

MCDB 153

## Developmentally Regulated Neuronal Survival and Neuronal Cell Death



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?

Target of  
Innervation

Lecture Set 6

*Chronologically, what has  
happened so far?*

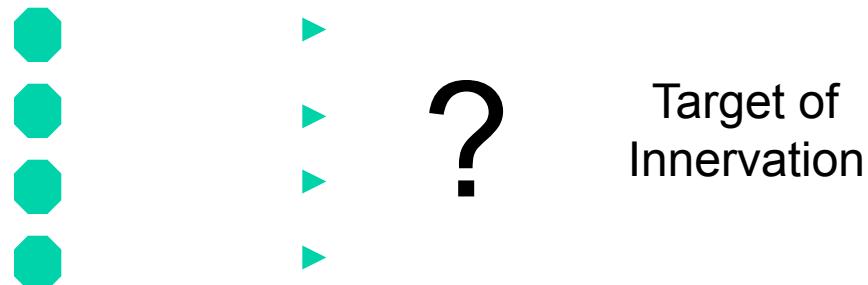
Fertilization --> Blastula --> Gastrula --> Neurula

- Neuroblast Proliferation
- Neuroblast Migration
- Axon Outgrowth
- Programmed Cell Death
- Target Selection & Synaptogenesis
- Synaptic Rearrangements
- Active Cell Maintenance

# Outline

1. The early days - “Recruitment Model”
2. Viktor Hamburger-Naturally Occurring Cell Death
3. Discovery of NGF and Related Factors
4. Defining Molecular Mechanisms
5. Current View: Target Mediated Regulation of Apoptosis

The First Big Question:  
Is there a relationship between targets of innervation and  
their pool of innervating neurons?



## 1. The Early Days....."Recruitment"

1909 - M.L. Shorey

Question: Is there a relationship between targets of innervation and the pool of innervating neurons?

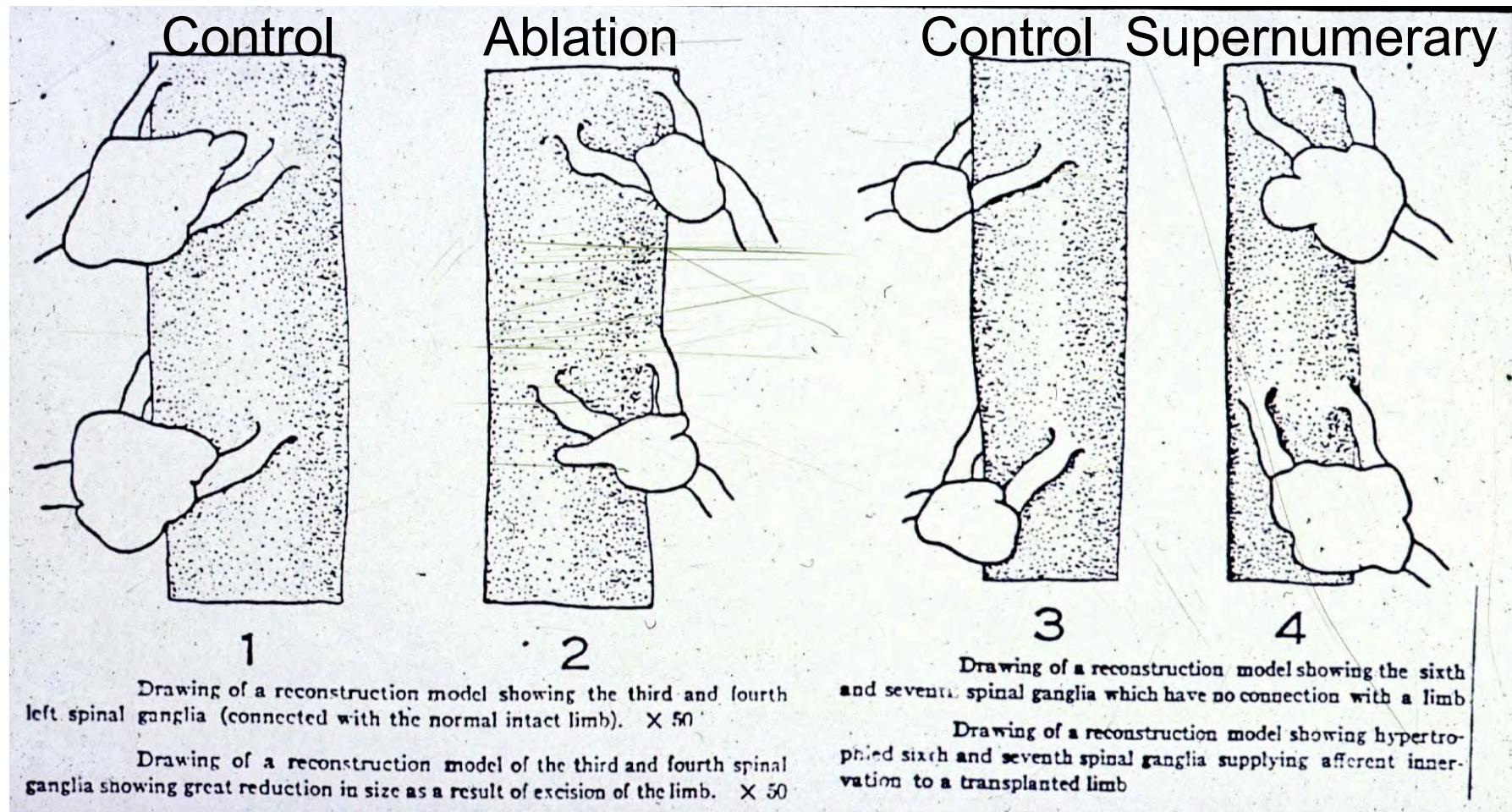
Expt: Removes limb bud from one side of a chick embryo and see what happens;

Result: Many fewer neurons in the region of the spinal cord that would ordinarily innervate the “missing” limb;

BUT, the expt was not done very quantitatively  
very difficult for technical reasons

1920 - Detwiler

does both limb ablation and limb addition  
(aka, "supernumerary limbs")

