Specific necrosis suppressor classes in *C. elegans* affect:

- Ca^{2+} homeostasis
- Proteolysis
- pH homeostasis
- Autophagy

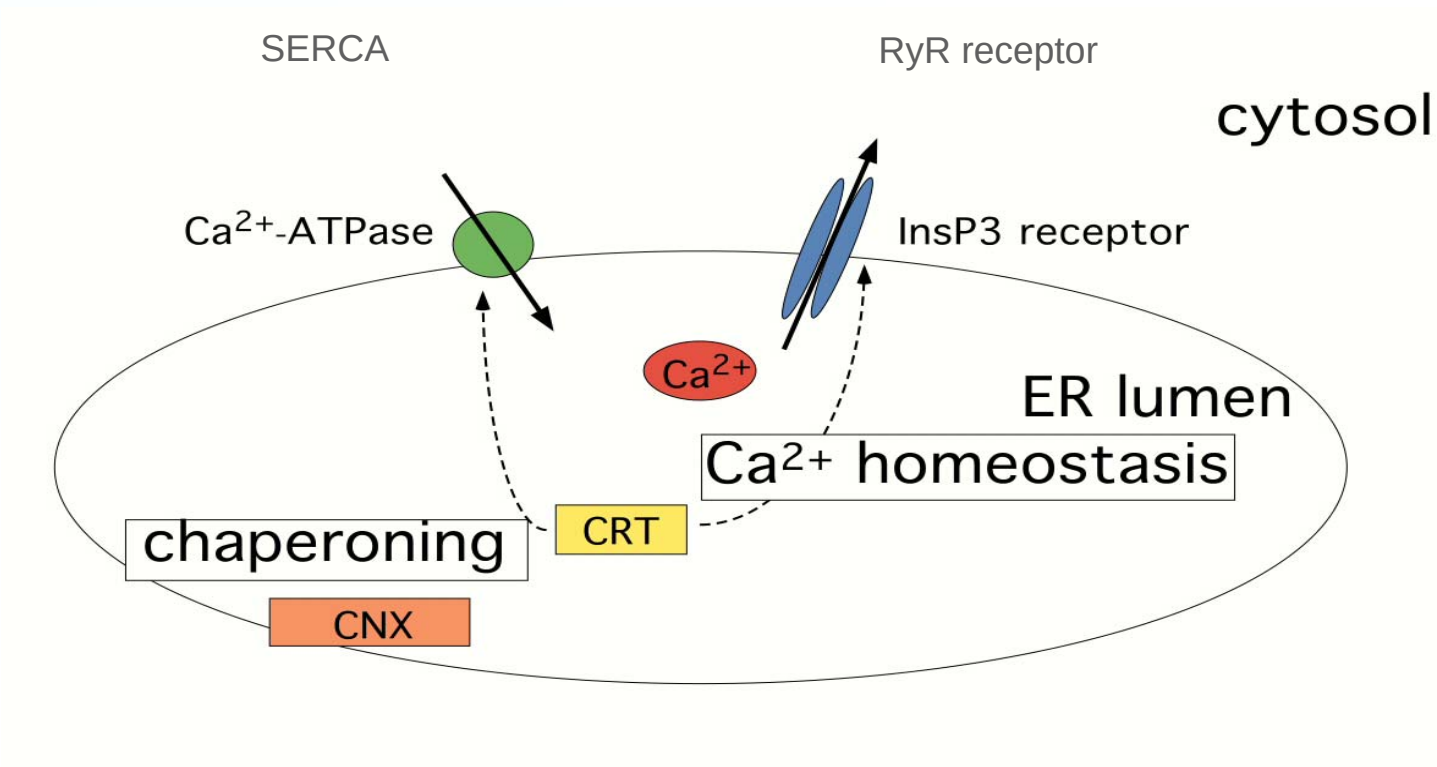
Ca^{2+} homeostasis

A death suppressor locus:

crt-1 **encodes Calreticulin**

a calcium-binding molecular chaperone (CRT-1)

Physiological role of Calreticulin



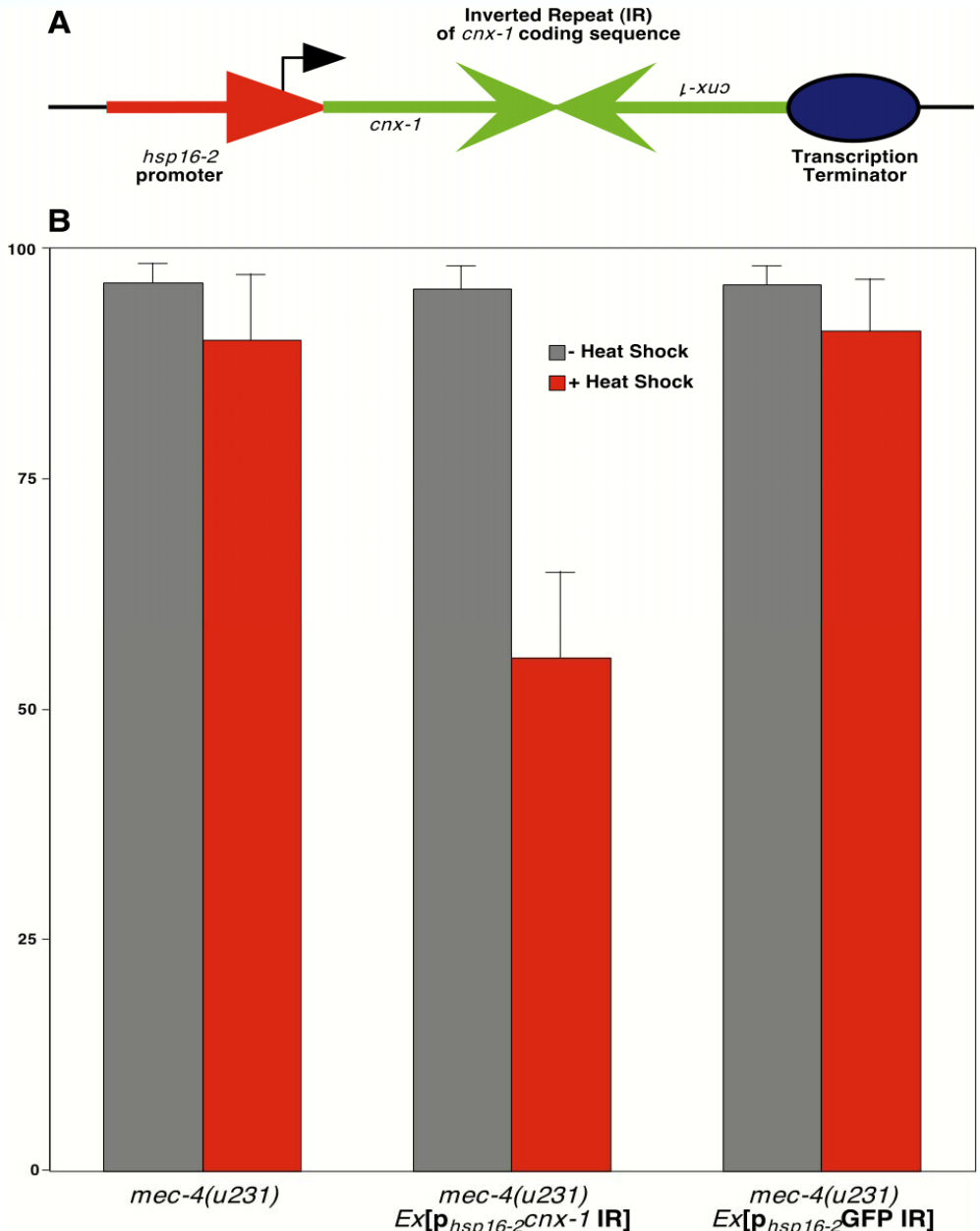
crt-1 mutations suppress the toxic effects of hyperactive degenerin channels expressed in neurons and muscles

	crt-1(+)	*crt-1(bz29)*	*crt-1(bz30)*	*crt-1(bz31)*	*crt-1(bz50)*
mec-4(u231) (swollen touch cell bodies)	95±1	3±2	3±2	95±2	82±4
bzIs3 (swollen touch cell bodies)	95±2	5±2	ND	ND	ND
mec-4(u231) (MEC-4::GFP)	4±3	84±7	ND	ND	ND
deg-1(u38) (swollen neuronal cell bodies)	86±7	13±11	ND	ND	ND
unc-8(n491) (inability to back)	100	0	0	100	100
unc-105(n1274) (paralysis)	100	0	ND	100	100

crt-1 mutations suppress degeneration/dysfunction induced by $G\alpha_s$(*gf*) or human $A\beta_{1-42}$, but not *deg-3(u662)*

	crt-1(+)	*crt-1(bz29)*	*crt-1(bz30)*	*crt-1(bz31)*	*crt-1(bz50)*
$p_{hsp16\text{-}41}$*gsα-1*(Q208L) (L1 arrest)	89±2	87±3	ND	41±4	48±3
$p_{glr\text{-}1}G\alpha_s$(Q227L) (PVC cell death)	87±4	39±5	ND	ND	ND
$p_{unc\text{-}54}A\beta_{1\text{-}42}$ (paralysis of 4d adults)	95±1	5±4	ND	32±5	80±3
deg-3(u662) (swollen cell bodies)	5.1±0.4	5.7±0.6	5.8±0.4	5.5±0.7	ND

RNAi with the related chaperone *cnx-1* partly prevents *mec-4(d)*-induced touch cell degeneration



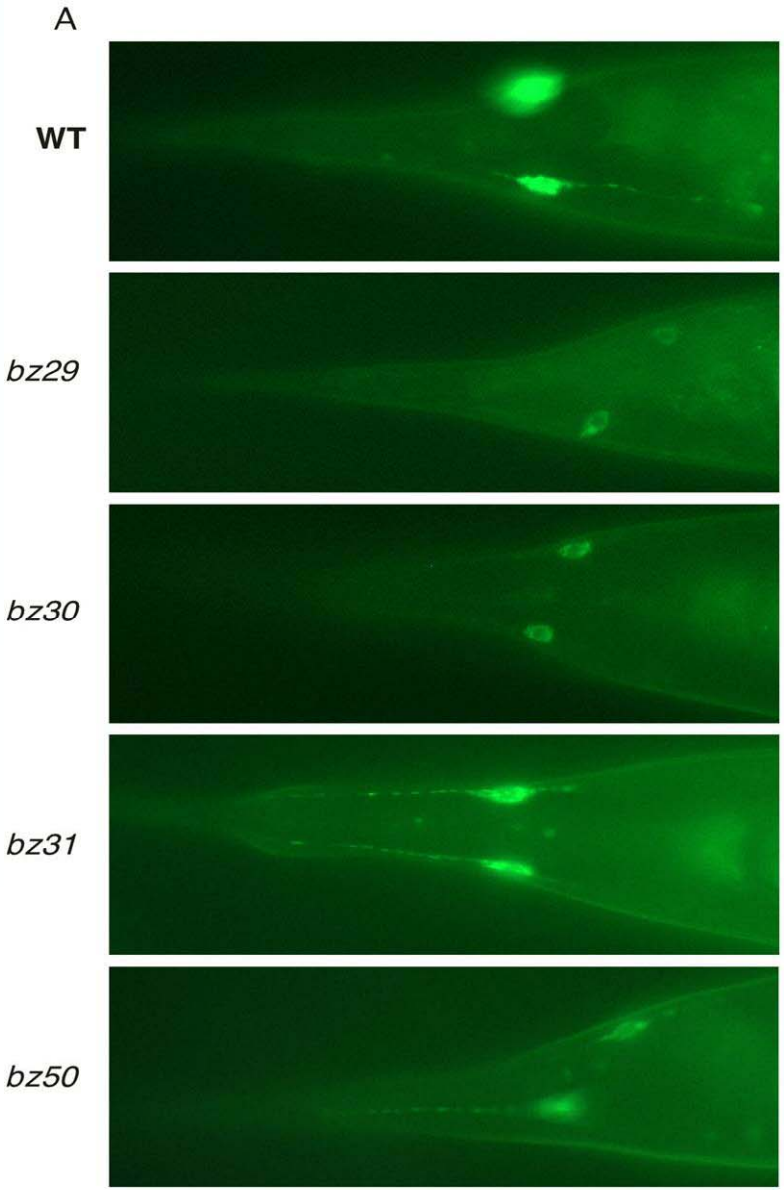
CRT (or CNX) deficiency suppresses necrotic cell death. Why?

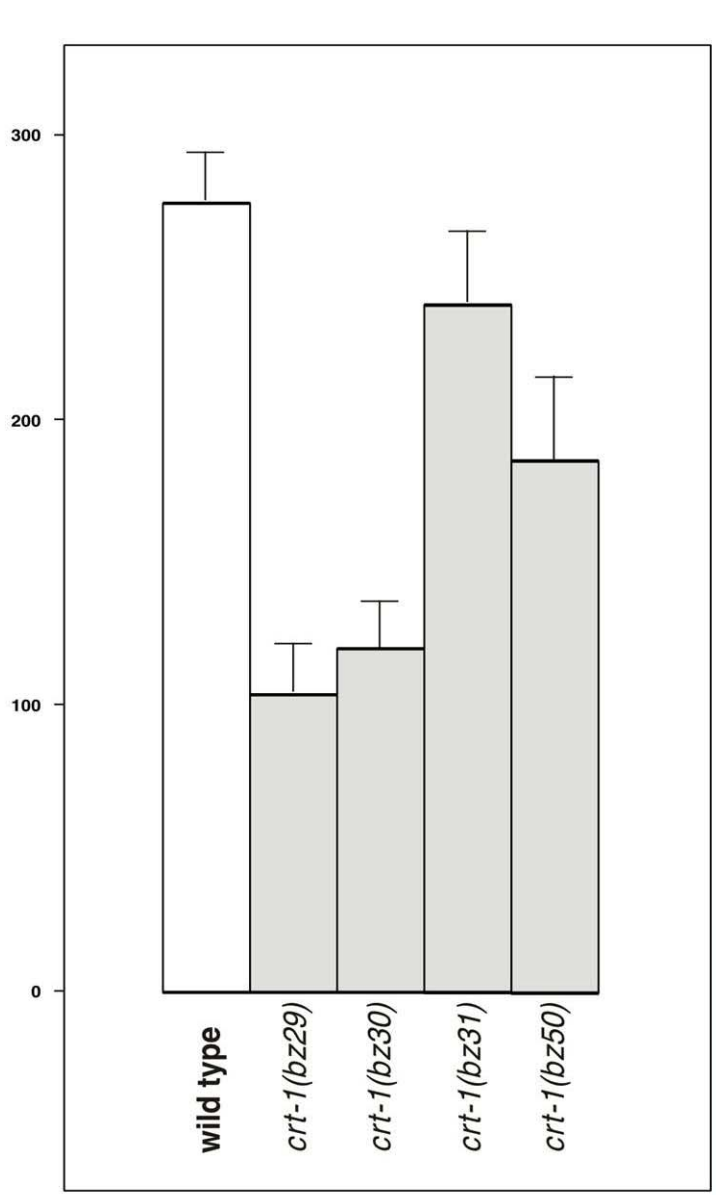
Defective chaperoning?

or

Disruption of intracellular Ca^{2+} homeostasis?

