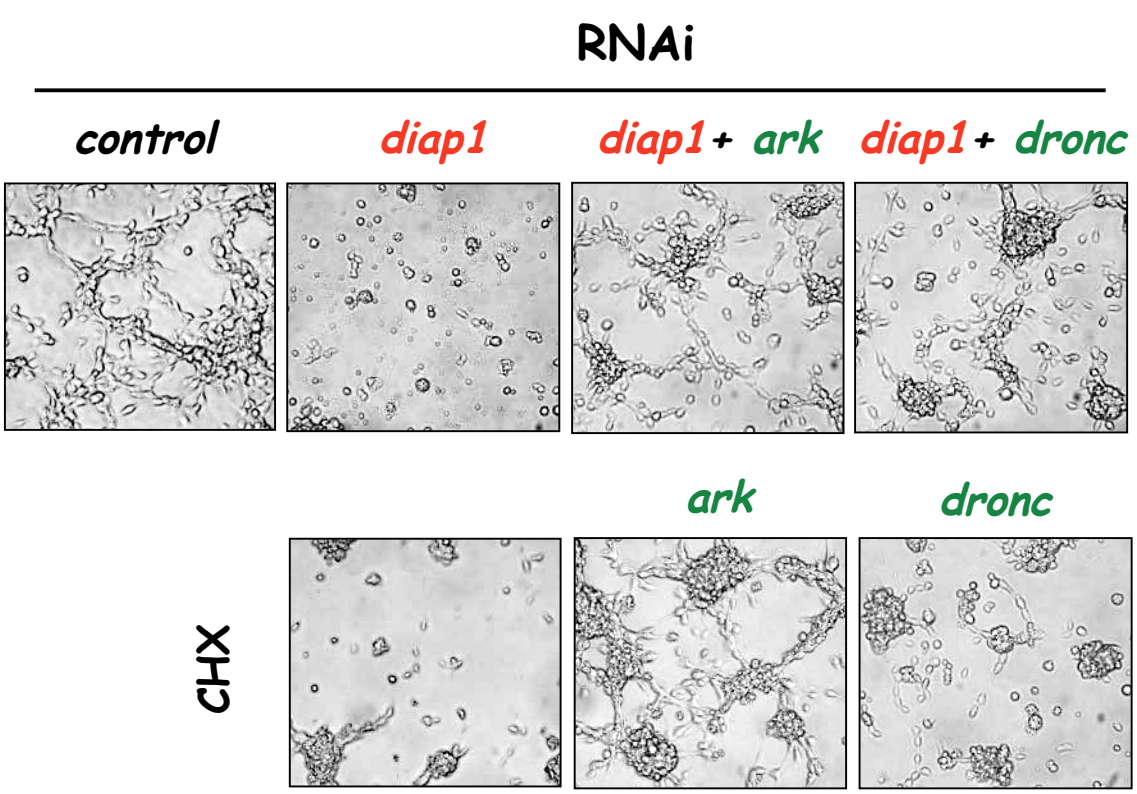
Acridine orange staining (eye disc)

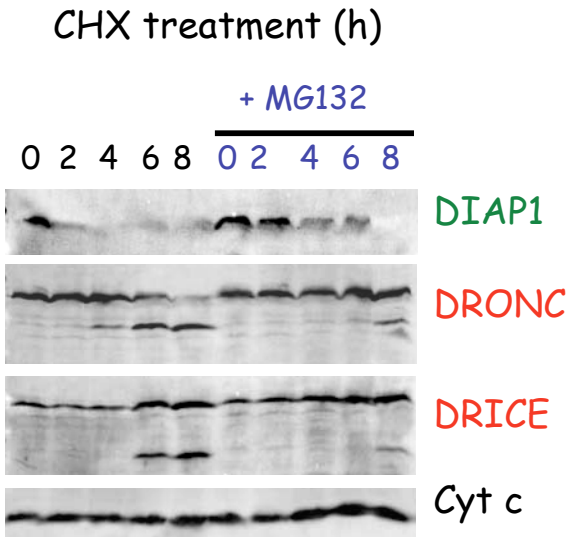
Adult eye phenotype

dronc Cys mut

dronc

Ablation of *ark* or *dronc* prevents cell death induced by *diap1* depletion or drug treatment

