The renaissance of caspase 2: The "Cinderella caspase"

General idea:

Caspase 2 may be a "master" initiator caspase

The evidence:

1) It induces cyt c release/C9/C3 activation when overexpressed

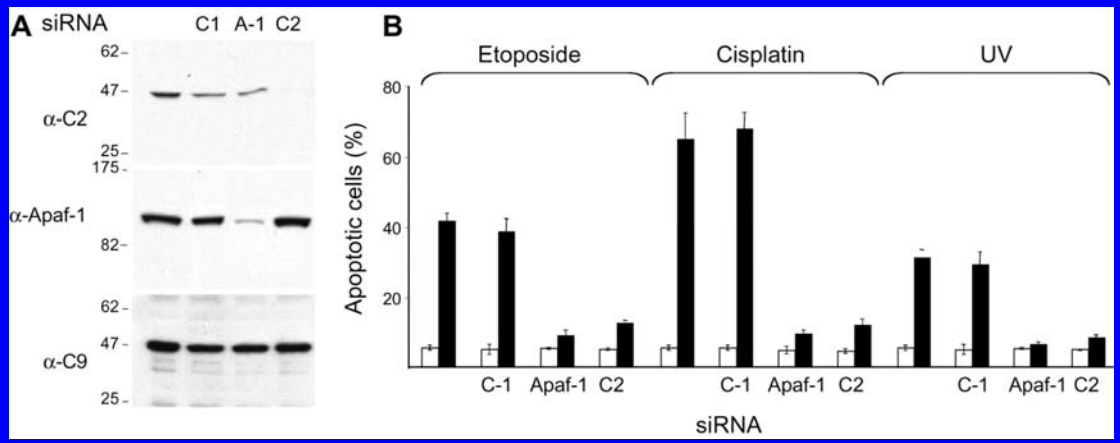
2) It may have such an action on isolated mitochondria

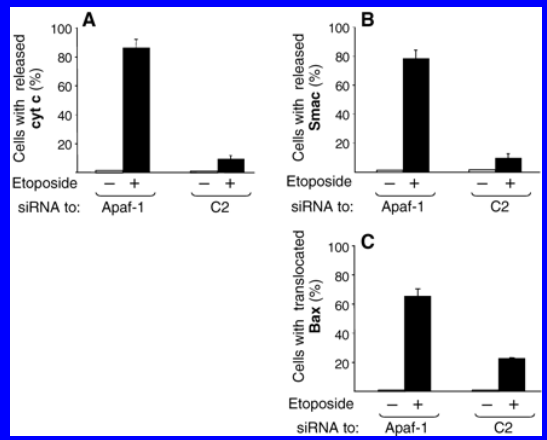
3) More importantly, in certain tumor cell lines, siRNA against caspase 2 protected from etoposide-induced death and cyt c release (Lassus et al., 2002)

4) It can dimerize, also form HMW complexes; in an in vitro system, formation of this complex was independent of the apoptosome and was associated with its enzymatic activation

Guo et al., 2002; Paroni et al., 2002; Lassus et al., 2002; Robertson et al., 2002; Kumar and Vaux, 2002; Read et al., 2003

Involvement of caspase 2 in DNA damage-induced death?

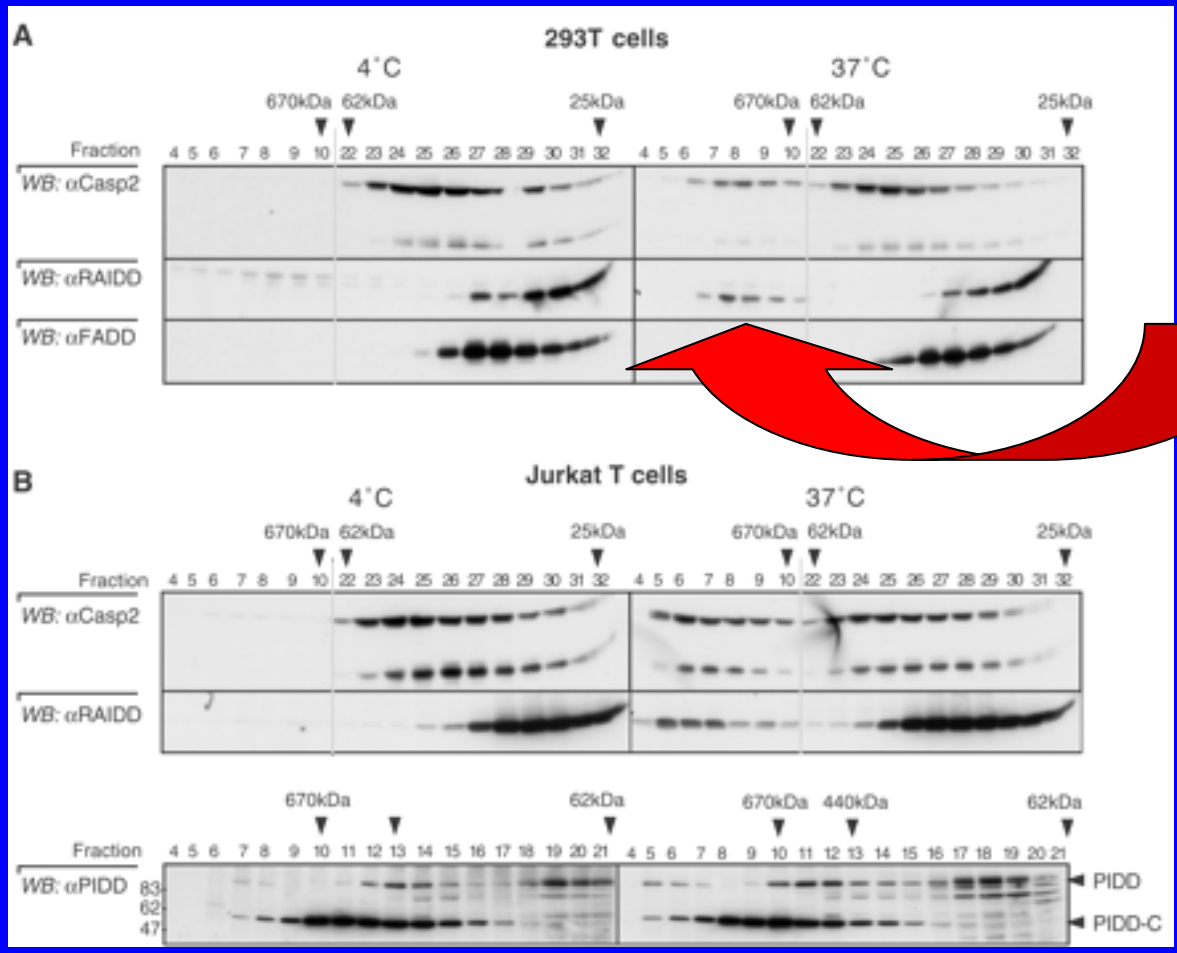




But another siRNA targeted against caspase 2 did not inhibit death……

Lassus et al., 2002

Is RAIDD present within the C2-containing HMW complex?

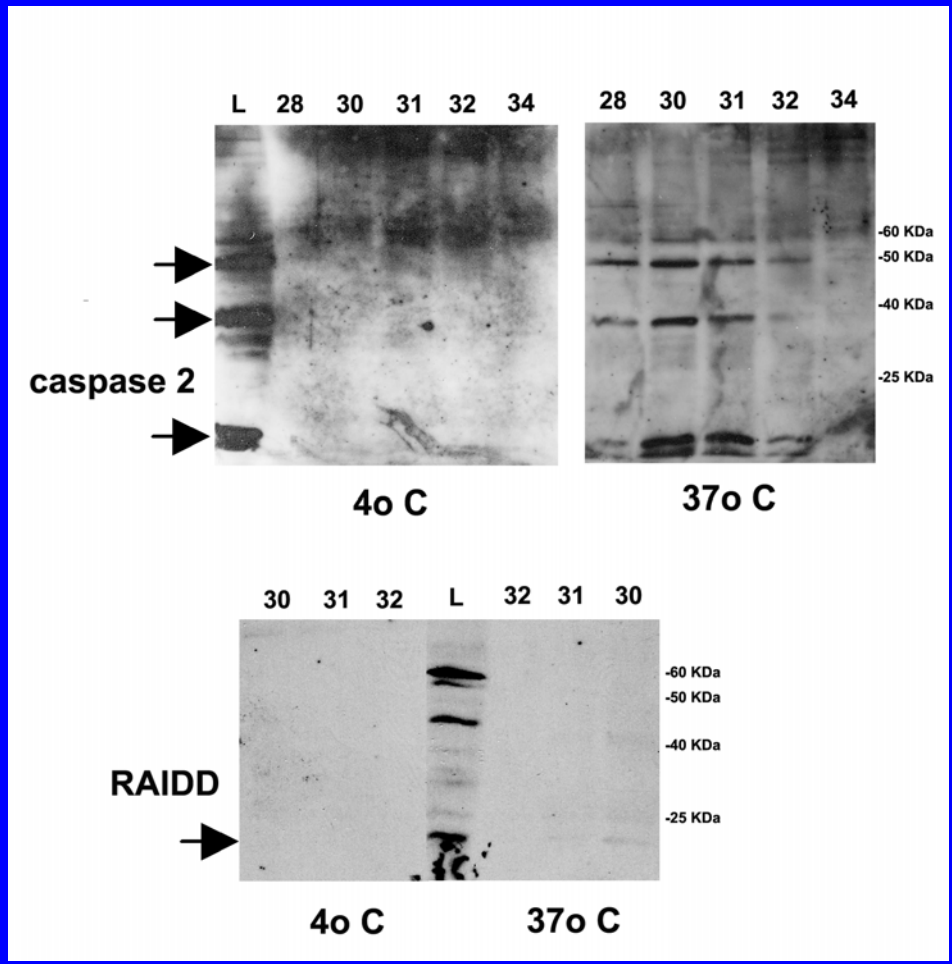


Yes!!

But, this contrasts with Read et al., 2003

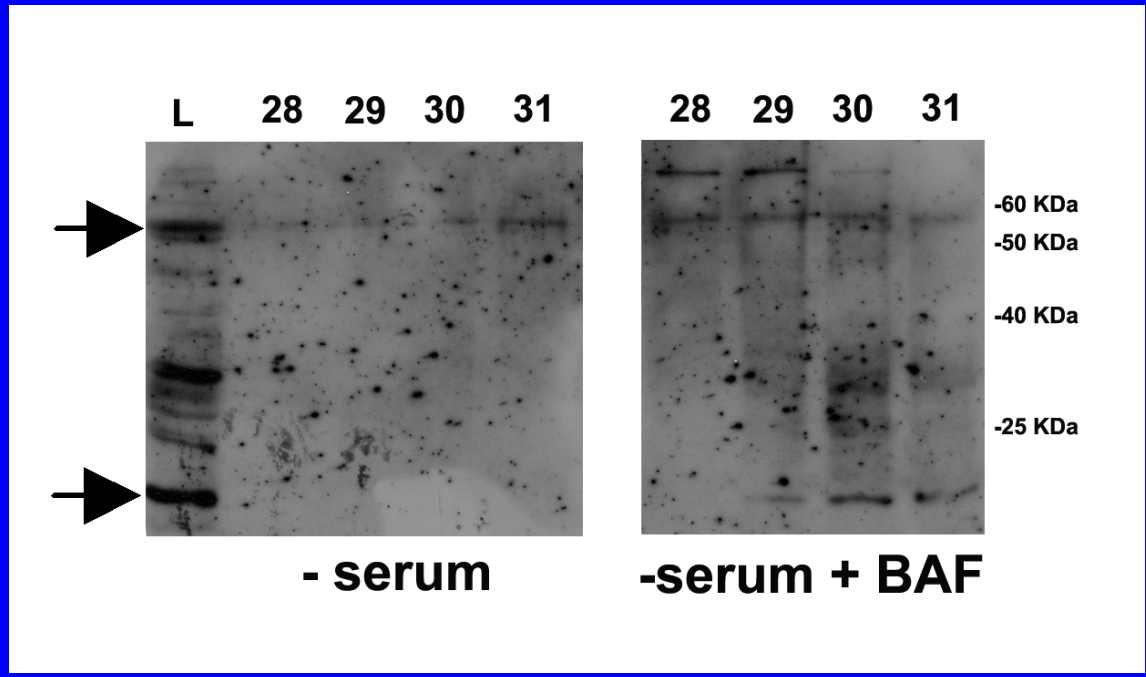
Tinel and Tschopp, 2004

Formation of HMW caspase 2-containing complex *in vitro* in PC12 cell lysates after incubation at 37oC



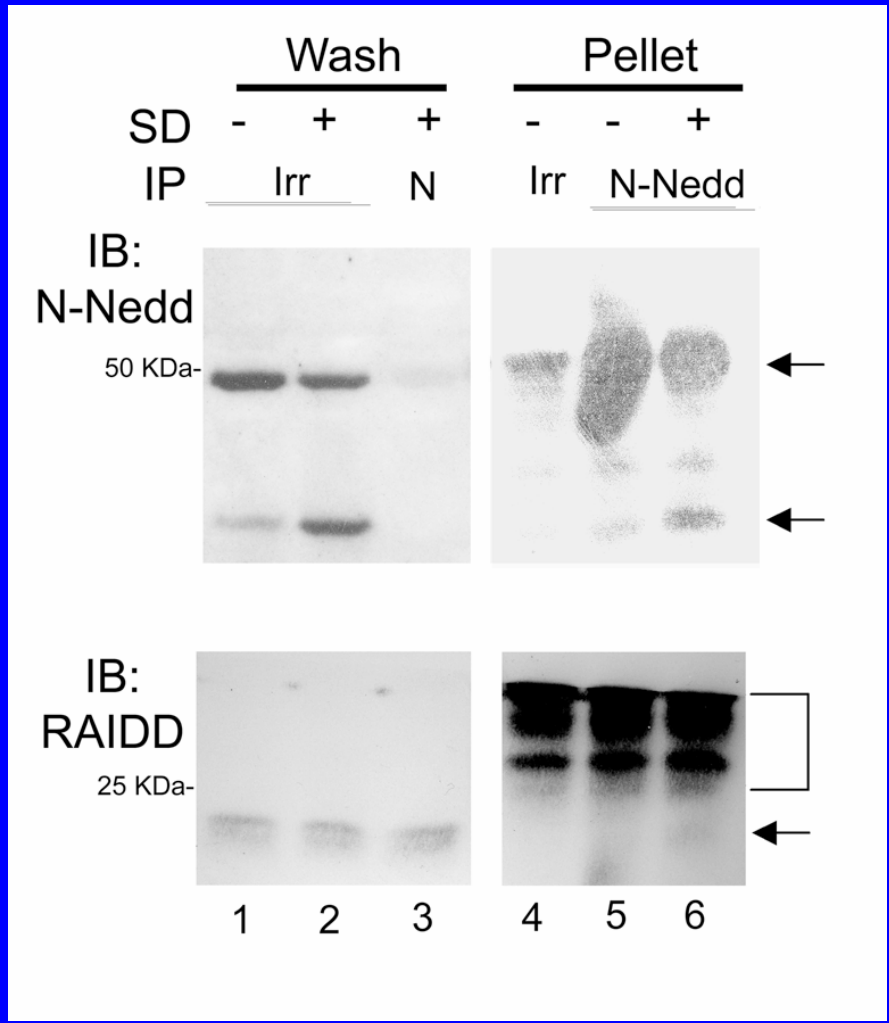
This complex also contains RAIDD

HMW caspase 2-containing complexes are also formed *within cells* following serum deprivation

