Σκύλλα και Χάρυβδη: Caspase-dependent and –independent neuronal death

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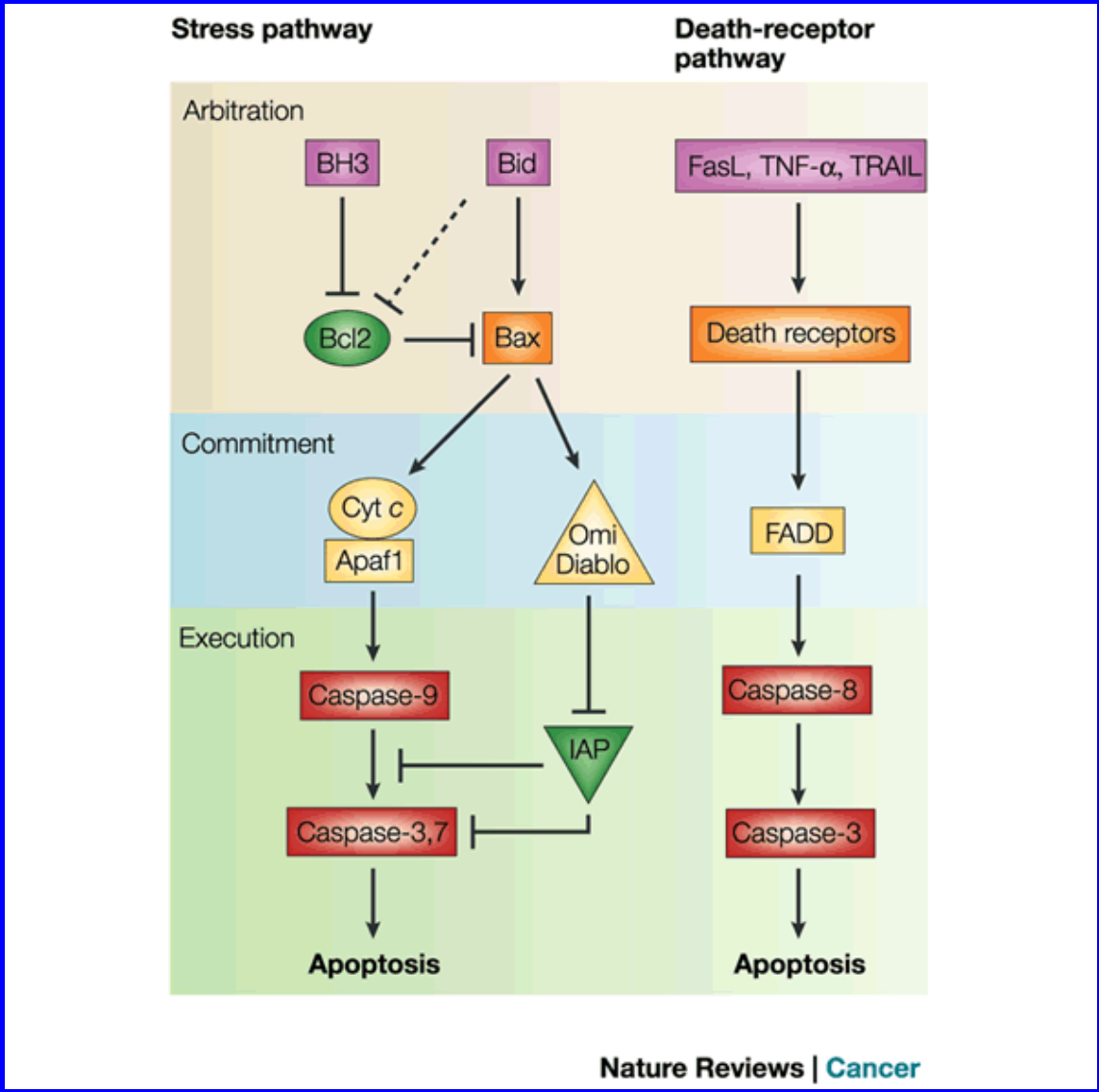
The two major pathways of apoptosis

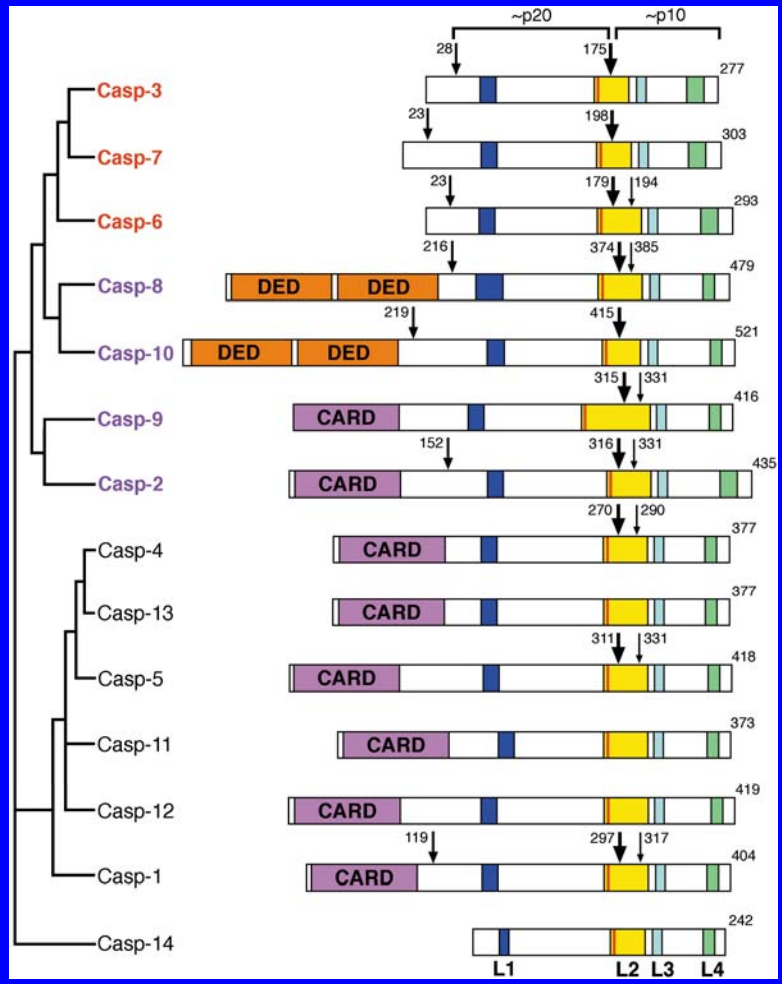


Cory and Adams, 2002

The caspases

- Cysteine proteases involved in cell death in vertebrate systems
- Homologous to the death protein *ced-3* in *C. elegans*
- Generally separated into “initiator” and “effector” caspases
- In intrinsic cell death pathways they are thought to act downstream of the mitochondria, through formation of the apoptosome

The caspases: Cysteine proteases involved in cell death



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

- Possibility that caspase 2 and its adaptor RAIDD may play a regulatory role in intrinsic, caspase-dependent cell death pathways in neuronal cells
- Caspase-independent death pathways when caspases are inhibited

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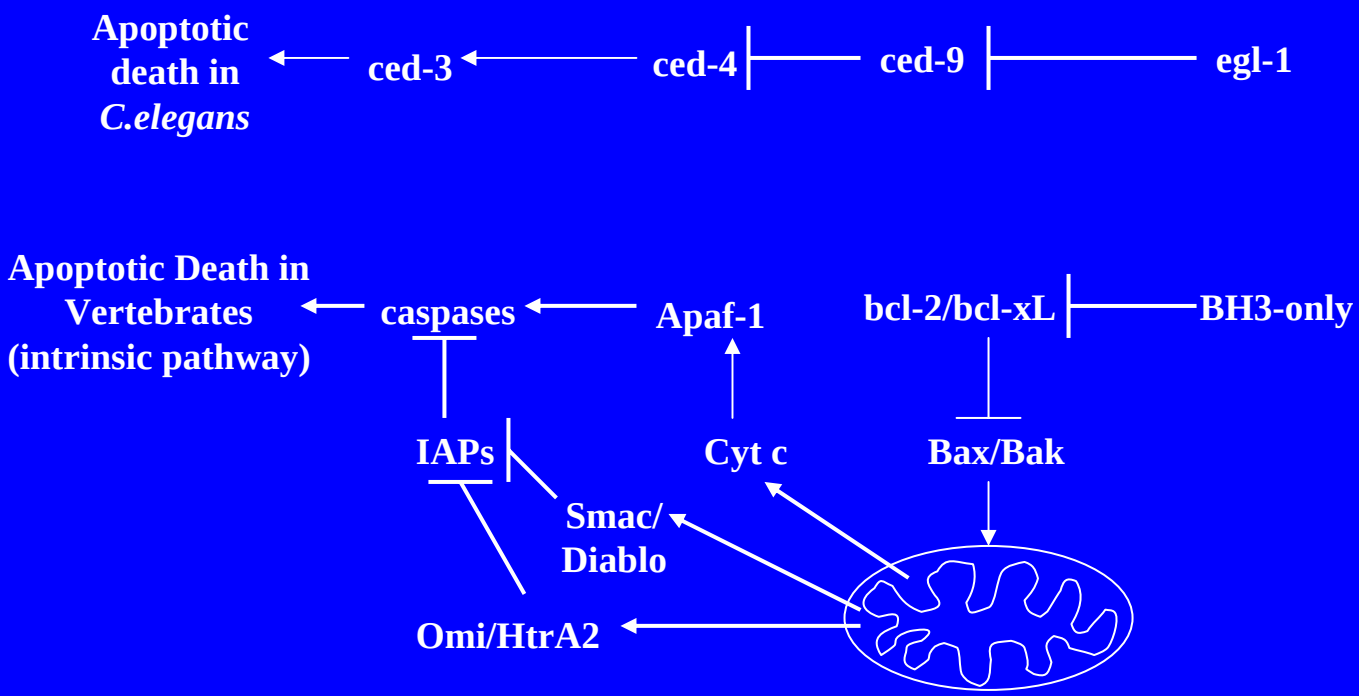
Biochemical pathways of apoptosis in *C.elegans* and vertebrates