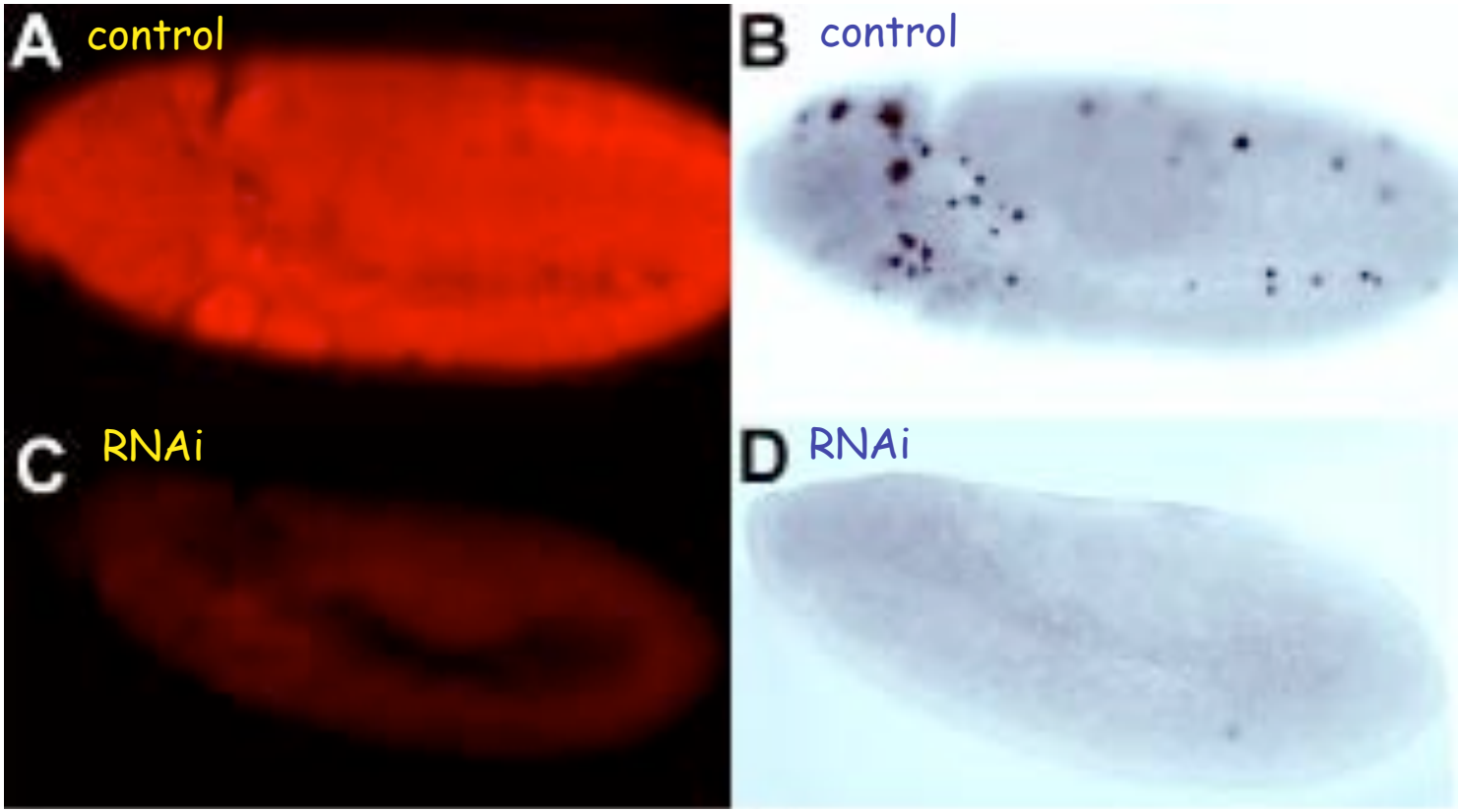




DIAP1 ⊣ DRONC → Apoptosis

ARK ↑

RNAi-mediated *dronc* ablation blocks developmental cell death in *Drosophila* embryos



DRONC protein

TUNEL (dead cells)