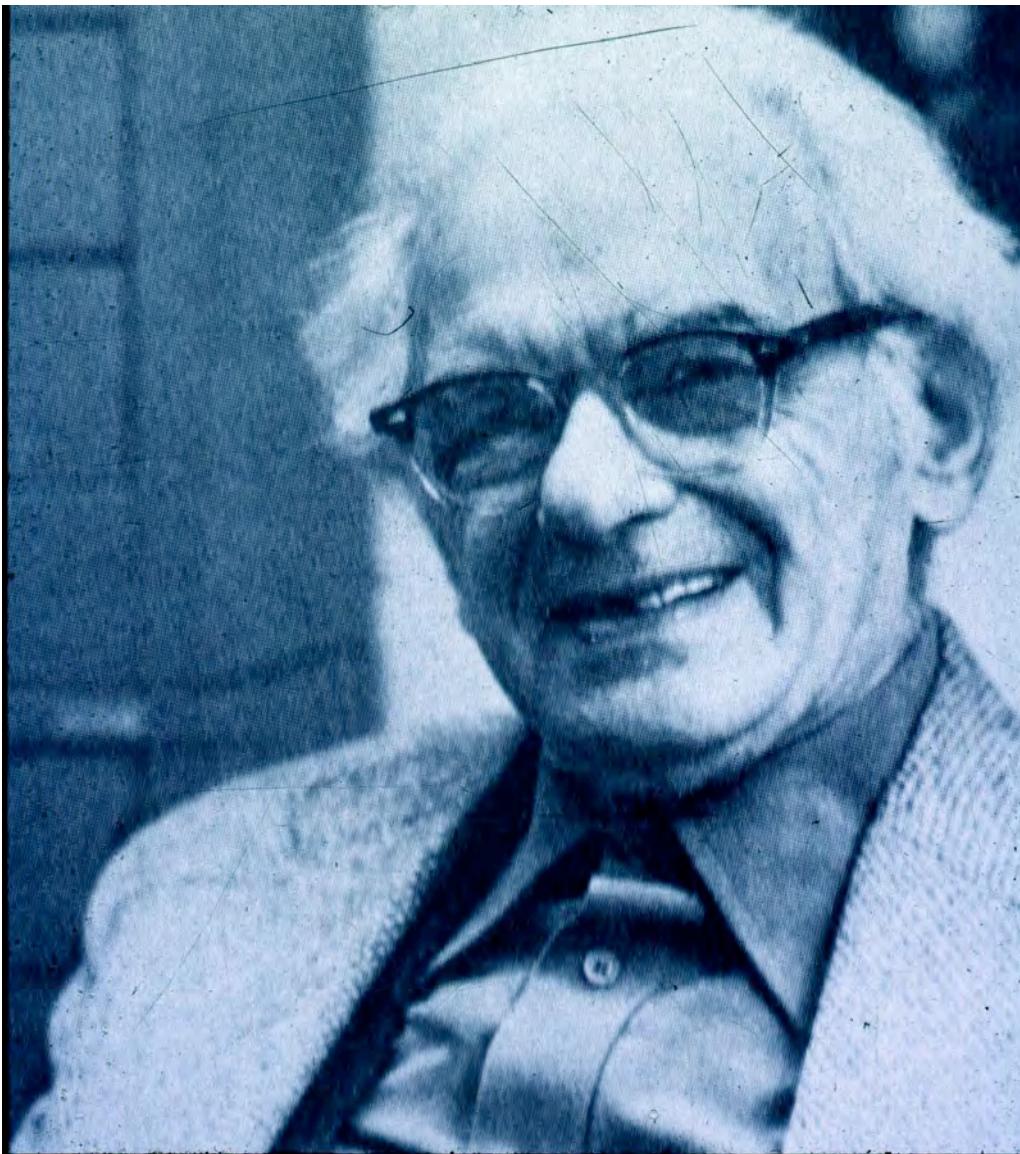


These data led to the “Recruitment Model” for the effects of targets upon neuronal fate determination.

Model: Targets of innervation “recruit” otherwise naïve cells to become neurons.

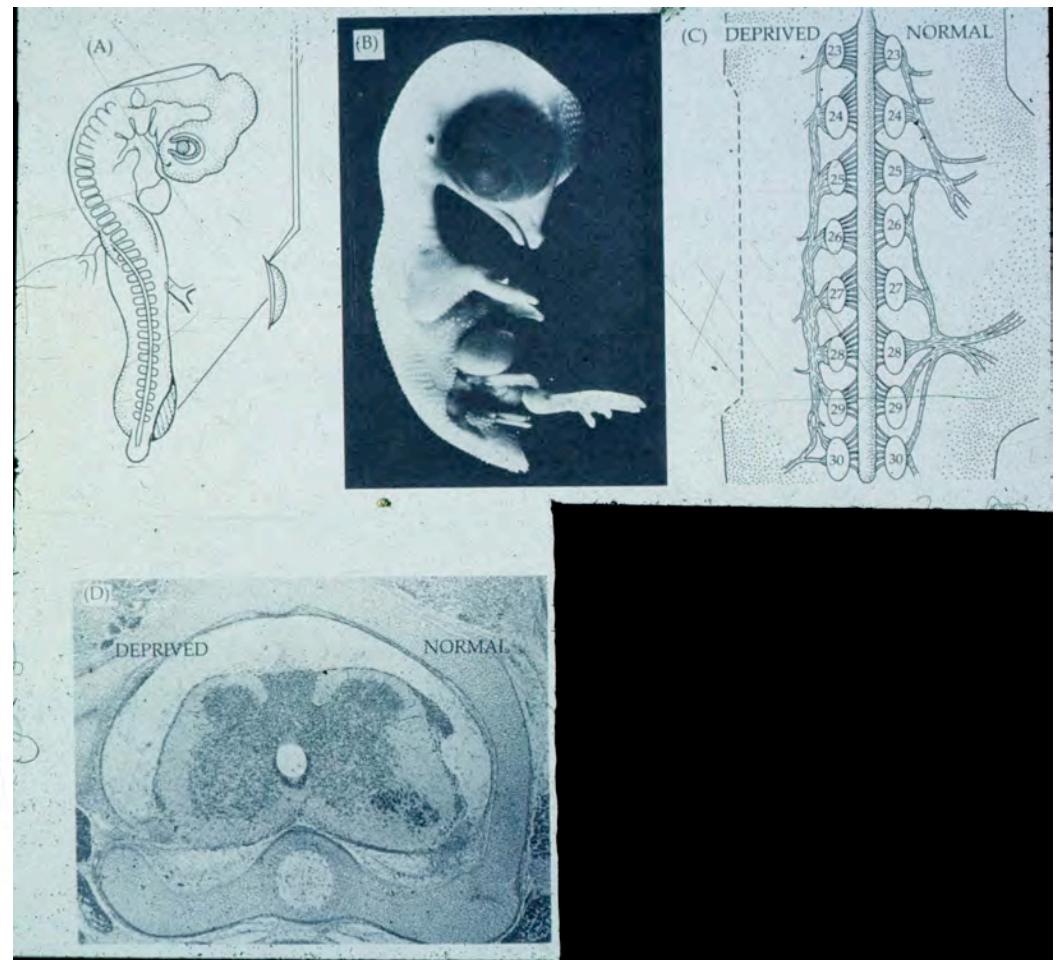
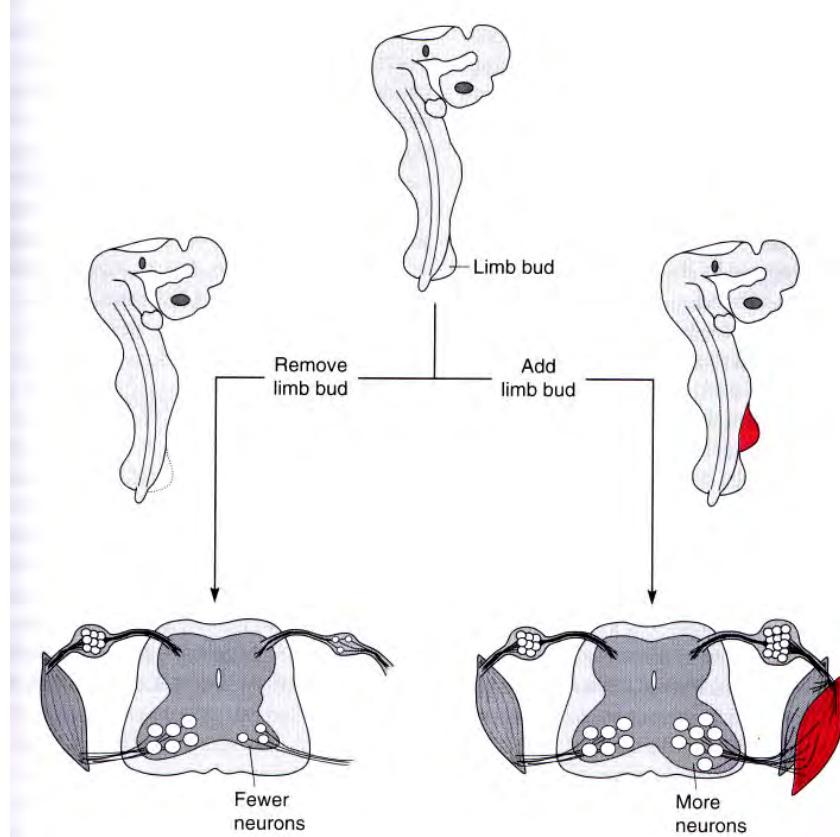
One possible mechanism is that targets release “recruitment factors” that influence fate determination in the naïve cells.