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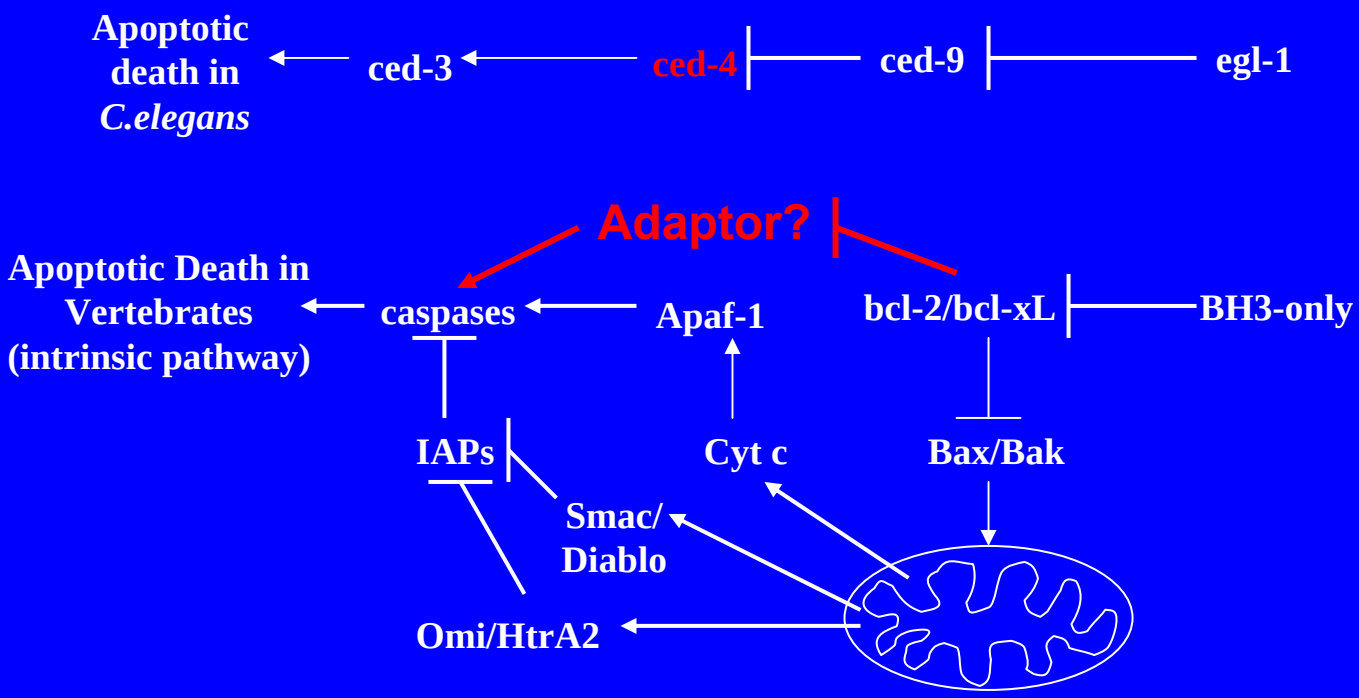
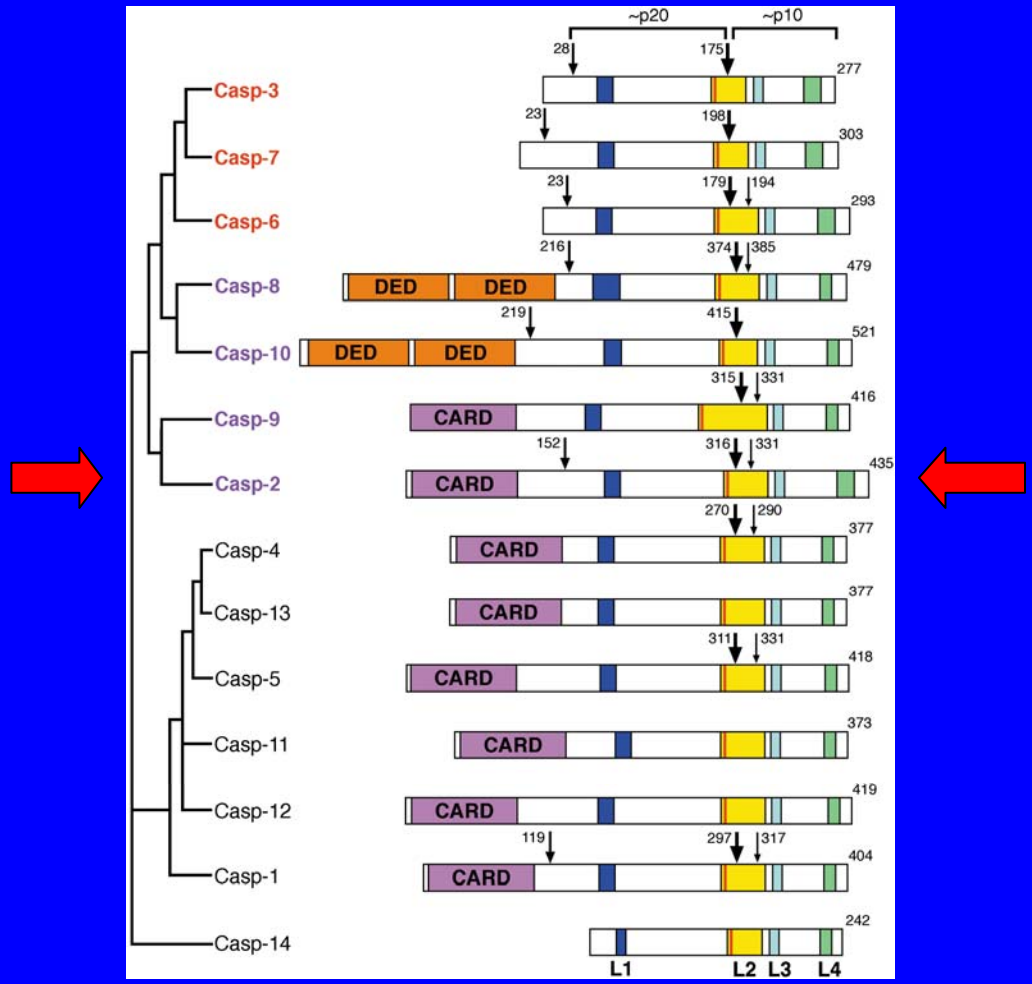
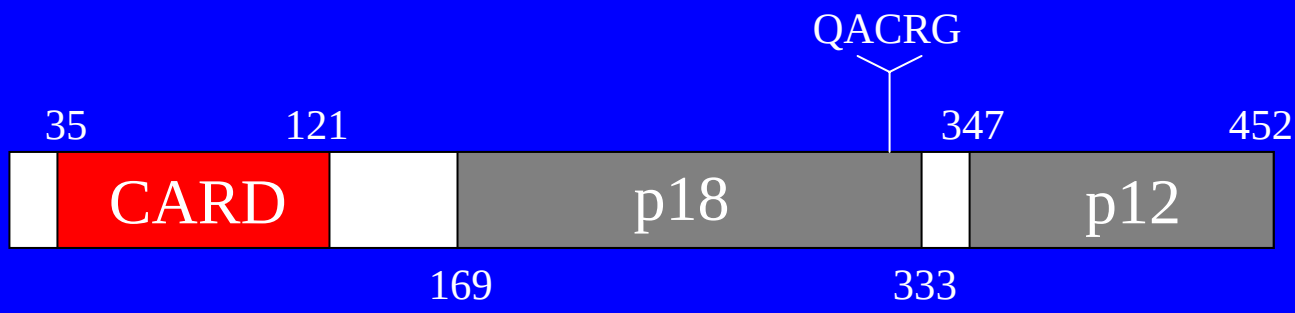


The caspases: Cysteine proteases involved in cell death



Structure of rat caspase 2



Caspase 2

- Initially identified as gene that is downregulated during brain development
- 2 isoforms: long (pro-) and short (?anti)-apoptotic
- Greatest homology with ced-3
- Large prodomain with CARD

Caspase 2

- Initially identified as gene that is downregulated during brain development
- 2 isoforms: long (pro-) and short (?anti)-apoptotic
- Greatest homology with ced-3
- Large prodomain with CARD
- Uncertain role in apoptosis, given the relative lack of a phenotype in null mice
- Debatable whether it is effector or initiator
- Cleavage profile most like caspase-3 and -7
- Unclear manner of activation
- Paucity of substrates, even among other caspases
- Poor inhibition by classical pharmacological caspase inhibitors
- Debated localization (cytoplasmic, nuclear, Golgi, mitochondrial)

The experimental system:

PC12 cells and neonatal rat sympathetic neurons

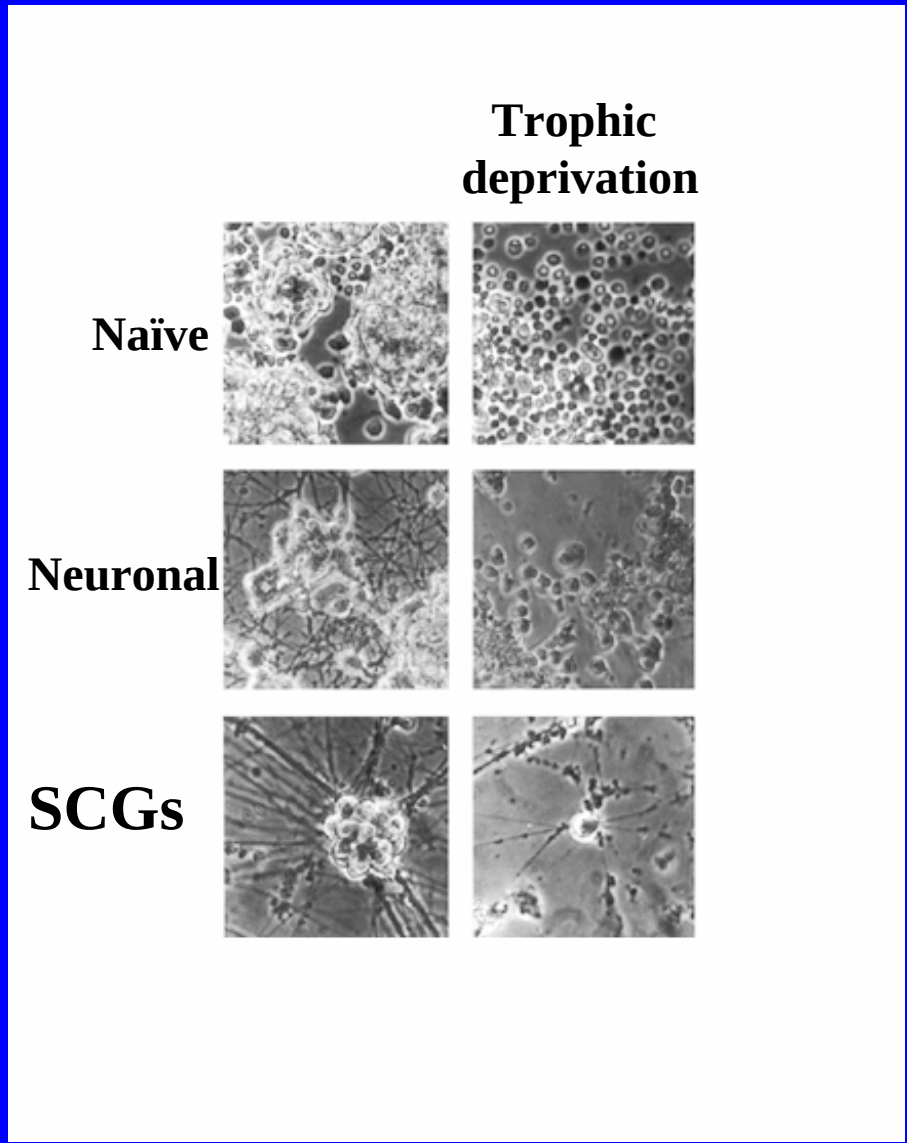
PC12 cells

- Rat pheochromocytoma cells
- In the undifferentiated state (naïve), they proliferate
- They release catecholamines and acetylcholine
- They differentiate into a neuronal (primed) phenotype upon treatment with physiologic concentrations of NGF
- When naïve PC12 cells are deprived of serum or neuronal PC12 cells are deprived of NGF, they undergo apoptotic cell death