Genetic analyses of the *ark/dronc* pathway

DRONC is required for most developmental PCD

dronc -/- animals are pupal lethal

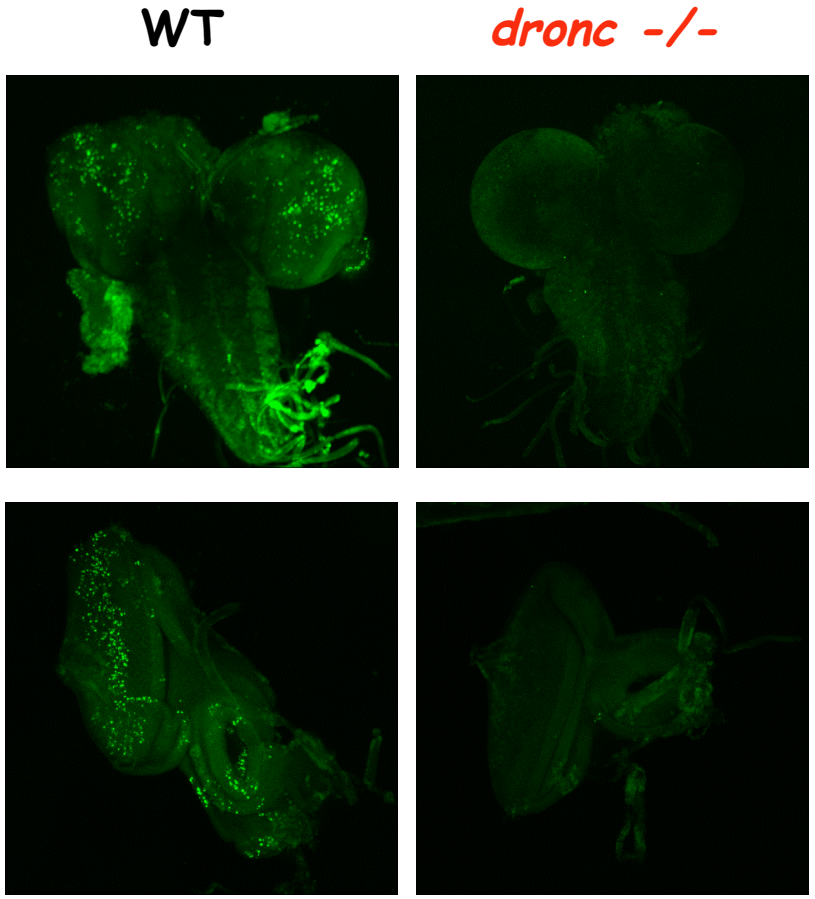
Slightly delayed development

Extra cells in embryos

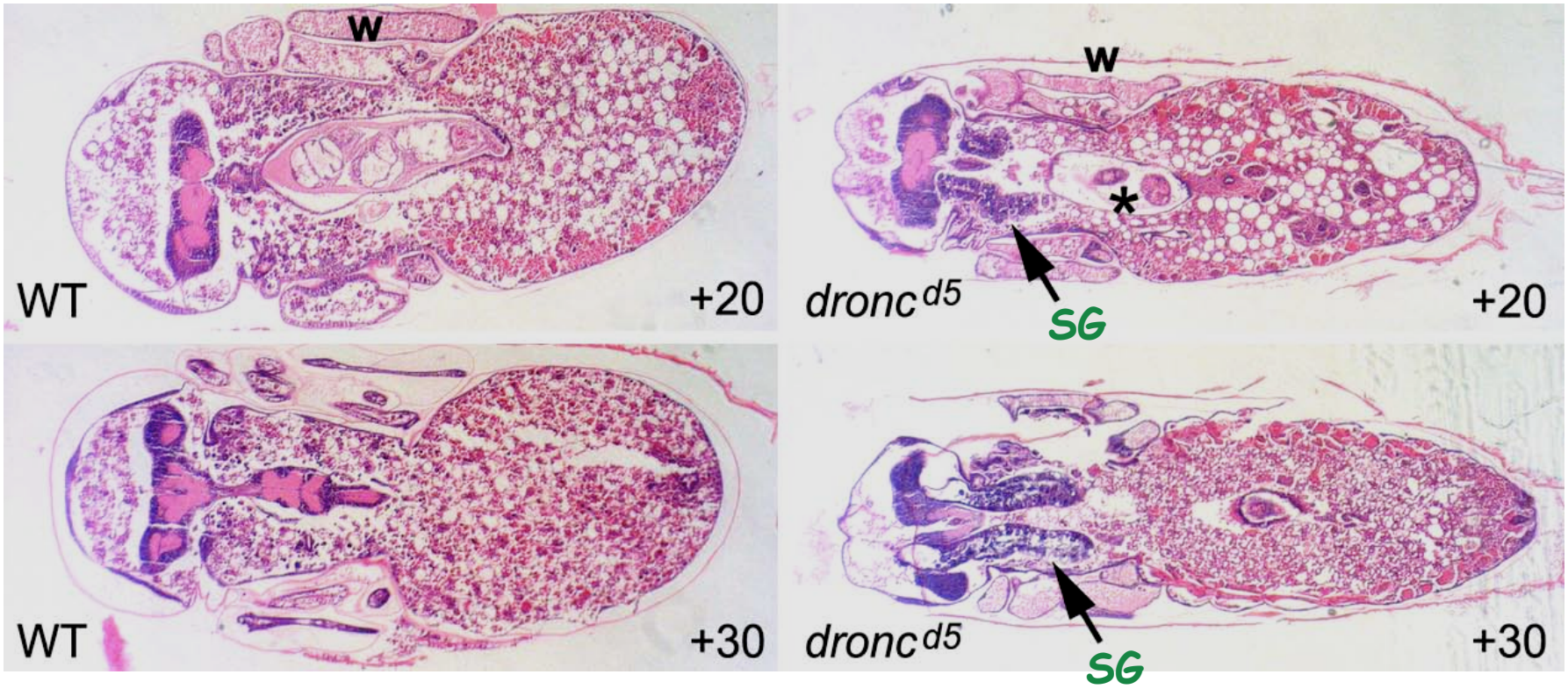
Enlarged CNS and imaginal discs

Hyperplasia in hemocytic compartment