## 2. Viktor Hamburger - Naturally Occurring Cell Death



# Key Hamburger Expts in 1940's

## Reduced and extra target material



Limb removal

The key experiment is to count neurons  
(hundreds of thousands of them)  
at various times during development

## Neuronal Cell Numbers in Control Ganglia and Ganglia Innervated by a Supernumary Limb

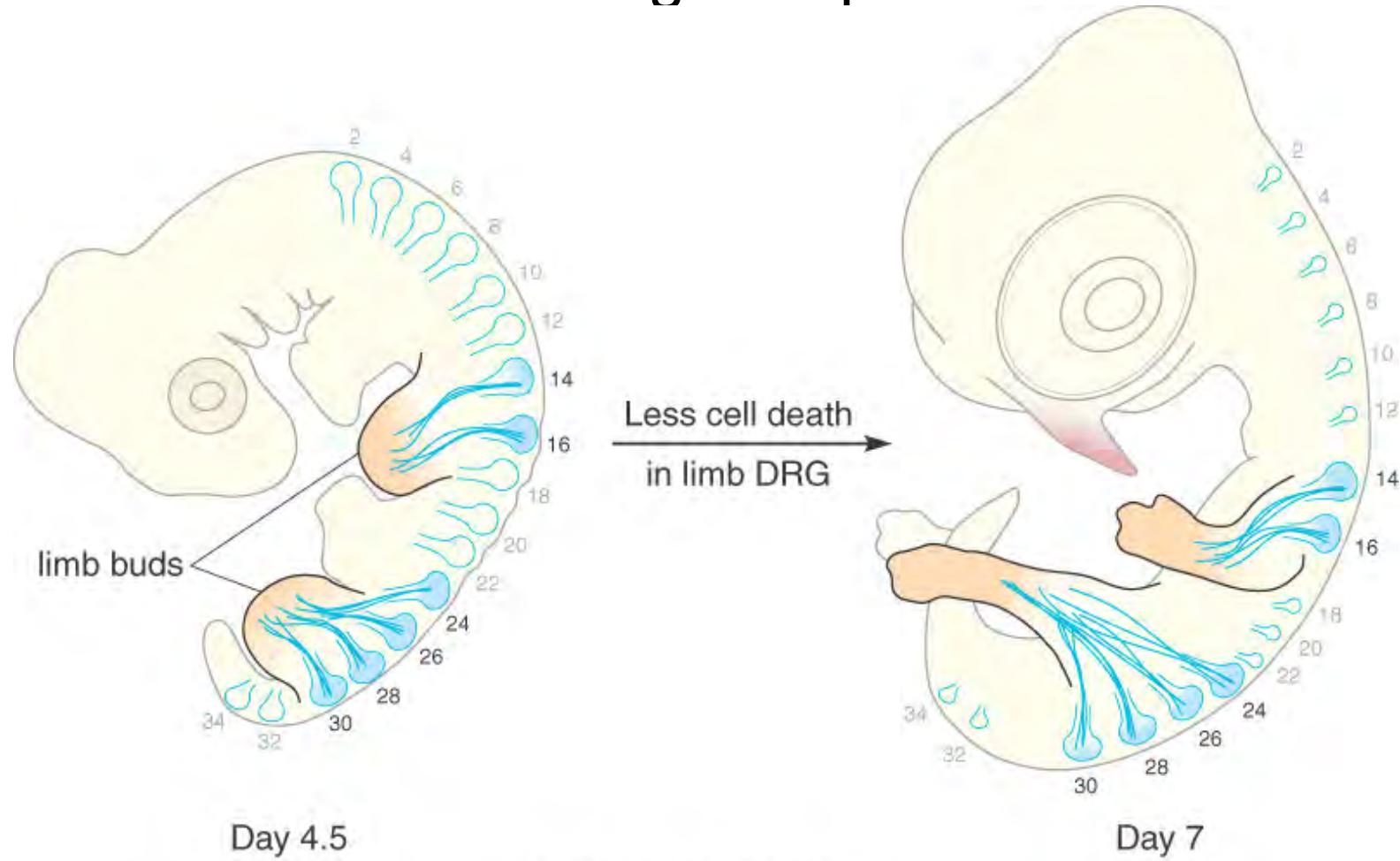
<u>Age and ID</u>	<u>LMC</u>		<u>Percent Difference</u>
	Control	Experimental	
6 day embryos			
6.6.1	15,694	15,570	-0.8%
6.19.1	16,262	16,276	+0.1%
6.19.2	15,615	15,587	-0.2%
7.1.1	16,568	16,489	-0.5%
9.8.2	18,114	17,445	-3.8%
12 day embryos			
2.14.3	10,930	12,372	+13.2%
2.14.4	10,815	12,346	+14.2%
2.21.1	10,313	11,598	+12.7%
3.5.1	10,252	13,068	+27.5%
3.27.1	9,512	11,610	+22.1%
7.24.4	9,909	11,919	+20.3%
9.7.1	10,973	12,175	+11.0%
9.8.1	10,903	12,460	+14.3%
9.13.1	9,701	11,171	+15.2%
9.13.3	10,938	13,165	+20.4%
19 day embryos			
9.11.5	9,978	11,186	+12.1%
11.20.1	9,648	10,727	+11.2%

Based on these and additional data, Hamburger proposes:

1. The decline in final neuronal numbers following target ablation occurs, at least in part, because many neurons that are initially present degenerate following ablation;  
“atrophy” causes reduced numbers, not lack of “recruitment”
2. “Cell death occurs not only after limb ablation, but as an ordinary and normal feature of embryonic development” throughout the nervous system

...not received by the community with much enthusiasm.....

# Additional Hamburger Expts in 1940's



(Adapted from Hamburger and Levi-Montalcini, 1949)

Neuron number is correlated with the amount of target tissue. The size of DRGs at segments 14-16 decreases only slightly during development (large target) whereas DRGs at segments 18-22 become much smaller (small target)...fits with Hamburger's model...