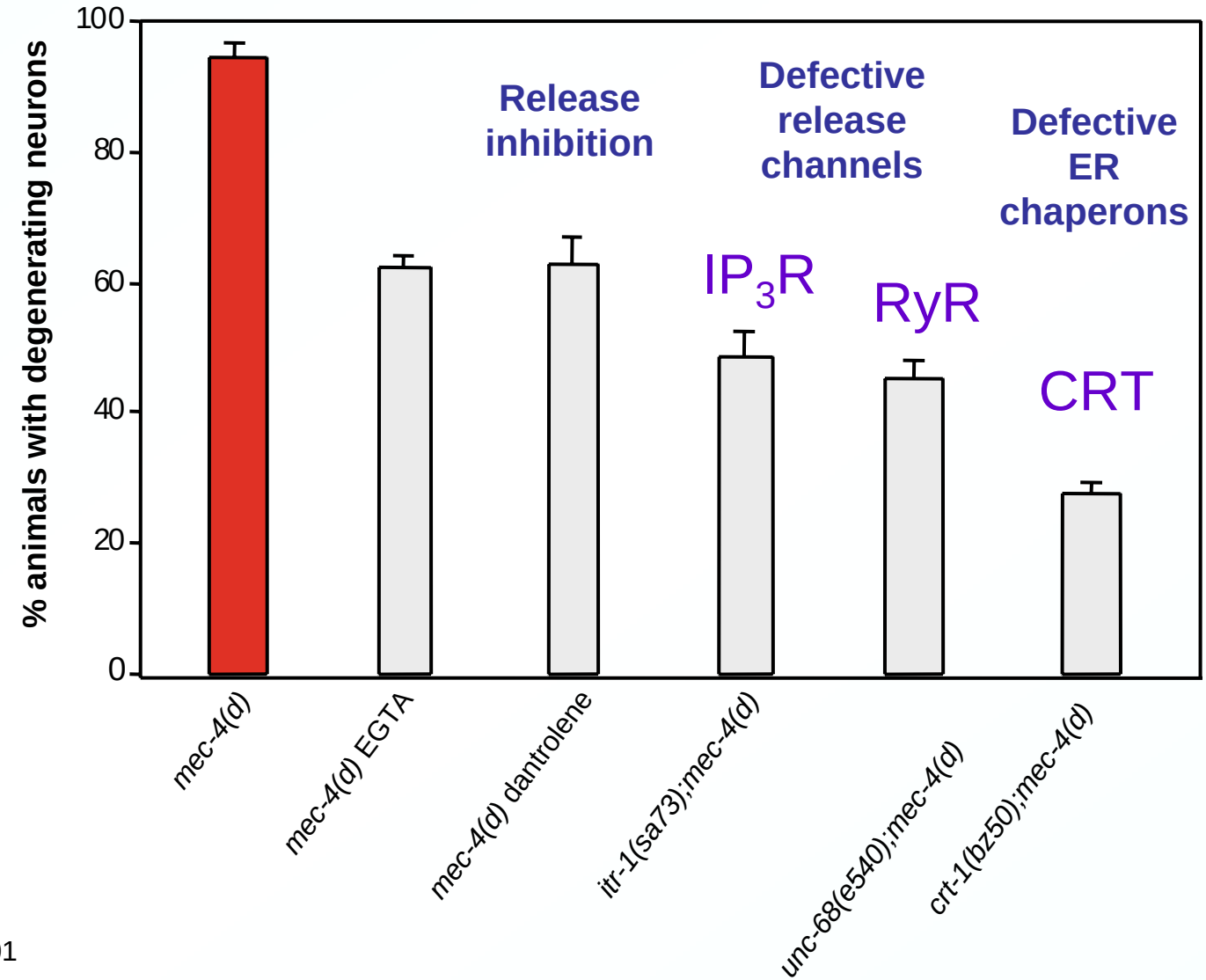
crt-1 mutations affect MEC-4::GFP levels





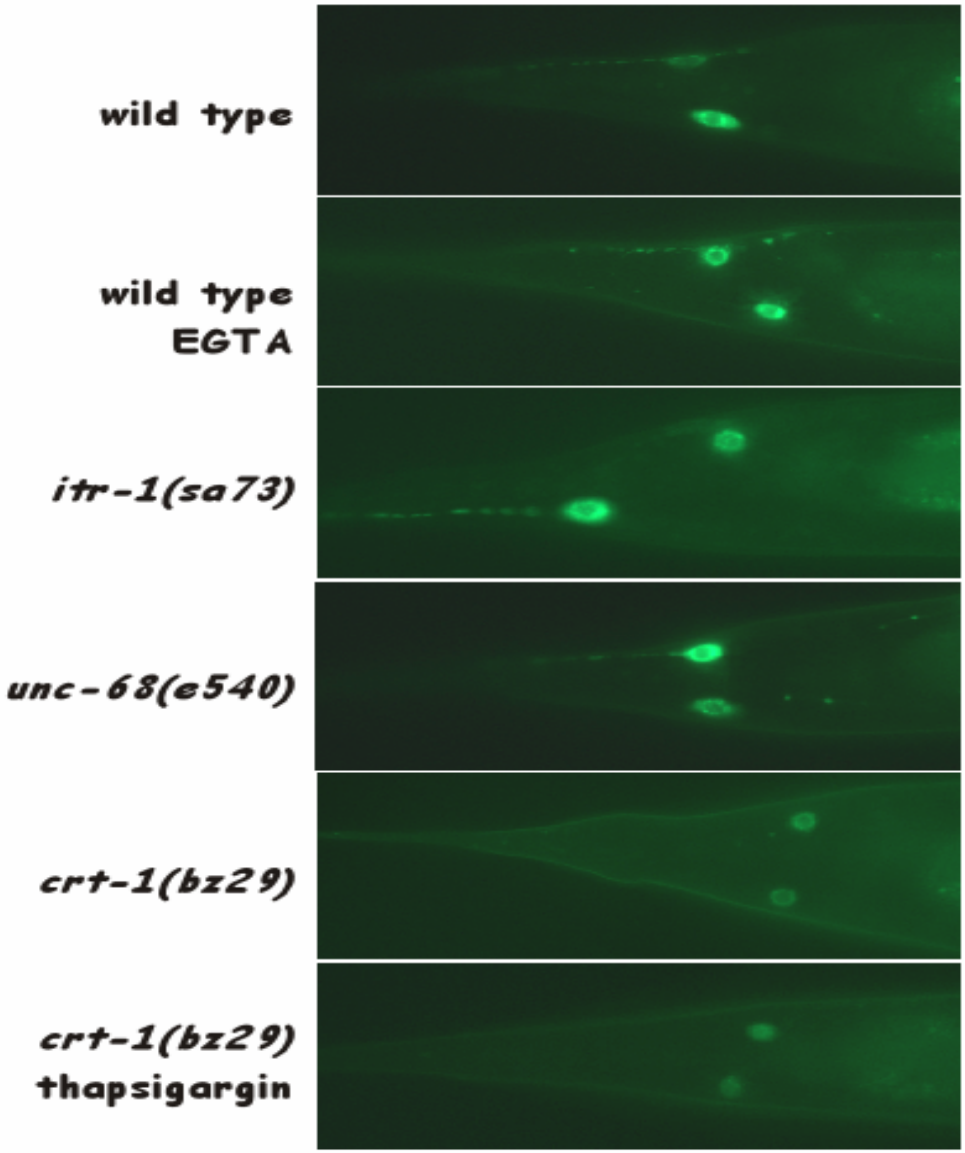
Down-regulation of expression contributes to degeneration suppression

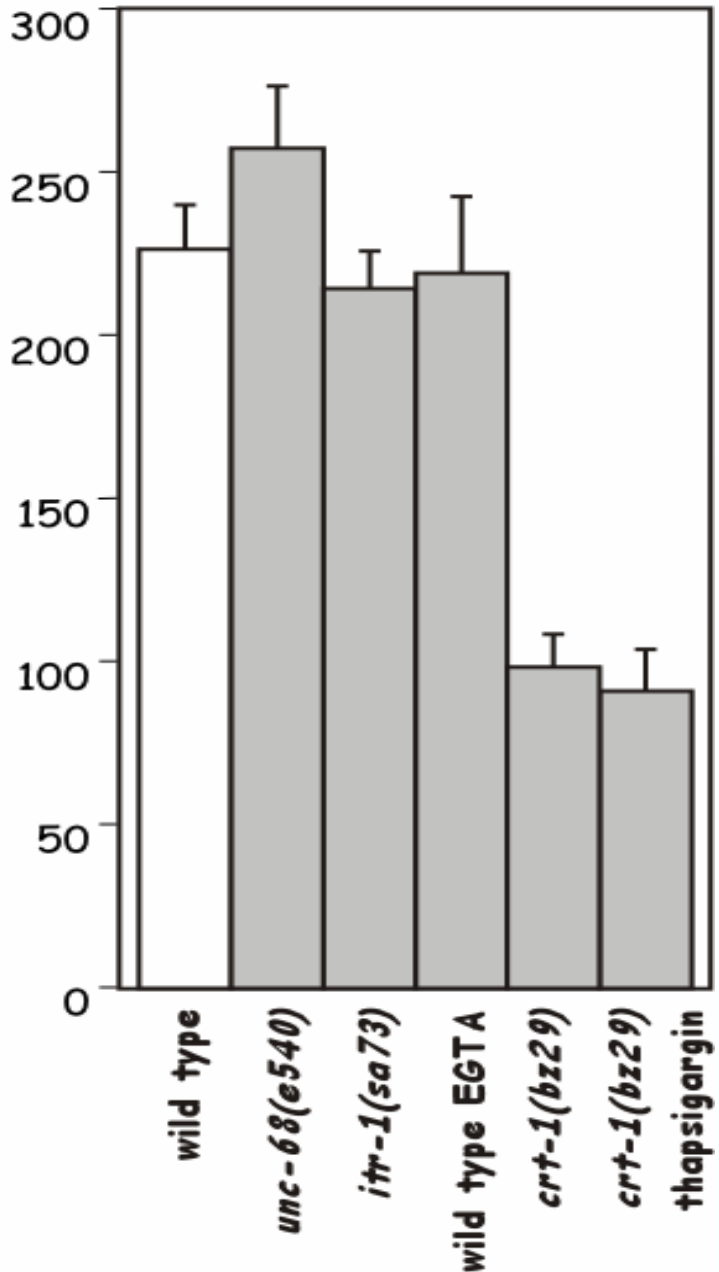
Manipulation of ER Ca^{2+} release partially suppresses *mec-4(d)*-induced cell death



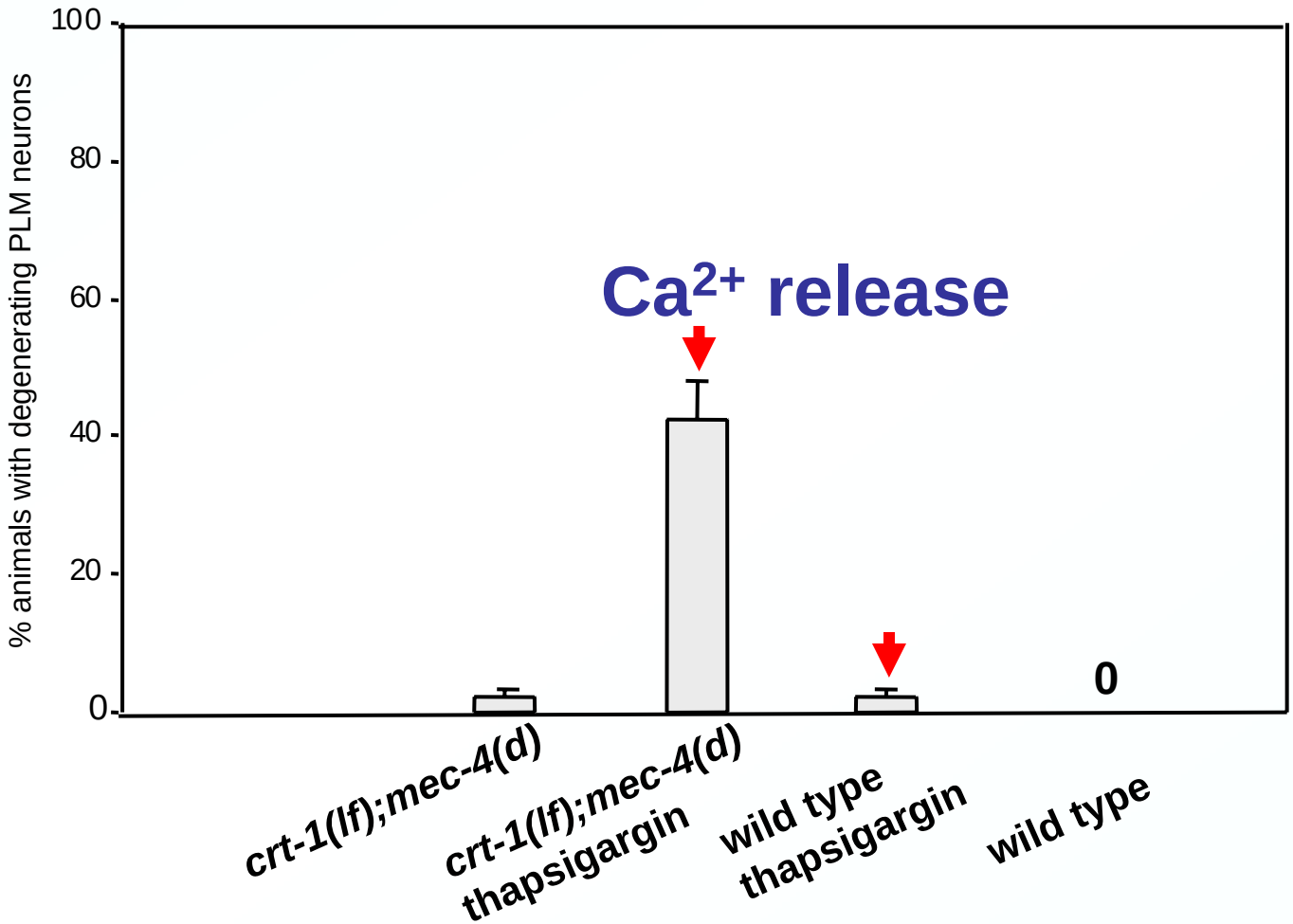
Xu et al., 2001

Neither genetic nor pharmacological manipulations of intracellular Ca^{2+} affect MEC-4::GFP levels