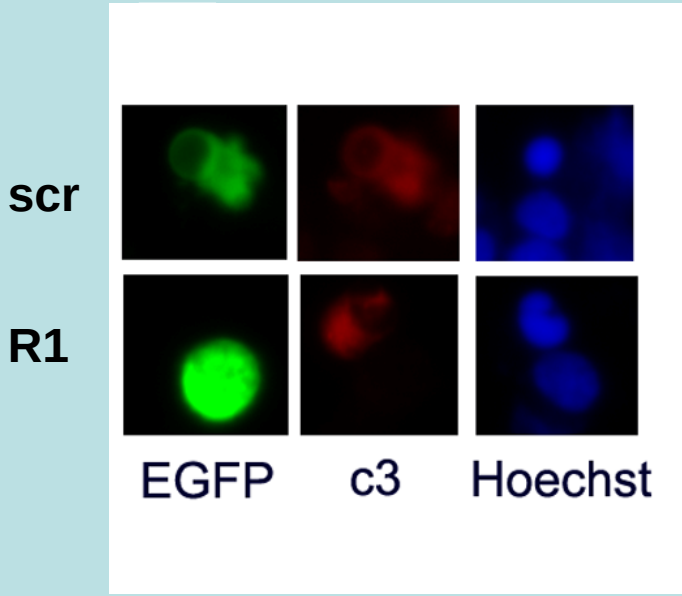


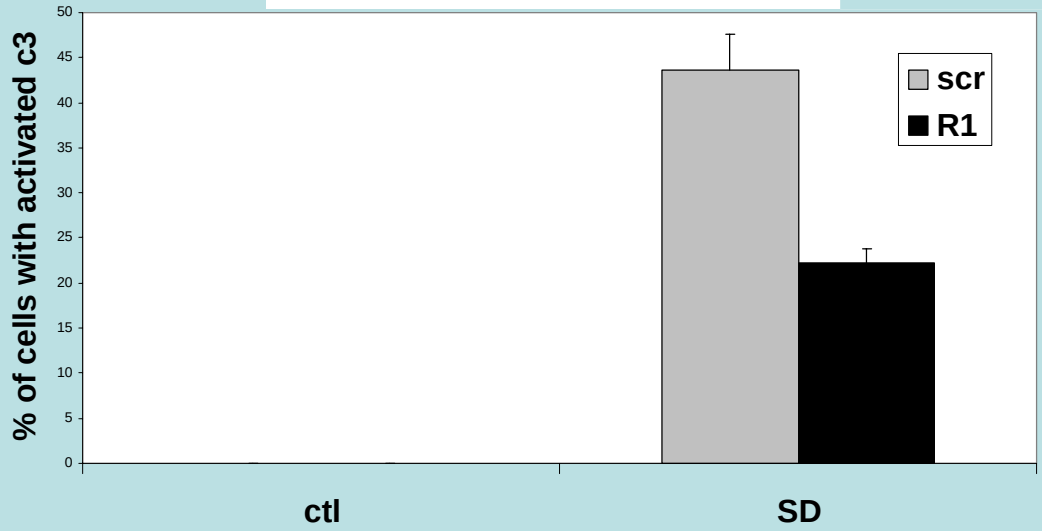
in a caspase-independent fashion

Caspase 2 co-IPs with RAIDD only after serum deprivation in PC12 cells

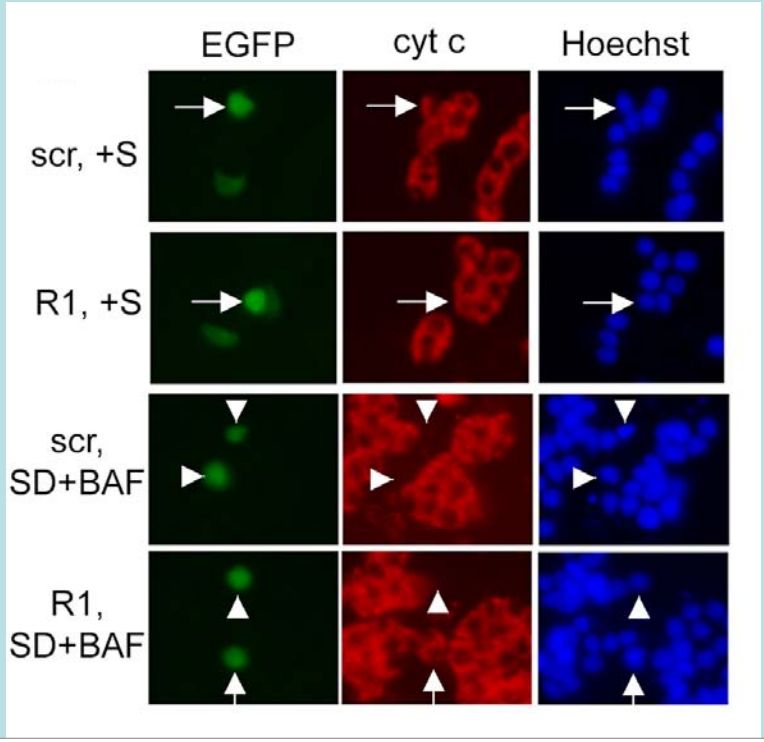


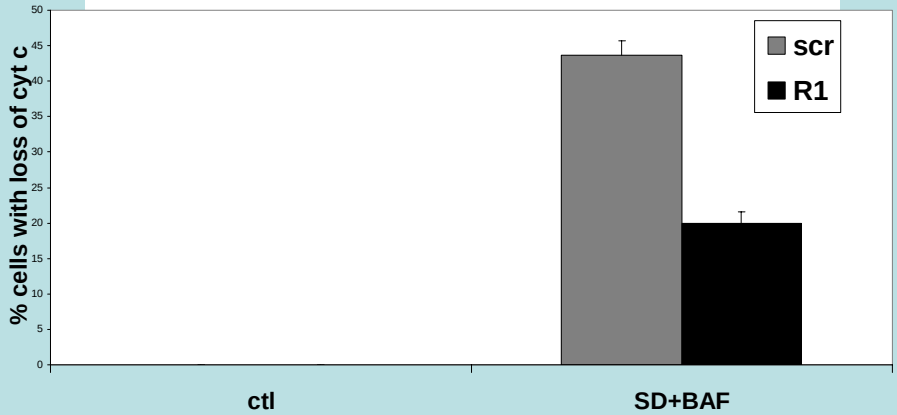
RAIDD siRNA diminishes caspase 3 activation in serum-deprived PC12 cells