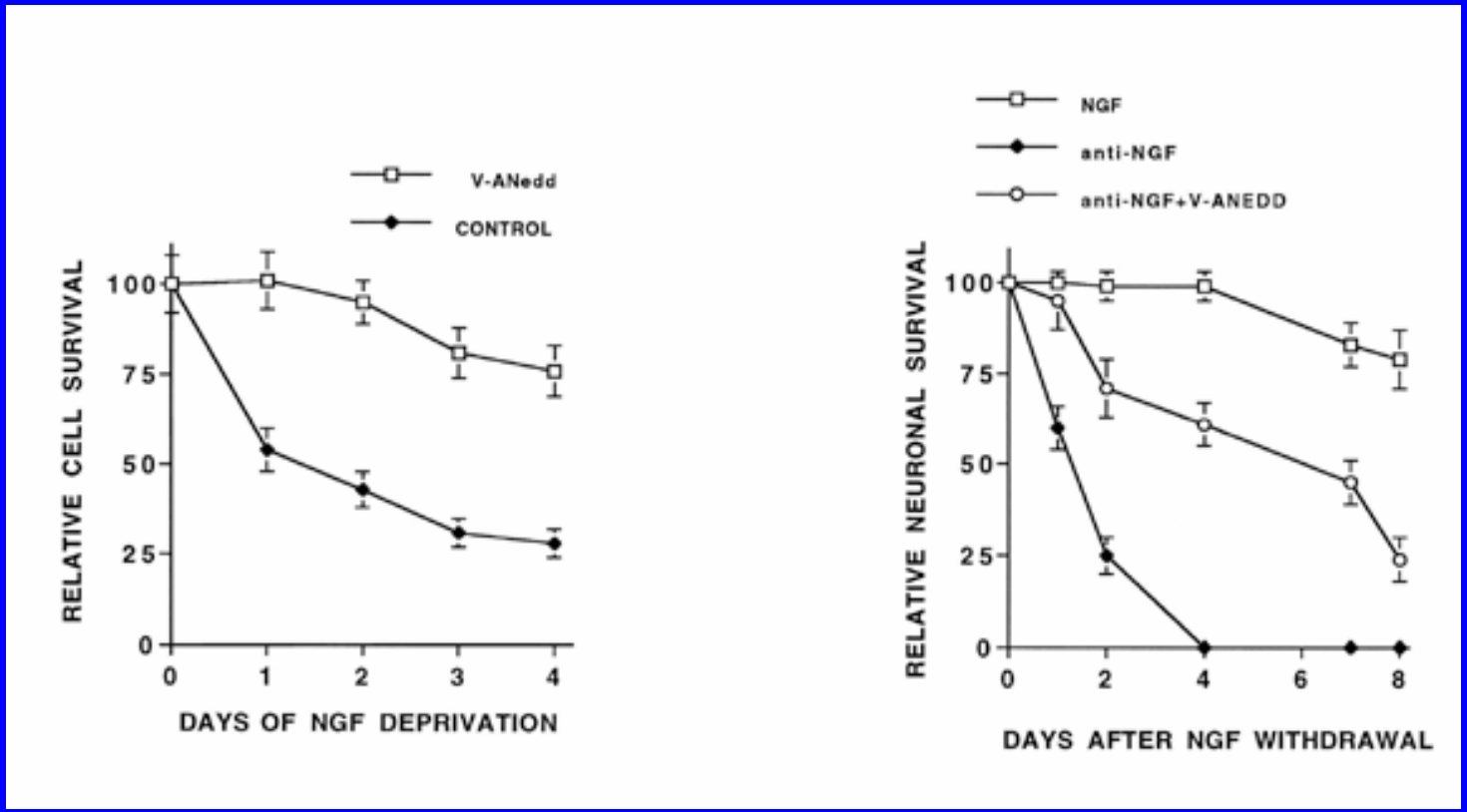
Sympathetic neurons

- Cultures derived from the superior cervical ganglia of neonatal rats (or mice)
- Primary post-mitotic catecholaminergic neurons
- Homogeneous population
- Die within 1-2 days of NGF deprivation
- Death is apoptotic
- This model replicates the in vivo phenomenon of total dependence of SCG neurons on NGF for survival during a critical perinatal period
- It is the best characterized cell culture model for neuronal cell death that occurs normally during development, ie neuronal PCD

Trophic deprivation-induced death of PC12 cells and sympathetic neurons



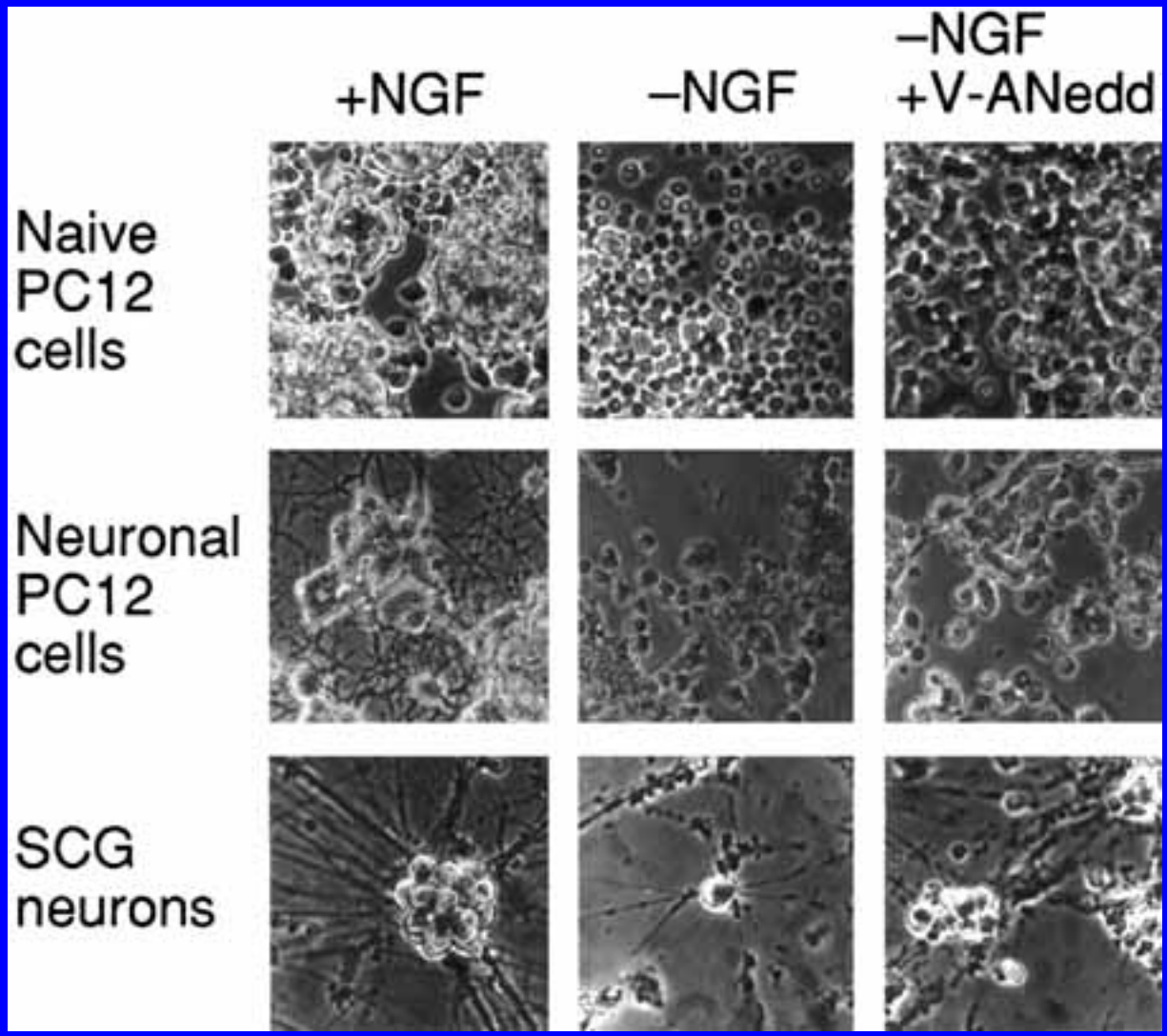
Application of an antisense oligonucleotide directed against caspase 2 attenuates trophic deprivation-induced death of PC12 cells and sympathetic neurons



Neuronal PC12 cells **Sympathetic Neurons**

Troy et al., 1997

Application of an antisense oligonucleotide directed against caspase 2 attenuates trophic deprivation-induced death of PC12 cells and sympathetic neurons