Forced ER Ca^{2+} release can bypass the *crt-1*-induced block of cell death



- **Ca^{2+} release from the ER**

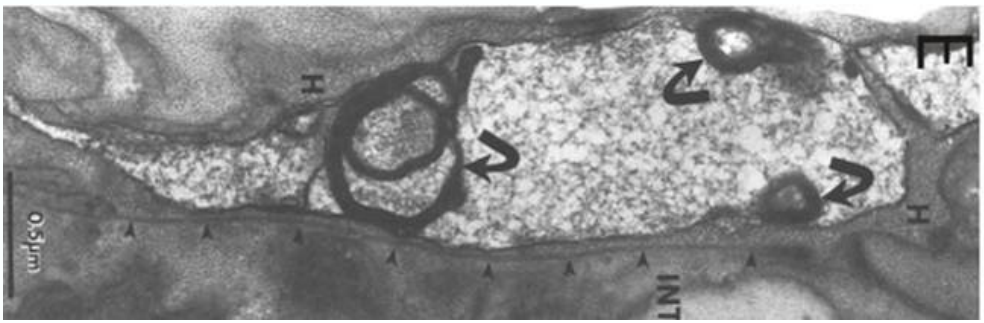
…and…

- **Regulation of the ER Ca^{2+} stores by calreticulin**

… are critical for neurodegeneration in *C. elegans*

Intracellular calcium elevation is required to trigger necrotic cell death

Proteolysis appears to play a major role in degenerin-induced cell death



Highly degraded cytoplasm of a touch receptor neuron soma at late degeneration stage

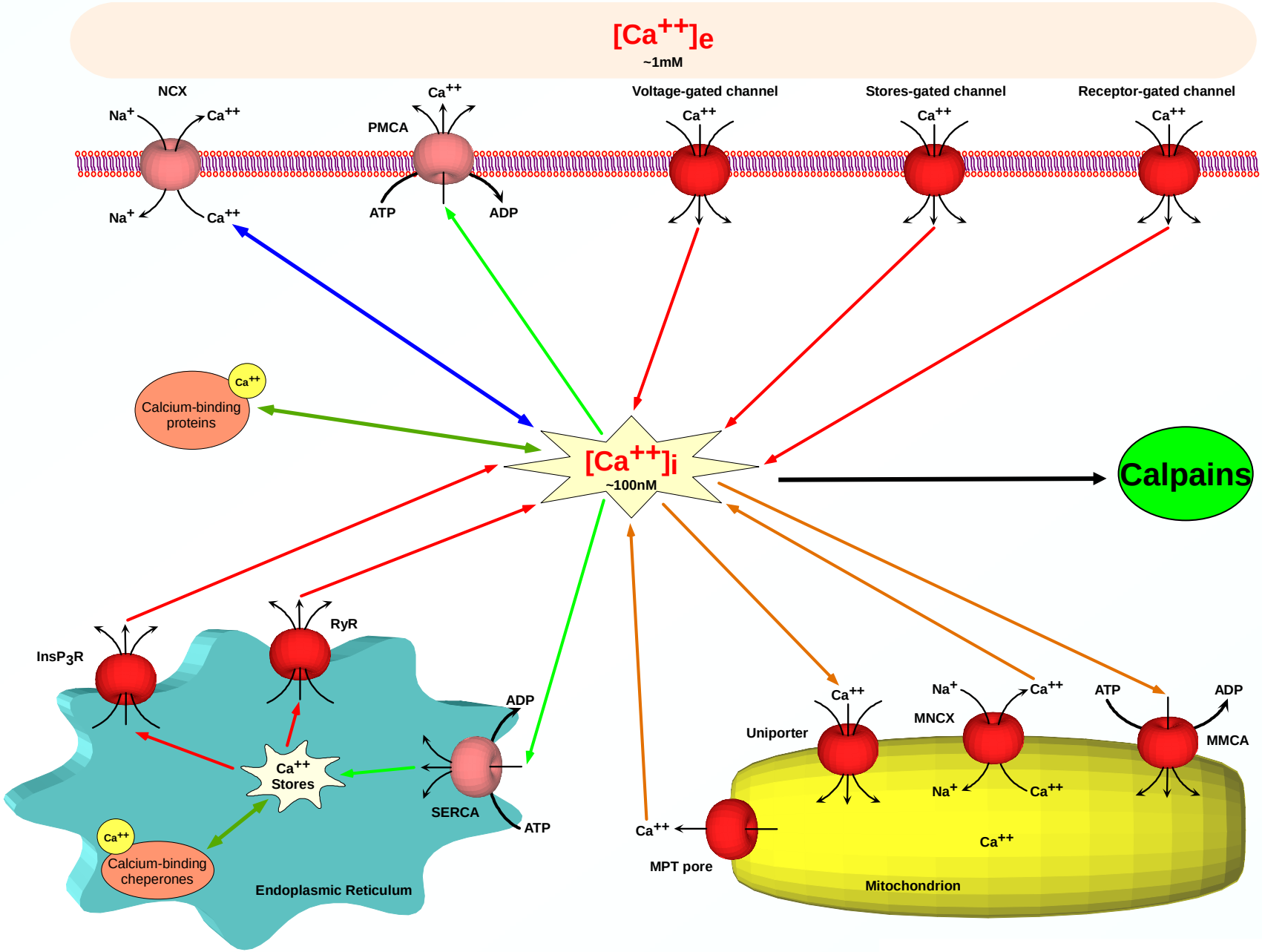
(Hall et al., 1997)

What proteolytic activities are involved in the destruction of a cell during necrotic cell death?

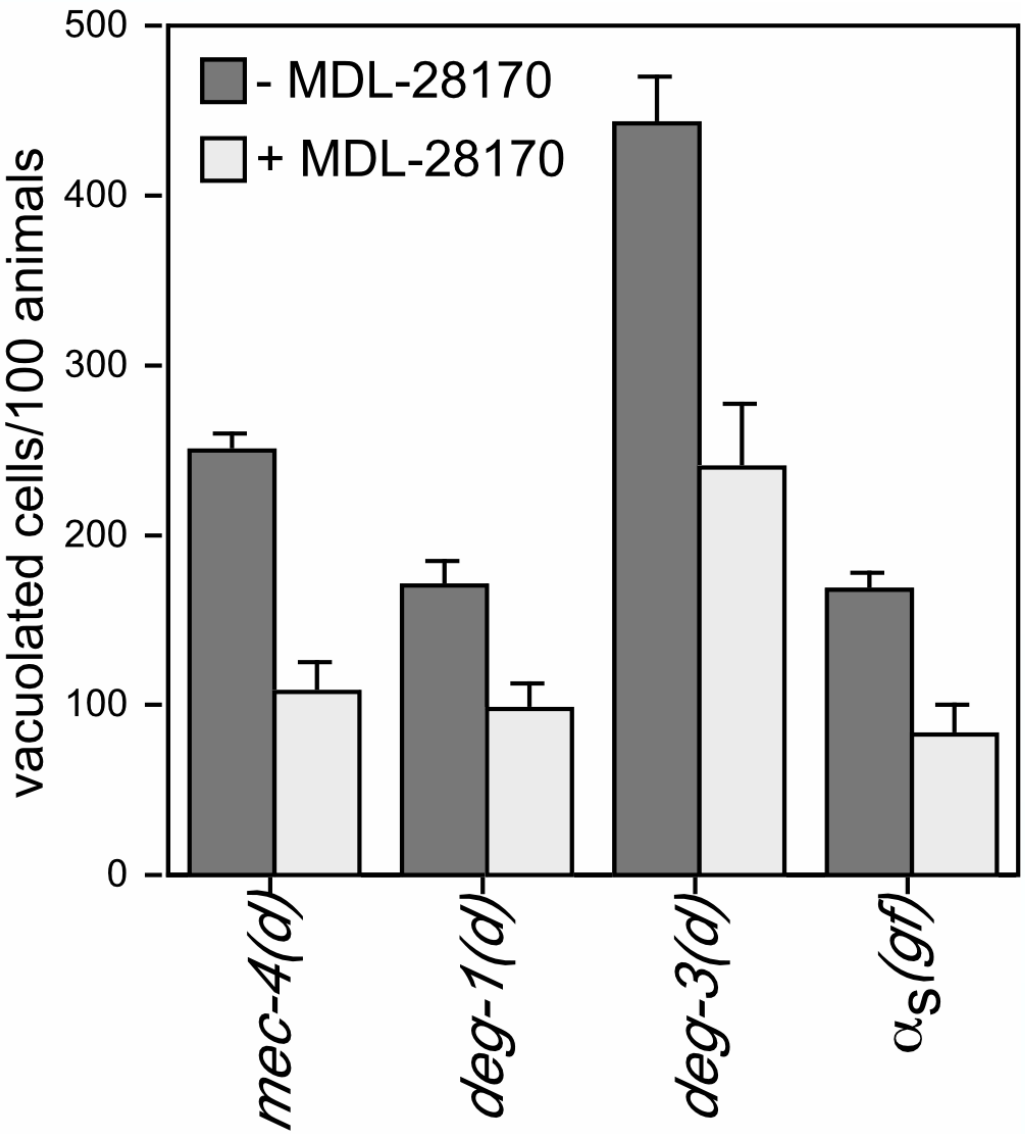
- **Caspase proteases, the mediators of apoptosis, are not required**
  (Syntichaki et al., 2002)

- **Are other cytoplasmic or lysosomal proteases necessary?**

Calcium: a central cell death signal

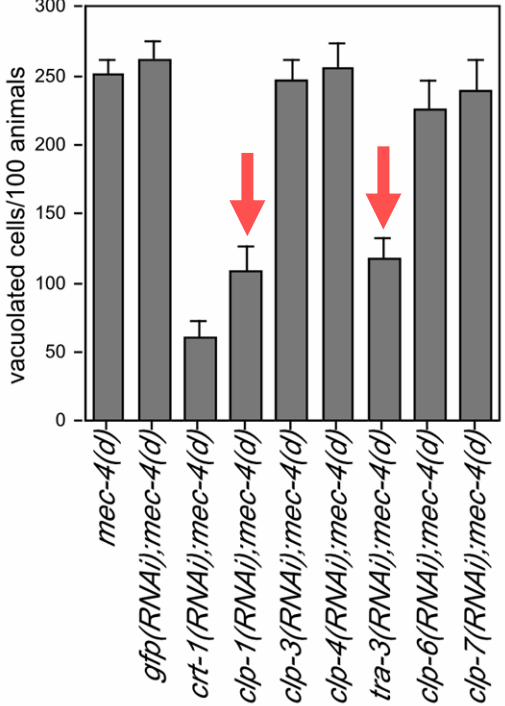


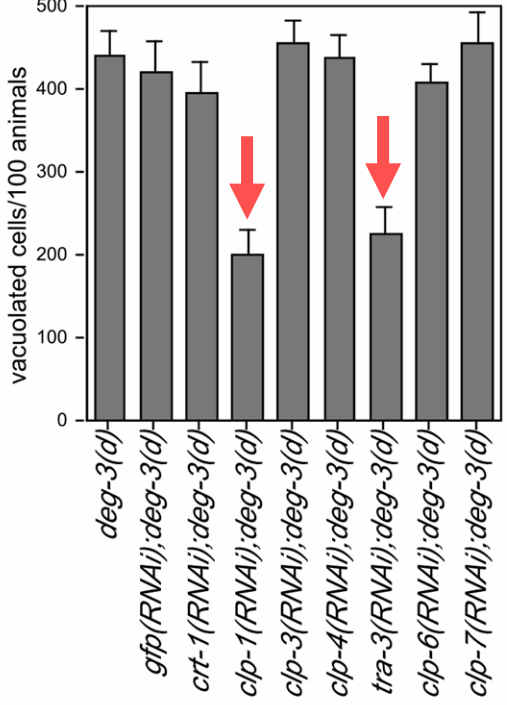
Calpain activity is involved in the necrotic cell death process in *C. elegans*

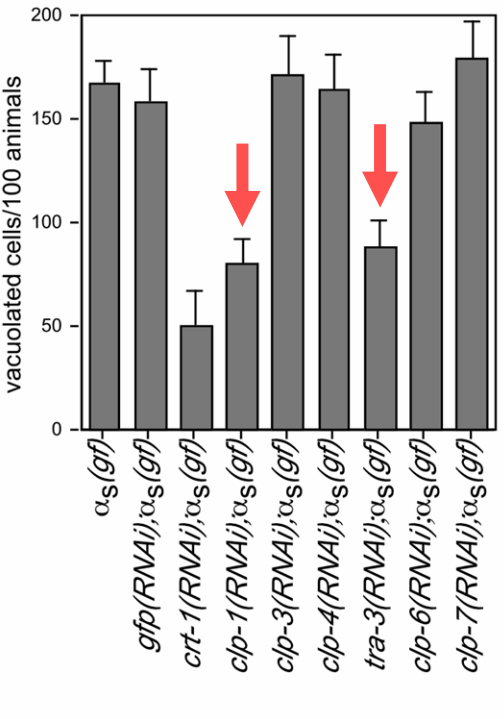


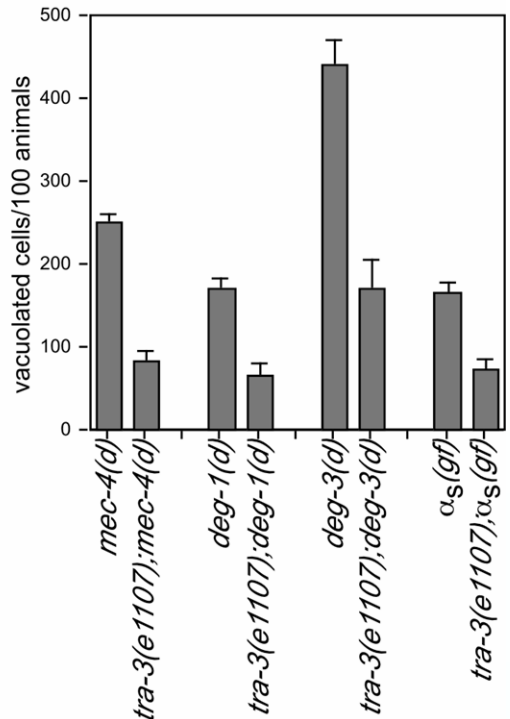
Which calpain?