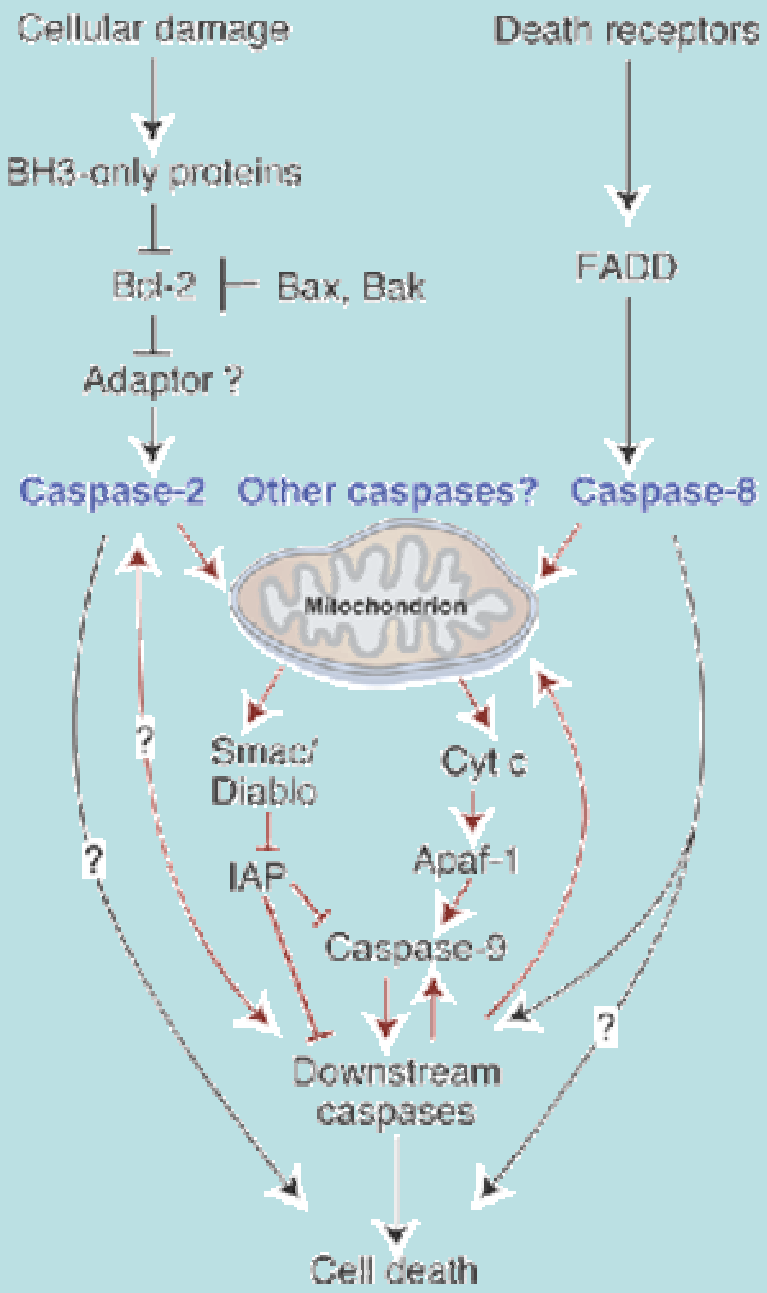


RAIDD siRNA diminishes cyt c release from serum-deprived PC12 cells



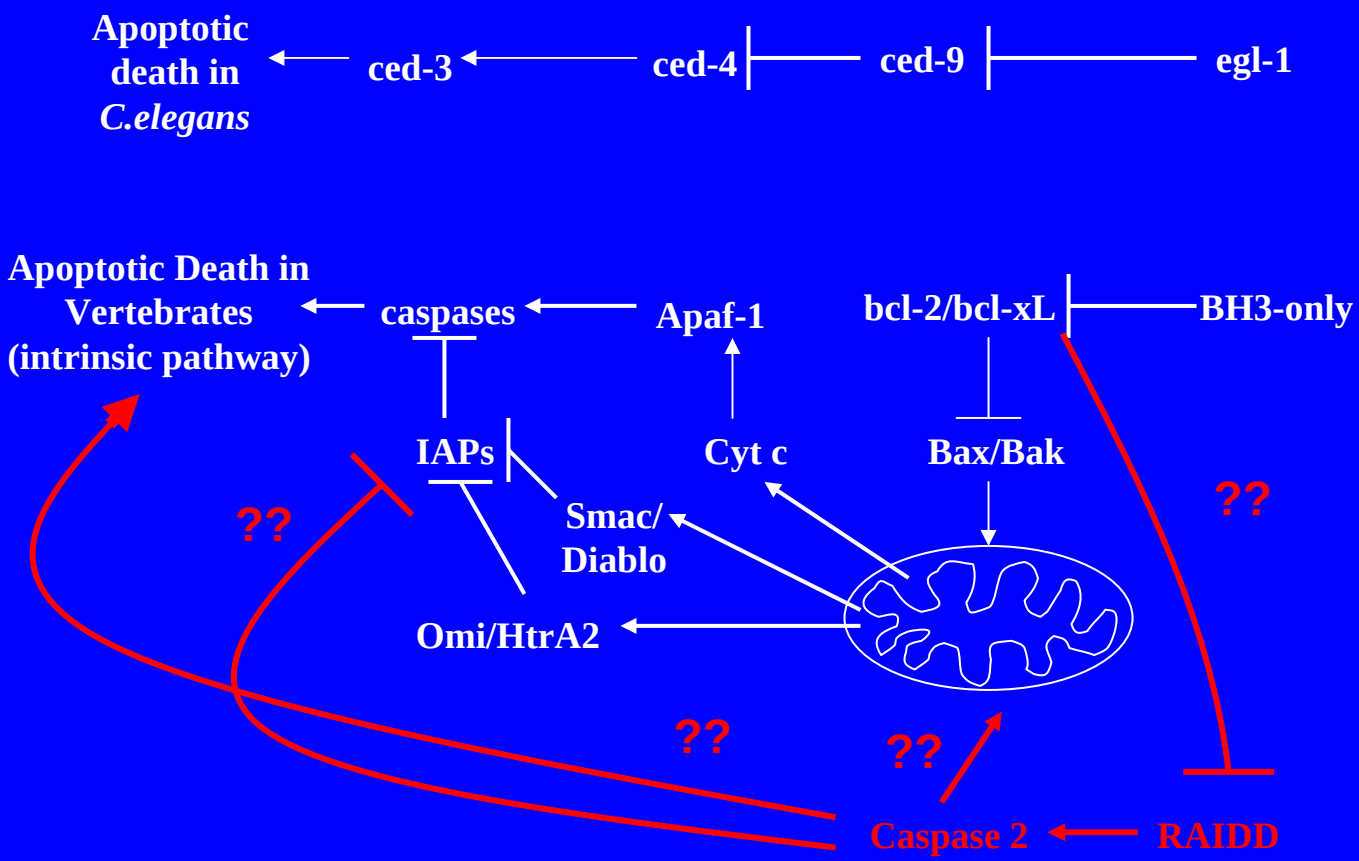


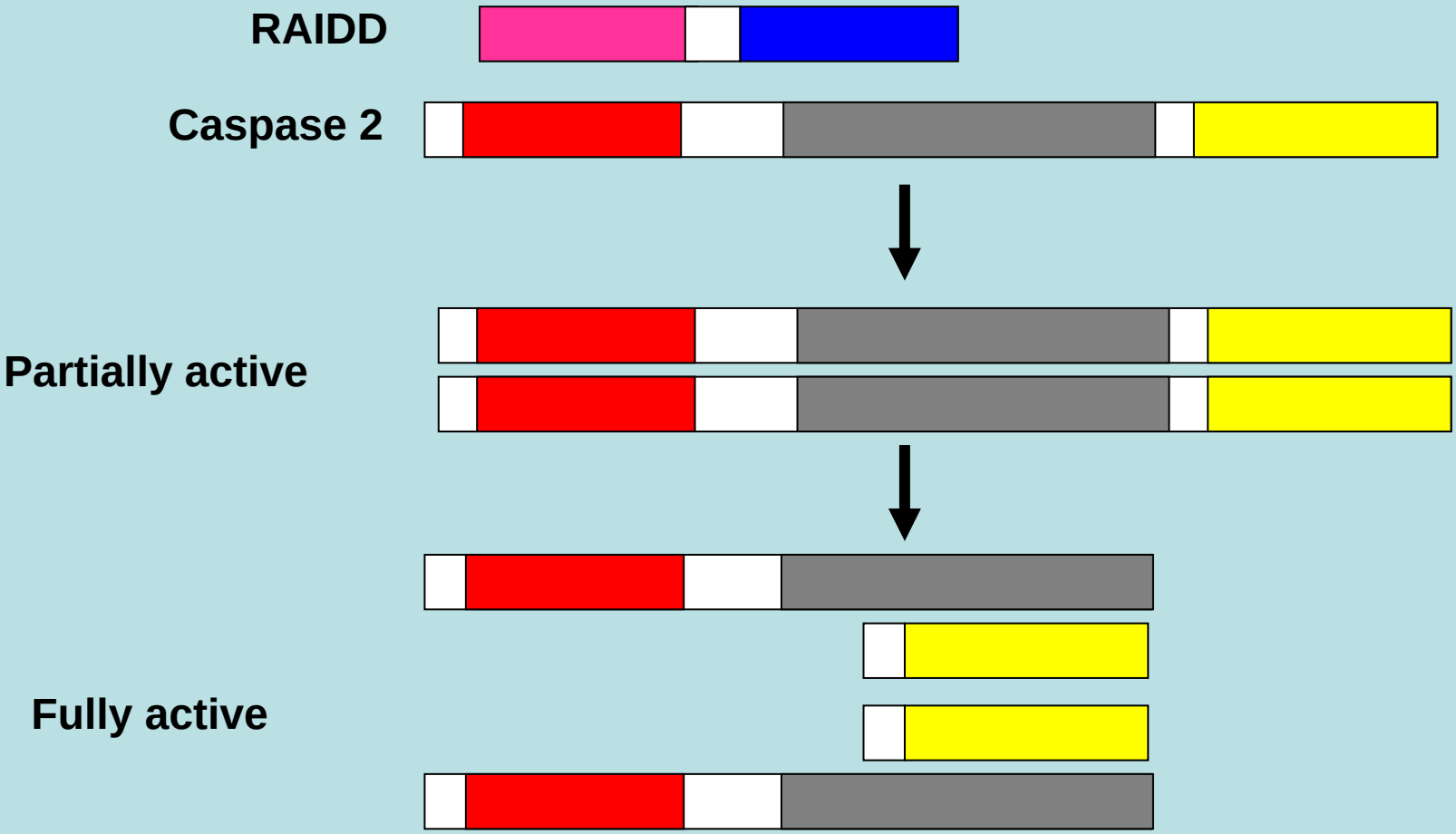
Working model for the involvement of caspase 2 in “intrinsic”apoptotic pathways



Kumar and Vaux, 2002

Biochemical pathways of apoptosis in *C.elegans* and vertebrates





Model of caspase 2 activation

(adapted from Baliga et al., 2004)

Lingering Questions

- How is the RAIDD/caspase 2 interaction initiated?
- What does activated caspase 2 do?
- How is caspase 2 activated?
- How widespread is the importance of the RAIDD/caspase 2 modulation in neuronal apoptotic pathways?
- Role in *in vivo* neuronal apoptosis?

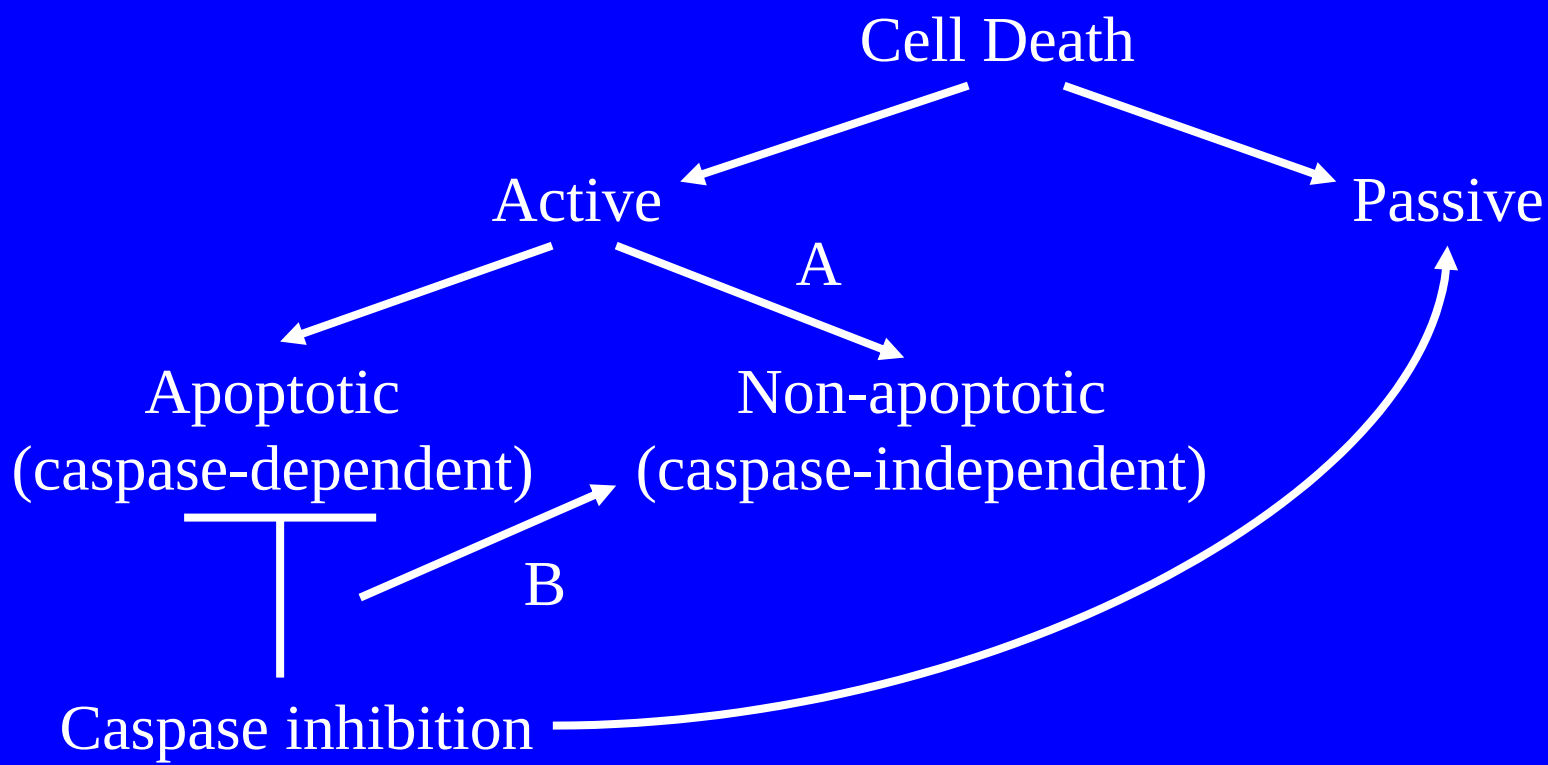
Why is all this C2/RAIDD stuff interesting?

- It adds a level of complexity and regulation to the system
- It is context-dependent
- Perhaps other initiator caspases, such as caspase 1, may play similar regulatory roles in other systems
- Inhibition of RAIDD-Caspase 2 may be more specific for certain death stimuli/neuronal cell types
- Such inhibition may also be more effective therapeutically, given the limitations of current pharmacological strategies that inhibit caspases by acting at the level of the apoptosome (see below)

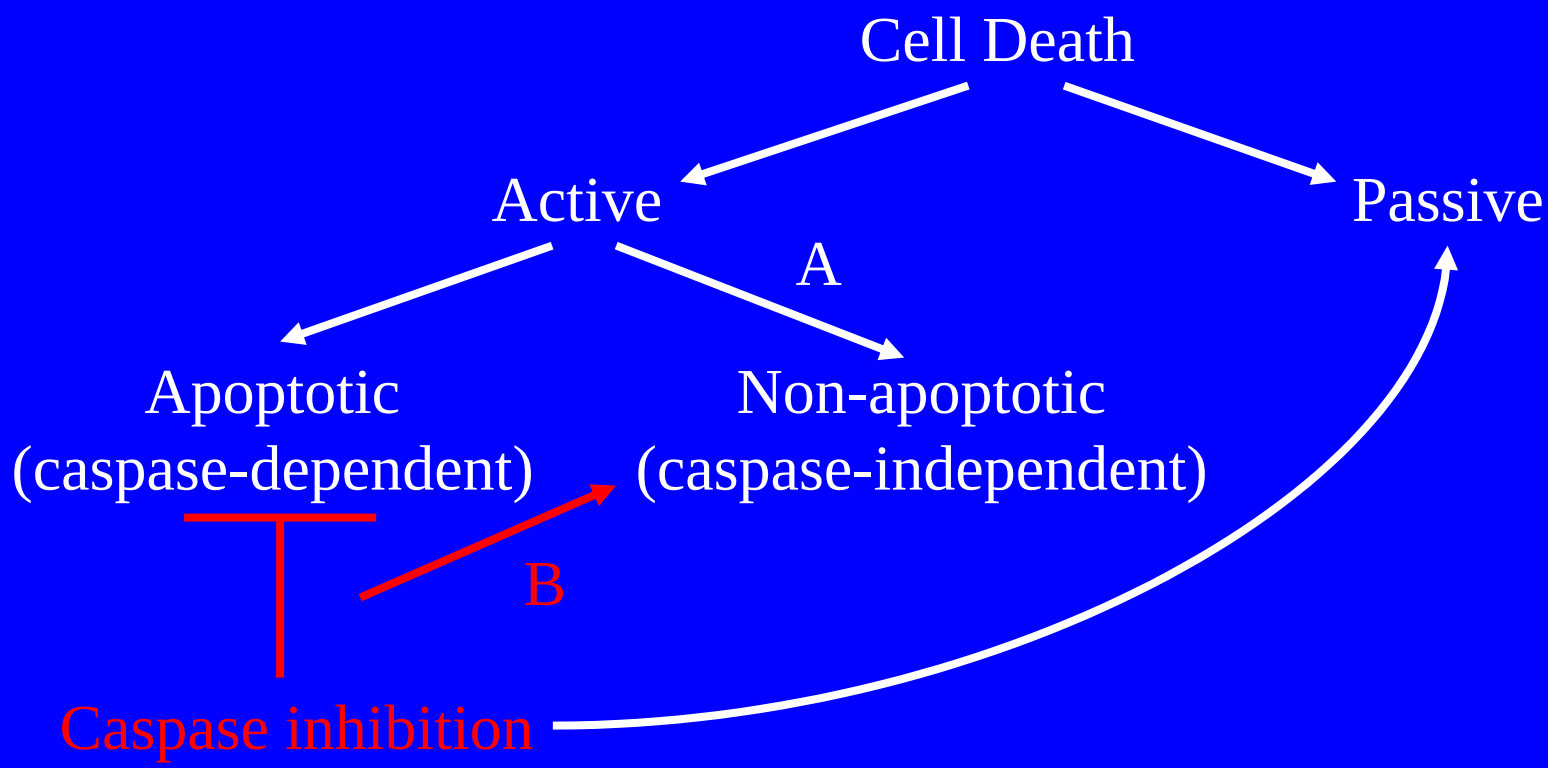
Today's talk:
Caspase-dependent and –independent neuronal death