Troy et al., 1997

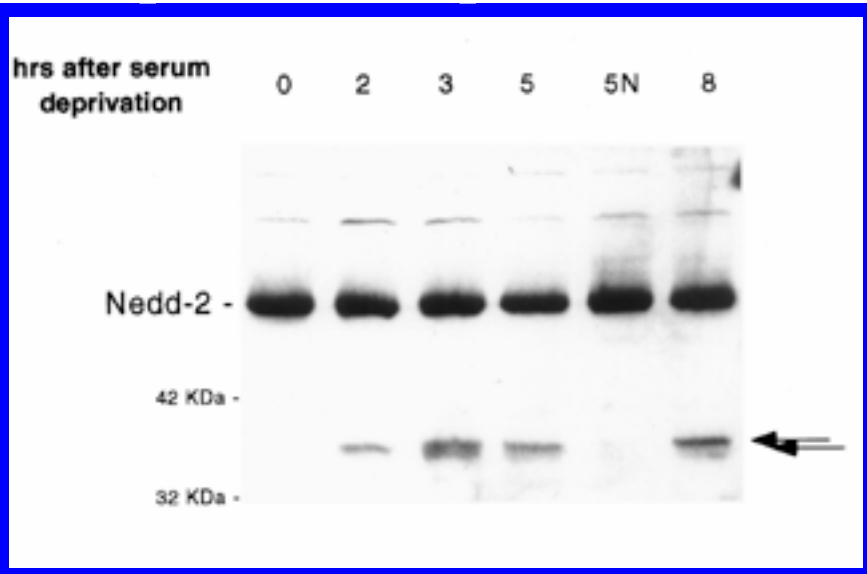
Therefore:

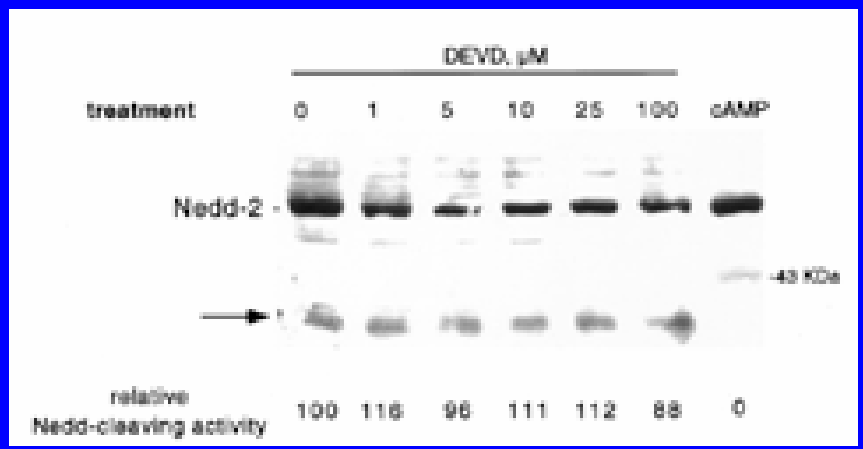
Caspase 2 is involved in apoptotic death in this model

Questions

1) Where does its action take place within the pathway?
2) How is it activated?
3) Is it processed?
4) What is its relationship to other events in the apoptotic pathway?
5) Is it an effector or an initiator caspase?
6) What is the role of the CARD/prodomain?

Processing of caspase 2 following trophic deprivation does not depend on caspase 3-like activity





Stefanis et al., 1998

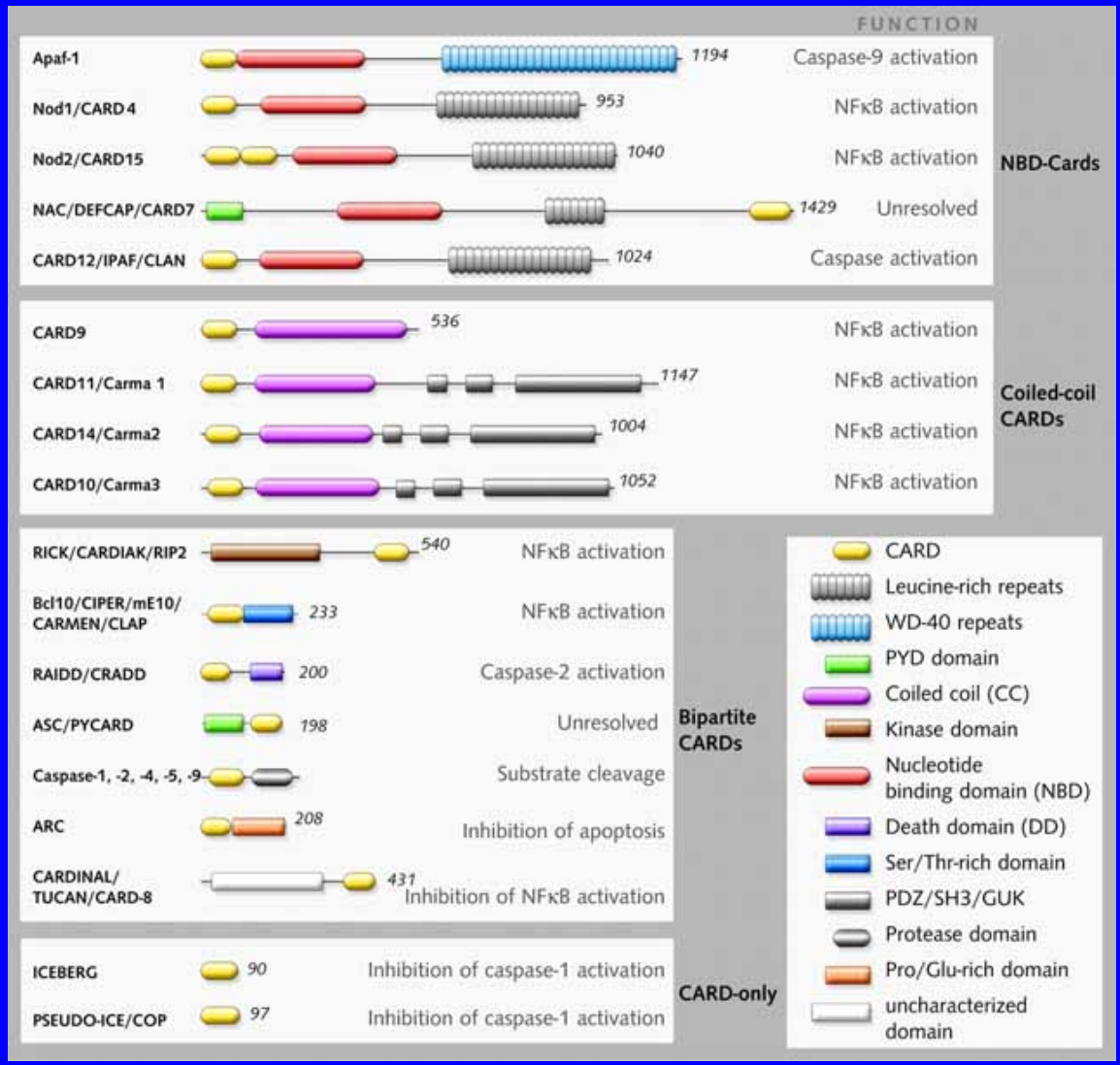
Therefore: Caspase 2 does not appear to be processed/activated (?) by effector caspases

How is it then activated?

A reasonable hypothesis is that it is activated through an interaction with other CARD-containing adaptor proteins

Such identified proteins include RAIDD and ARC ;
PACAP is another caspase 2-binding protein,
but it does not have a CARD

Proteins with CARDs

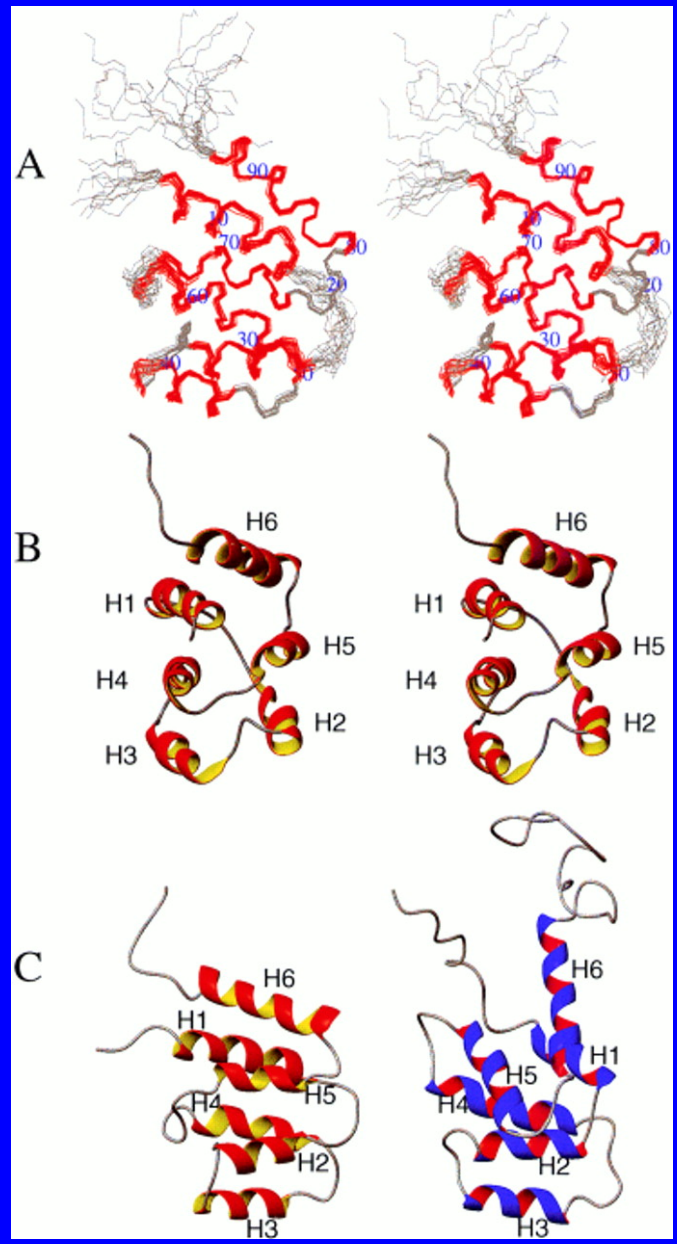


Caspase 2-interacting molecule: RAIDD

- Cloning of human RAIDD/CRADD (Duan and Dixit, 1997; Ahmad et al., 1997)
- Has a N-terminal CARD and a C-terminal DD
- Binds to caspase 2 CARD through its own CARD
- Induces death upon overexpression
- Death inhibited by DN C2
- Wide tissue and cell type expression
- Has not been implicated in any known apoptotic pathway