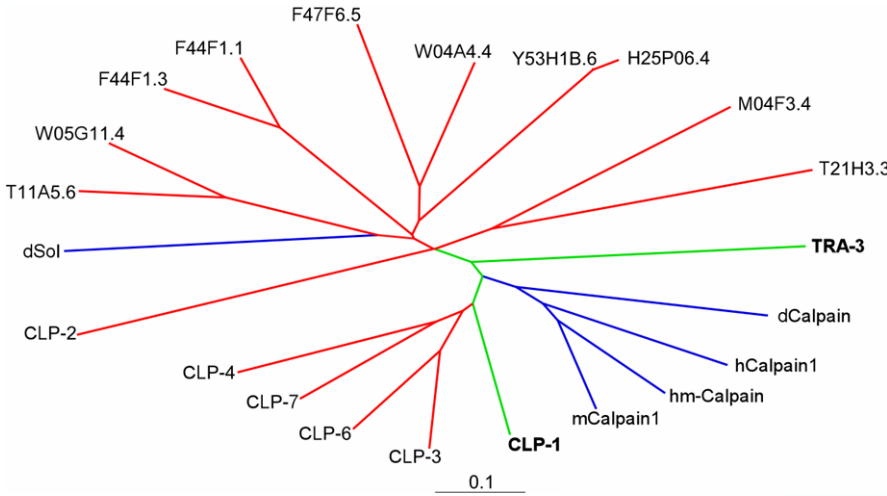
Two specific Calpains, CLP-1 and TRA-3, are required for neurodegeneration in *C. elegans*



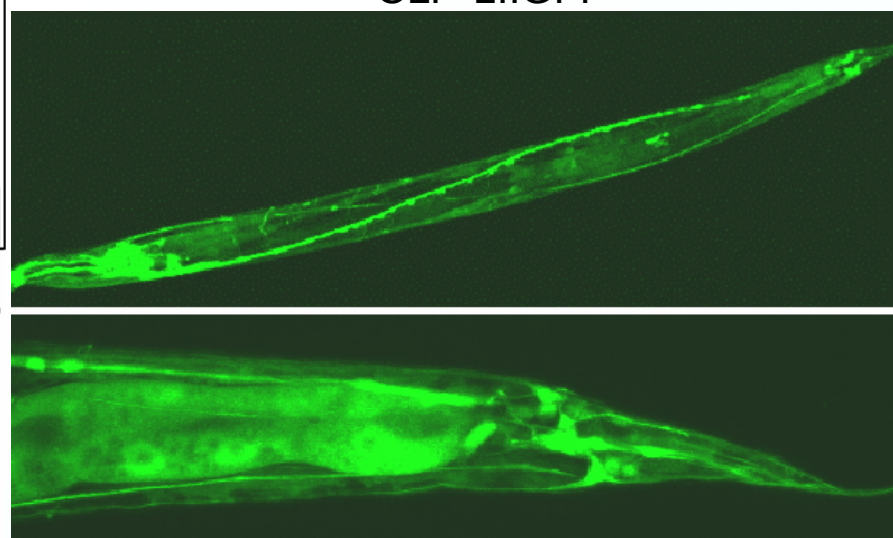




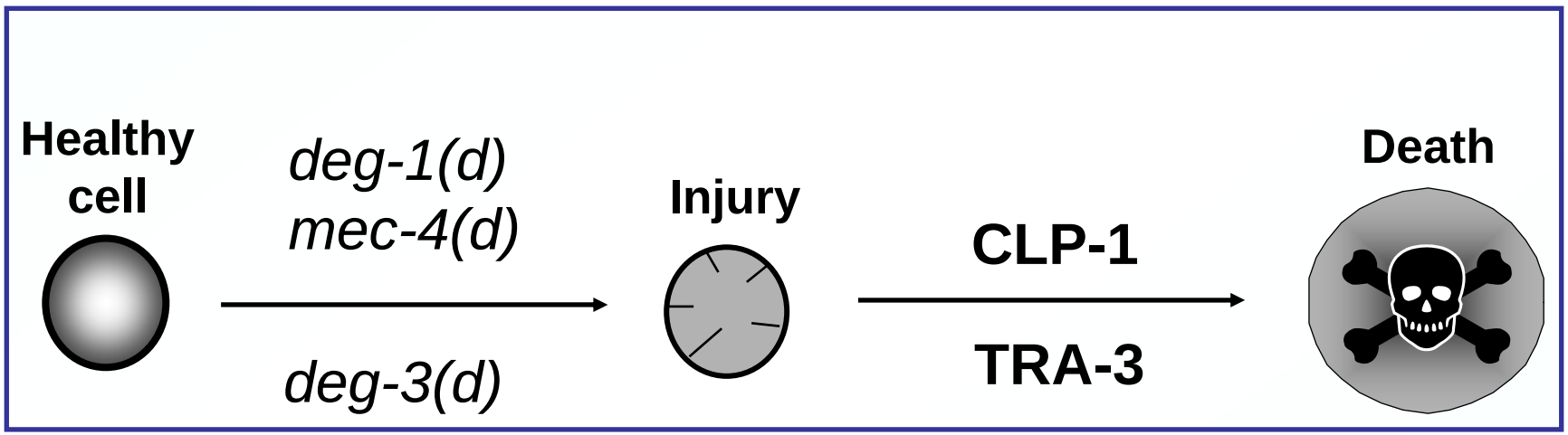




CLP-1::GFP

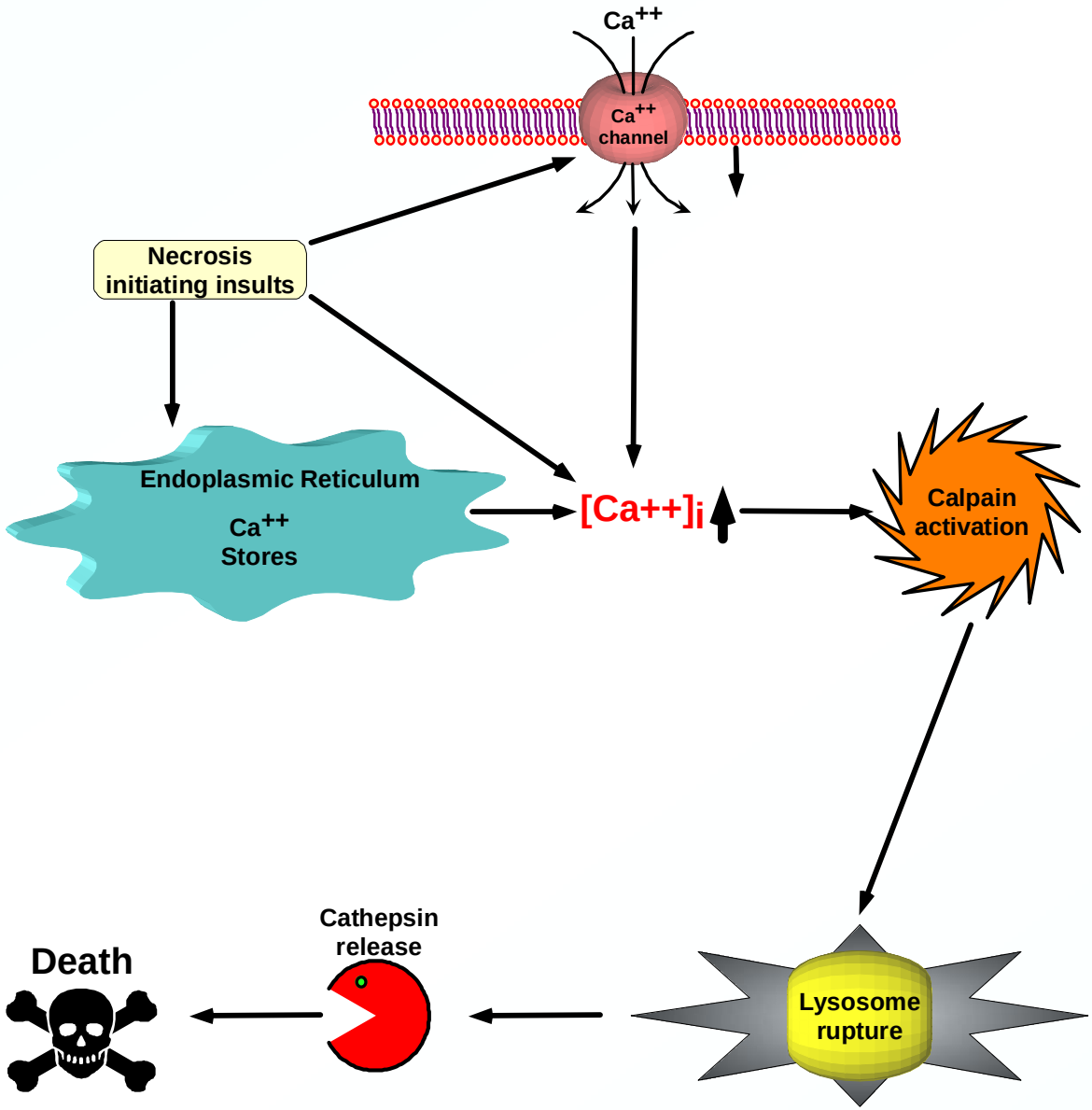


Calpain protease activity is necessary for necrotic cell death in *C. elegans*



What happens next?

The calpain-cathepsin hypothesis



(Yamashima, 1998)

Aspartyl protease activity is required for *mec-4(d)*-induced neurodegeneration

***C. elegans* mutants with diminished aspartyl protease enzymatic activity:**

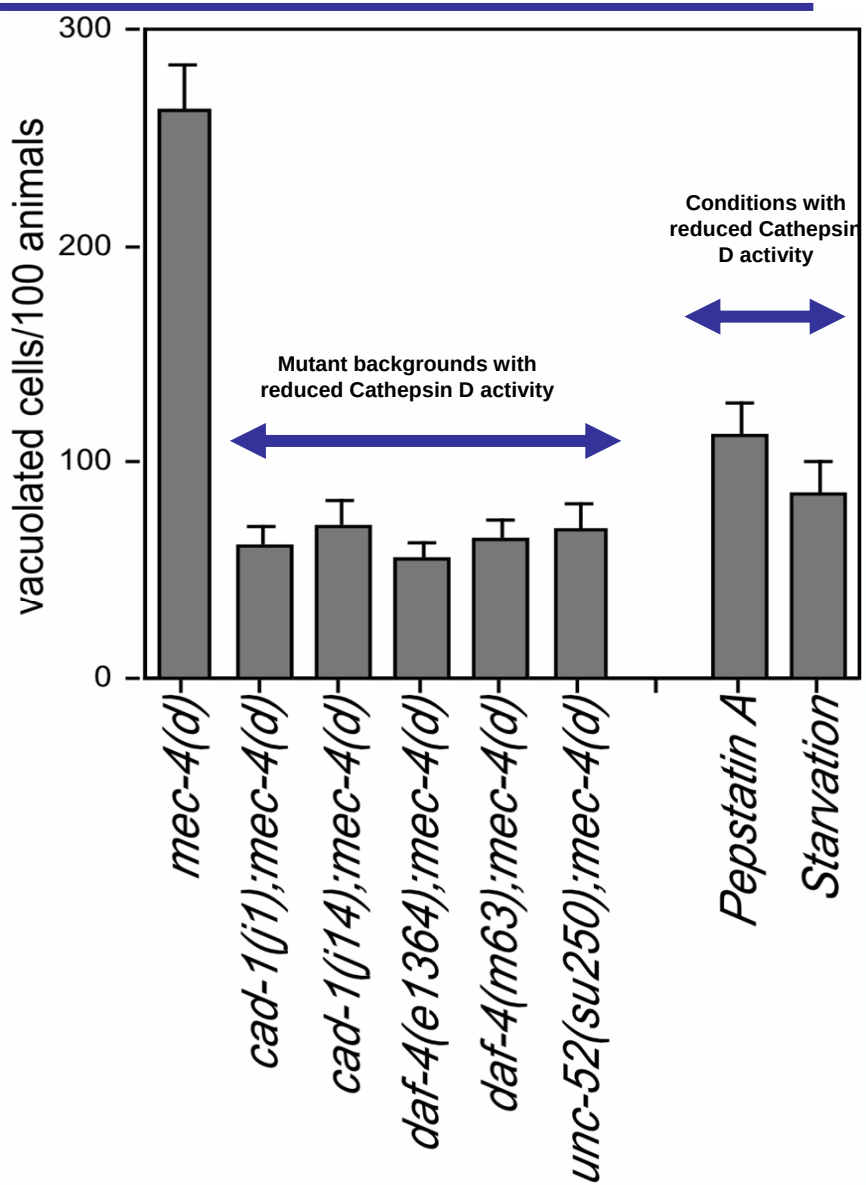
1. ***cad-1****(j1), (j14)* Cathepsin D regulator?
2. ***unc-52****(su250)* Similar to Perlecan
3. ***daf-4****(e1364), (m63)* Type II TGFb receptor kinase

(Jacobson et al., 1988)

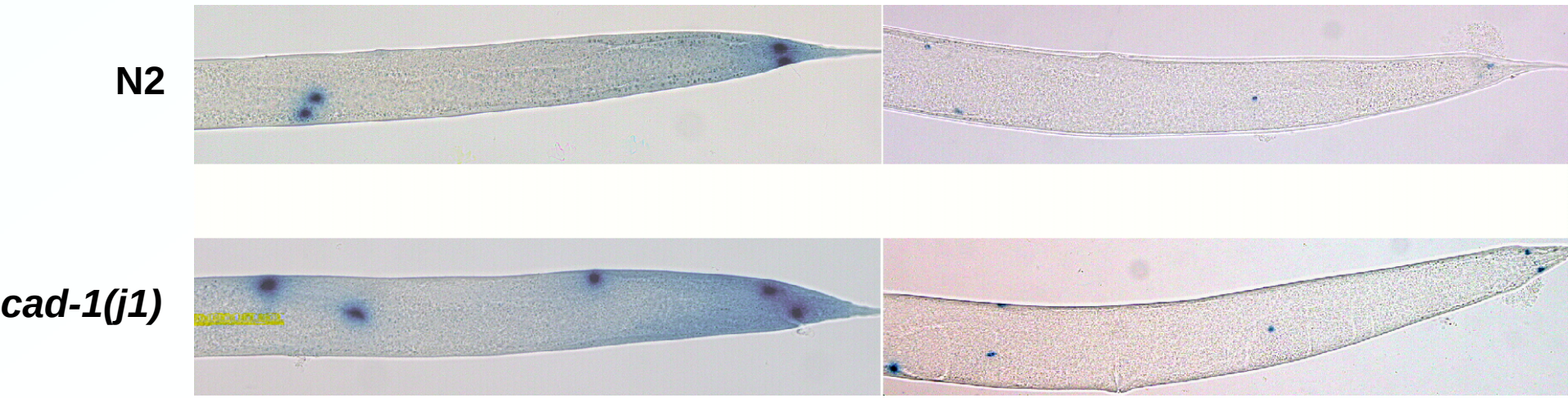
Conditions that reduce aspartyl protease activity in *C. elegans*:

1. **Pepstatin A treatment**
2. **Starvation**

(Hawdon et al., 1989)

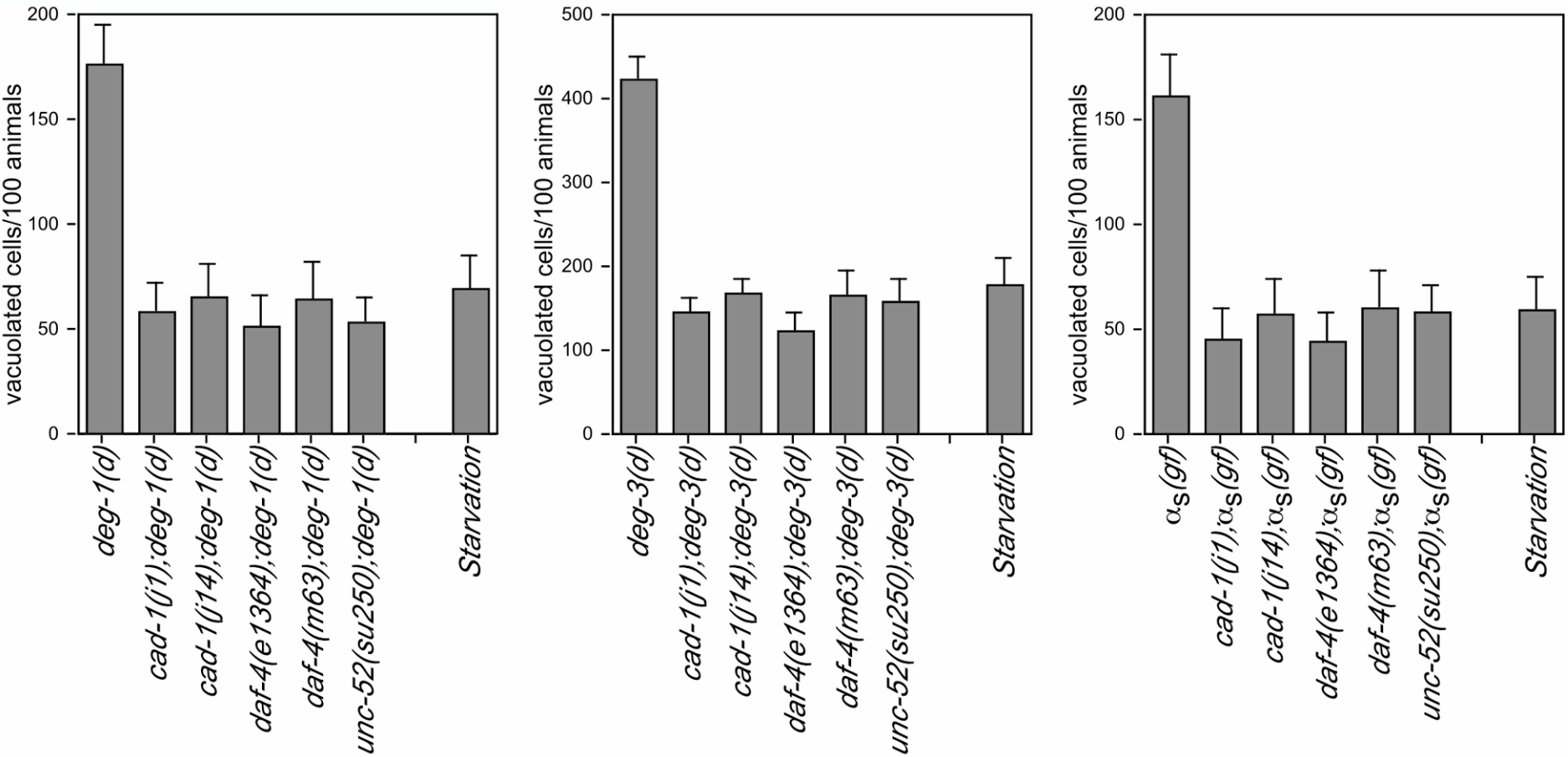


Expression/stability of MEC-4 is not reduced by Cathepsin D depletion



Suppression of necrotic cell death inflicted by *mec-4(d)* is not a result of lower levels of toxic MEC-4 when Cathespin D is depleted

Aspartyl protease activity is required for necrotic cell death inflicted by various genetic lesions in *C. elegans*

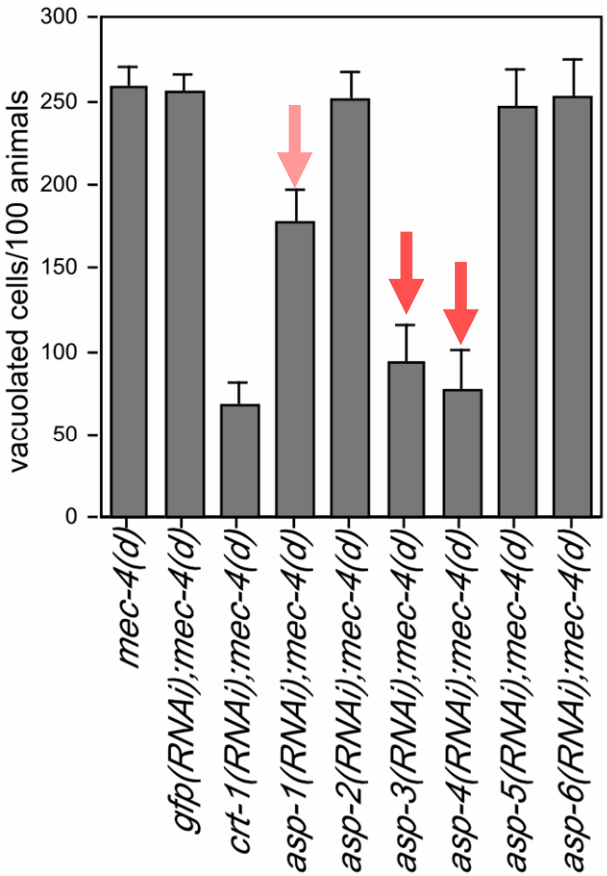


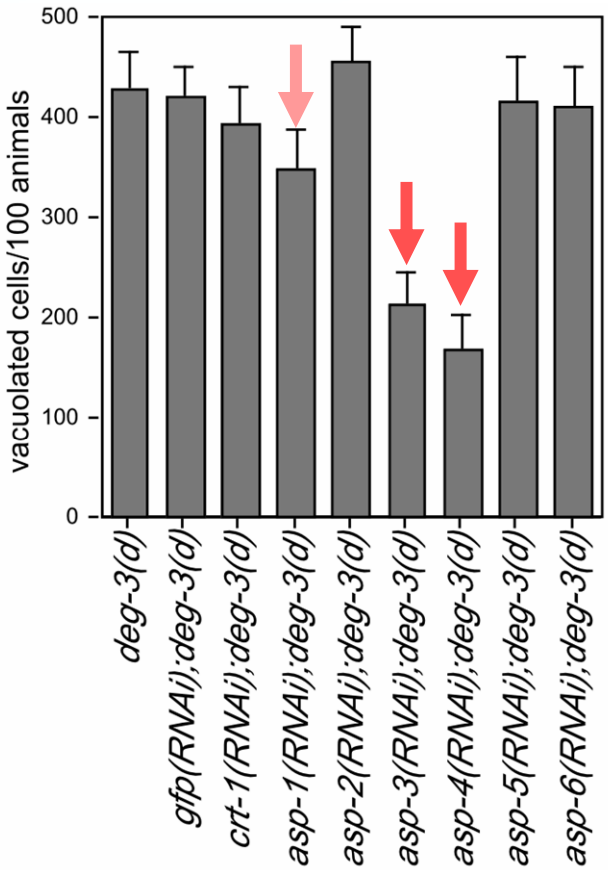
At least six expressed ASpartyl Proteases (Cathepsin Ds) are encoded in the *C. elegans* genome

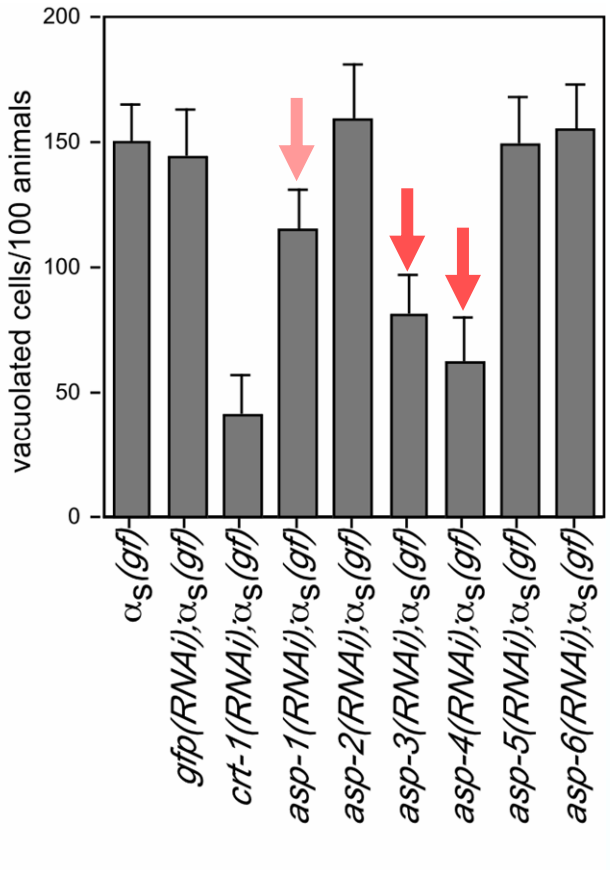
(Tcherepanova et al., 2000)

Which aspartyl protease?

ASP-3 and ASP-4 are Mostly Required for Necrotic Cell Death in *C. elegans*

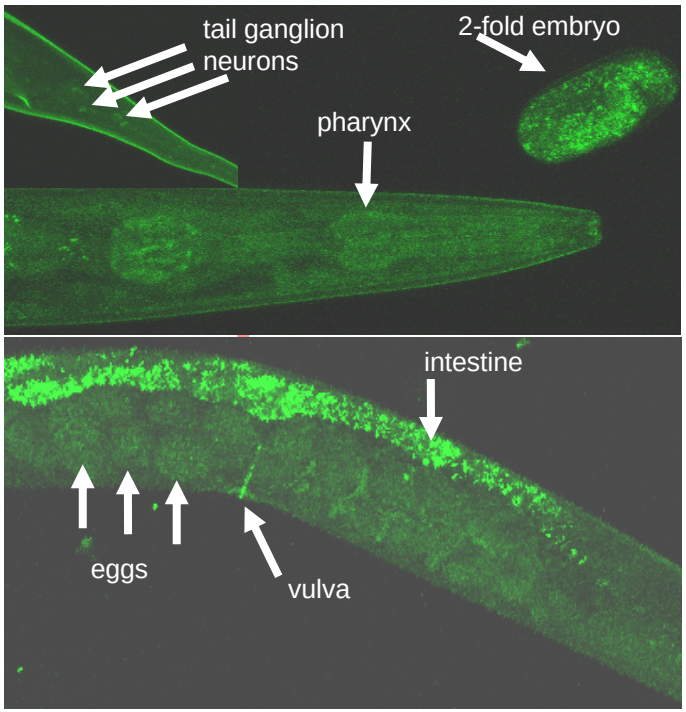




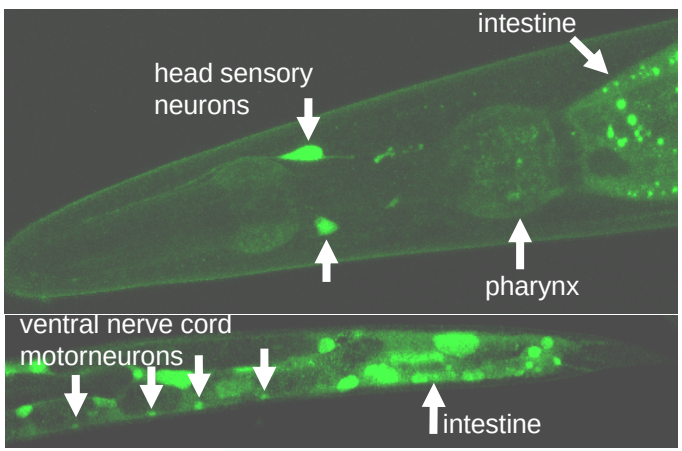


The bulk of aspartyl protease activity required for execution of necrotic cell death in *C. elegans* is contributed by the cytoplasmic ASP-3 and ASP-4 aspartyl proteases