- **Caspase-independent death pathways when caspases are inhibited**

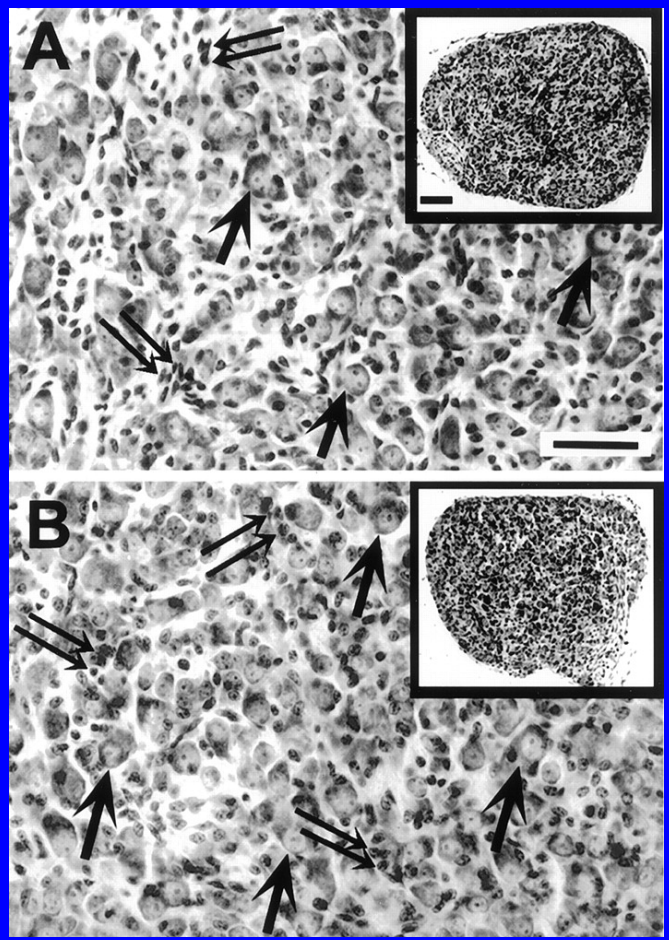
Caspase-independent neuronal death



Caspase-independent neuronal death



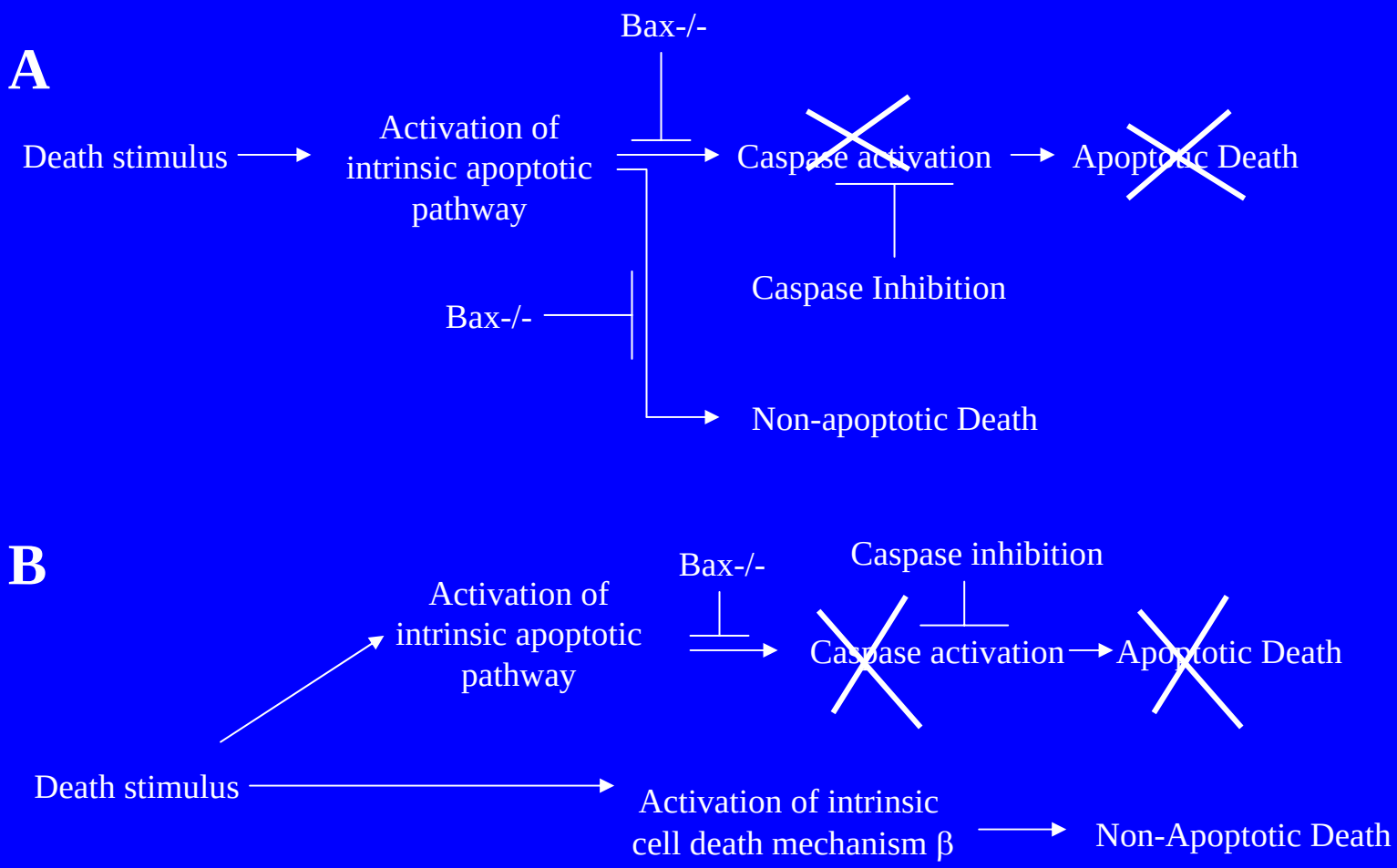
Sympathetic Ganglia from caspase 3 null mice are indistinguishable from WT



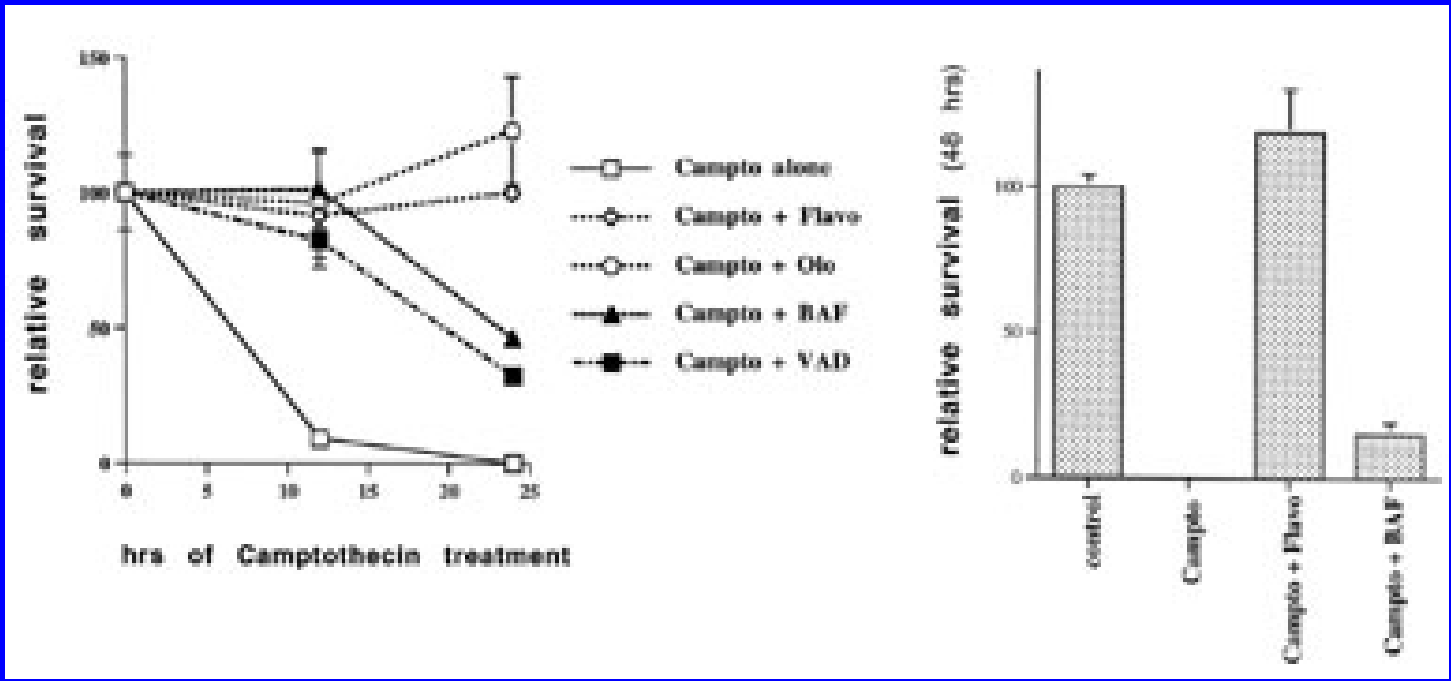
WT

C3 KO

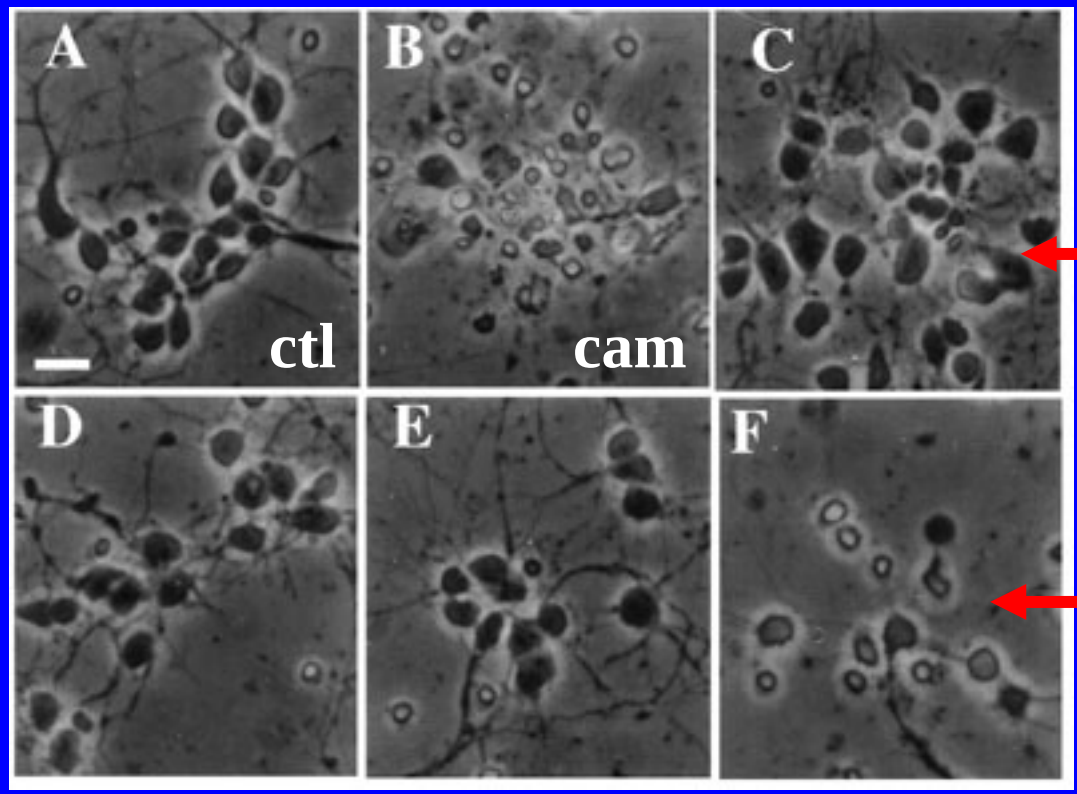
Oppenheim et al., 2001


A
Bax-/-
Death stimulus
Activation of intrinsic apoptotic pathway
Caspase activation
Apoptotic Death
Caspase Inhibition
Bax-/-
Non-apoptotic Death
B
Bax-/-
Caspase inhibition
Activation of intrinsic apoptotic pathway
Caspase activation
Apoptotic Death
Death stimulus
Activation of intrinsic cell death mechanism β
Non-Apoptotic Death

Pharmacological caspase inhibition delays, but does not prevent, neuronal cell death



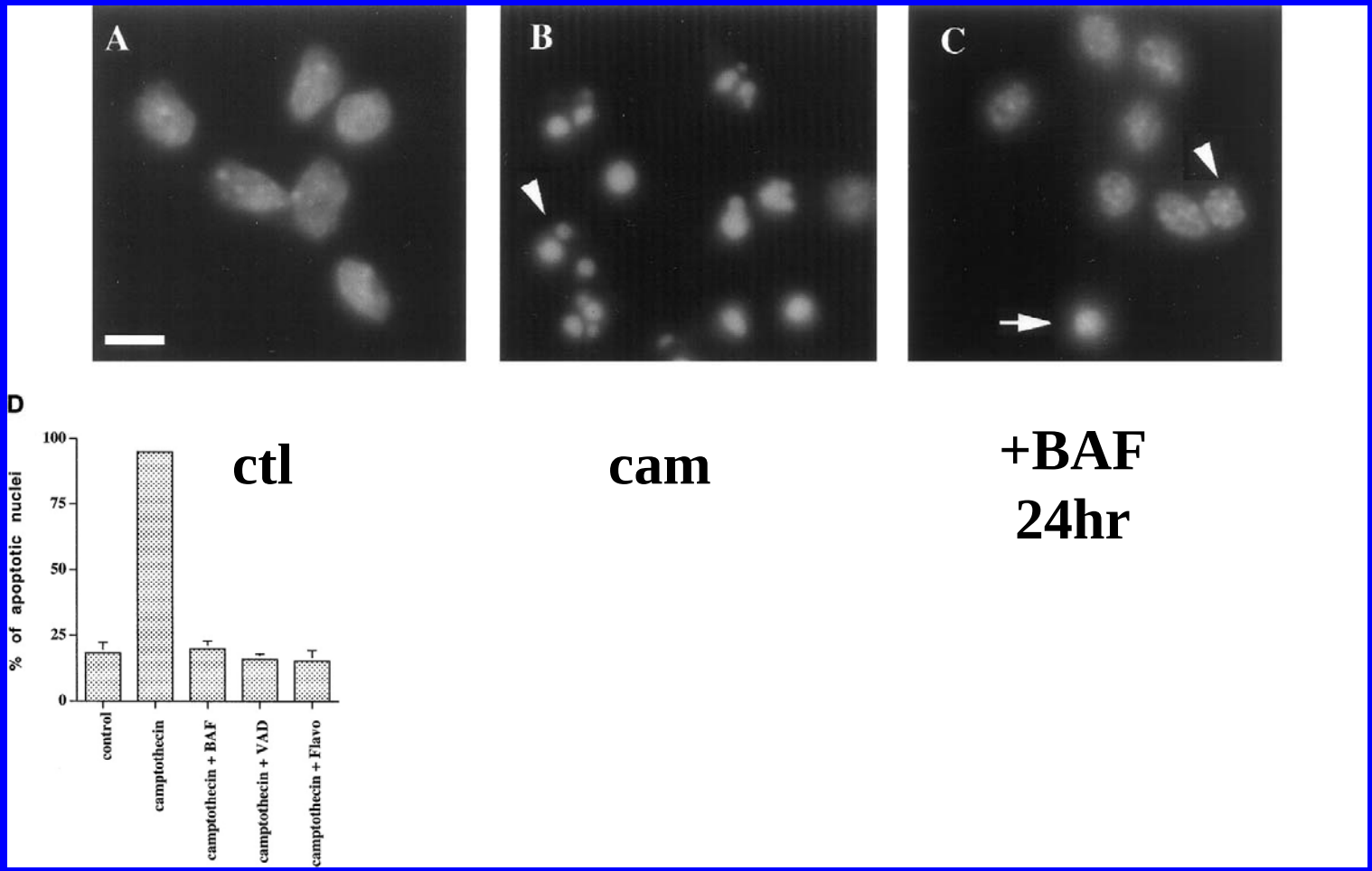
Pharmacological caspase inhibition delays, but does not prevent, Neuronal cell death



Caspase-independent neuronal death when caspases are inhibited

- What is the morphology?
- What are the mechanisms?
- Is it an “active” form of cell death?
- Can it be modulated?