Lots more data starts being acquired (idealized data below)

Situation	% cell death	% cell survival
Normal	50	50
Ablate target	100	0
Partial ablation	>50	<50
Enlarge target	<50	>50

Obvious conclusion is that there is “target dependent” or “target regulated” cell death

What is the molecular mechanism(s) by which targets can regulate neuronal cell death?

Clues:

Cell death occurs over a relatively brief window of time, when growth cones are newly arrived at the target;

Early cell biology of all neurons seems the same, i.e. all neurons appear to have an equal chance of survival

## Summary

1. Cells send axons to targets;
2. Some percentage of them die;
3. More die if target is ablated;
4. Less die if target is enlarged;
5. Death begins ~when axons arrive at targets.

The most straight-forward model is that neuronal survival involves acquisition of a target-derived “survival factor” which is present in limiting supply, leading to competition for it by the pool of innervating neurons.

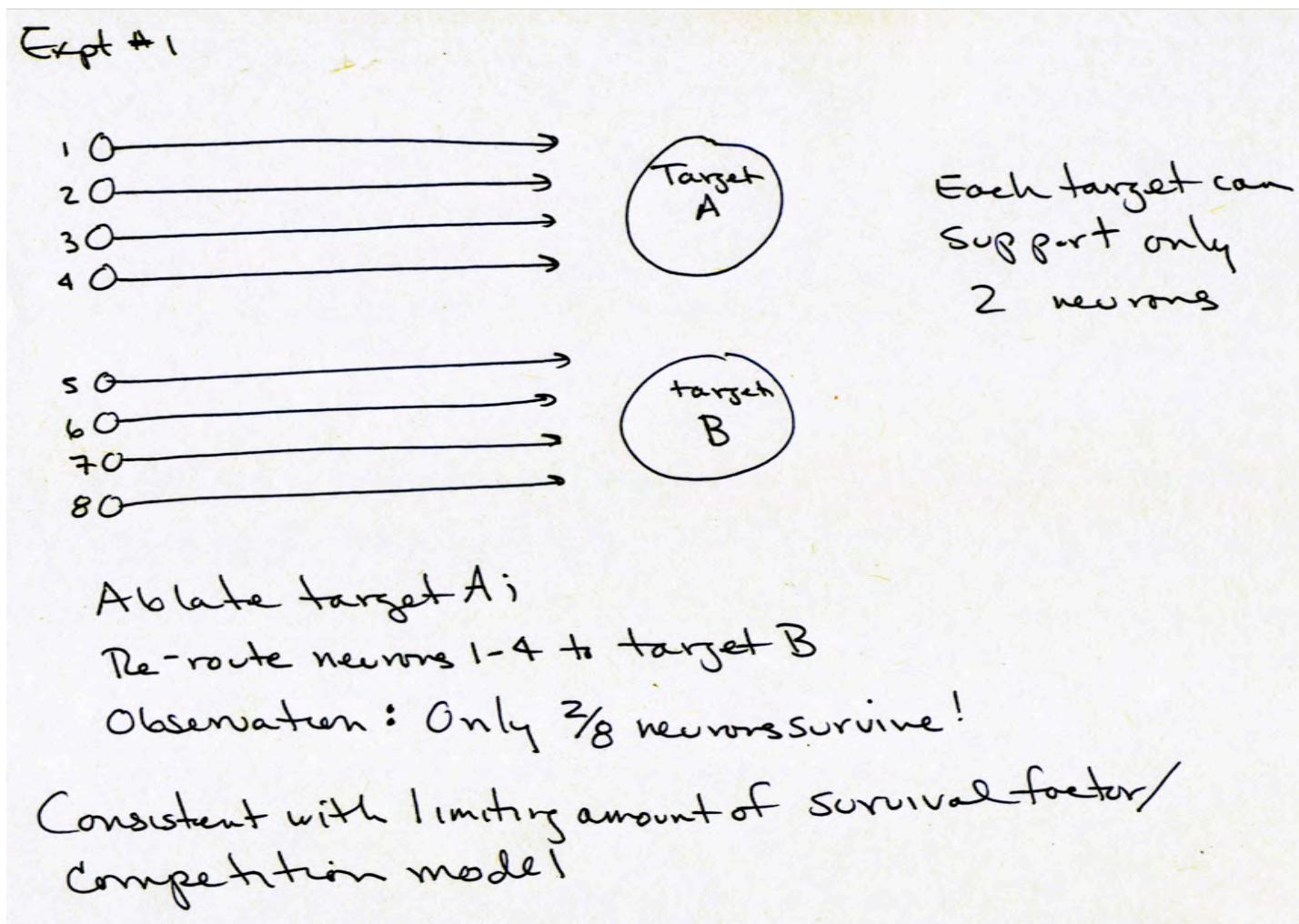
Cells/axons that acquire a sufficient (threshold) amount survive; all others die.

Model makes testable predictions:

1. If the number of neurons arriving at a single target increases, the percentage of survivors should go down;
2. If the number of neurons arriving at a single target decreases, the percentage of survivors should go up.

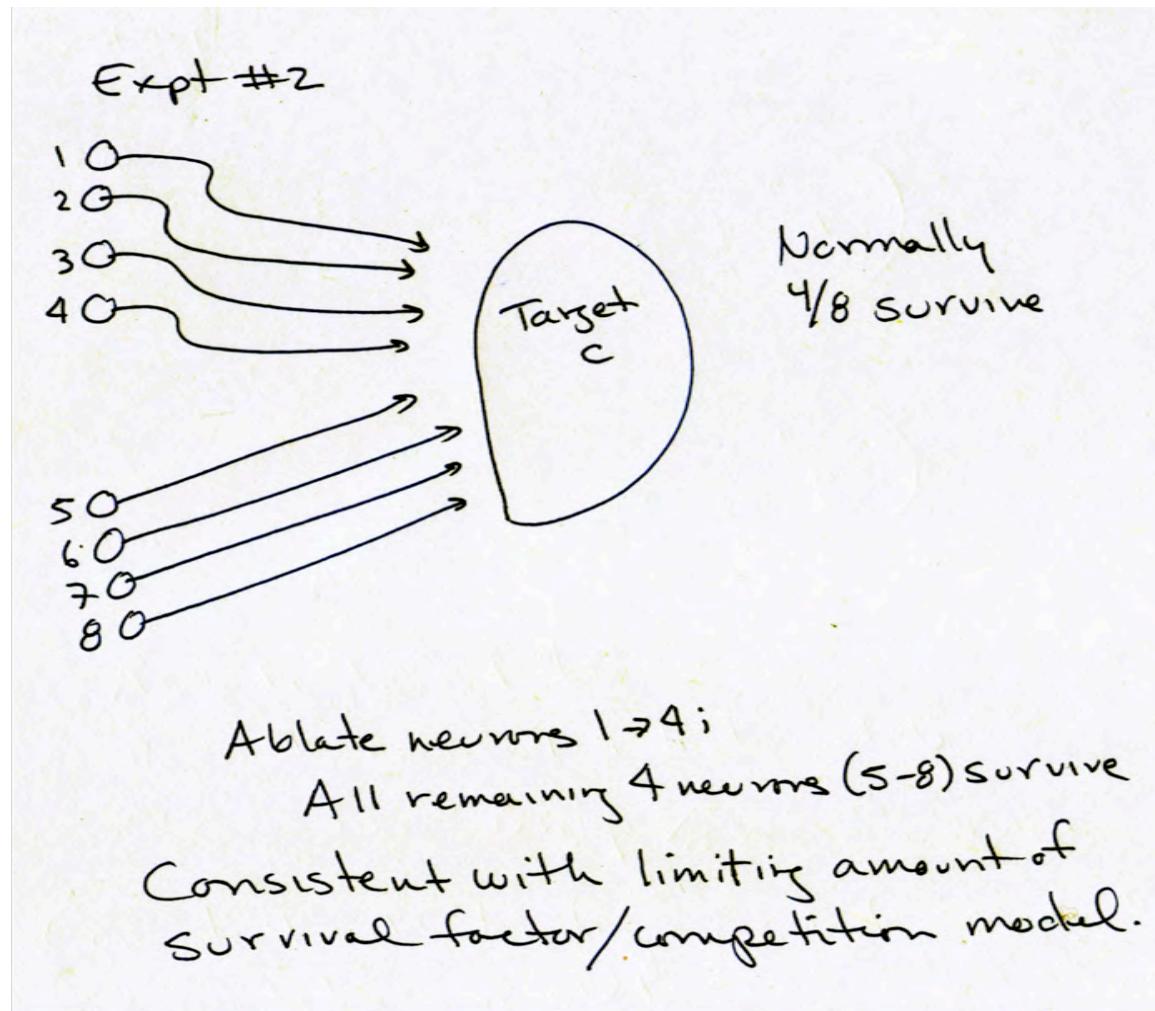
Model makes testable predictions:

1. If the number of neurons arriving at a single target increases, the percentage of survivors should go down;



Model makes testable predictions:

2. If the number of neurons arriving at a single target decreases, the percentage of survivors should go up.



What is the rationale for this cell death?

....nervous system is so important for the survival of the species and the individual....this is a good mechanism to match the size of the innervating pool of neurons with the needs of the target;

Also an element of quality control.

### 3. Defining Molecular Mechanisms –

...need to biochemically isolate the survival factor(s).....what molecules are regulating neuronal cell life and death? Are there lots of them? How do they work?

Originally called “trophic factors” implying a “nutritive” function.....

The Discovery of Nerve Growth Factor (NGF)

1948 - Bueker, working with Hamburger

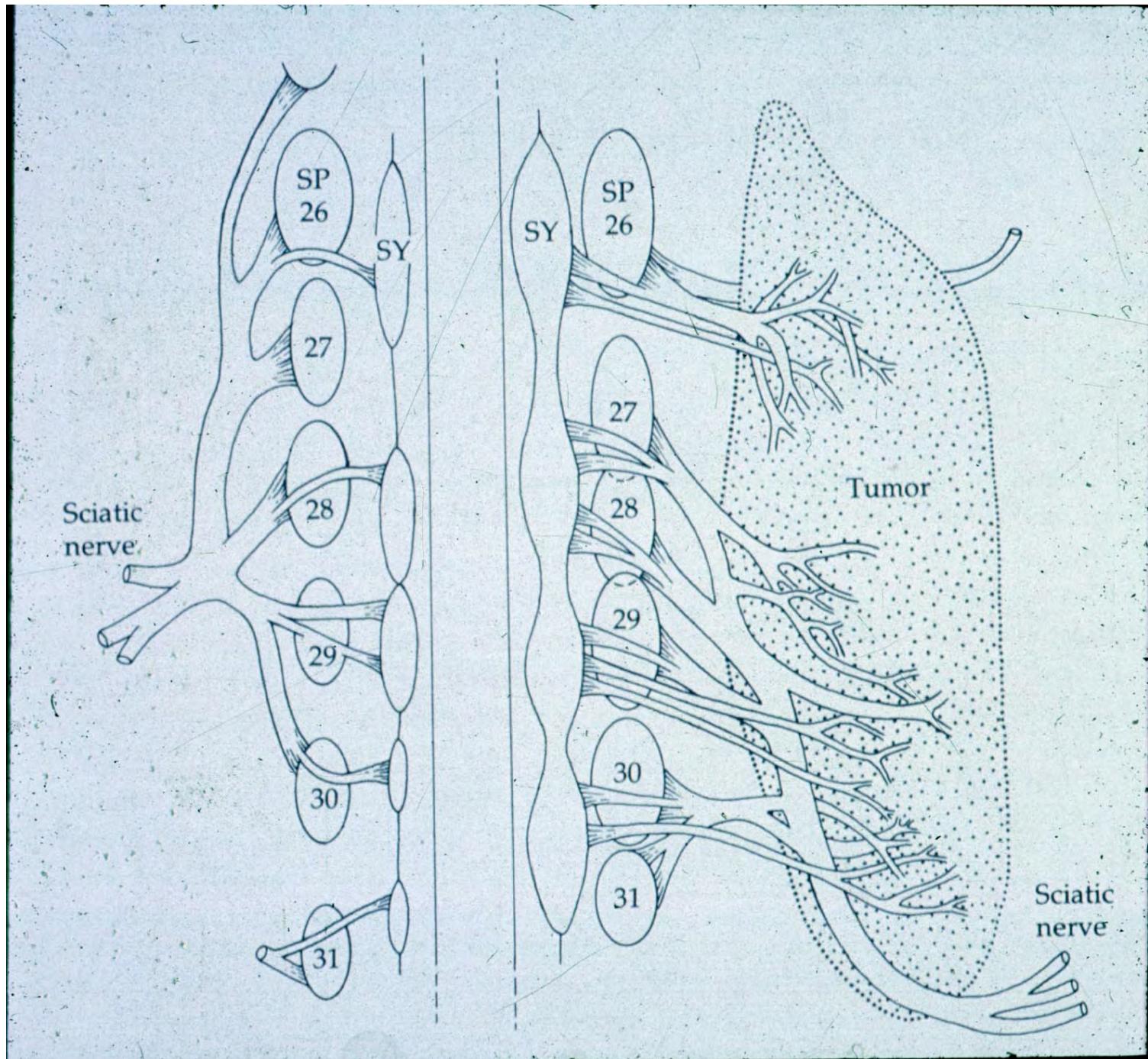
Big Goal: identify the molecules that regulate  
neuronal cell death in development

Need a good “starting material” for a “biochemical fractionation” or “purification” effort to identify and then purify enough of the survival factor to study

Is there something better than using many thousands of chick embryonic limbs? (which is not practical)