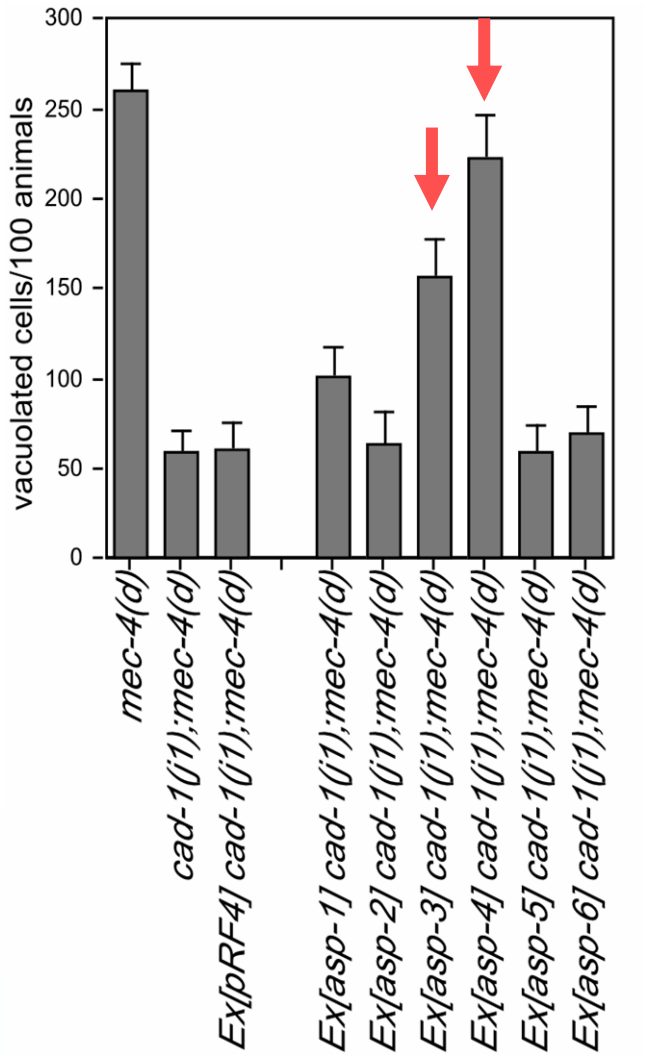
ASP-3



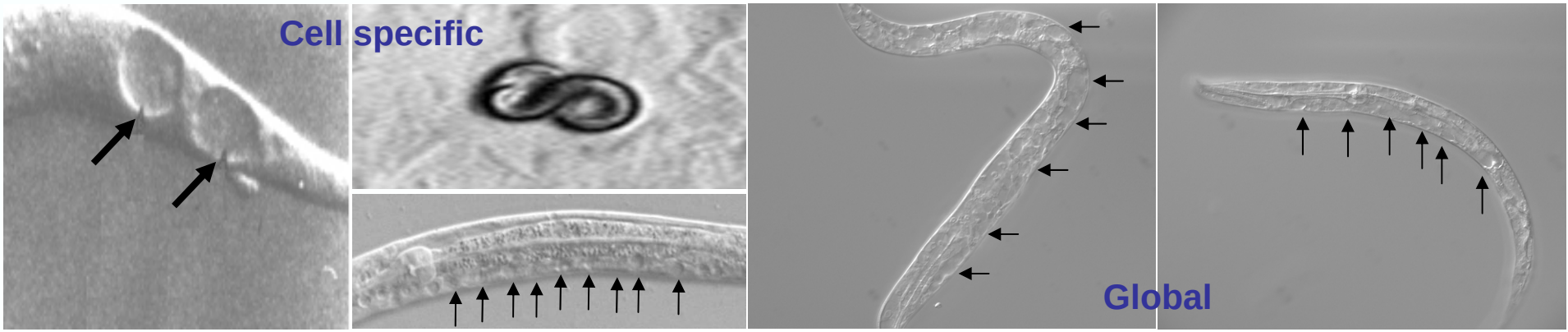
ASP-4

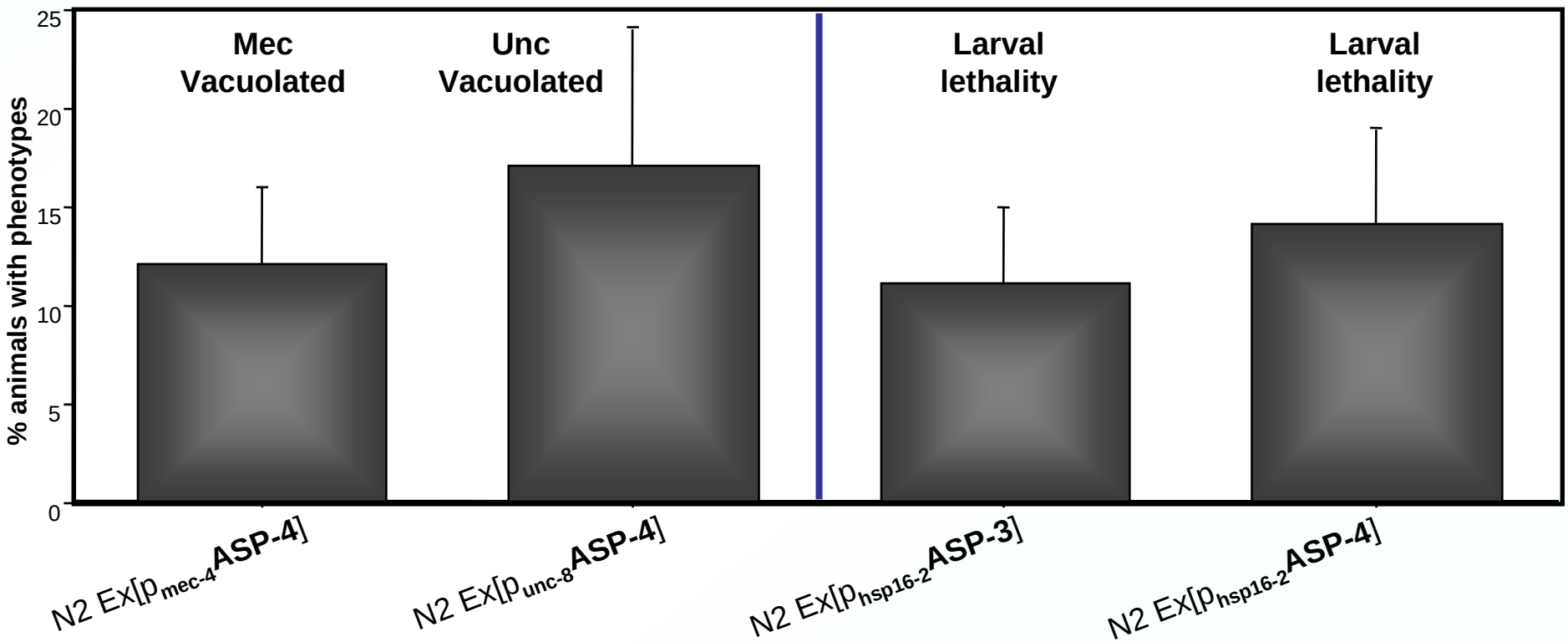


Lysosomal and cytoplasmic localization

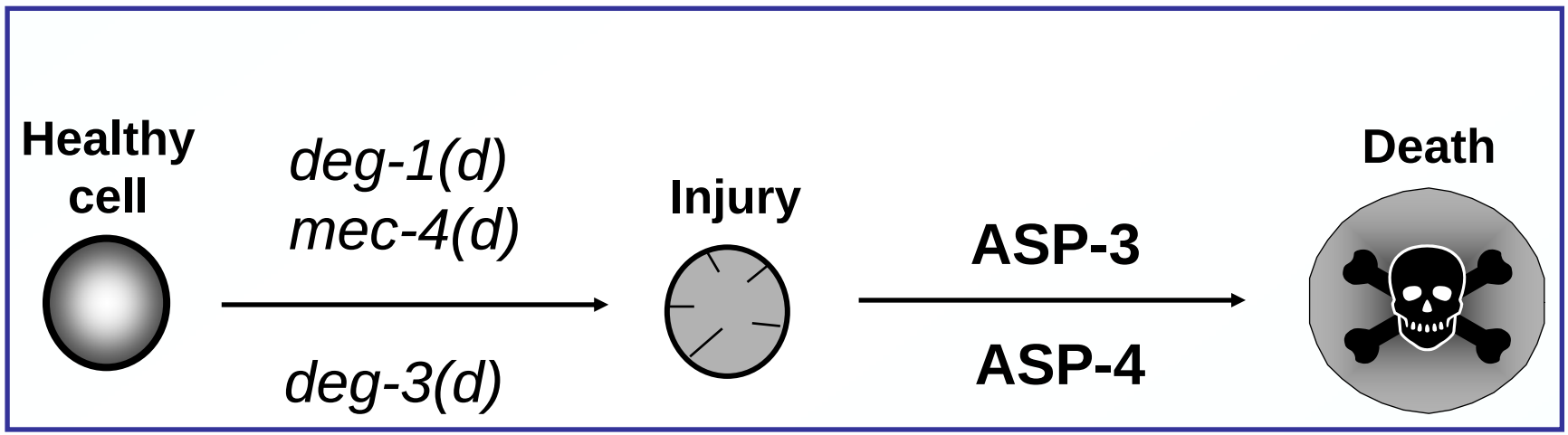


Overexpression of aspartyl proteases is sufficient to induce necrotic cell death in the absence of upstream insults

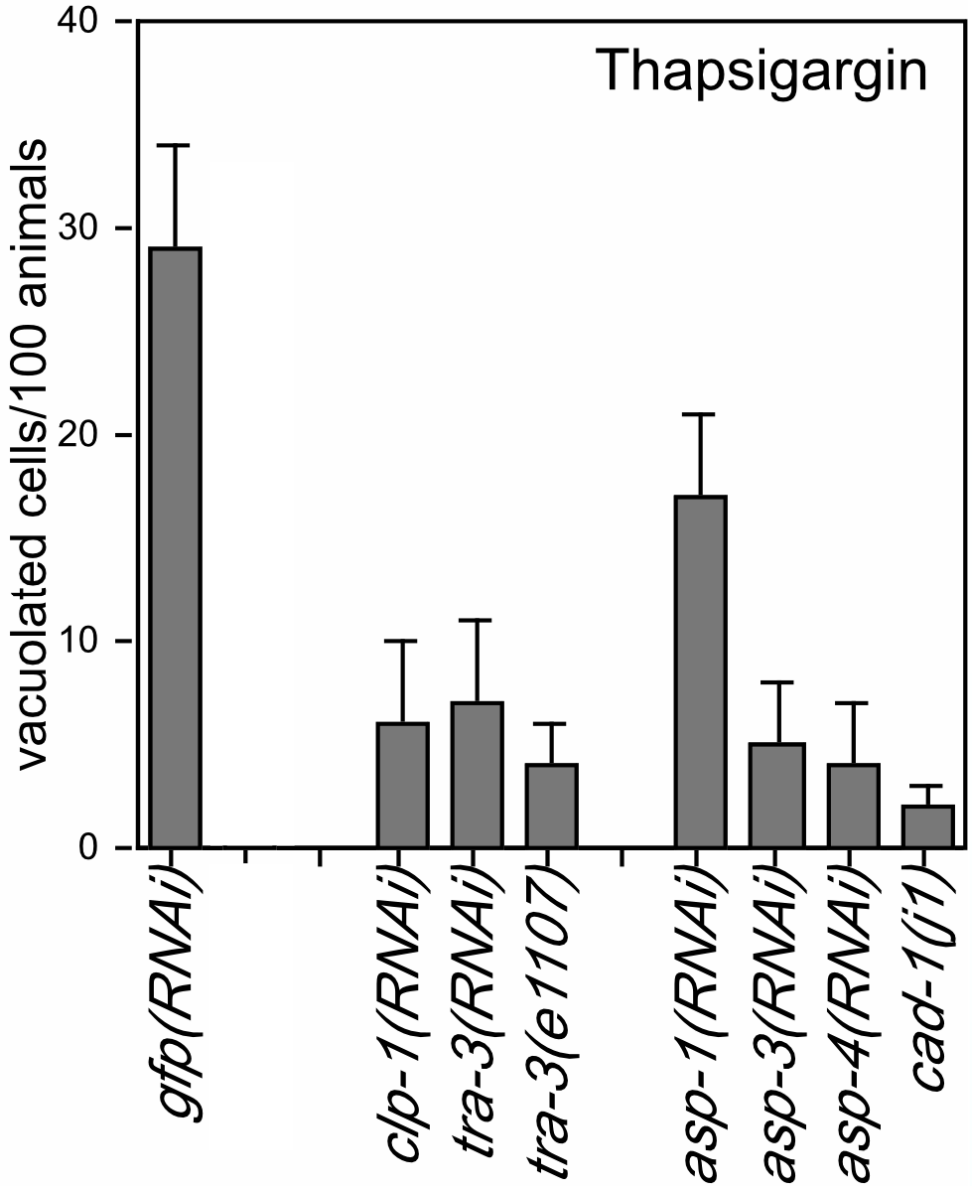




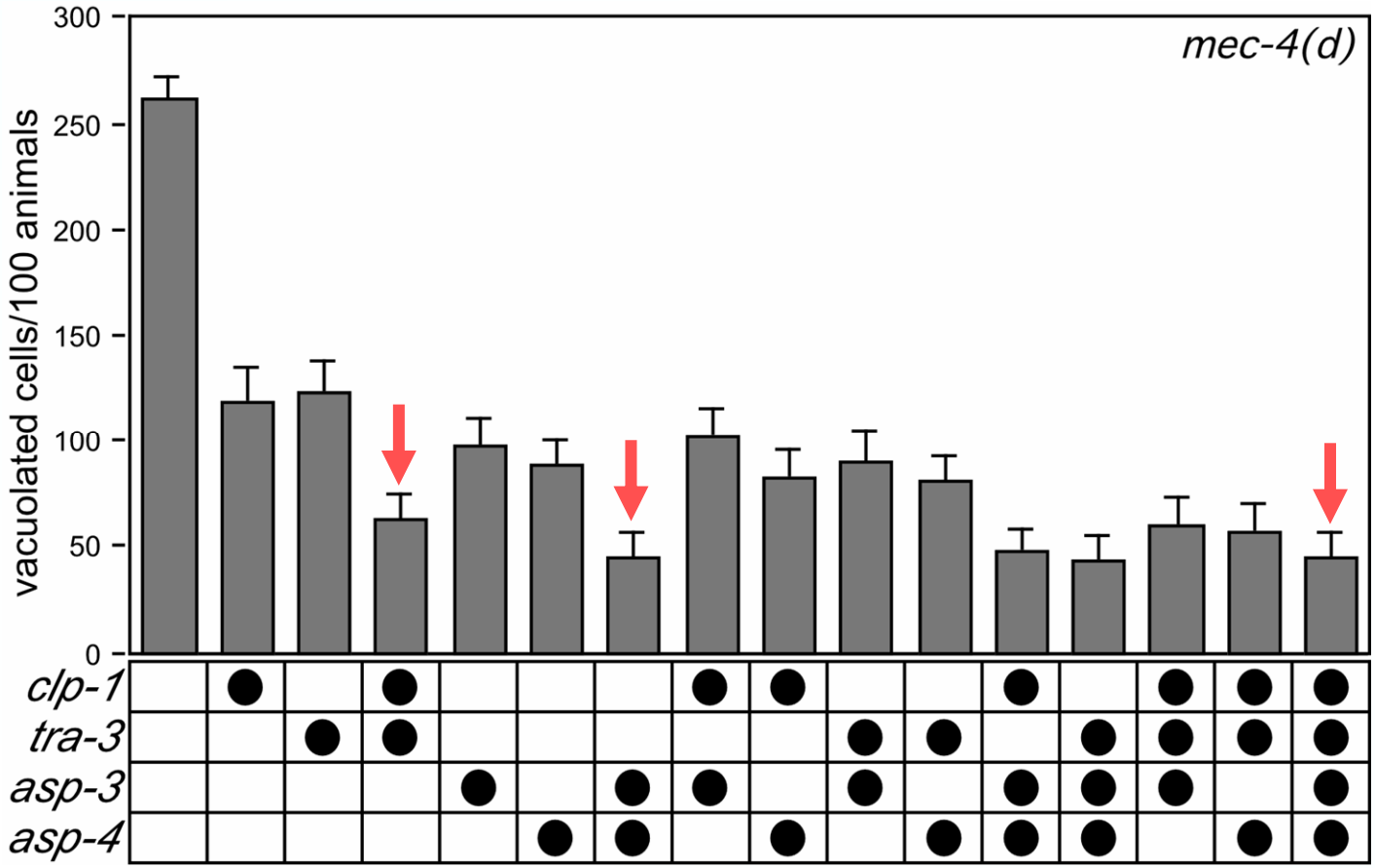
Aspartyl protease activity is both necessary and sufficient for necrotic cell death in *C. elegans*