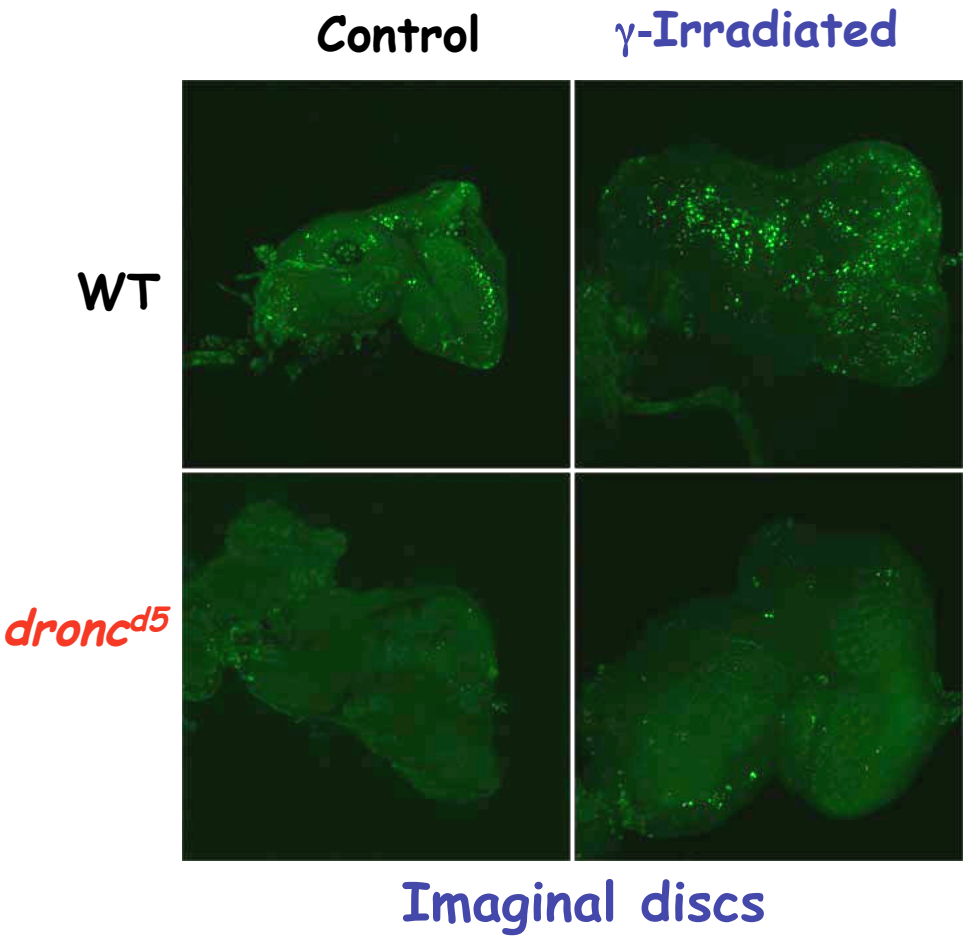
Resistance to stress-induced apoptosis

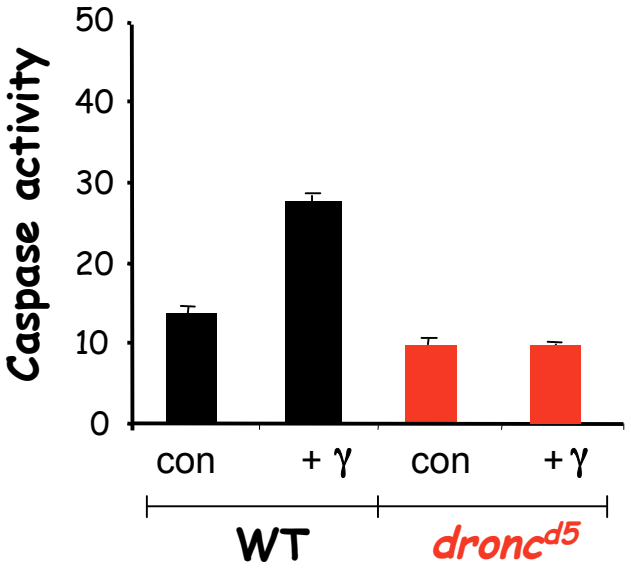


Delayed larval salivary gland removal in *dronc* mutant animals

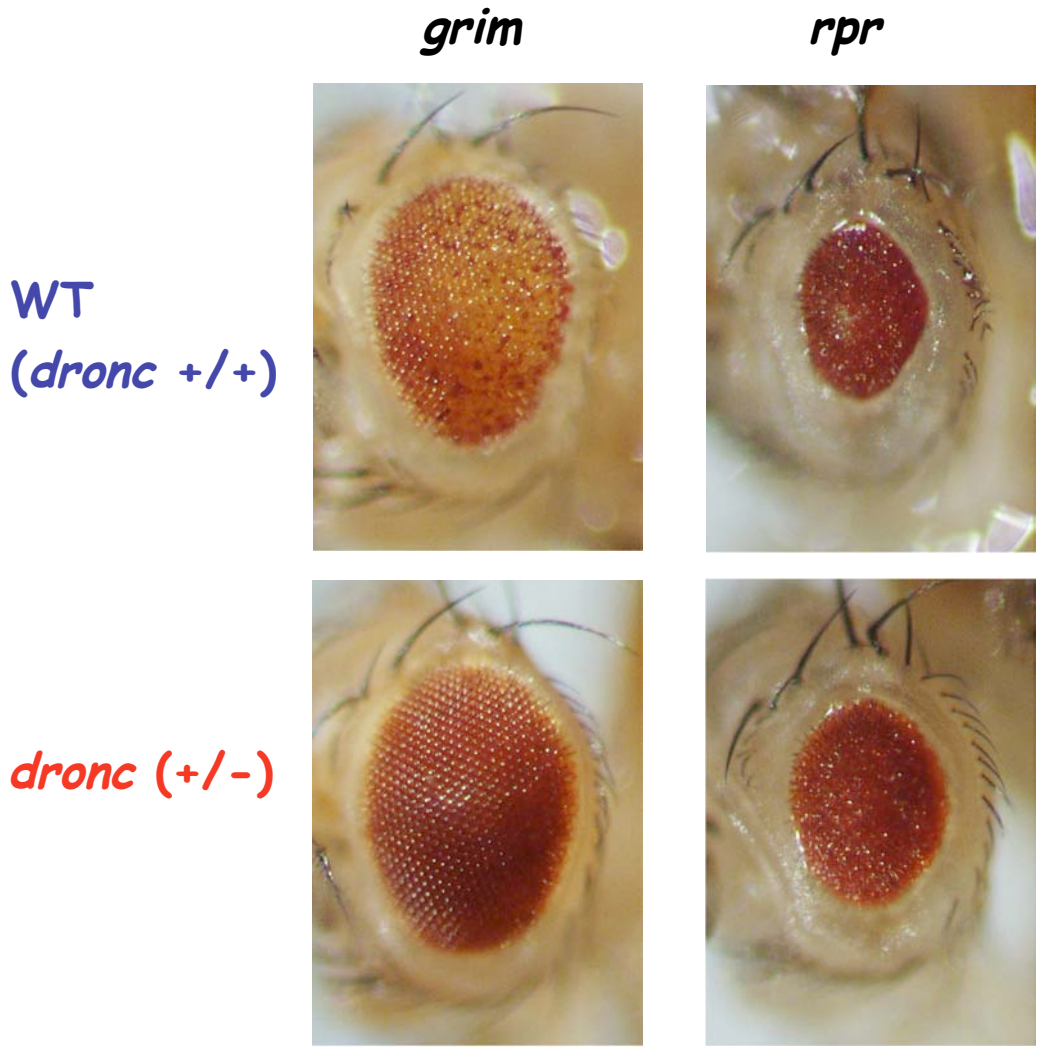


DRONC is required for radiation-induced cell death





Disruption of *dronc* suppresses *grim* and *rpr*-induced cell death in the fly eye