Approach: try out tumors cultured in animals

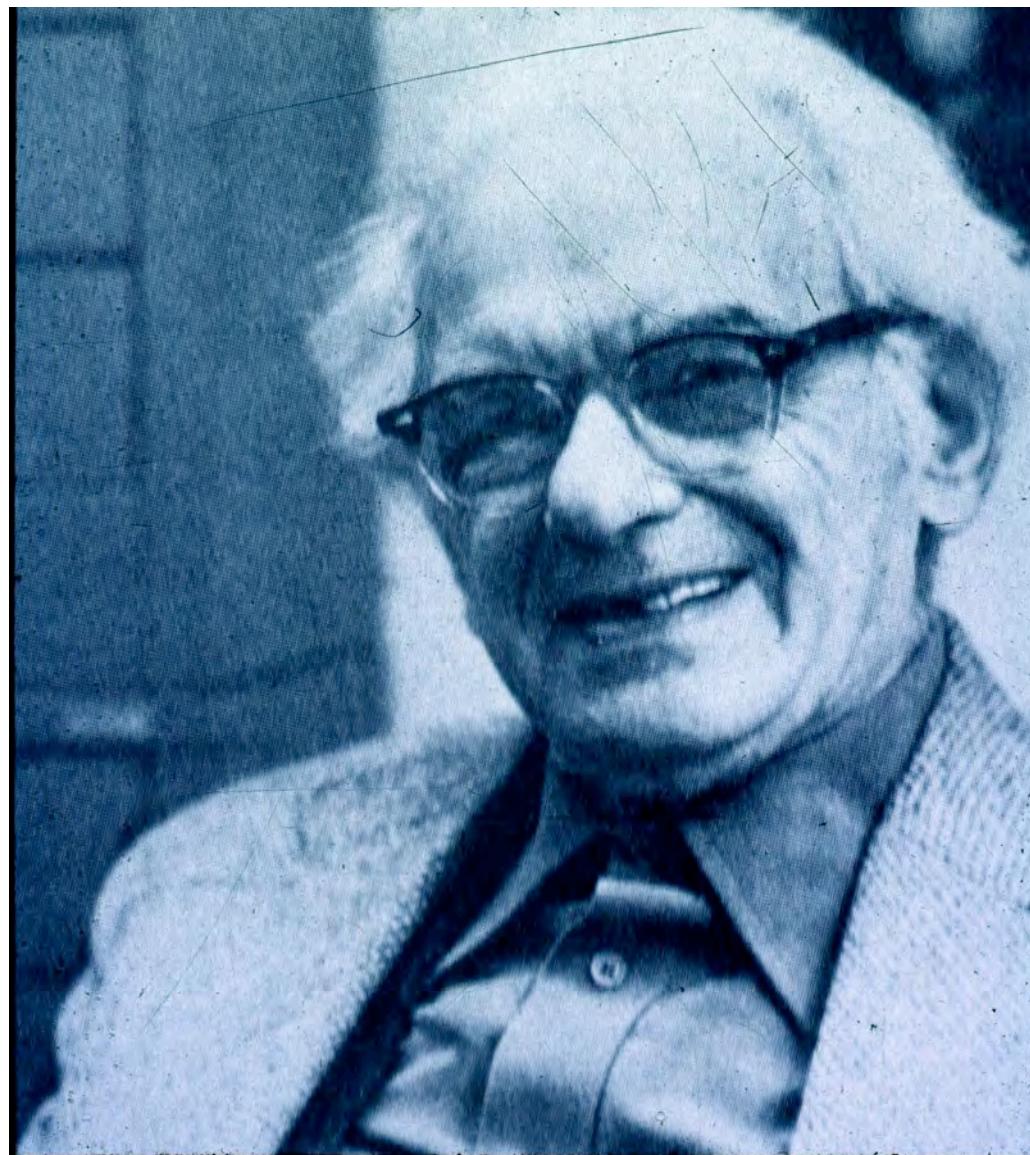
- mouse adenocarcinoma
  - fails to grow
- Rous Sarcoma
  - extensive hemorrhage
- Mouse sarcoma 180
  - grew well (see next slide!)



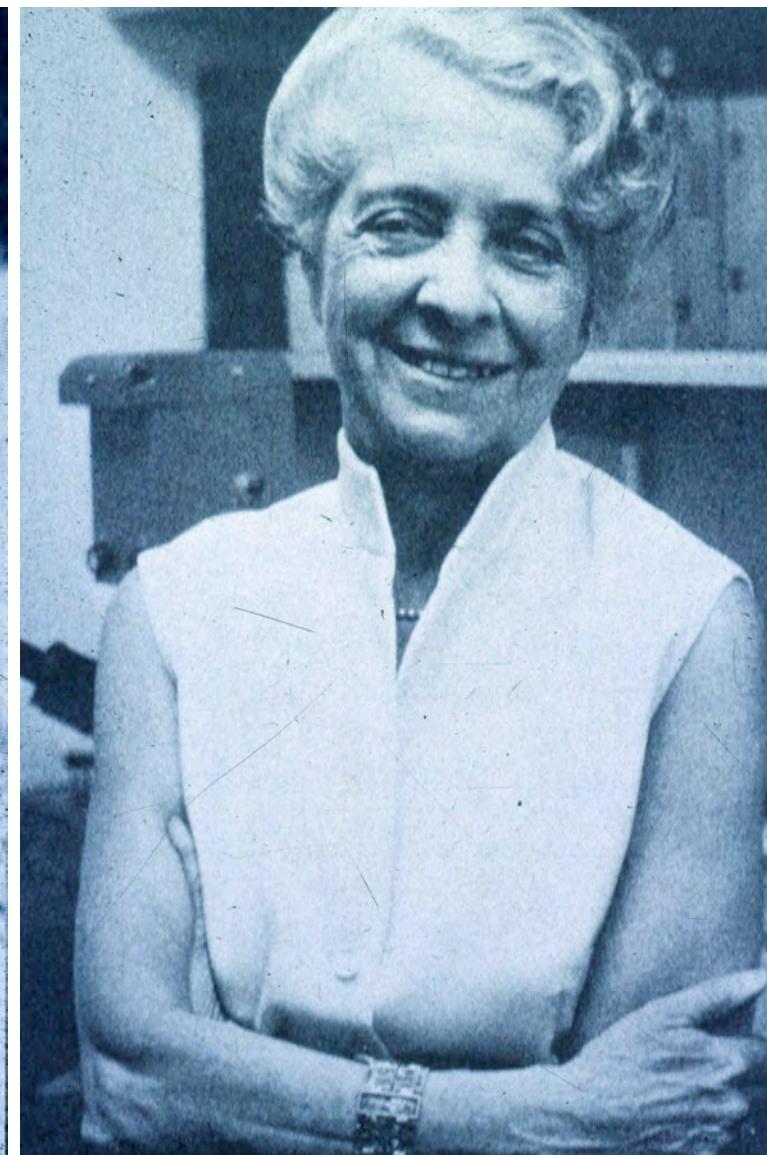
As the sarcoma 180 tumor grows:

- it gets invaded by nerves (chemoattraction!)
- DRG closest to tumor get very large  
(maybe increased survival?)
- more distant DRG are less affected  
(distance effect....maybe a soluble/diffusible factor?)
- no effect on spinal motor ganglia  
(shows specificity)

REALLY EXCITING RESULTS!!!