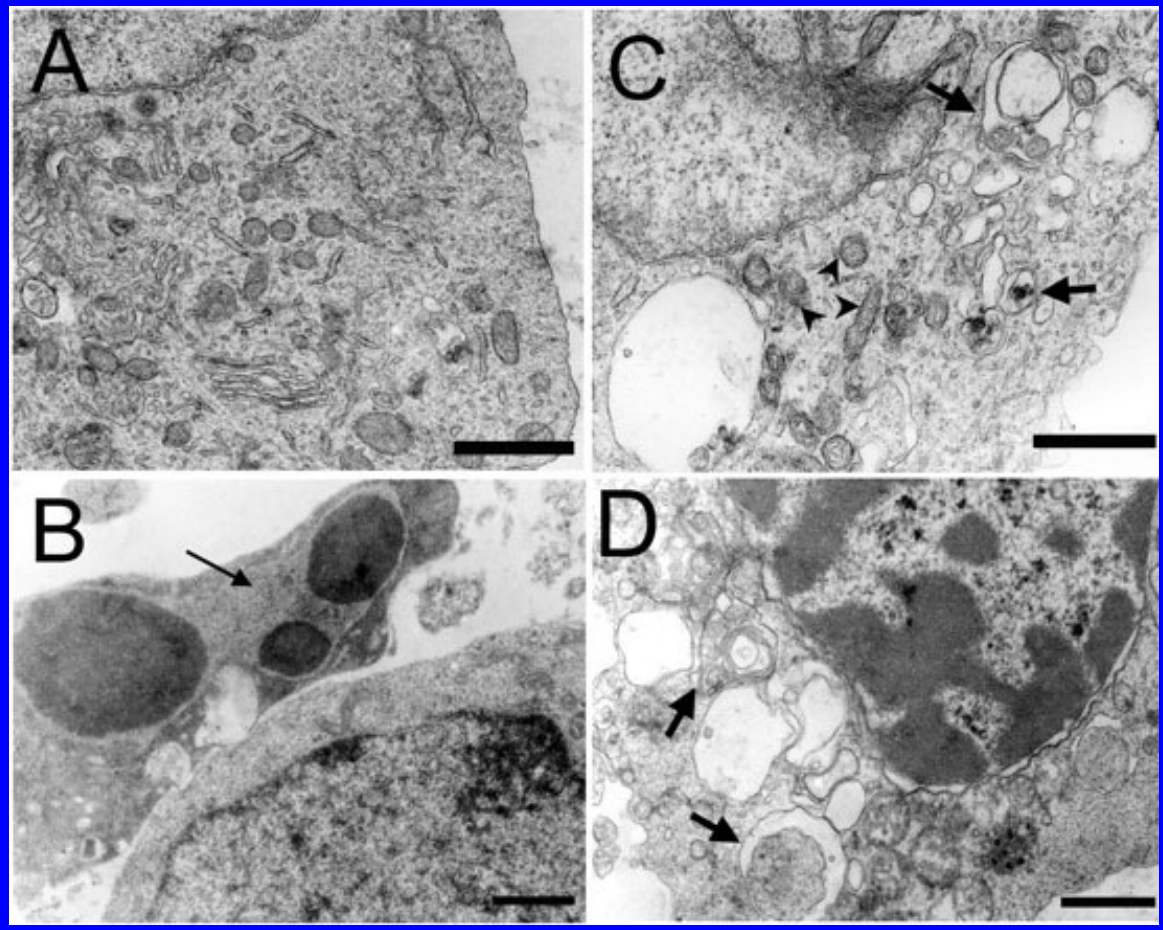
Death morphologically is non-apoptotic



Caspase-independent neuronal death is morphologically autophagic cell death

ctl

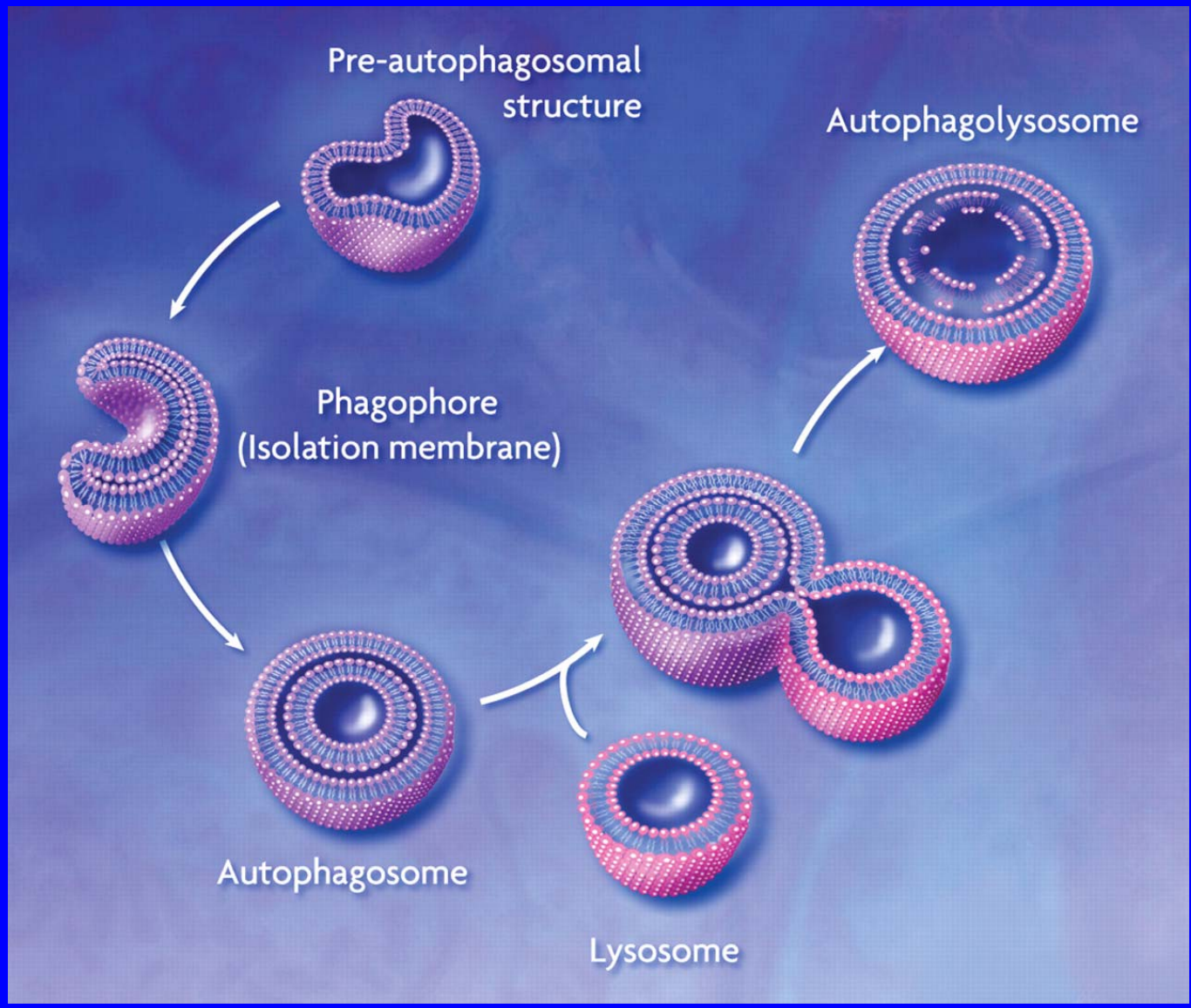
Cam



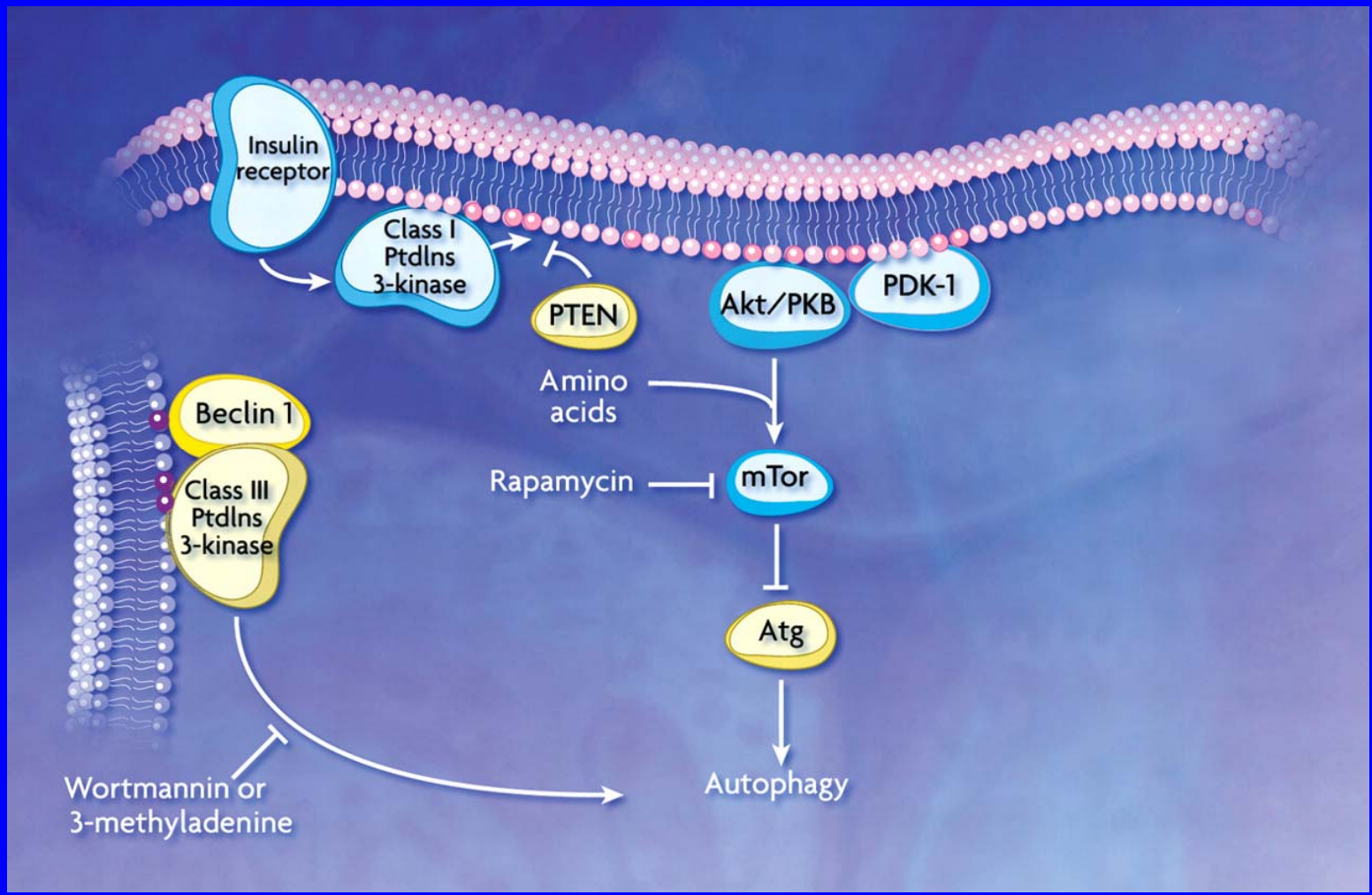
Cam+ BAF

Cam+ BAF

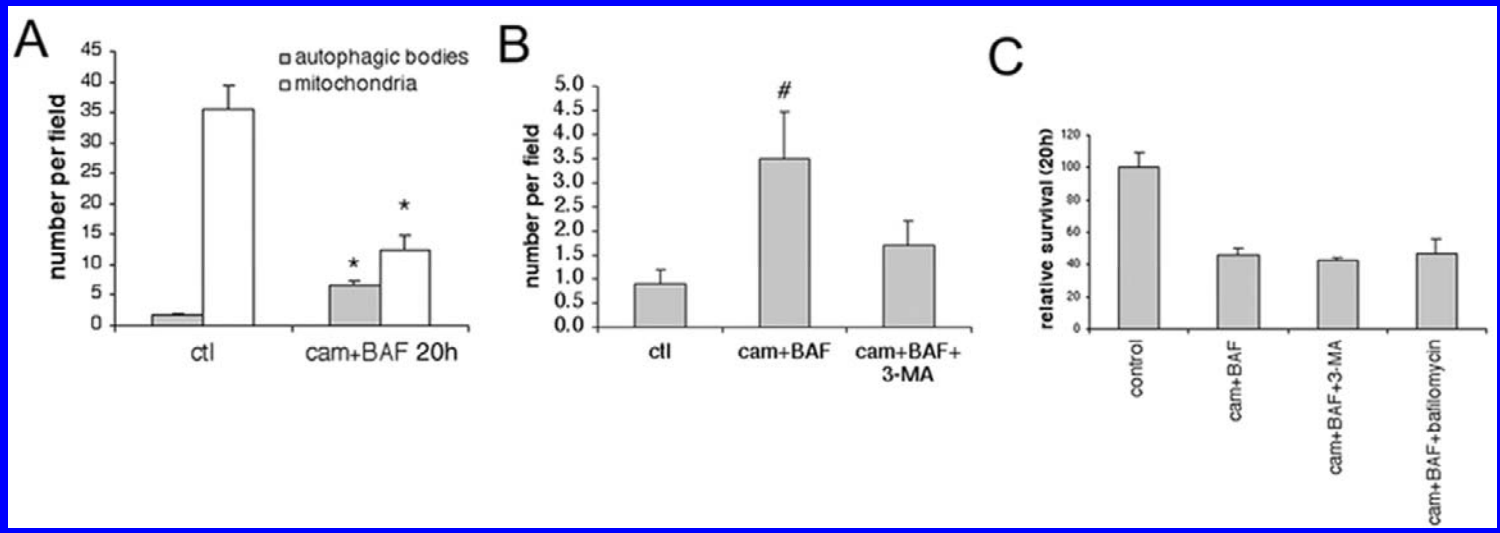
Process of macroautophagy



Macroautophagy: The molecular players



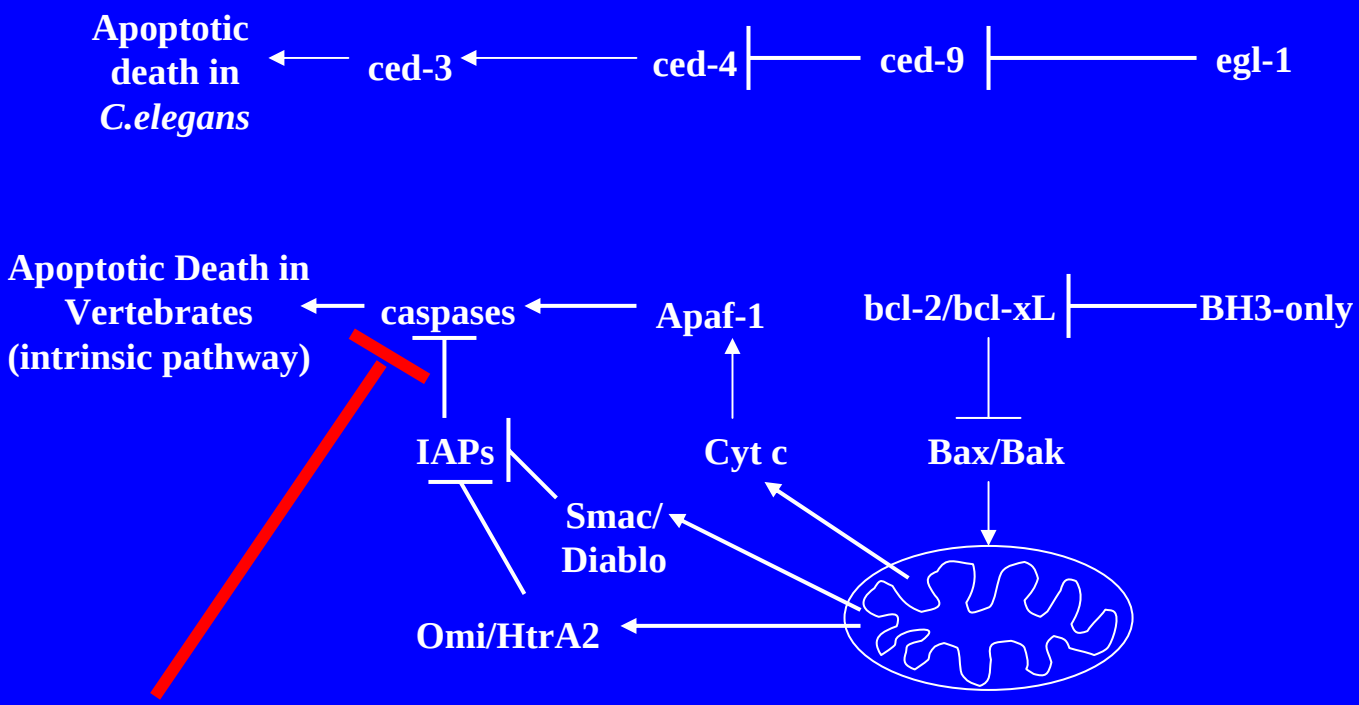
Inhibition of autophagy has no effect on Caspase-independent neuronal death



Relationship of autophagy to apoptosis and cell death

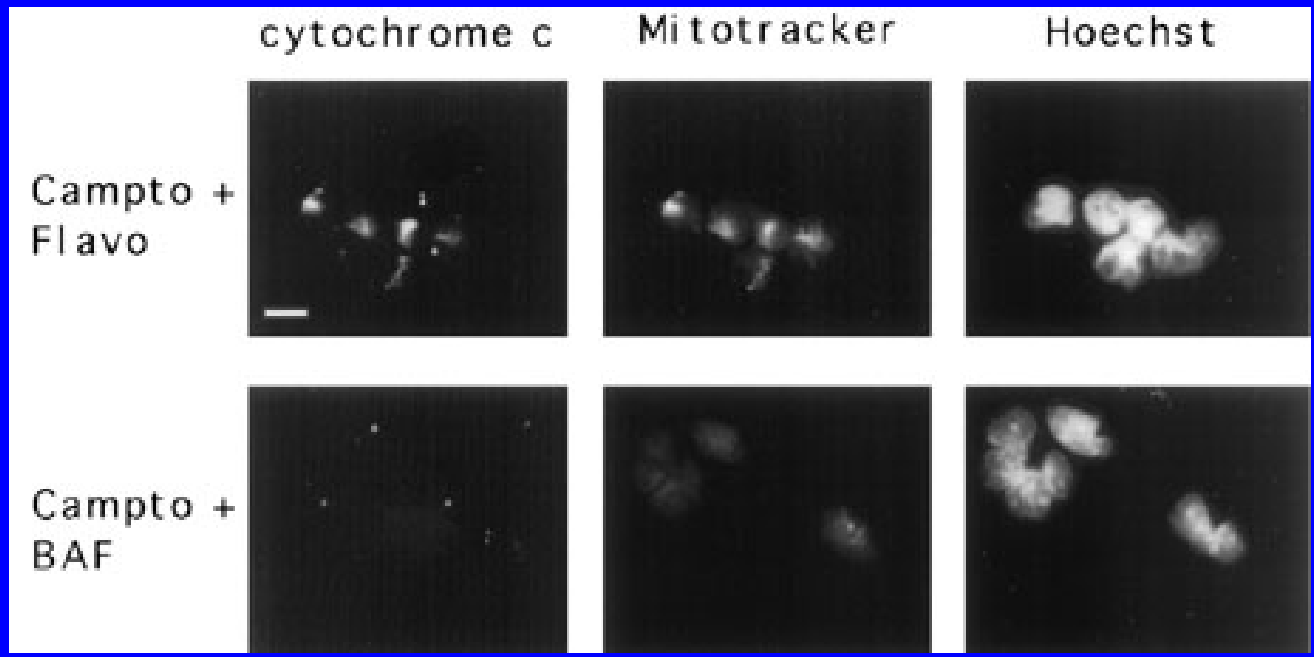
- In some models, like the above, these processes are completely separable even at the cell population level