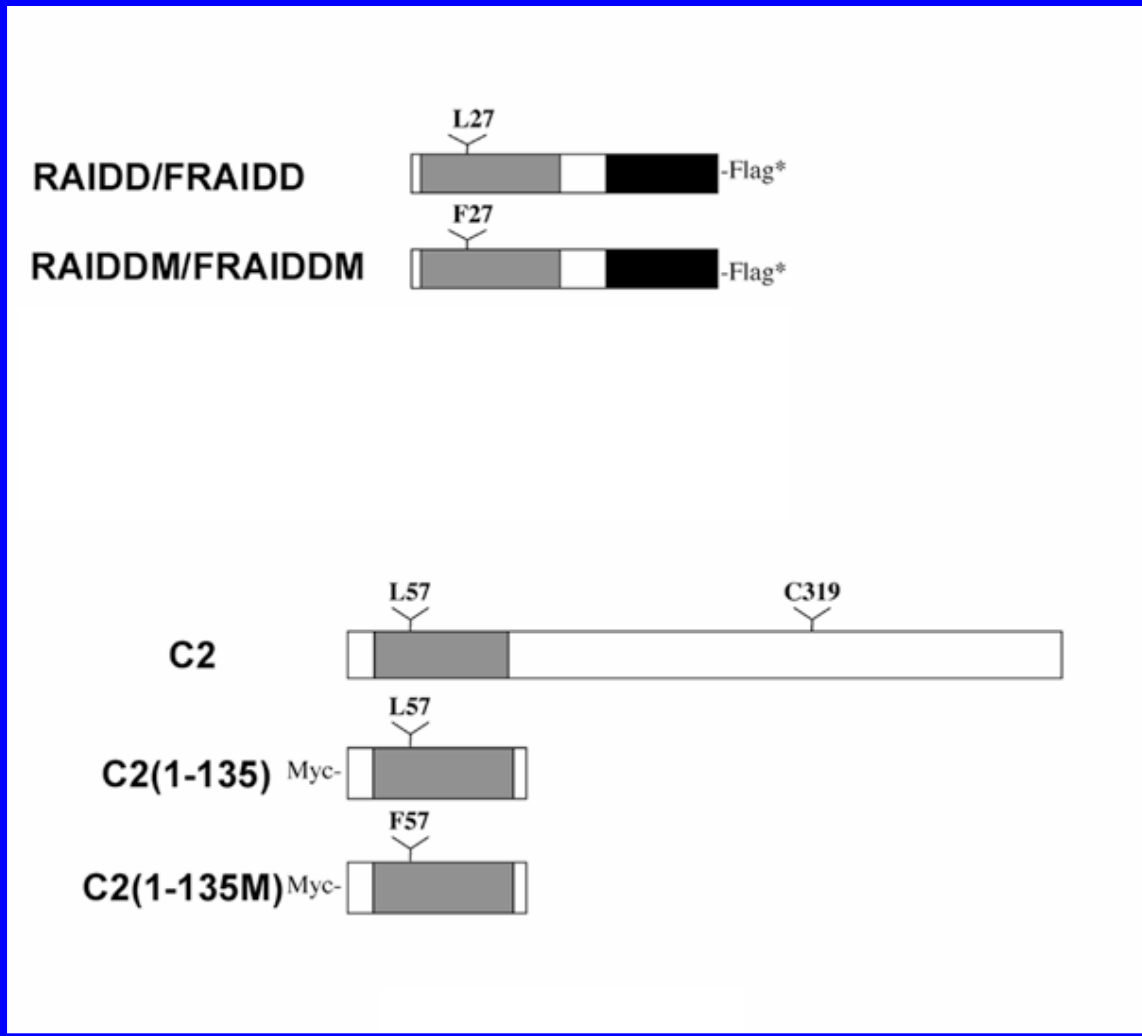
Three-dimensional model of the structure of the RAIDD CARD