ark mutants phenocopy *dronc* deficiency

ARK

↓

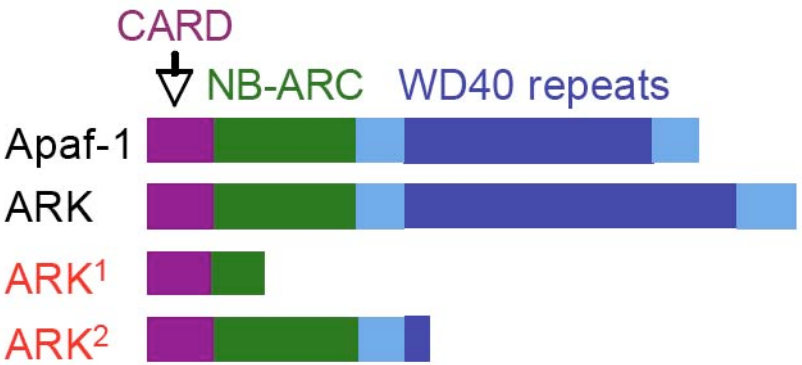
DRONC

↓

DRICE

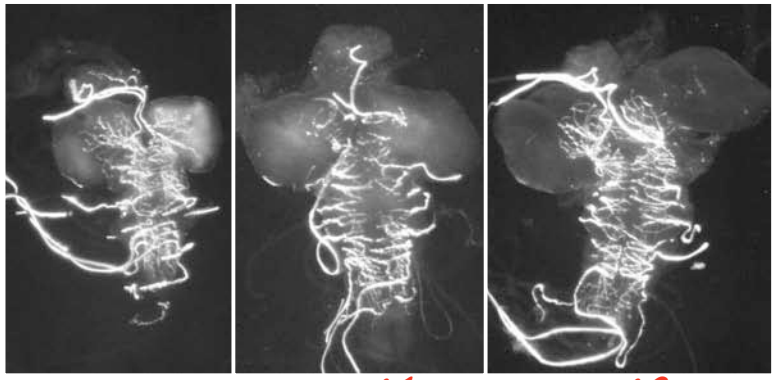
↓

apoptosis



ark mutants are pupal lethal

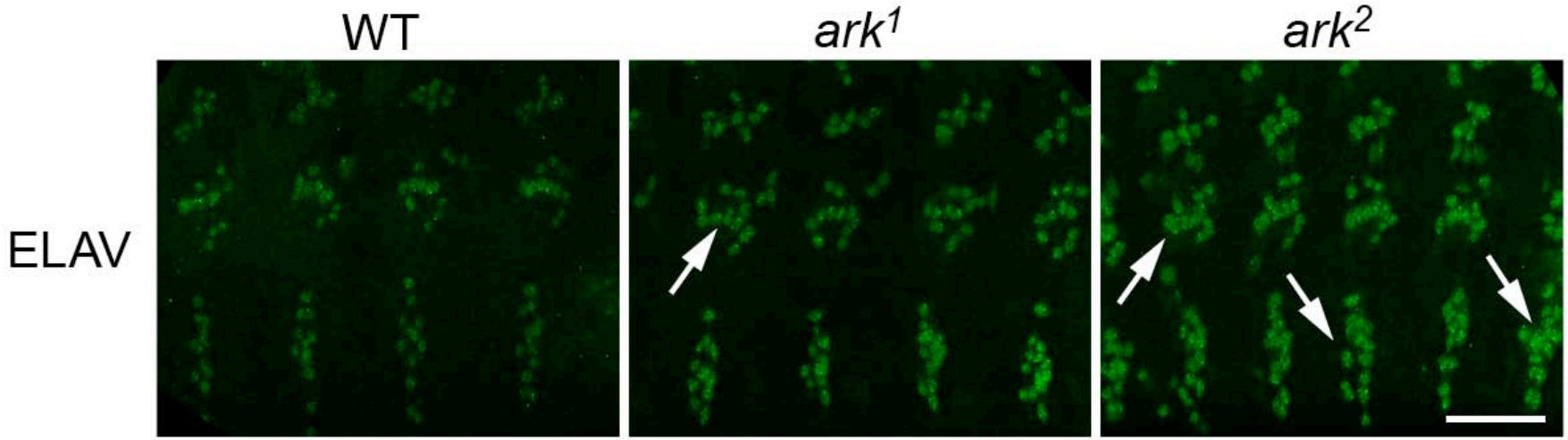
ark[1] *ark*[2]



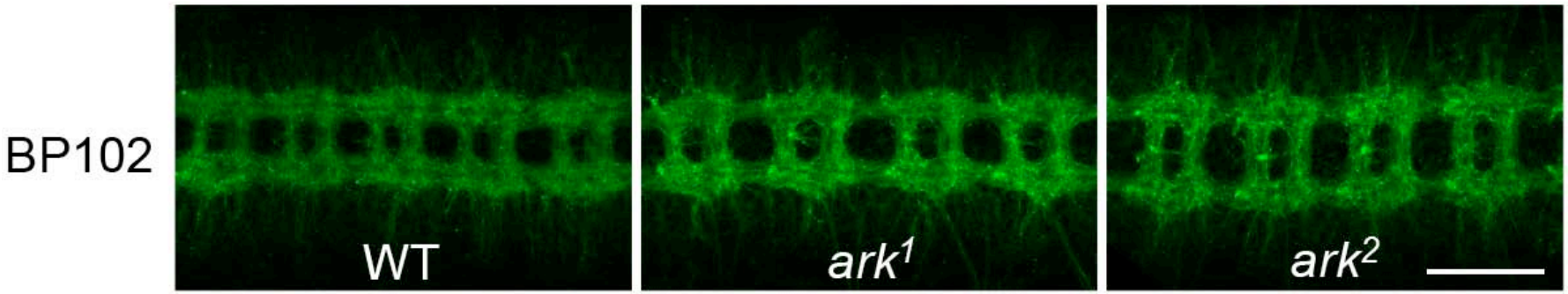
WT *ark*[1] *ark*[2]