- In others, both morphologies occur concurrently at the level of a cell population, or even at the single cell level
- There are now a couple of instances where activation of the mechanism of autophagy is shown to lead to cell death
- More commonly, autophagy appears to be a survival mechanism
- In cortical neurons exposed to cam + BAF, autophagy is the morphological mode of death, but it does not influence survival

Biochemical pathways of apoptosis in *C.elegans* and vertebrates

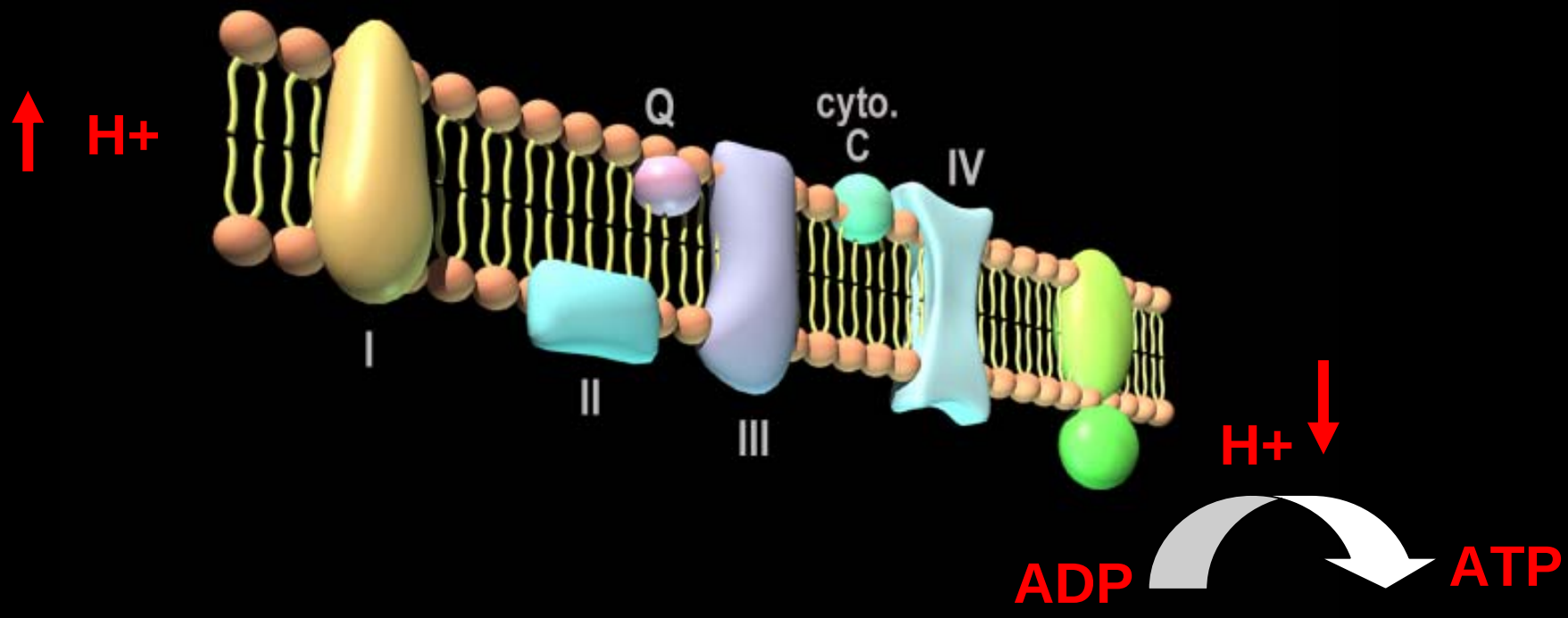


Generally, caspase inhibition does not prevent mitochondrial alterations

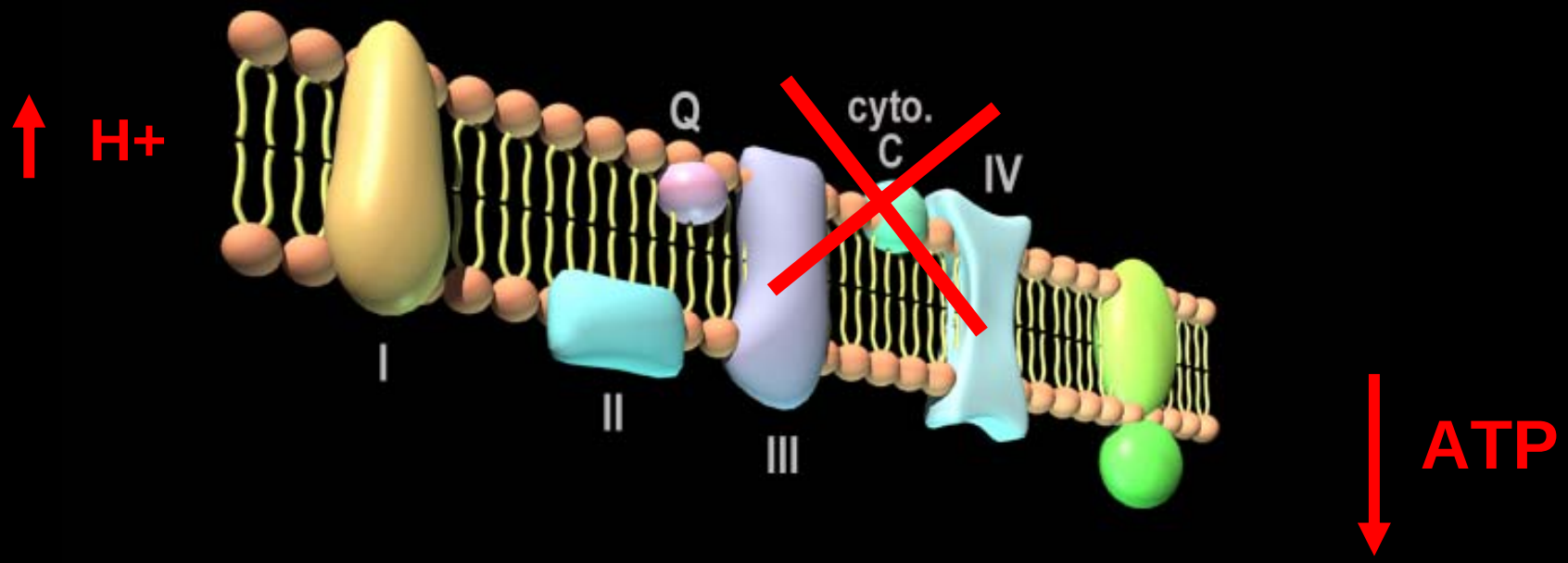
Loss of cyt c and of Δψm in caspase-independent neuronal death