Chou et al., 1998

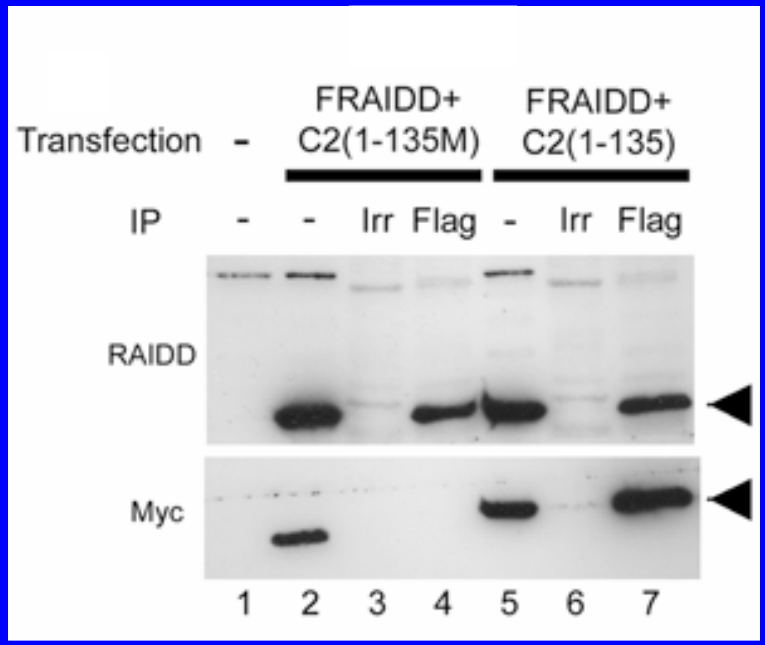


Rat RAIDD

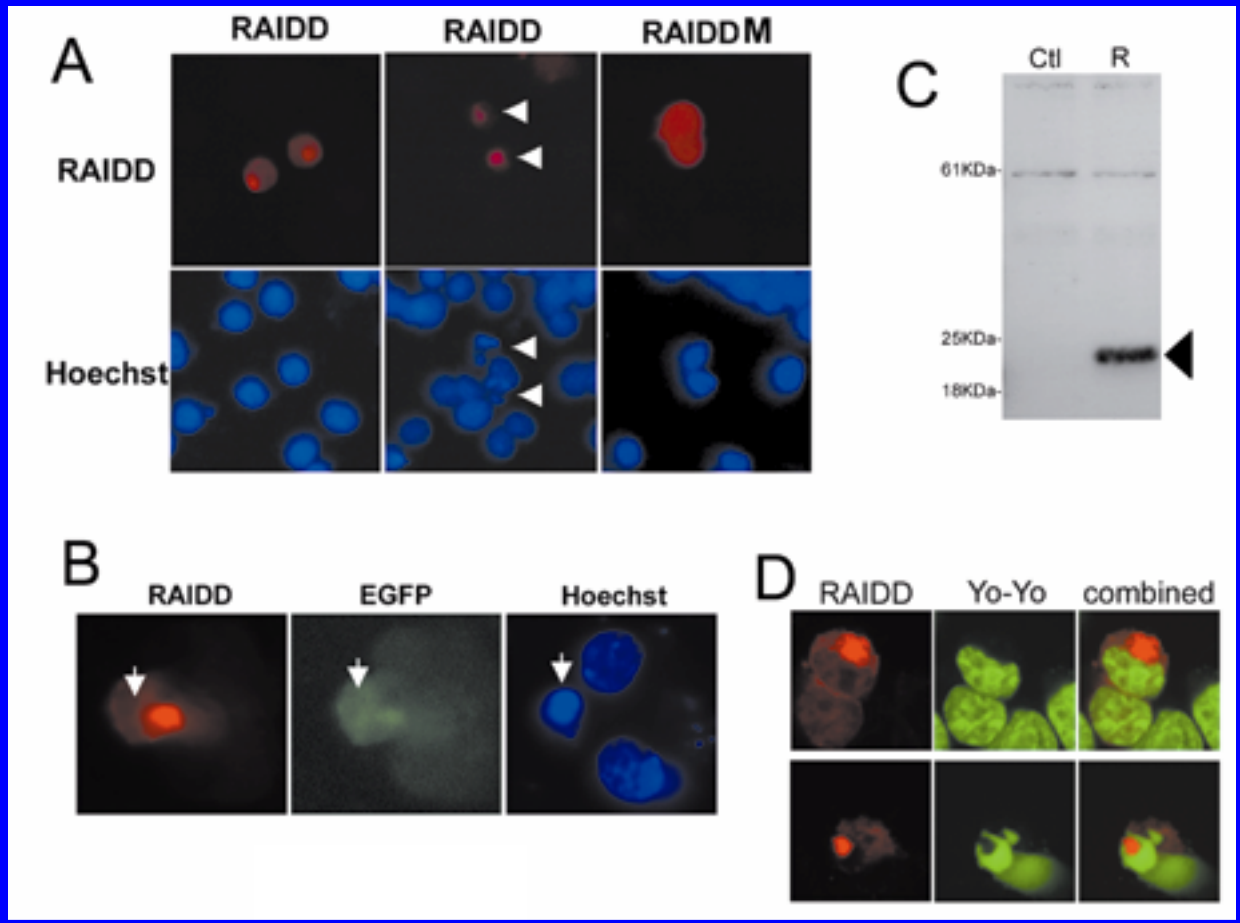
Rat Caspase 2 and CARD-only constructs



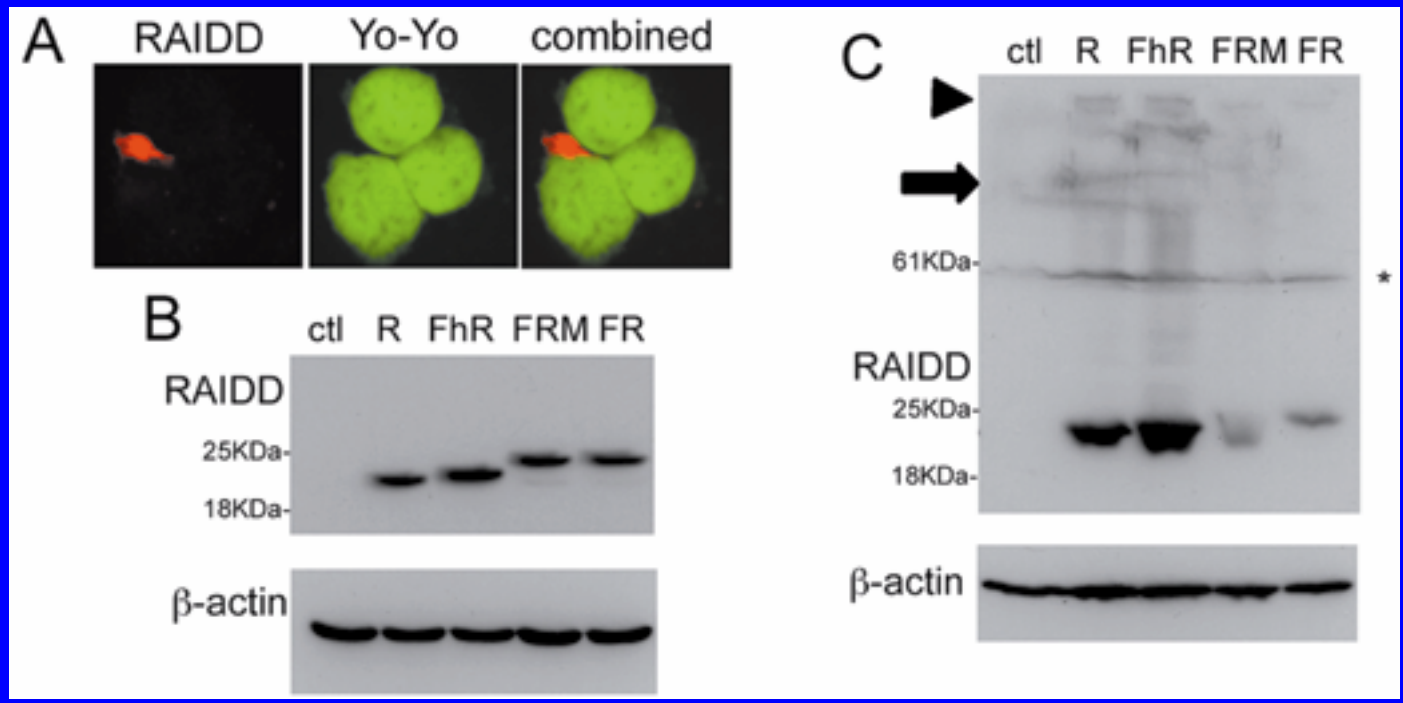
Rat RAIDD and the rat caspase 2 CARD associate upon overexpression in 293T cells



Overexpressed RAIDD forms aggregates and induces apoptosis in PC12 cells and sympathetic neurons

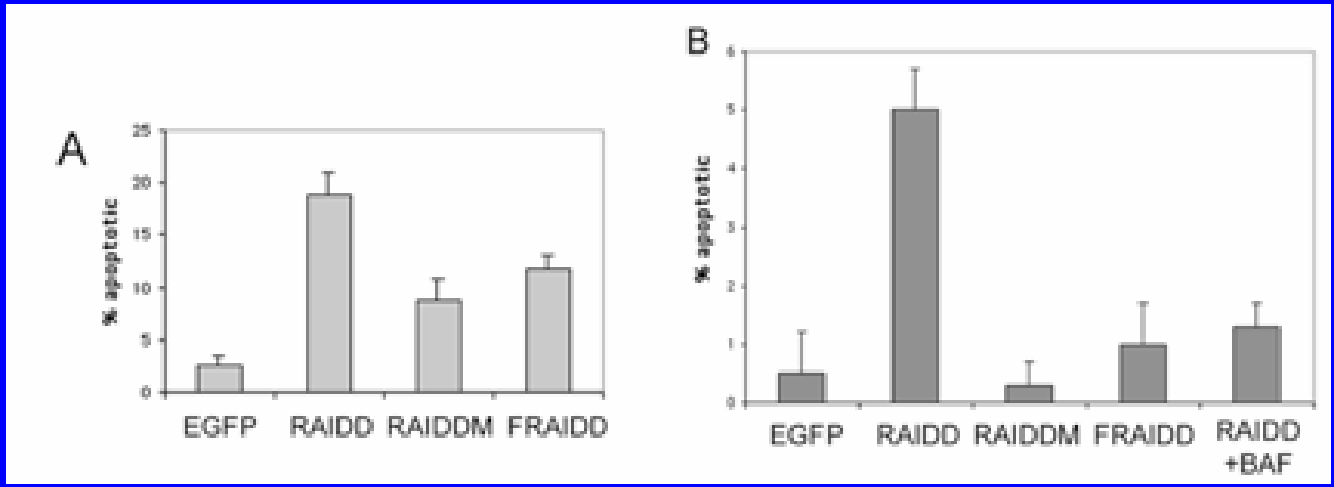


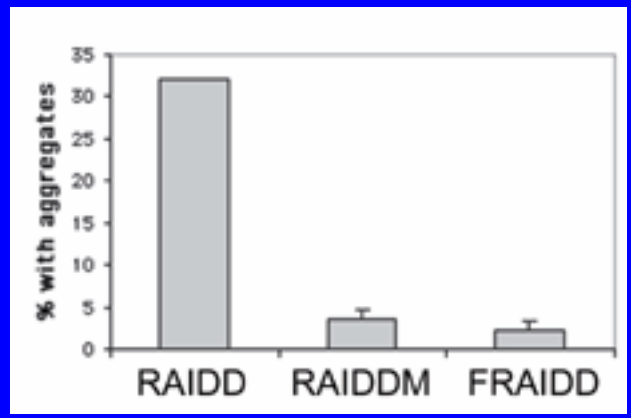
RAIDD aggregates are detergent-insoluble



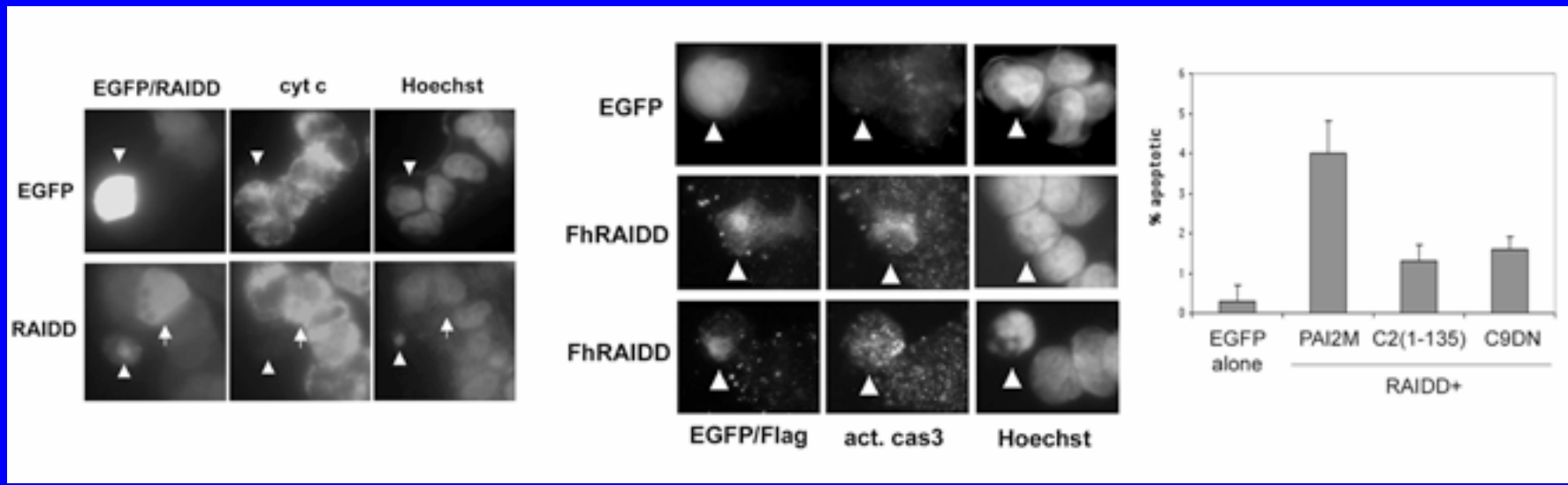
Detergent-soluble **Detergent-insoluble**

Apoptosis induced by RAIDD overexpression in PC12 cells is variable, but correlates with the ability to induce aggregate formation

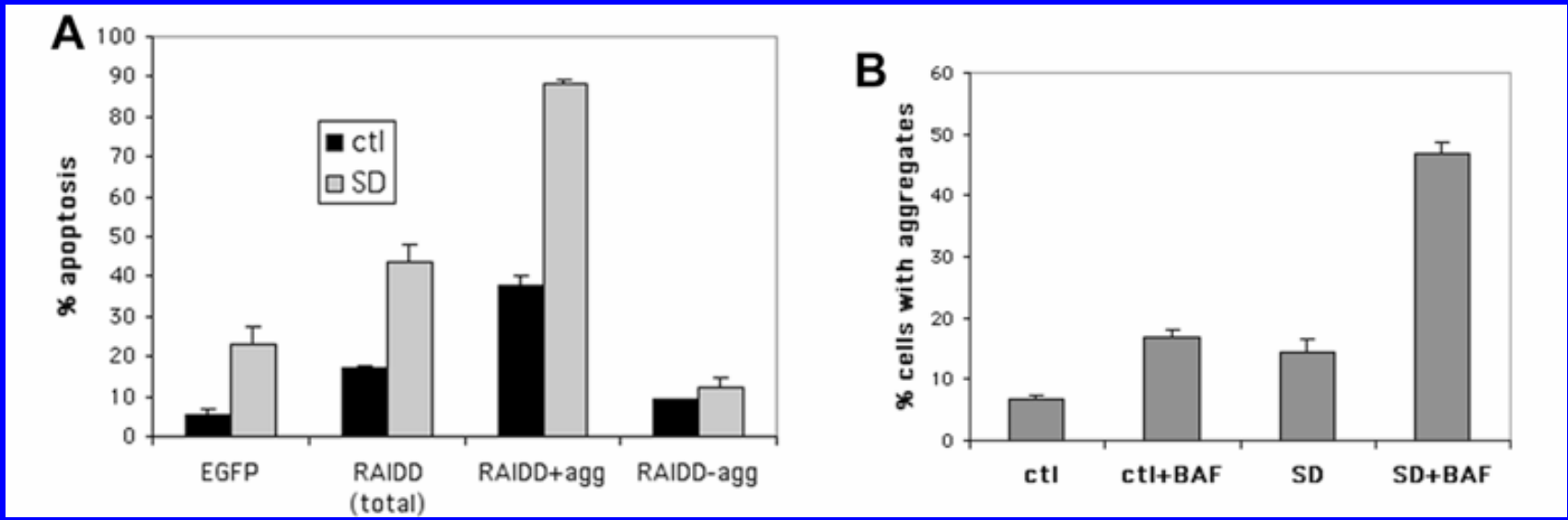




RAIDD overexpression activates the “intrinsic” mitochondrial pathway, but death also depends on an interaction with the caspase 2 CARD