ark mutants show CNS hyperplasia

Extra cells in *ark*-deficient embryos

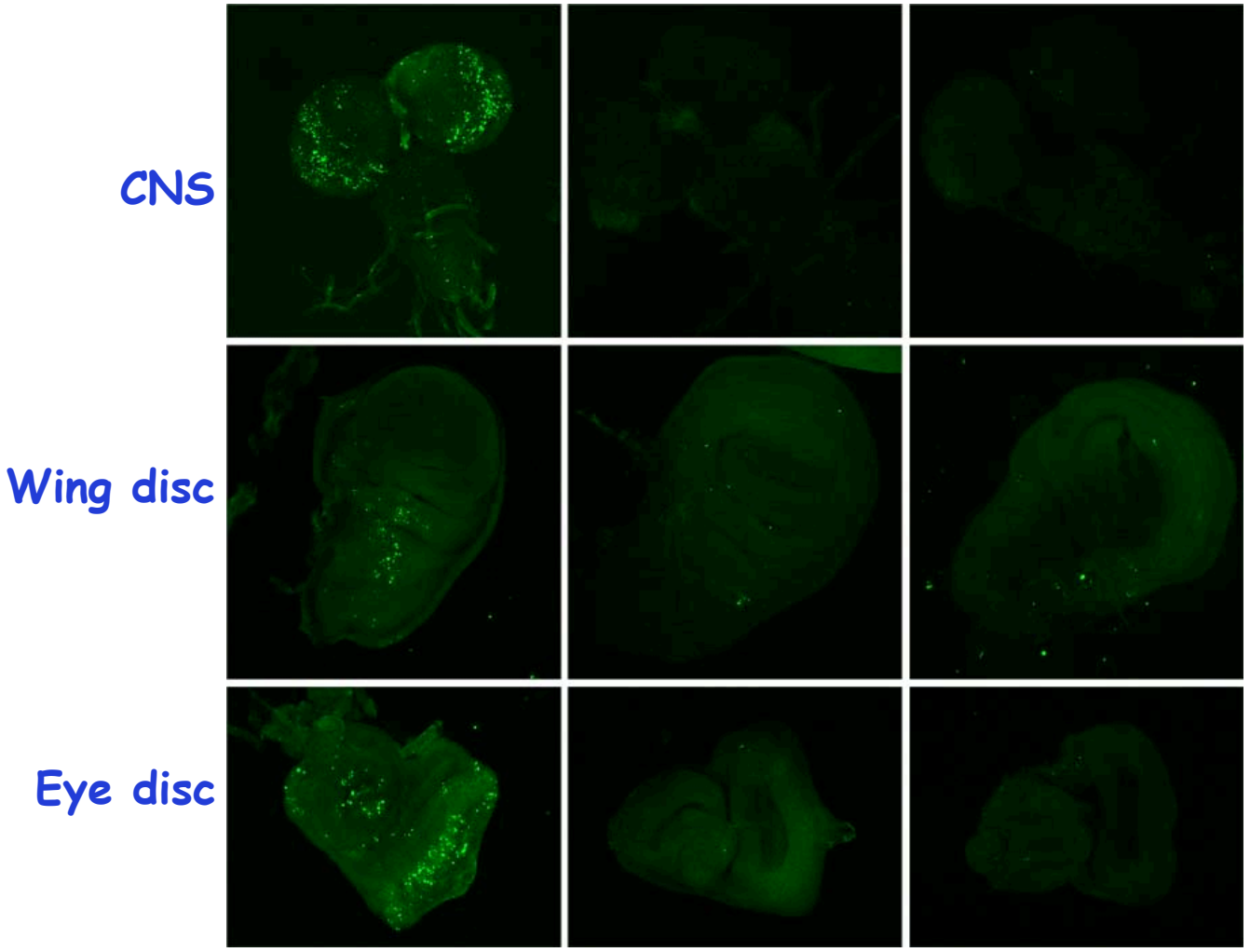


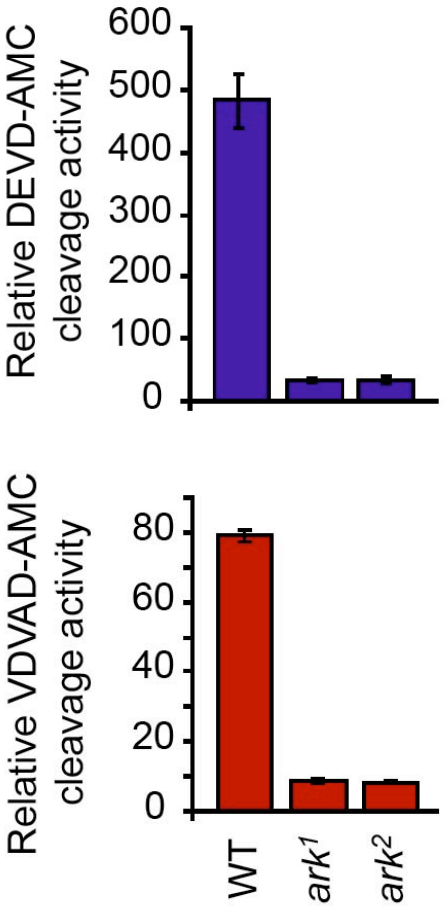
Abdominal hemisegments of the PNS with extra cells



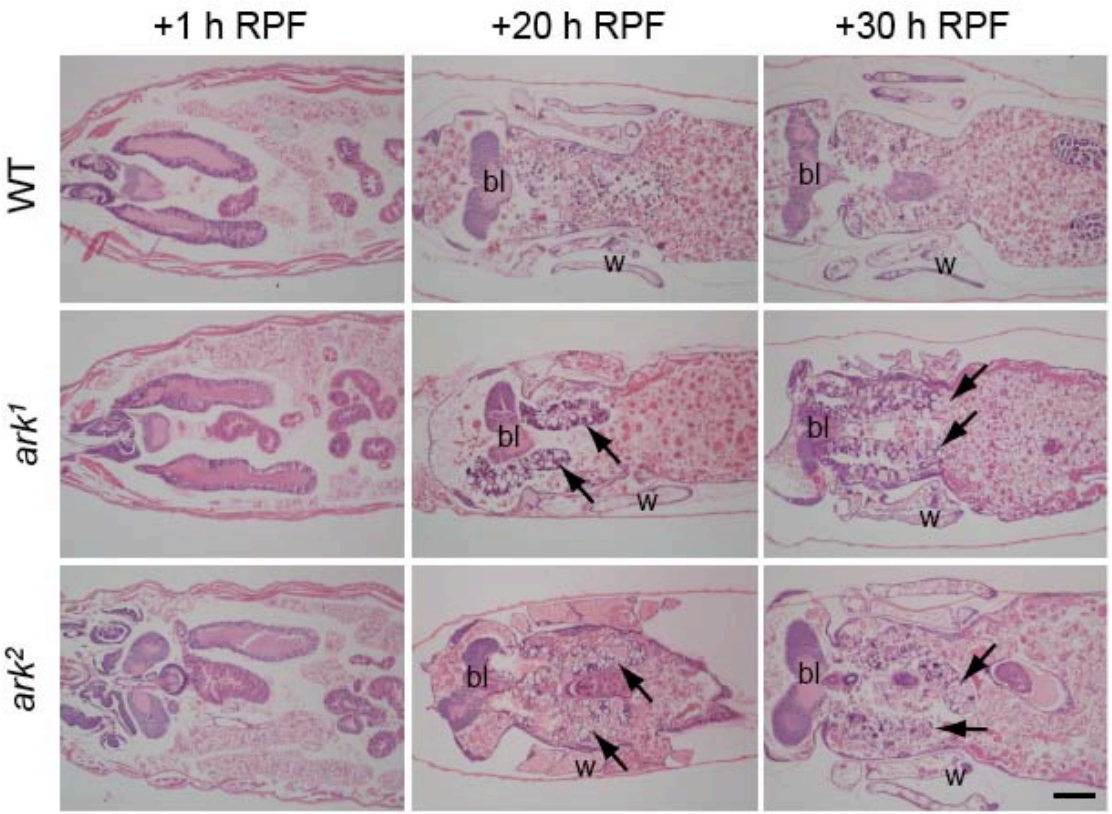
Ventral nerve cords with wider commissures

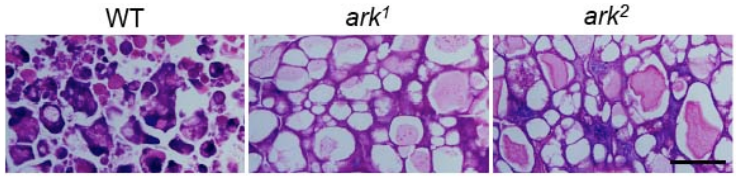
Reduced PCD and caspase activity in *ark*-deficient larvae