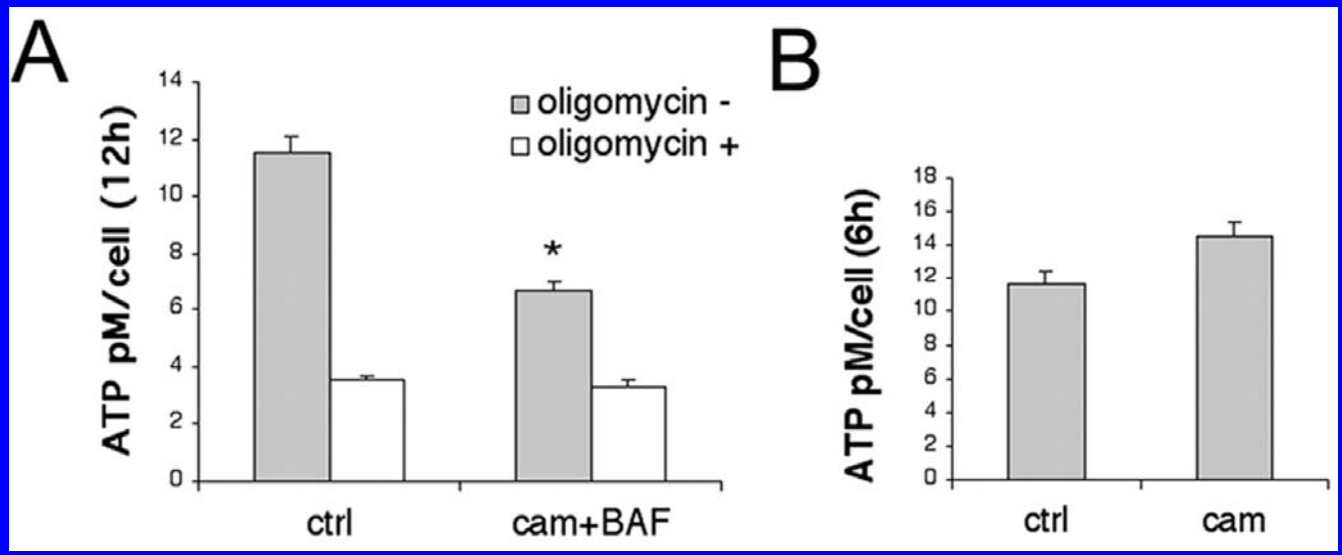
Mitochondrial respiration



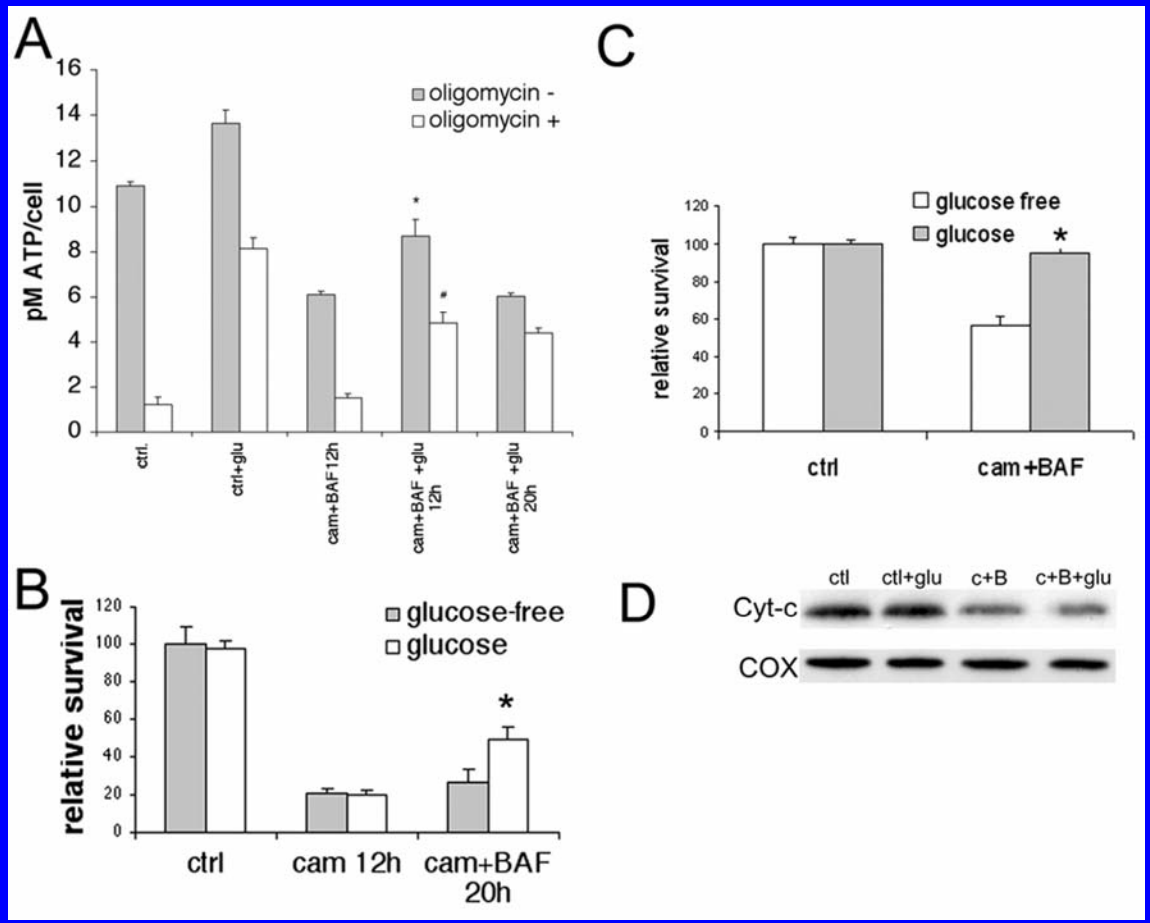
Effects of cyt c and Δψm loss



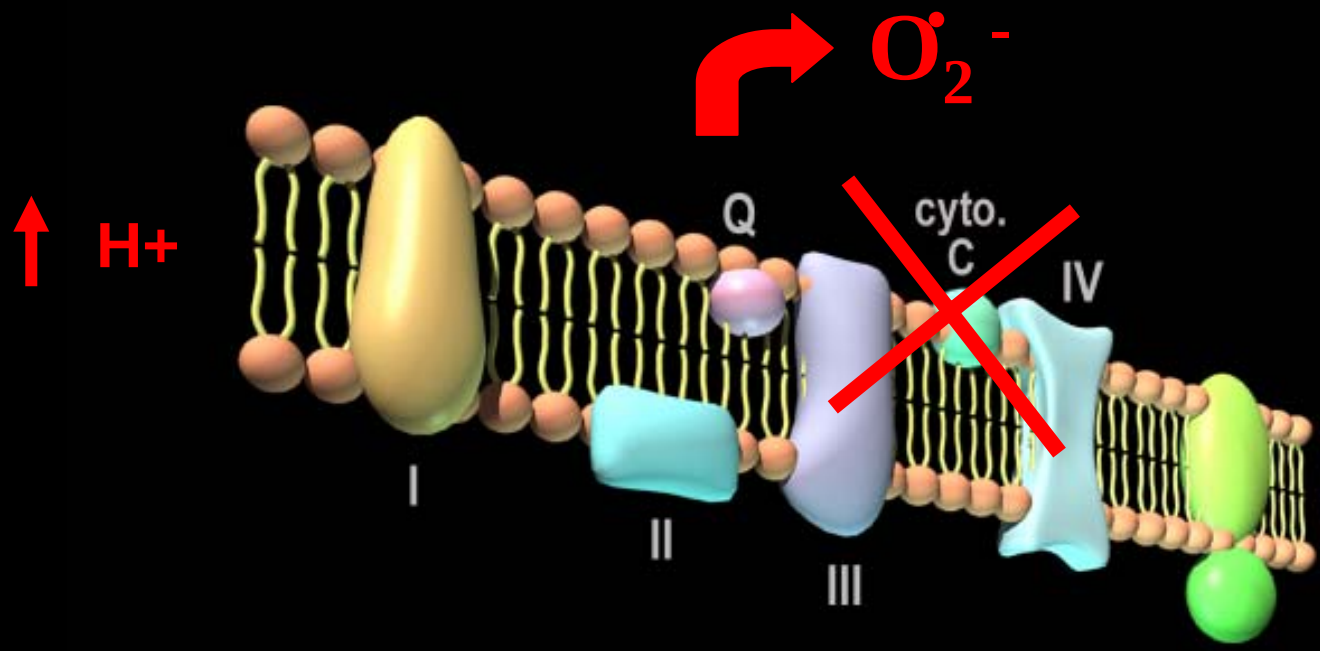
Loss of mitochondrial ATP in Caspase-independent neuronal death



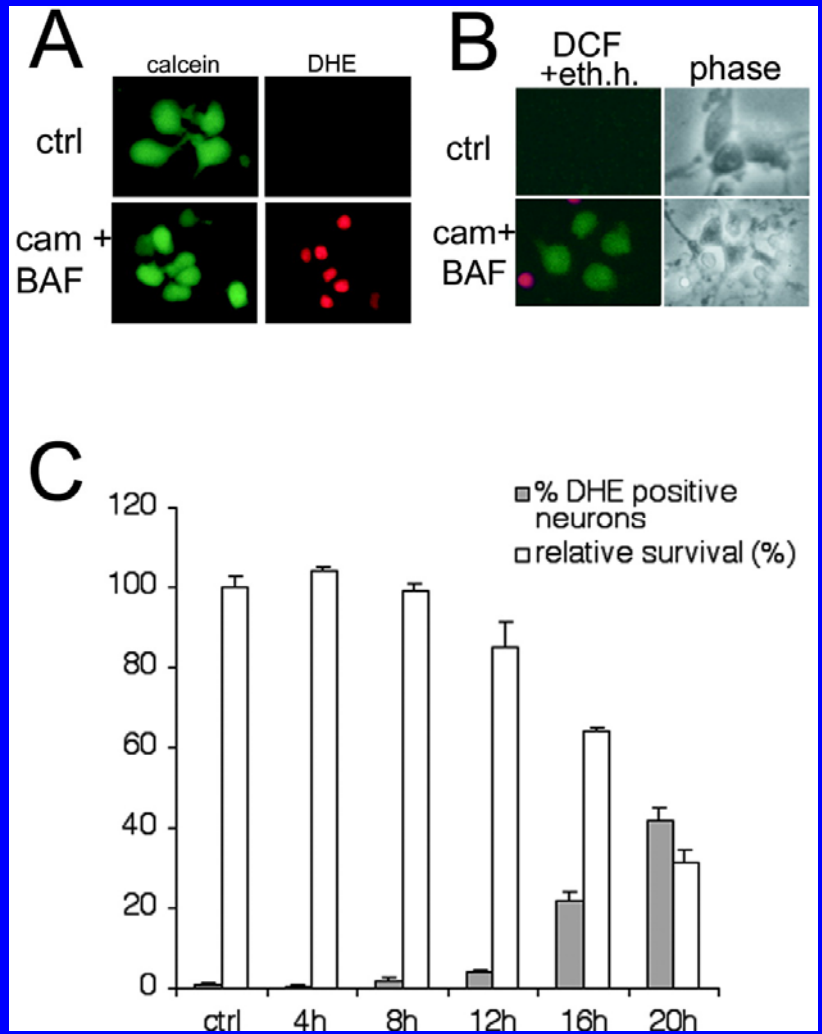
Increase of ATP leads to increase of survival in caspase-independent neuronal death



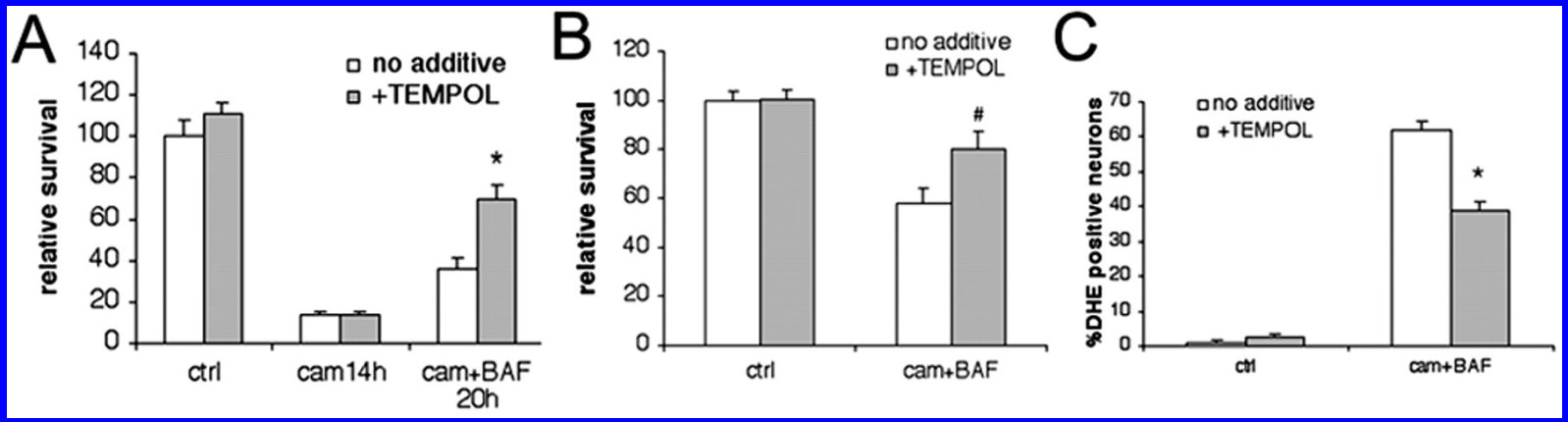
Effects of cyt c and $\Delta\psi m$ loss