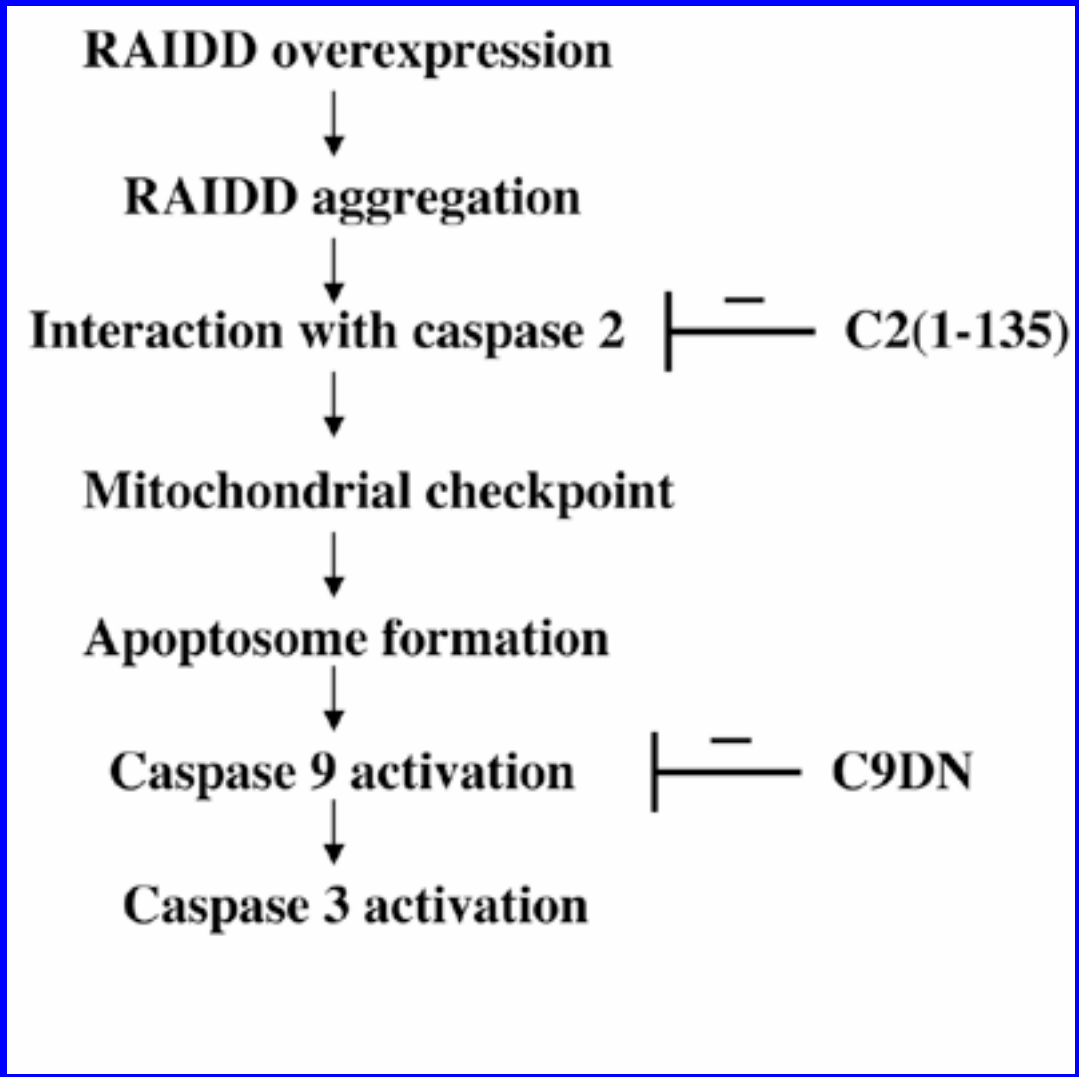
RAIDD overexpression acts synergistically with serum deprivation to induce death of PC12 cells



Cells with aggregates are much more likely to be apoptotic

Aggregates increase with serum deprivation in a caspase-independent fashion

Model of RAIDD overexpression-induced death of PC12 cells

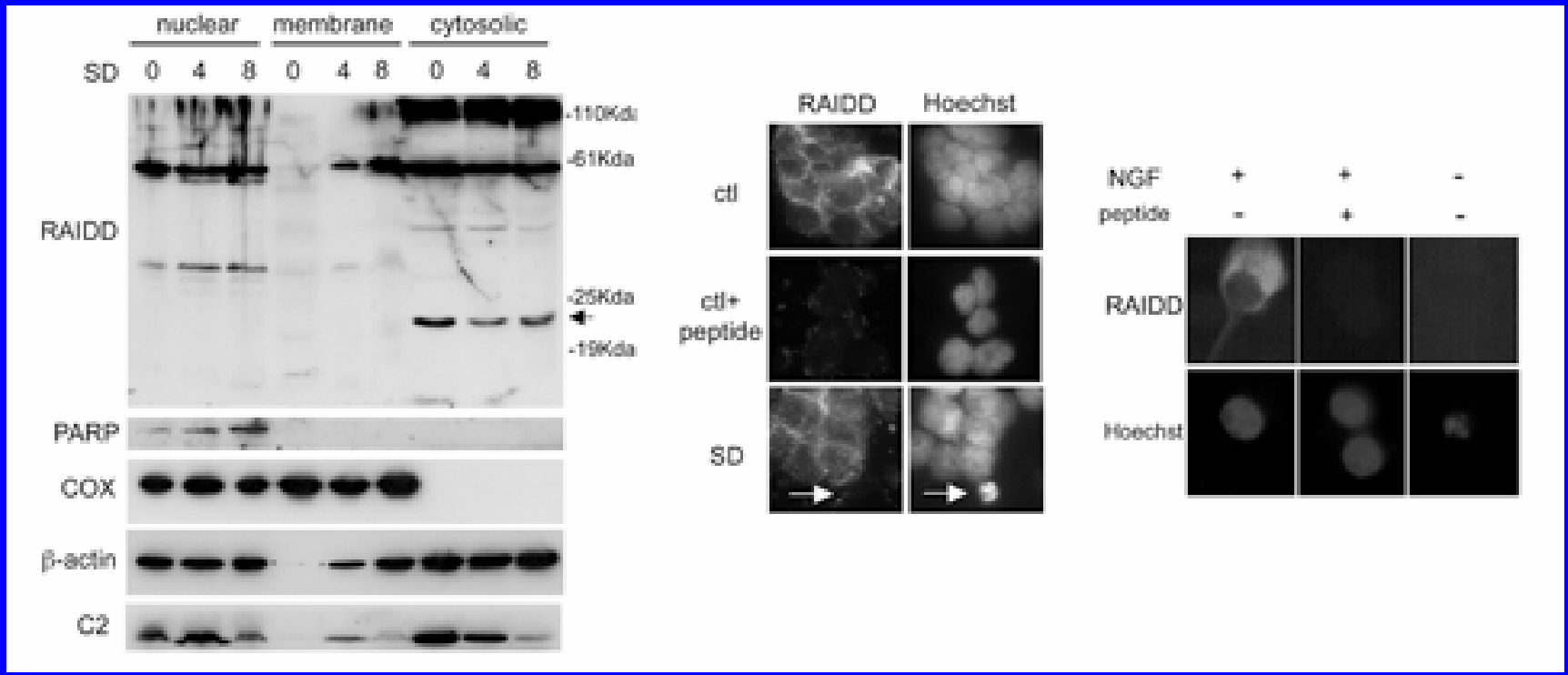


RAIDD overexpression: Conclusions

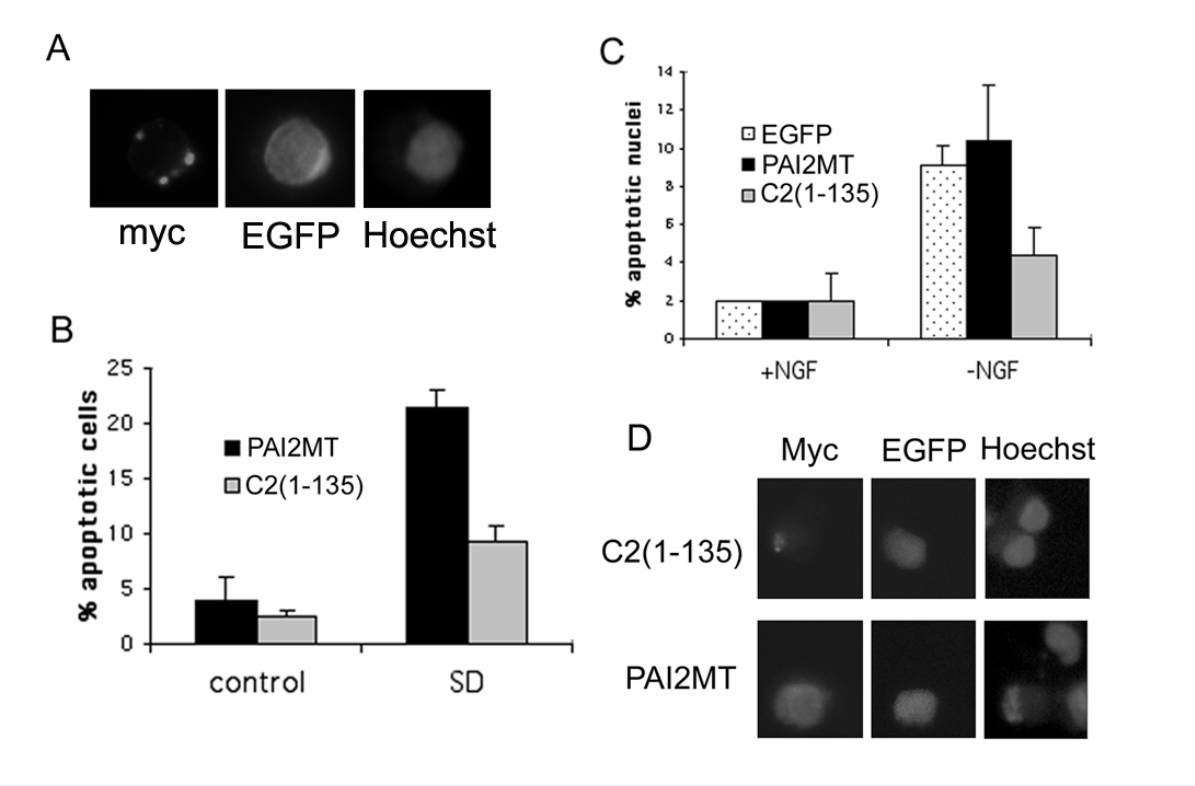
- RAIDD oligomerization in discrete perinuclear aggregates correlates with cell death
- Serum deprivation enhances RAIDD oligomerization in a caspase-independent fashion
- Serum deprivation and RAIDD expression act synergistically in inducing death

What about endogenous RAIDD?