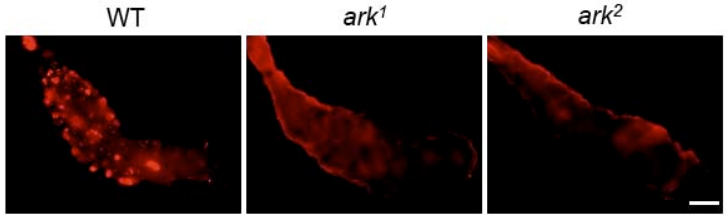


ark mutants contain persistent larval salivary glands



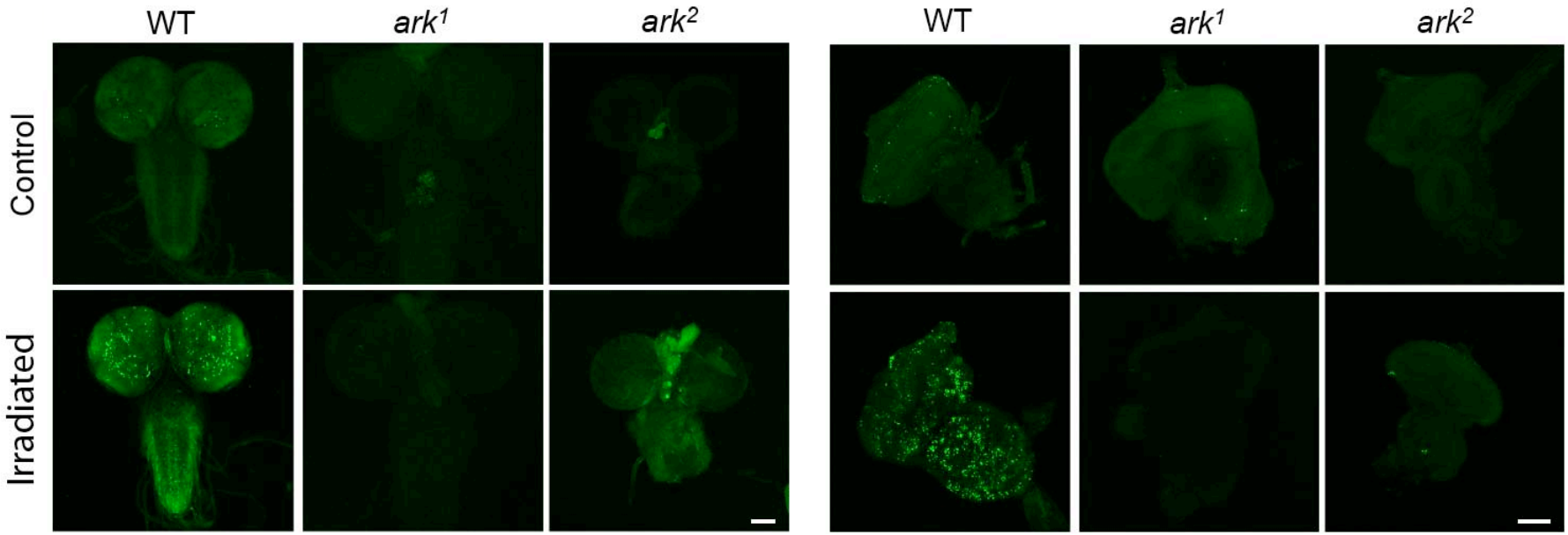


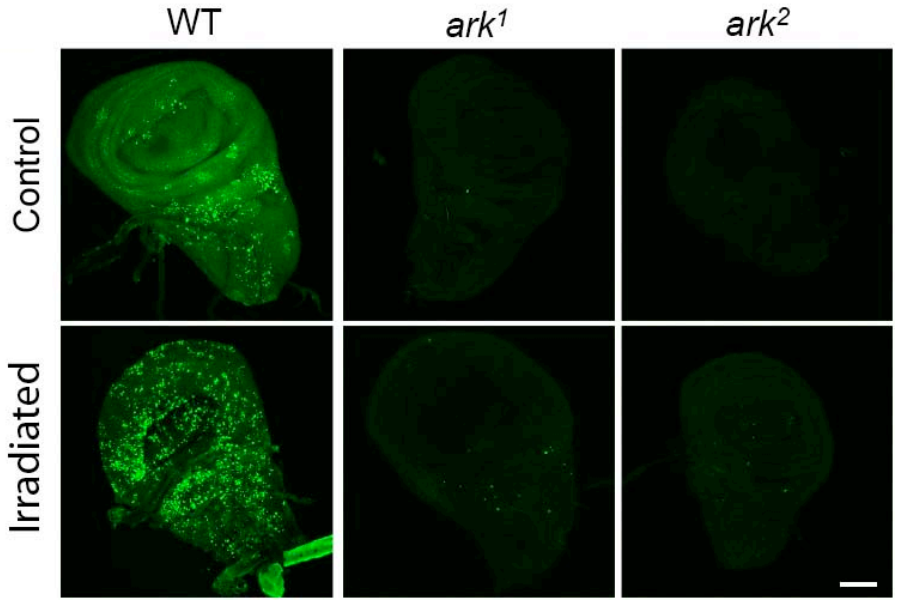
Histology



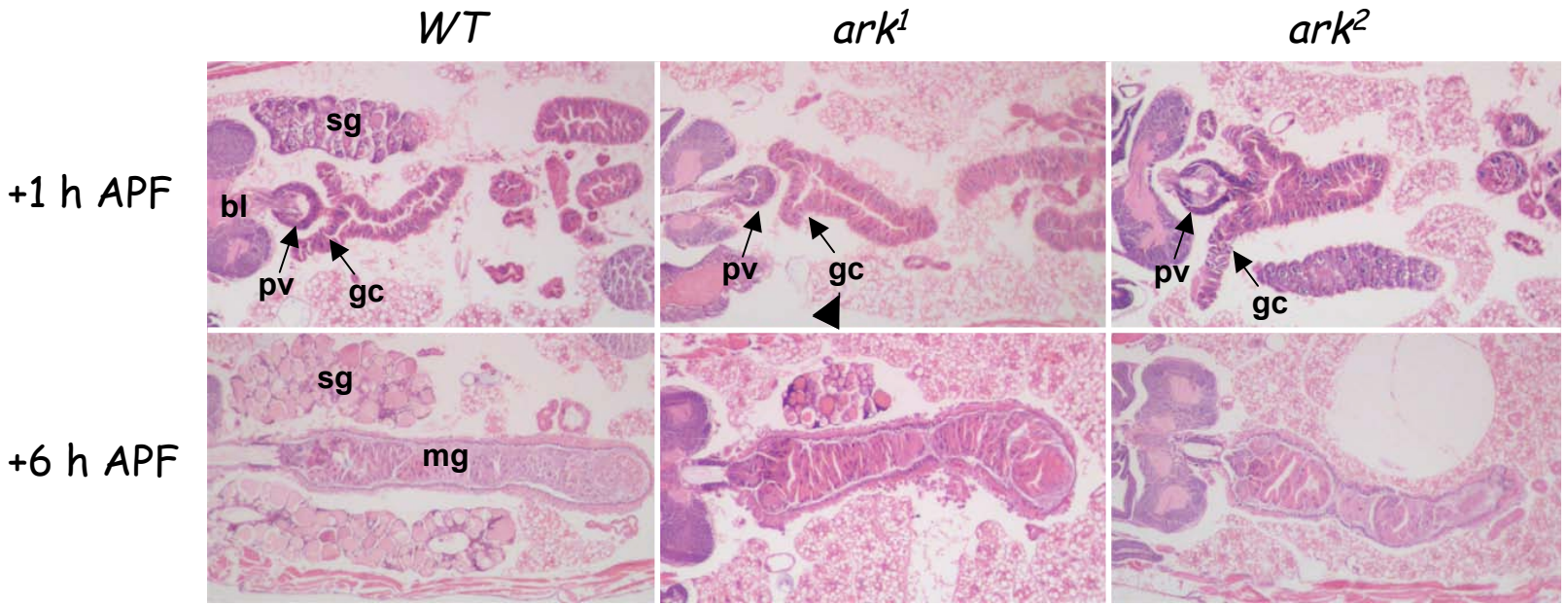
TUNEL

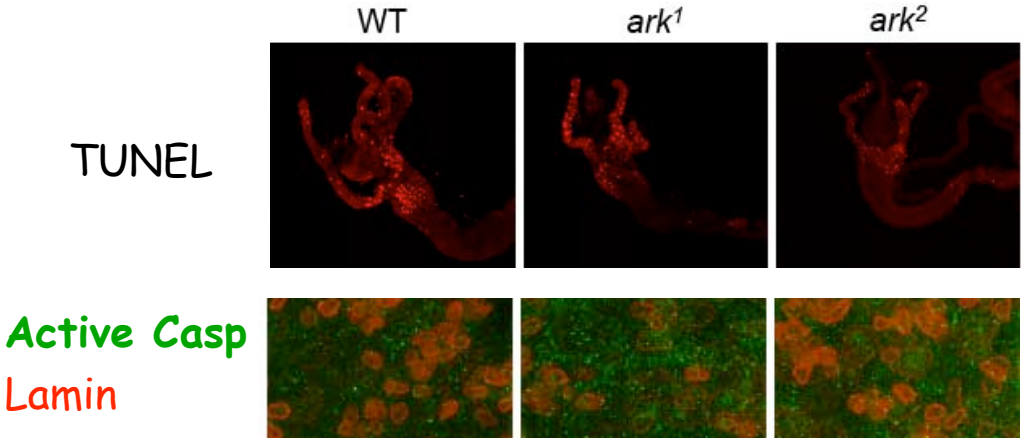
ARK is required for radiation-induced cell death

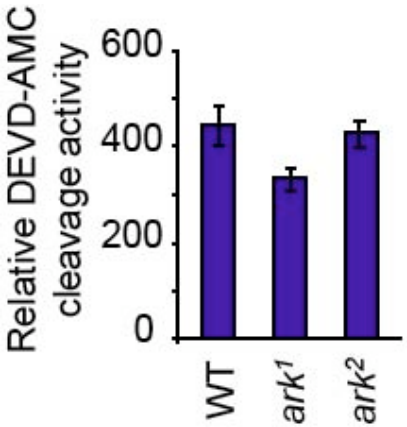




ARK is not essential for midgut PCD







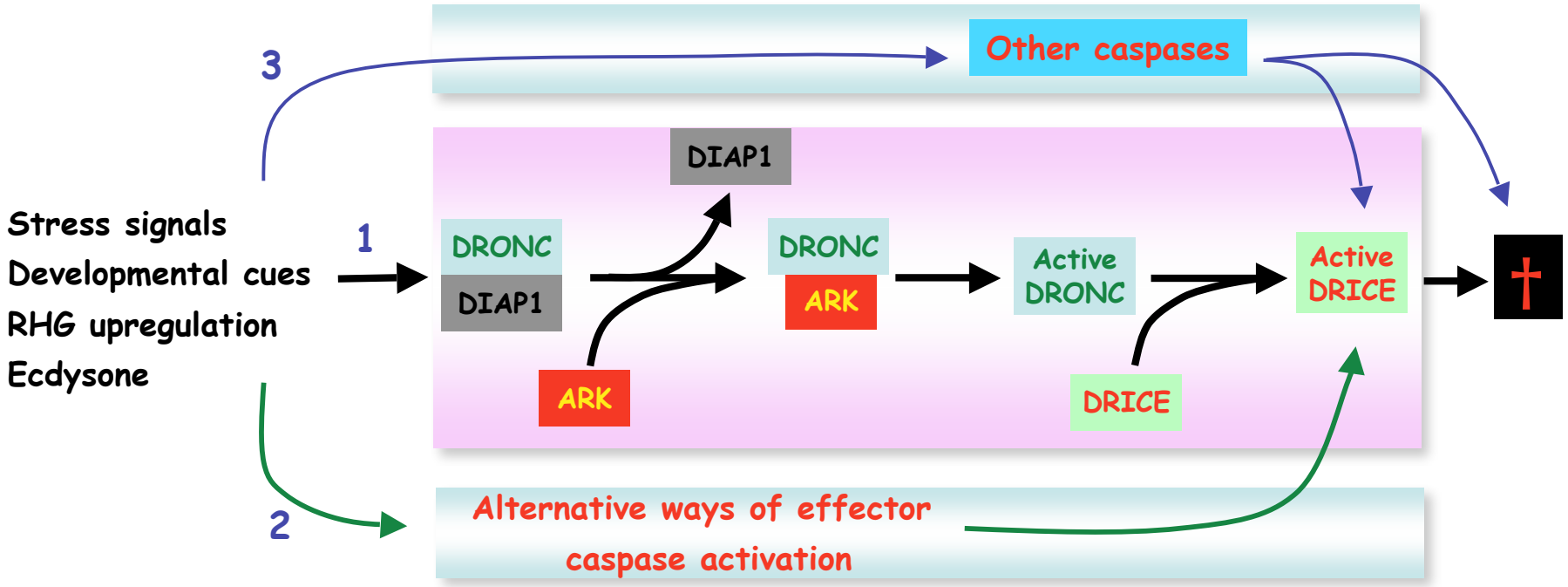
Lessons of caspase function from fly studies

Canonical ARK/DRONC axis is essential for most developmental PCD in fly

ARK/DRONC pathway is essential for stress-mediated apoptosis

Alternative pathways of effector caspase activation in some tissues/contexts

Loss of caspase/ caspase activation results in long-term cell survival



Future studies on caspases: what remains to be explored

1. Caspase redundancy and compensation
2. How some caspases can function in both apoptotic and nonapoptotic pathways
3. Alternative pathways of caspase activation in the absence of initiator caspases
4. Cell death in the complete absence of caspases
5. What caspase targets are important for dismantling cells
6. How precisely are caspases activated and regulated in response to different signals (apoptotic and nonapoptotic)
7. Potential roles of caspase dysfunction in human pathologies