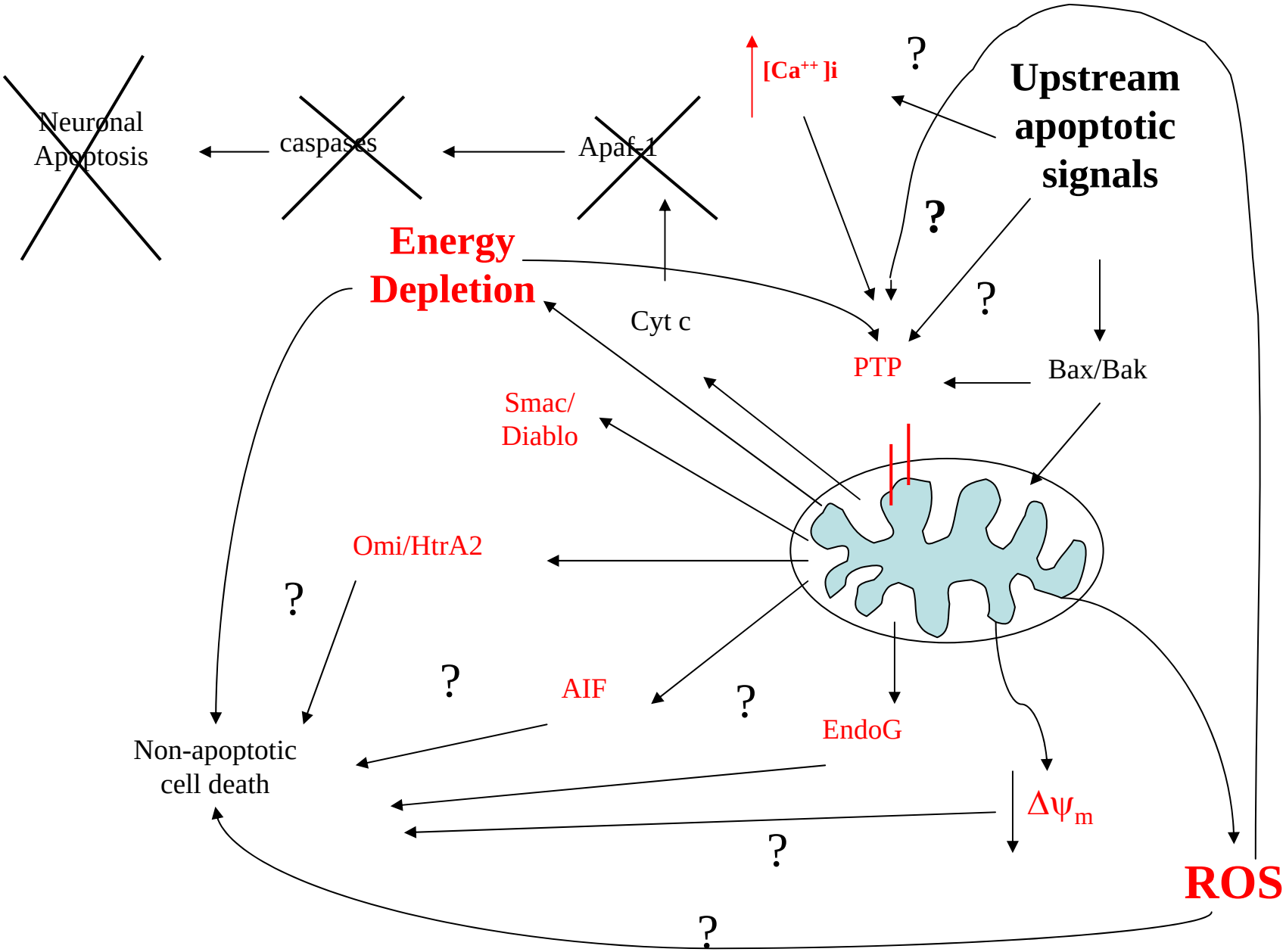


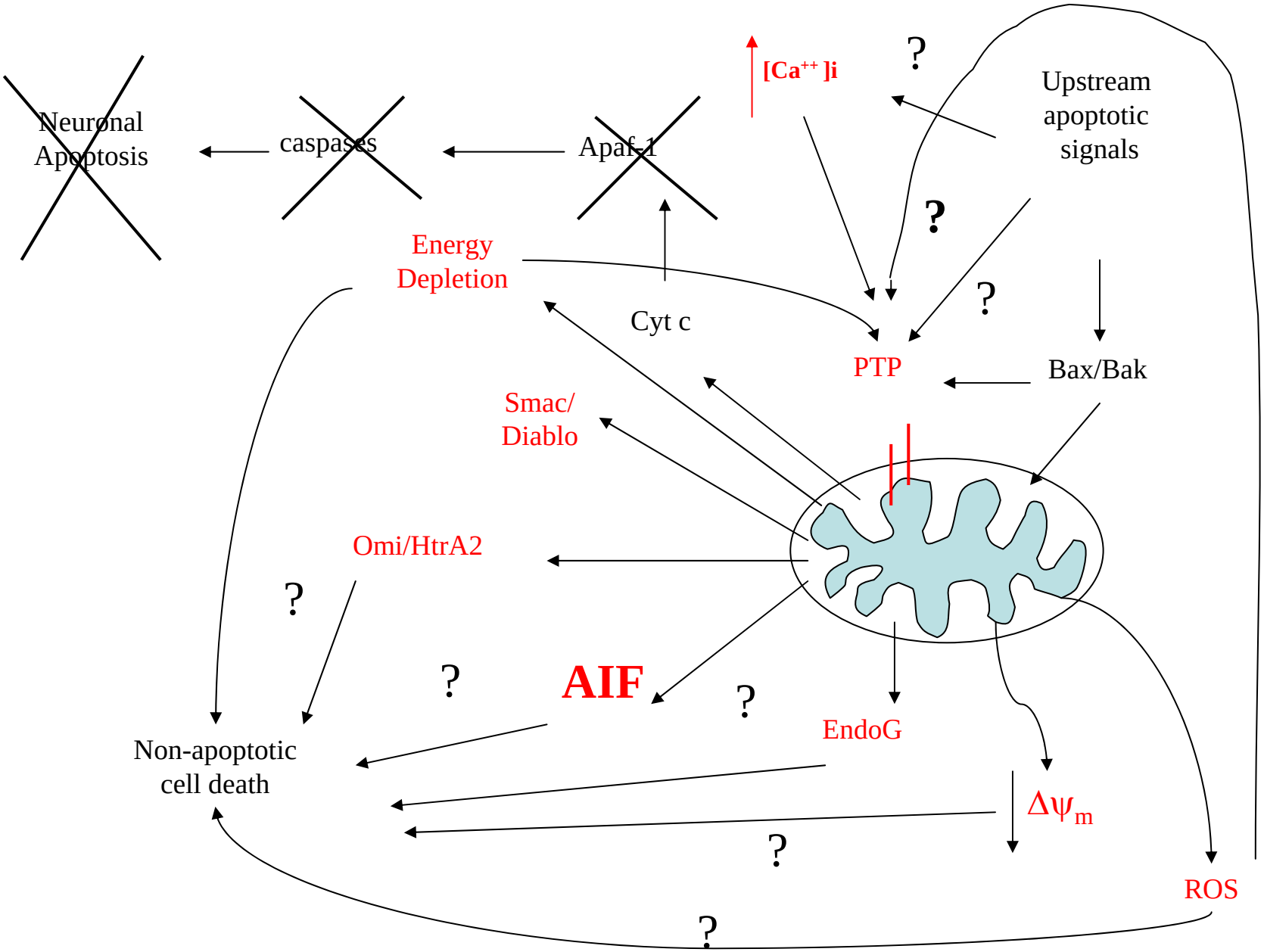
ROS induction in caspase-independent neuronal death



Decrease of ROS with the free radical scavenger TEMPOL mitigates caspase-independent neuronal death




Neuronal Apoptosis
caspases
Apaf-1
[Ca++]i
?
Upstream apoptotic signals
?
Energy Depletion
Cyt c
?
PTP
Bax/Bak
Smac/ Diablo
Omi/HtrA2
?
?
AIF
?
EndoG
Non-apoptotic cell death
Δψm
?
ROS
?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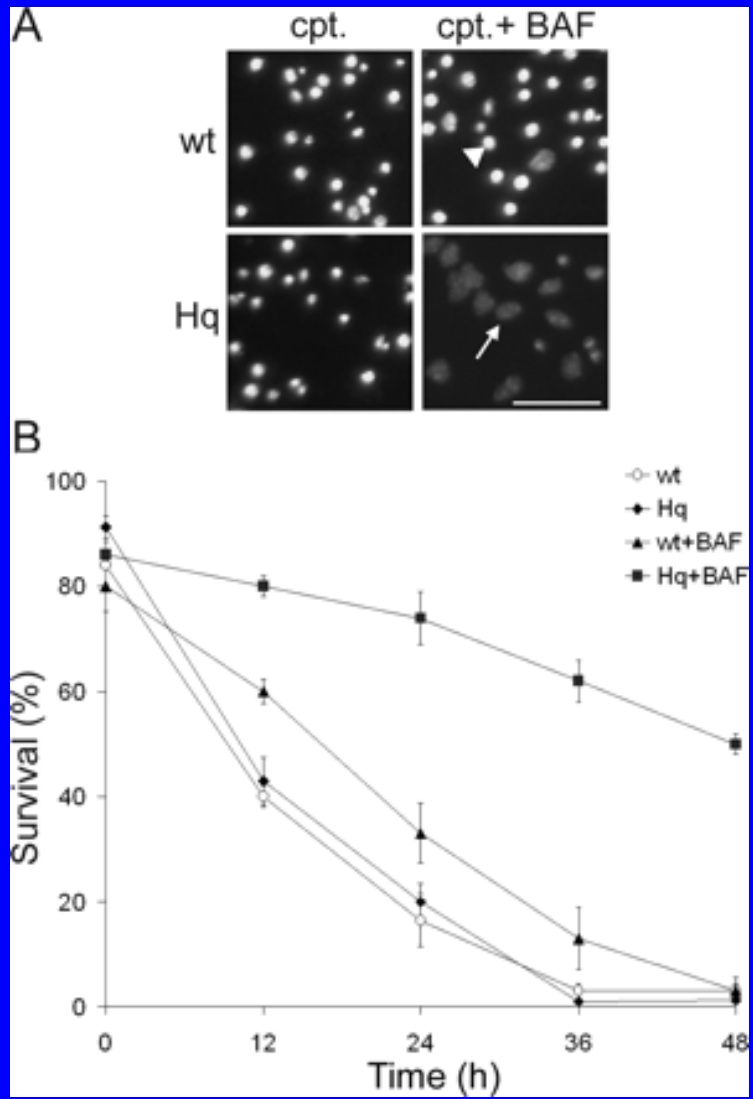
Smac/ Diablo
Omi/HtrA2
?
?
AIF
?
EndoG
Non-apoptotic cell death
Δψm
?
ROS
?

Diminution of AIF expression mitigates caspase-independent, but not caspase-dependent, neuronal deah



Cheung et al., 2005

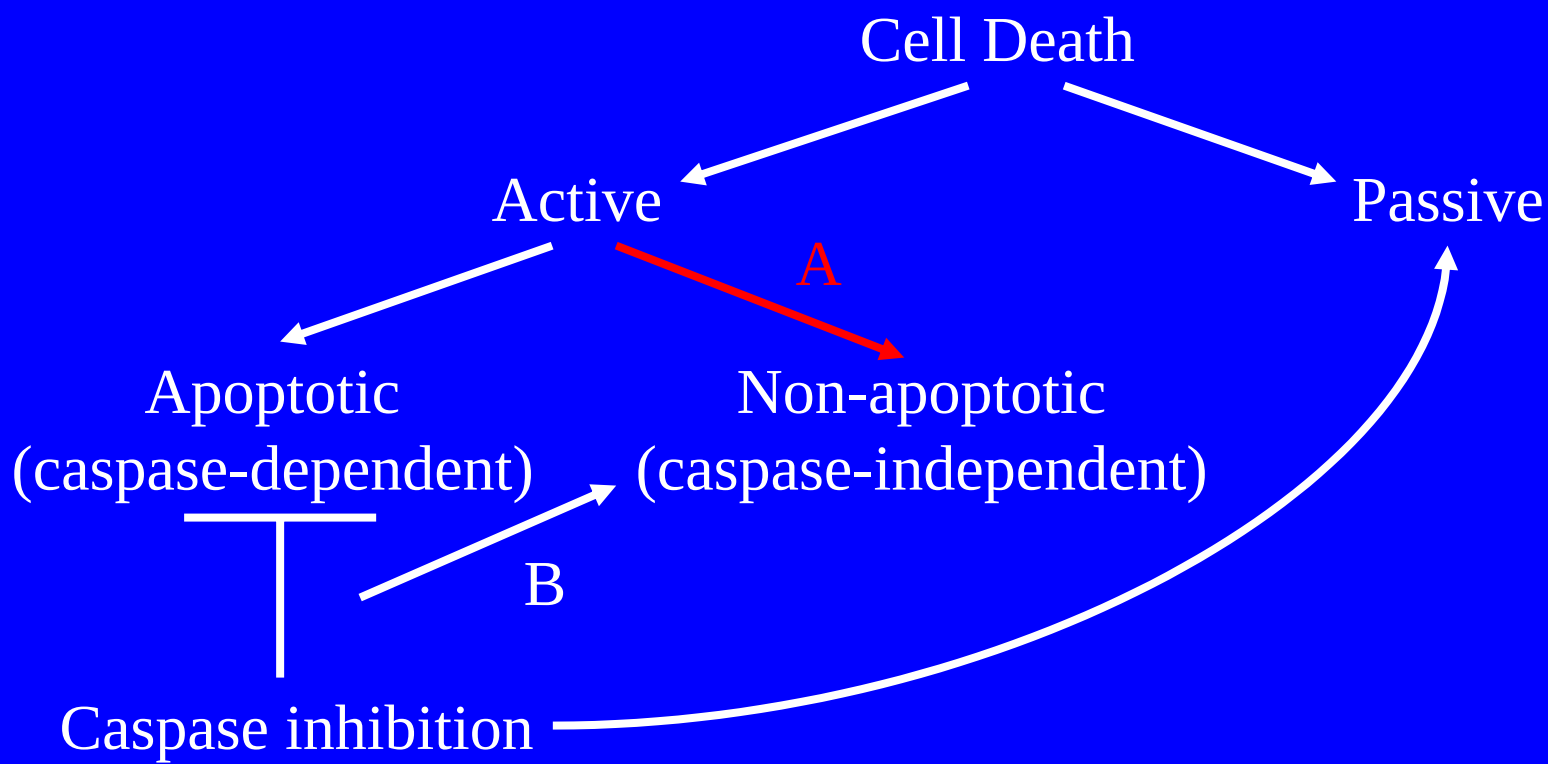
Neuronal death when caspases are inhibited: Mechanisms of death

- Energy depletion due to lowering of mitochondrial ATP production
- Free radical generation: Oxidative and/or nitrative stress?
- AIF translocation from mitochondria
- PTP opening??

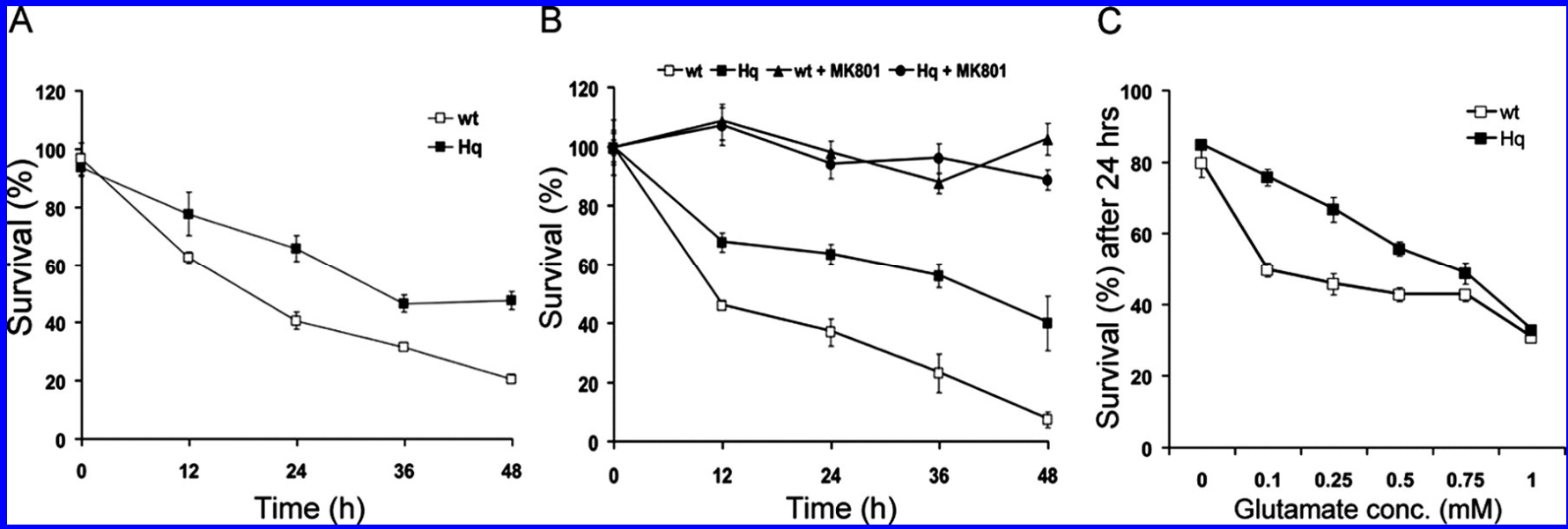
Why is this interesting?

- Knowledge of such mechanisms may help to augment/supplement therapies targeting caspases downstream of the mitochondrial checkpoint
- Such mechanisms may also be applicable to situations in which neuronal death occurs in a caspase-independent fashion, irrespective of exogenous intervention
- Such situations may be more applicable to neurodegenerative conditions

Caspase-independent neuronal death



Diminution of AIF expression mitigates caspase-independent excitotoxic neuronal deah



Cheung et al., 2005

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