Viktor Hamburger



Rita Levi-Montalcini

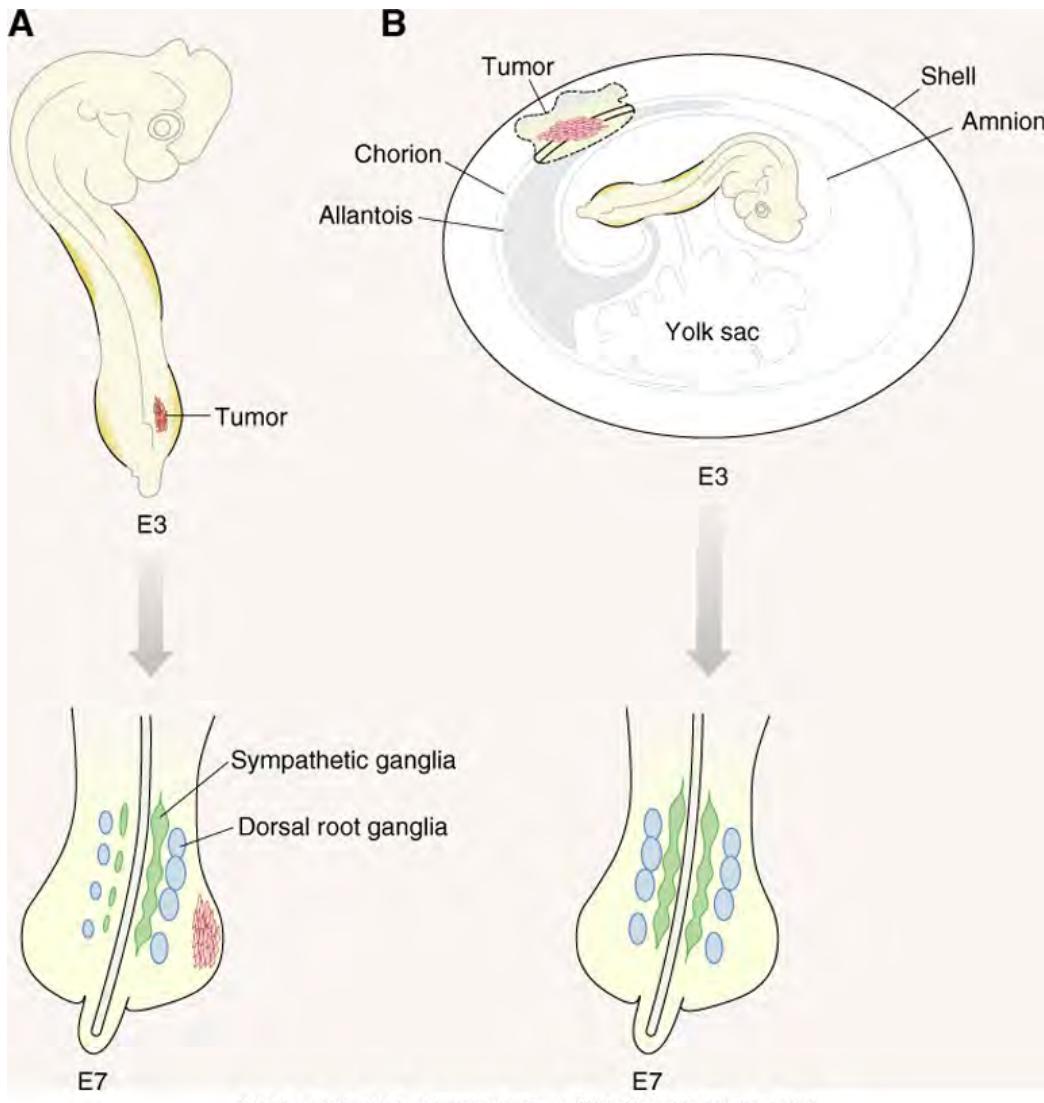
## Hamburger and Levi-Montalcini – early 1950's

- repeat Bueker's tumor work to confirm it;
- more detailed analyses

both sensory and sympathetic neurons invade the tumor;

the corresponding ganglia are enlarged;

many more neurons are present, i.e., cell death was reduced;



(Adapted from Bueker, 1948; Levi-Montalcini and Hamburger, 1951; Levi-Montalcini and Hamburger, 1953)

Tumor in embryo

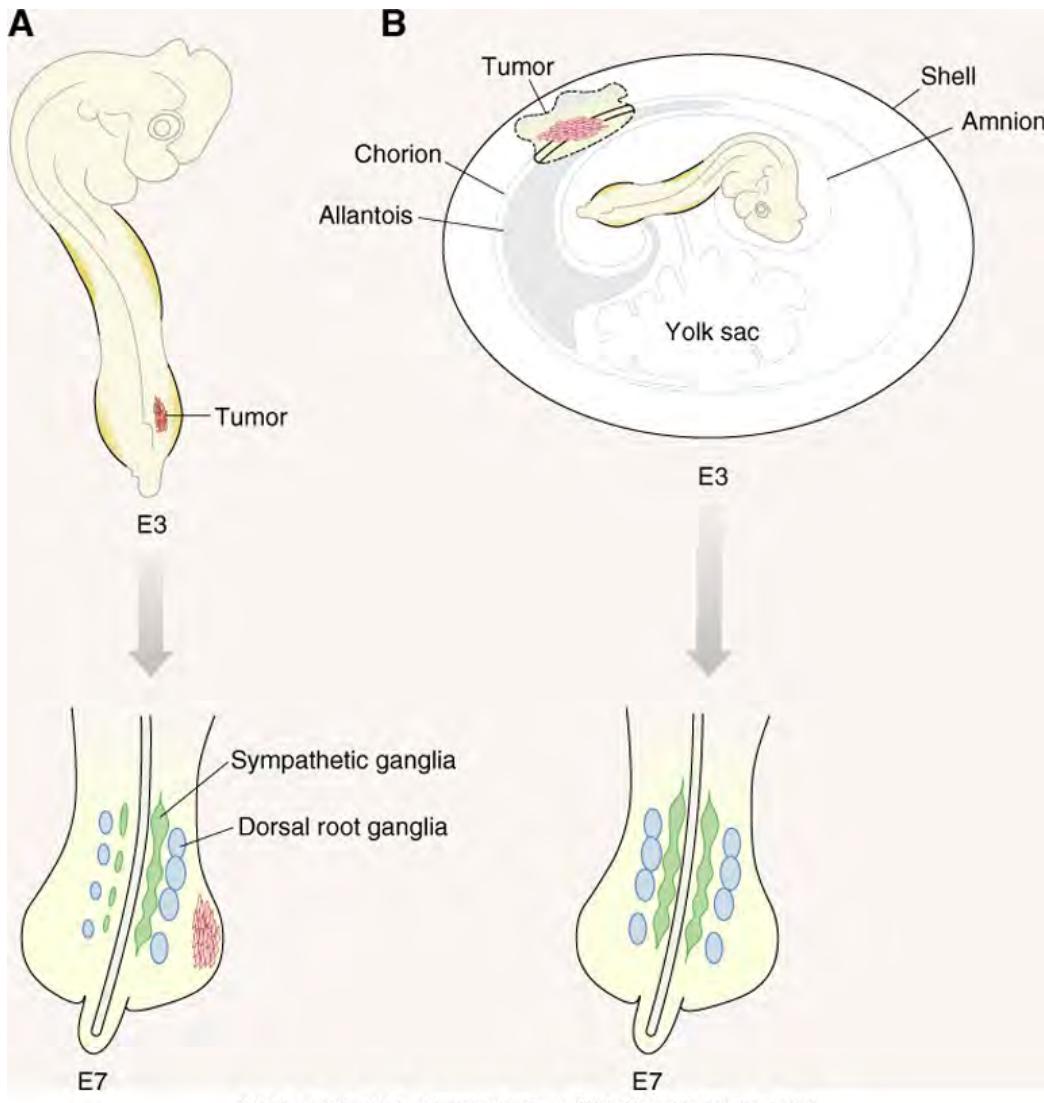
tumor not in physical contact with embryo

## Hamburger and Levi-Montalcini – early 1950's

- Is the survival factor a diffusible/soluble substance, as opposed to an insoluble factor like an ECM component?