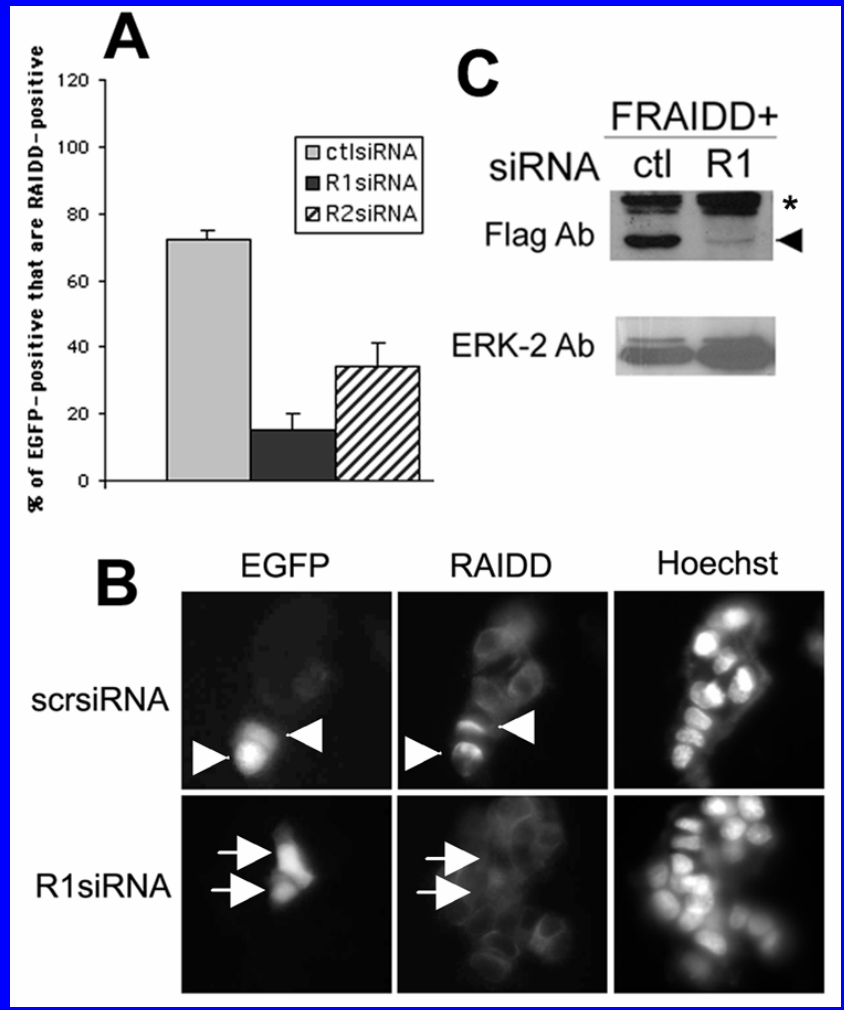
The endogenous 23 Kda RAIDD is cytosolic, and its levels or localization do not change appreciably with trophic deprivation



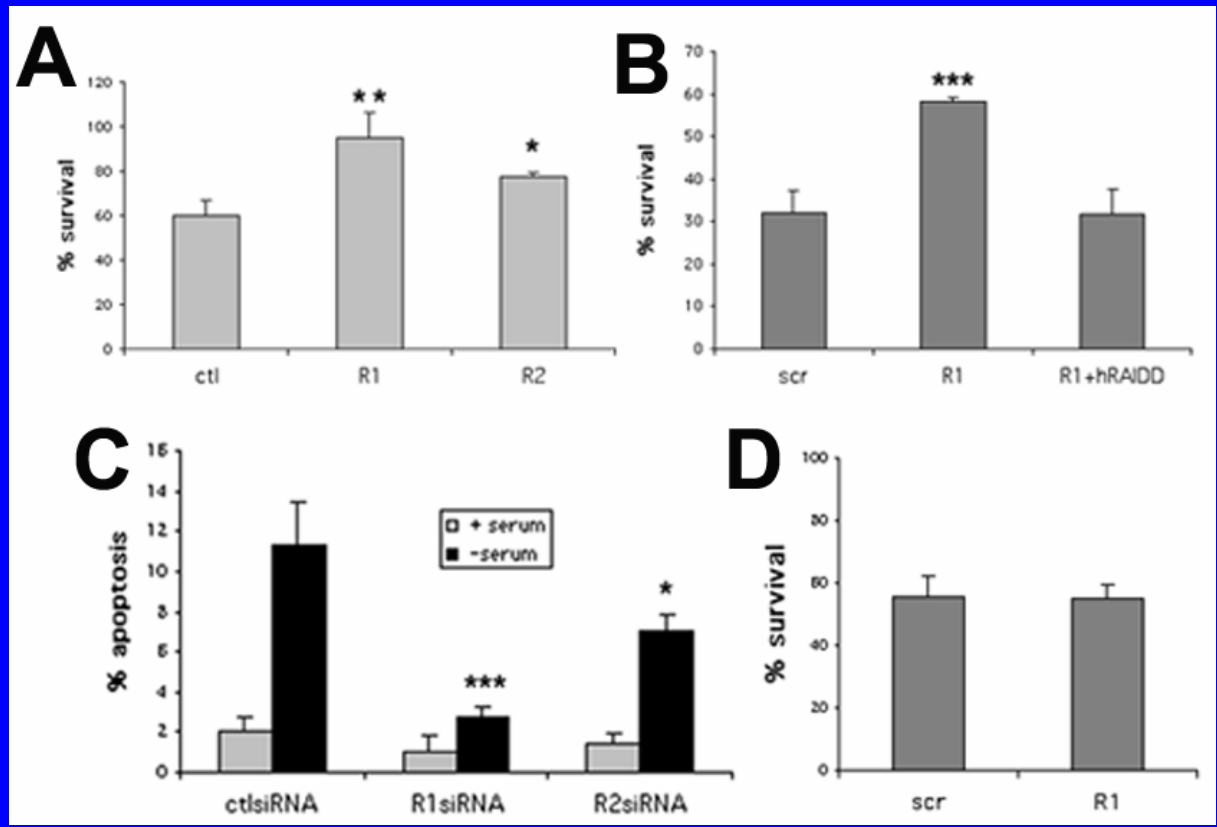
Overexpression of CARD-only caspase 2 attenuates trophic deprivation-induced neuronal death



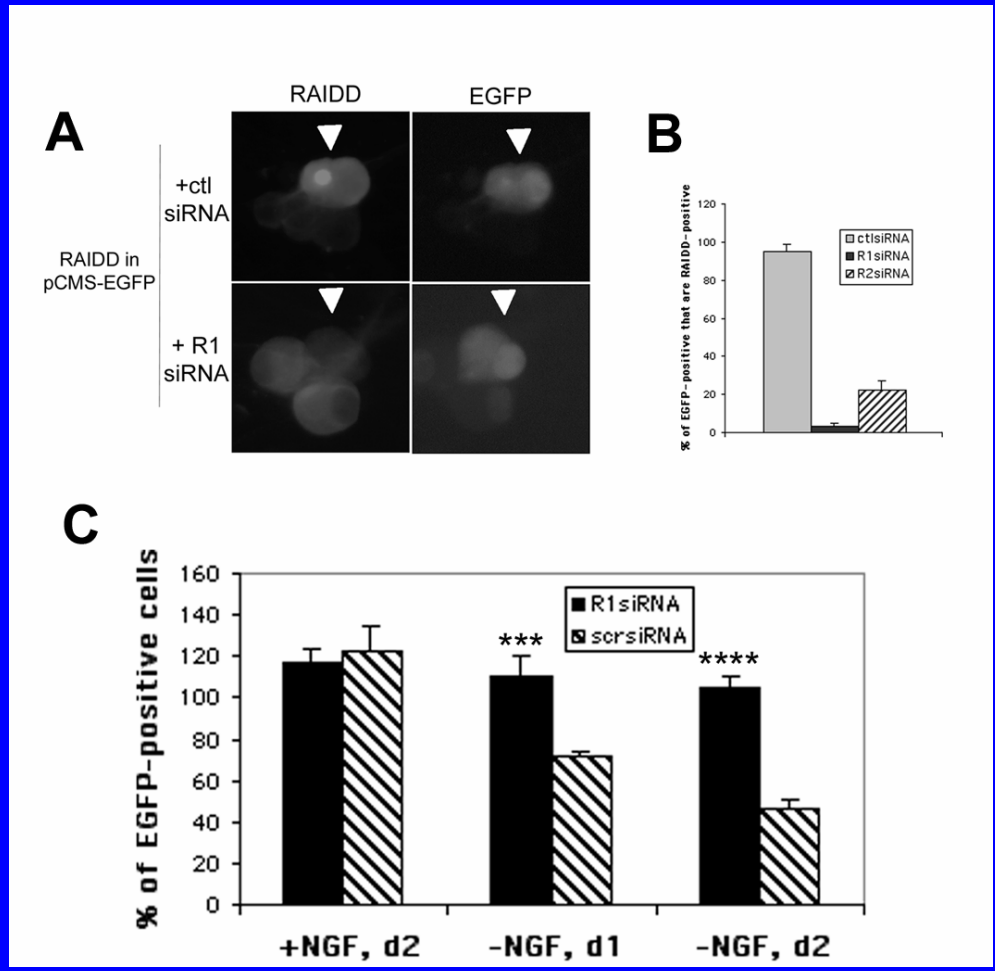
siRNAs targeted against RAIDD diminish its expression in PC12 cells



siRNAs targeted against RAIDD diminish serum deprivation-, but not DNA damage-induced death of PC12 cells



siRNAs targeted against RAIDD also diminish NGF deprivation-induced death of sympathetic neurons



Conclusions

- RAIDD is localized to the cytosol, where caspase 2 is also predominantly localized
- RAIDD is involved in trophic withdrawal-induced death of PC12 cells and sympathetic neurons
- This involvement is apparently linked to its interaction with caspase 2 (based on the inhibition of death with C2(1-135))
- RAIDD (or caspase 2) does not appear to be involved in DNA damage-induced death of PC12 cells

Further Questions

- How is the RAIDD/caspase 2 interaction mediated?
- At what point in the apoptotic pathway does it occur?
- Are there other molecules involved in this interaction?

Meanwhile…….