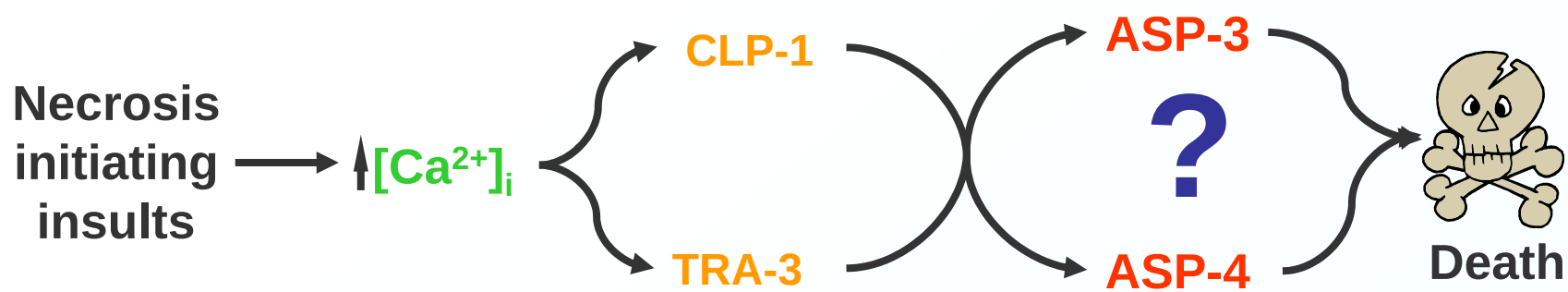


Calpains and Aspartyl proteases function downstream of calcium signaling to mediate death

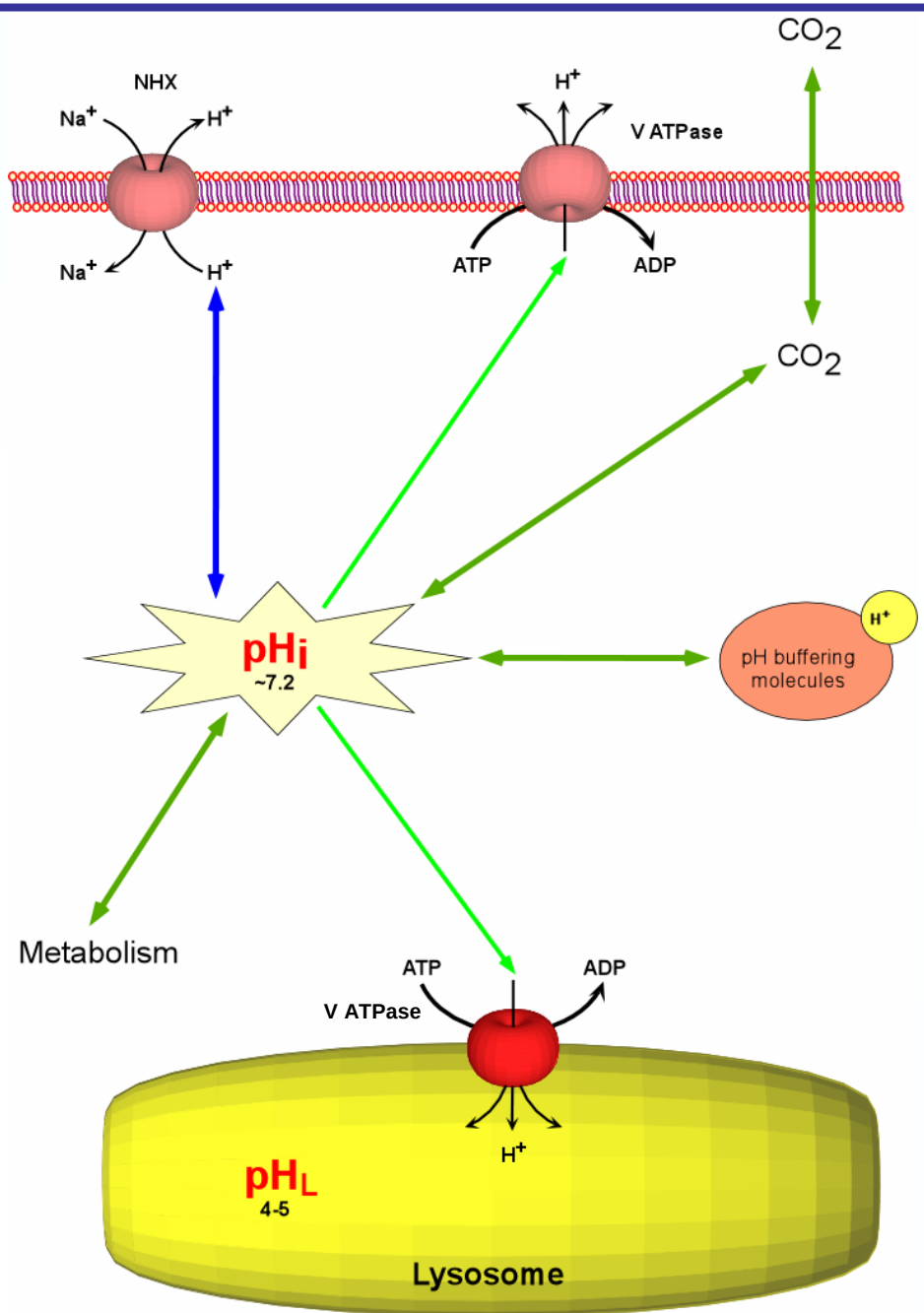


Calpains act sequentially with aspartyl proteases to facilitate cell death



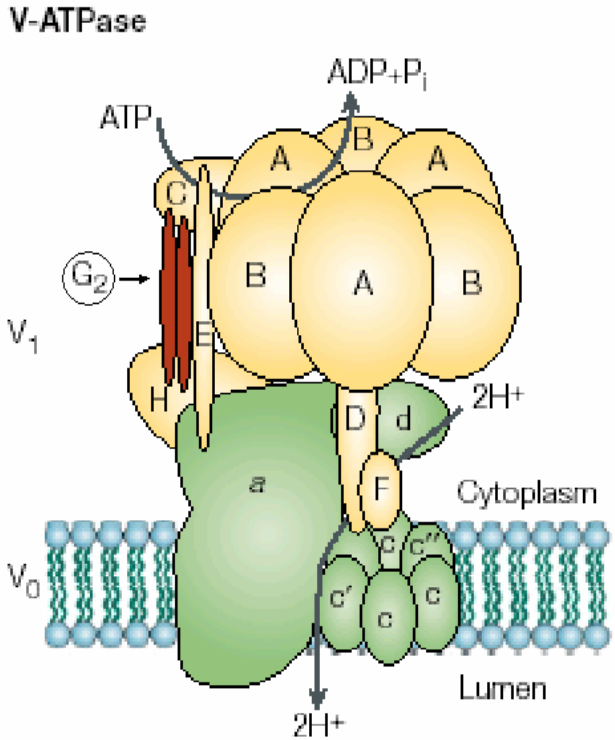


Cellular pH homeostasis



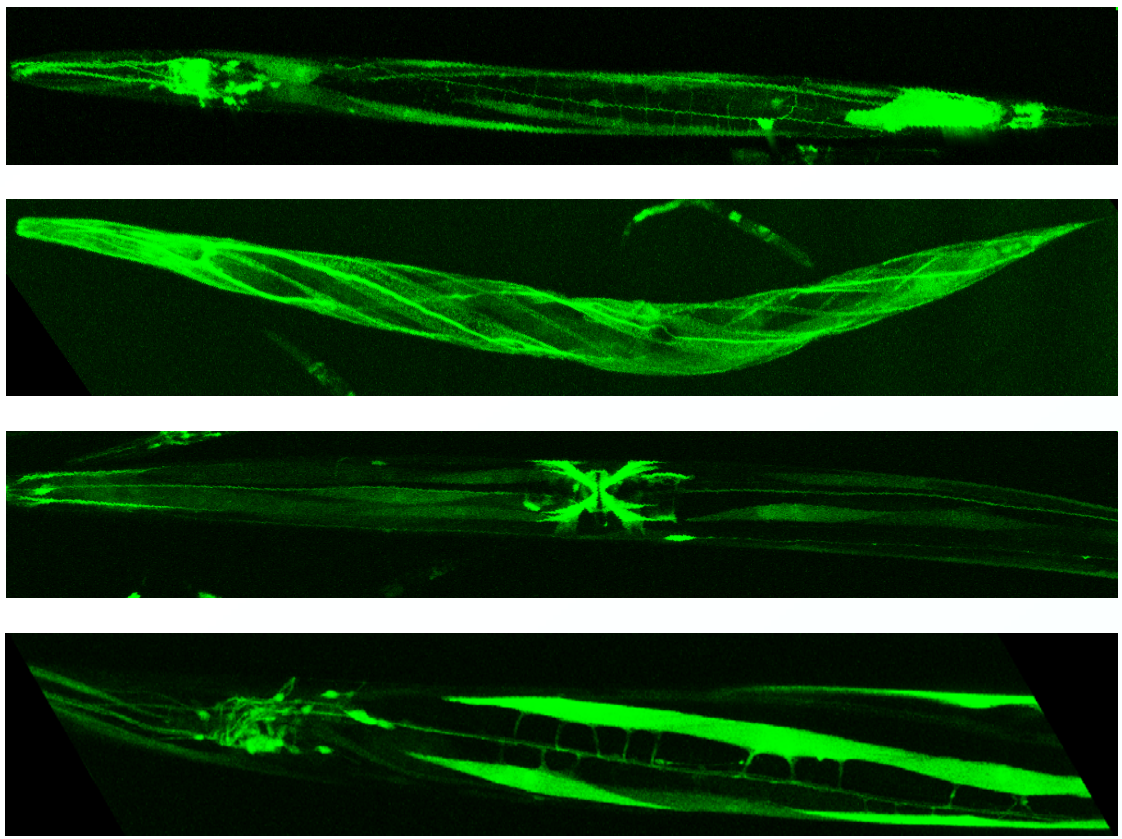
V ATPase: **Vacuolar H^+ ATPase**
NHX: **Na^+/H^+ exchanger**

The V-ATPase: A universal multi-subunit proton pump



Nishi and Forgac, 2002

Expression of the regulatory G subunit in *C. elegans*



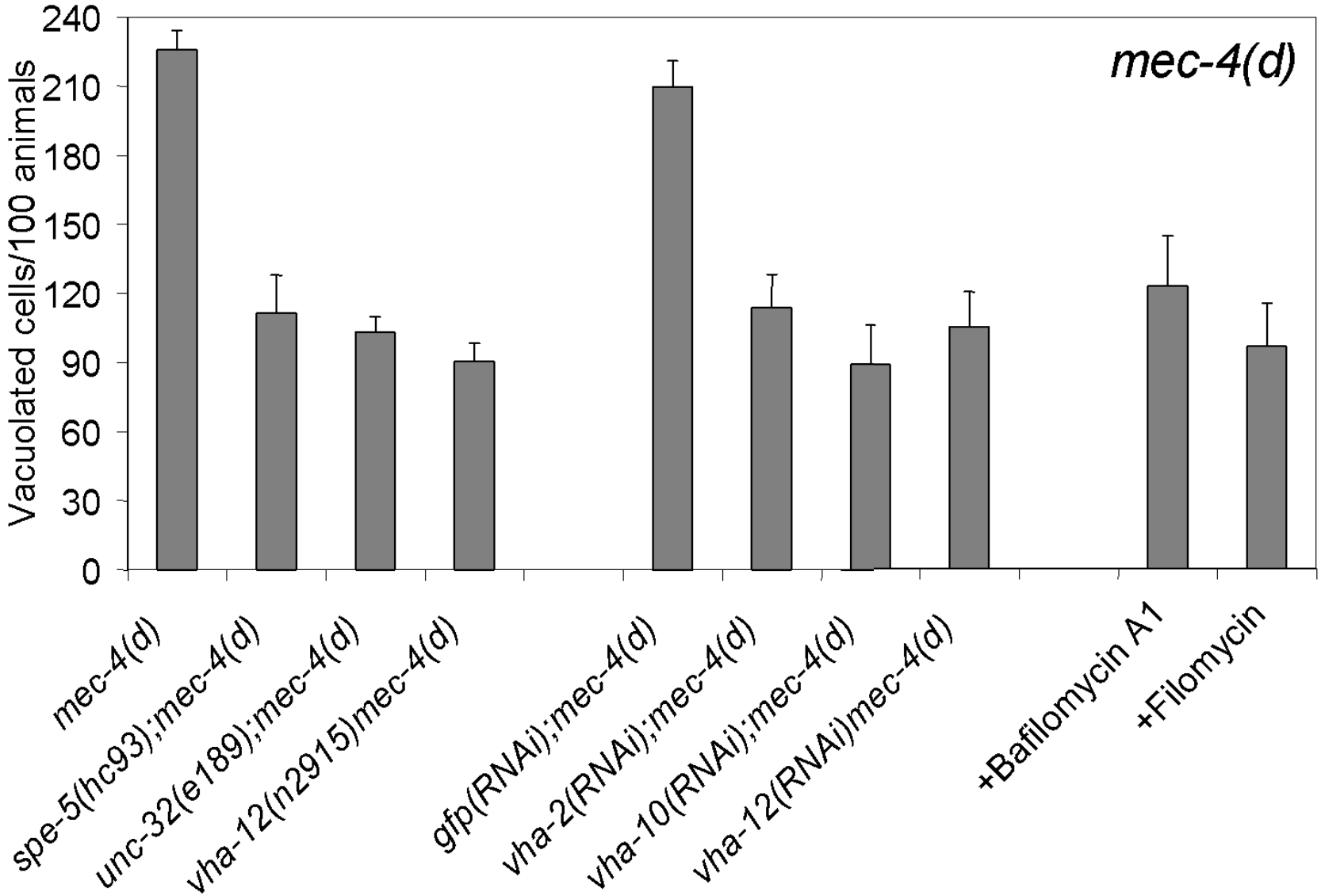
p*vha-10*GFP

V-ATPase G subunit

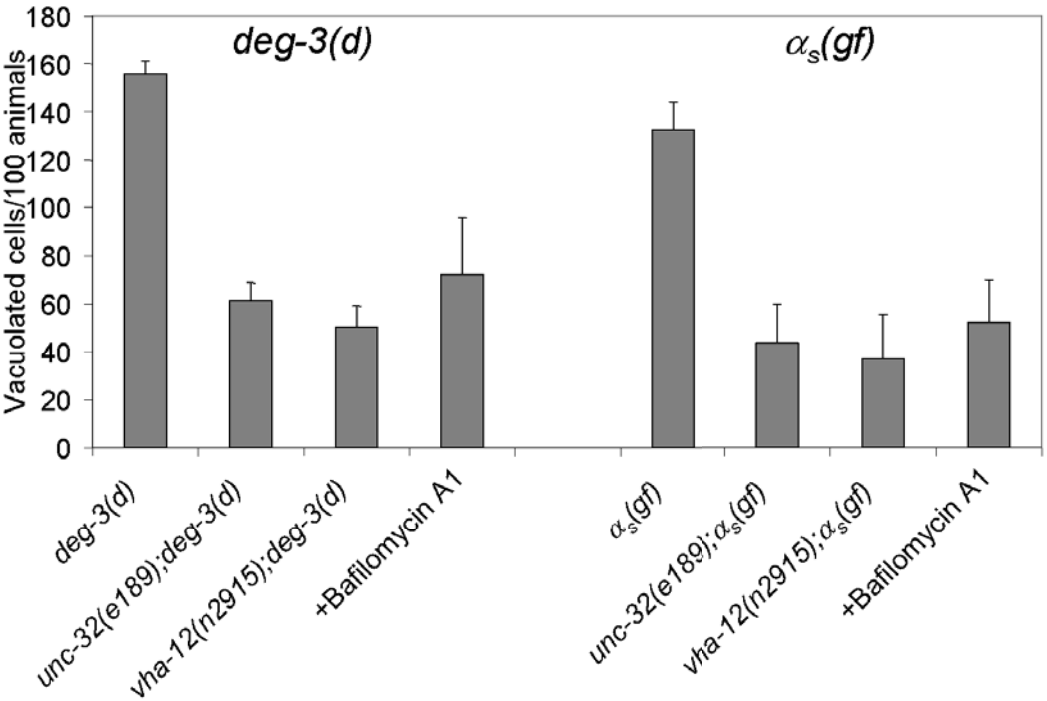
C. elegans V-ATPase genes:

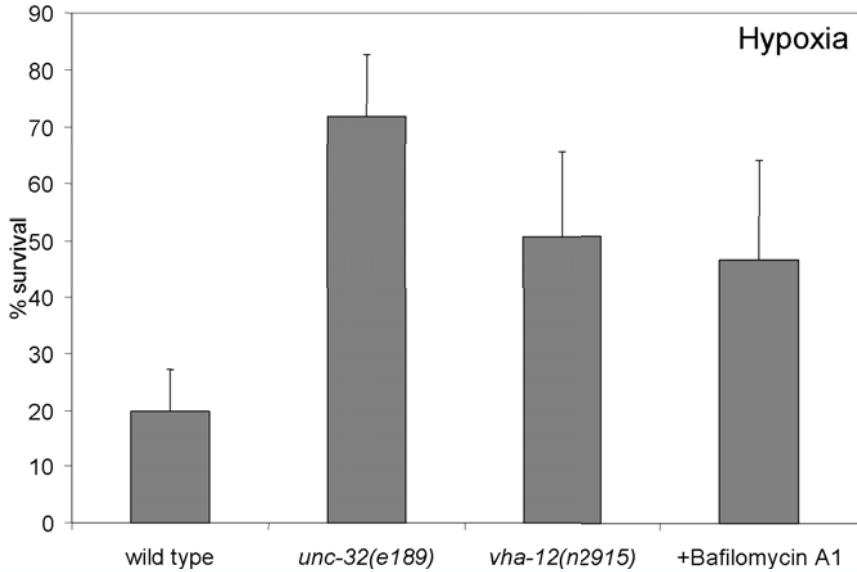
- *vha-1 to 16*
- *unc-32*
- *spe-5*

mec-4(d)-induced neurodegeneration is attenuated when V-ATPase activity is reduced



V-ATPase activity is generally required for full-blown neurodegeneration



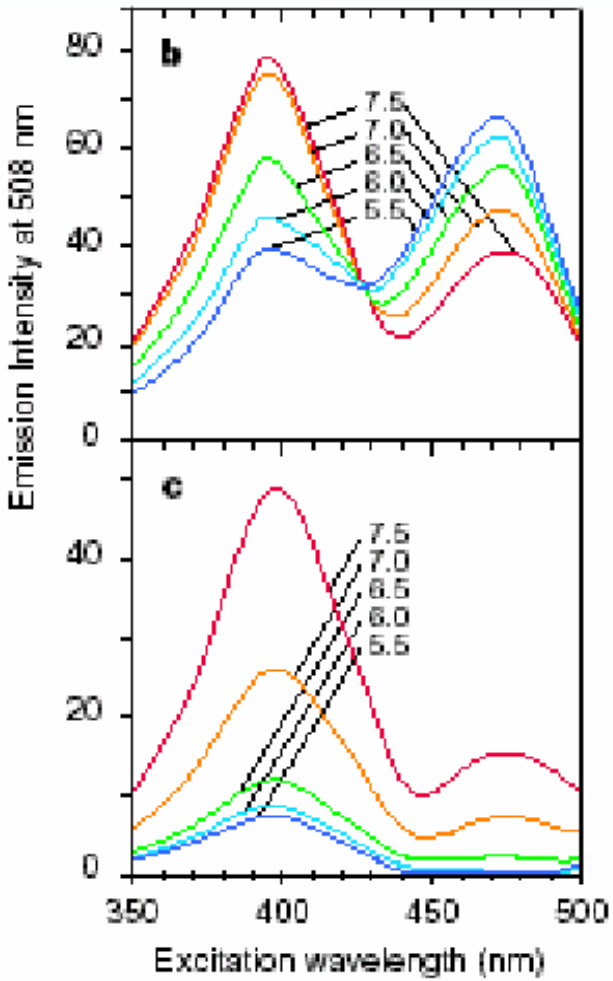


V-ATPases are required for the proper execution of necrosis in *C. elegans* neurons…

…implicating cellular pH homeostasis in neuronal demise

Monitoring organelle and cytoplasmic pH during necrosis with pHluorins

Excitation ratio change of ratiometric and super-ecliptic pHluorins between pH 7.5 and 5.5



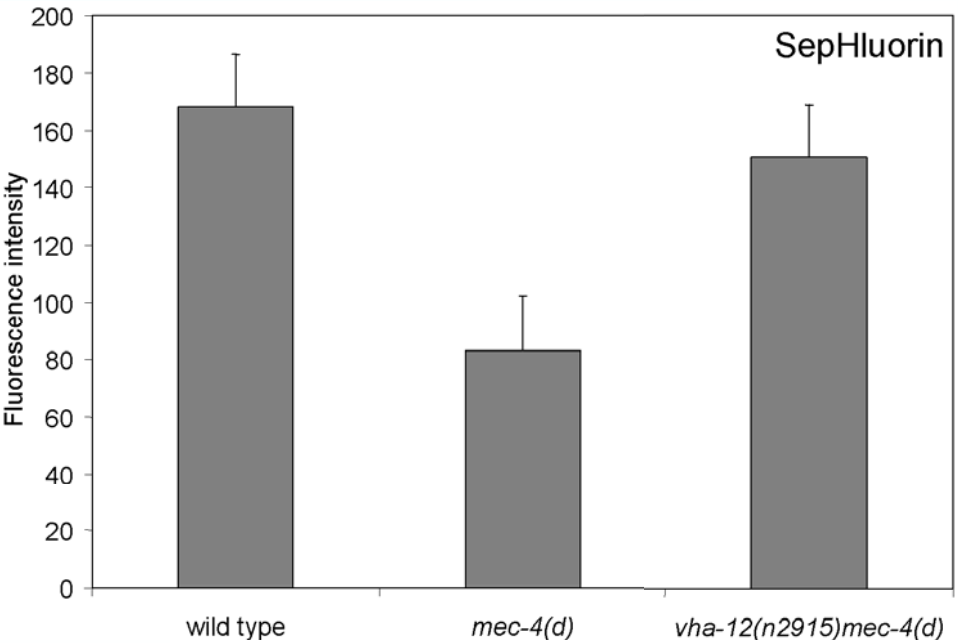
Fluorescence excitation spectra of…

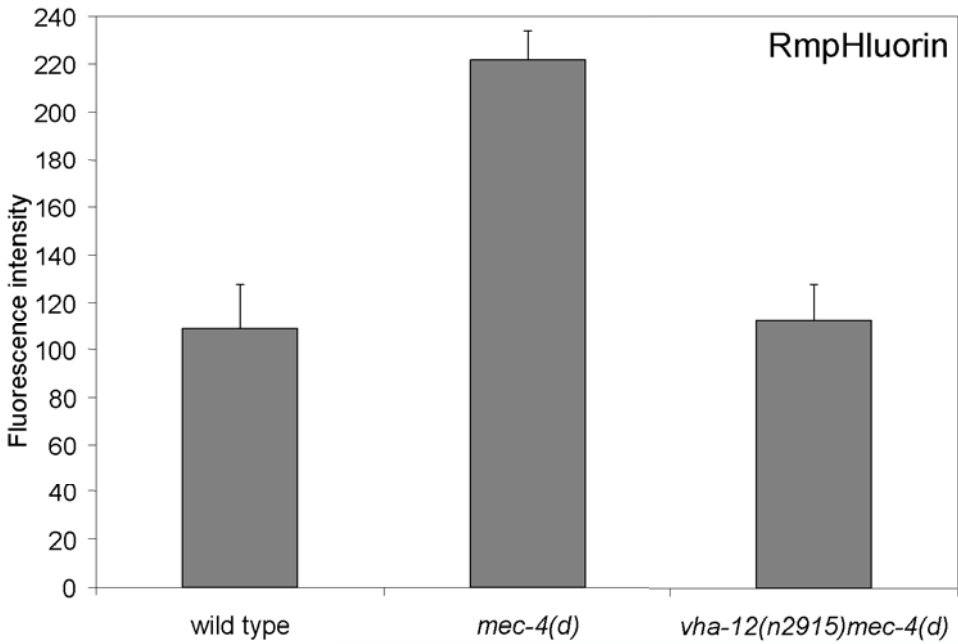
…Ratiometric pHluorin

…Super-ecliptic pHluorin

(Miesenbock G. et al., 1998)

Cytoplasmic acidification of neurons undergoing necrotic destruction





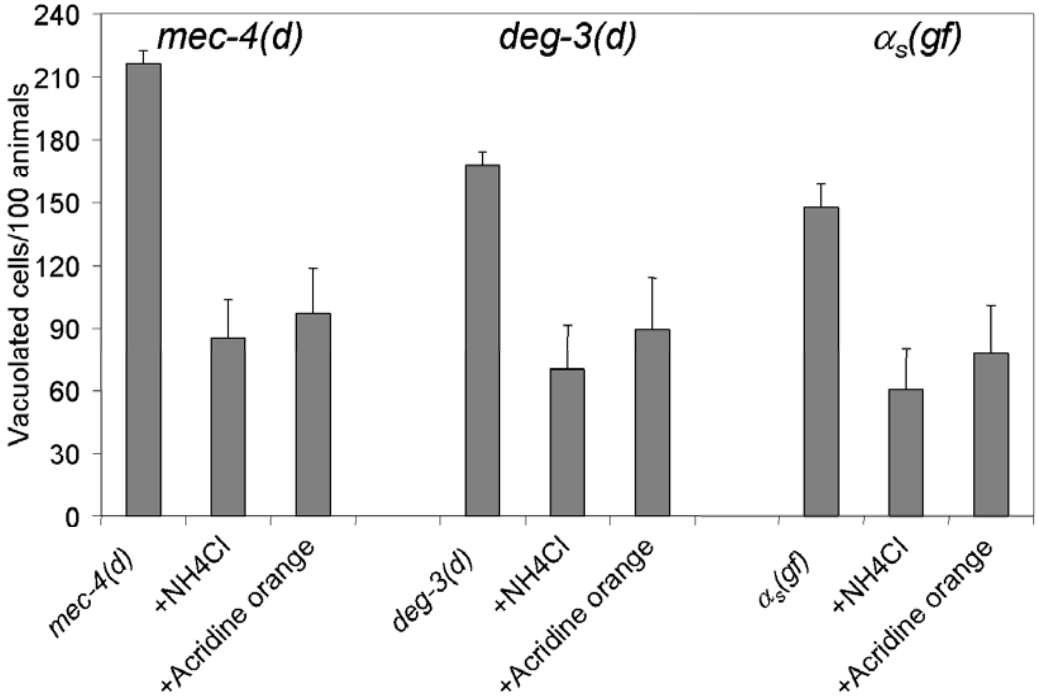
✓ Acidification is partly alleviated when the function of V-ATPases is compromised

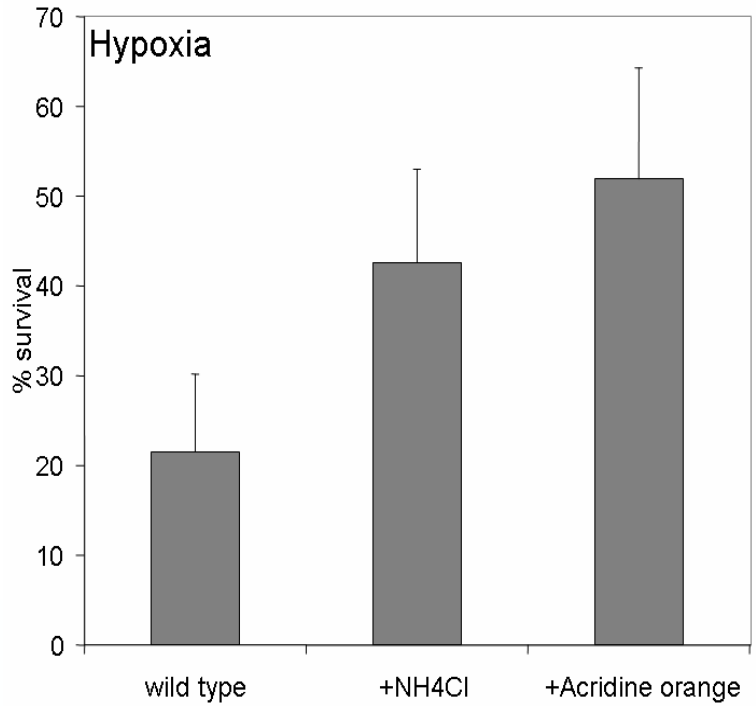
Necrotic cell death is accompanied by a marked decrease in cytoplasmic pH

Dysfunction of V-ATPase…

- …prevents acidification of neurons
- …suppresses neurodegeneration

Alkalization of endosomal compartments ameliorates necrotic cell death





Intracellular and lysosomal pH is an important determinant of necrotic cell death in *C. elegans*

A working model of a deadly cascade in *C. elegans*