Expt: put tumor on other side of the allantoic membrane instead of in the embryo; now the survival factor is everywhere; still get enlarged ganglia even though there is no direct contact between the tumor cells and the embryo.

Conclusion: survival factor is almost surely a soluble factor.



Tumor in embryo

tumor not in physical contact with embryo

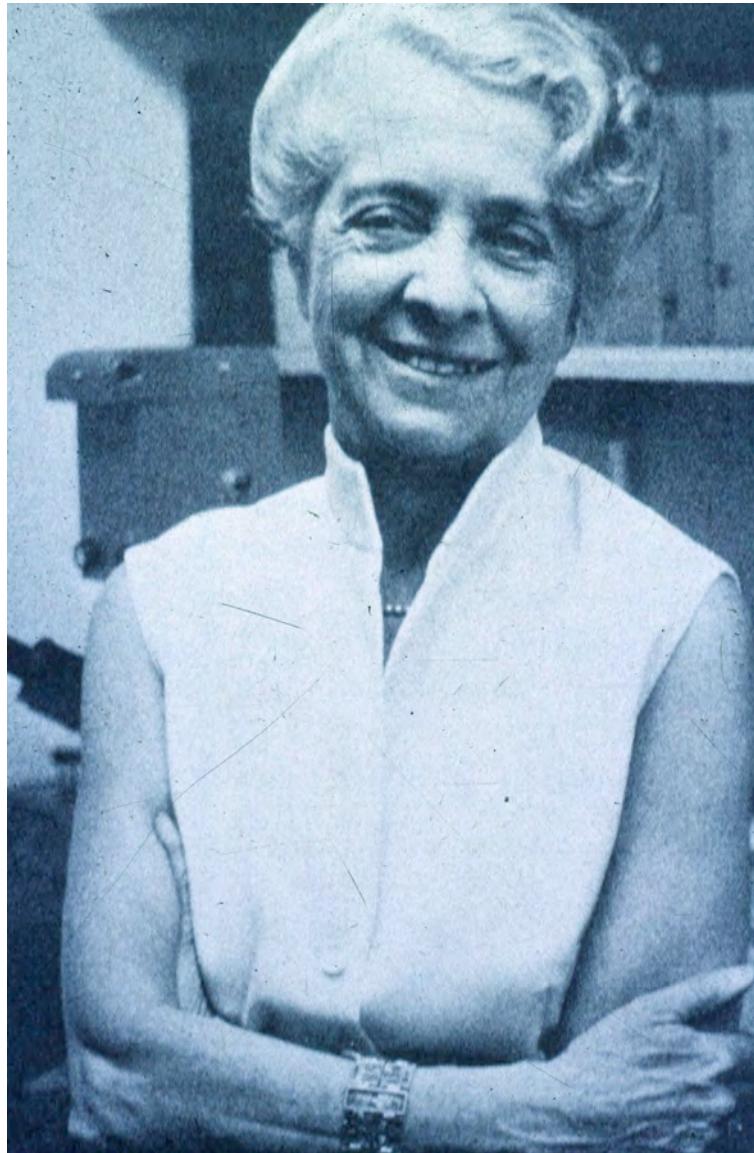
So.....at this point, we have a “biological activity”....  
but we don’t have a molecule “in hand”....

What is the molecular basis of these effects?

Think biochemical purification!!!

What do we need in order to purify the survival factor?

1. Abundant starting material – sarcoma 180
2. Fractionation procedures (they were crude, but OK)
3. A reliable, quick and at least semi-quantitative assay
4. A really good biochemist – enter Stanley Cohen



Rita Levi-Montalcini



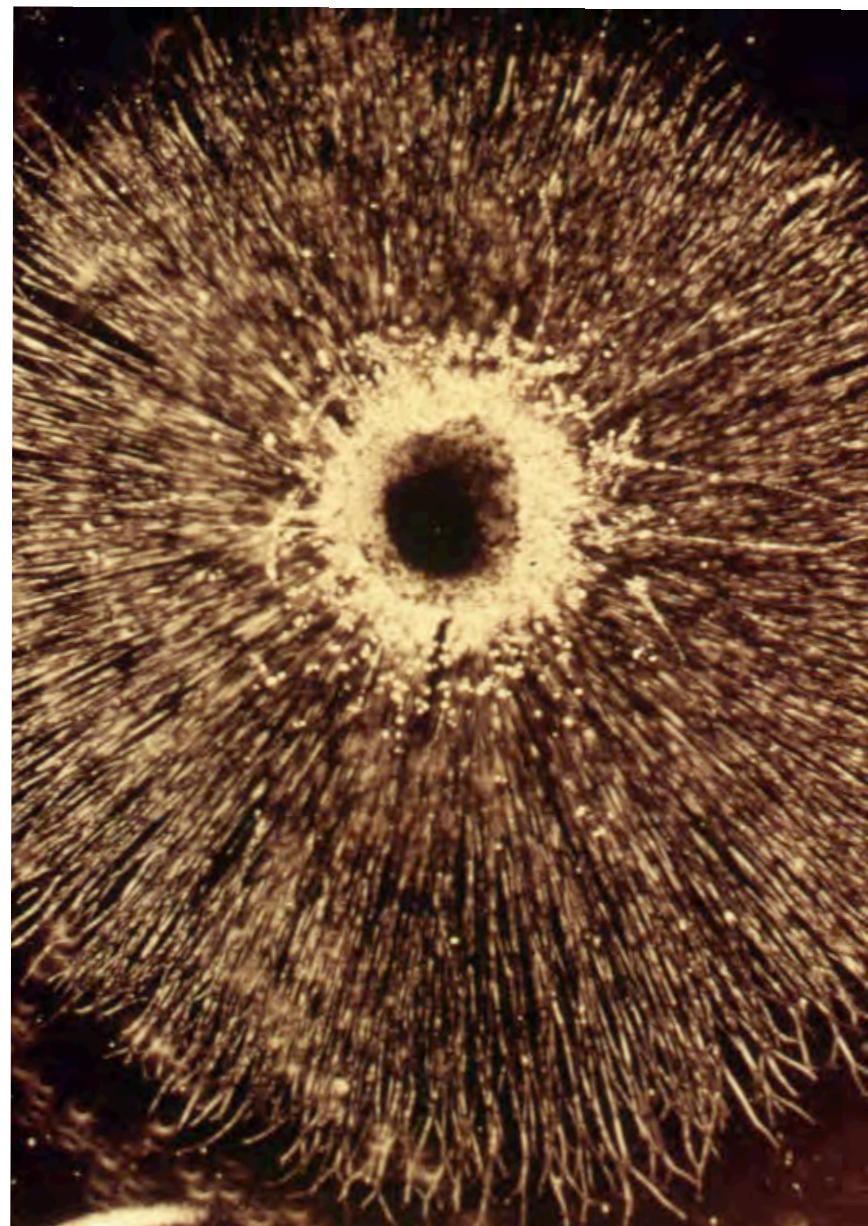
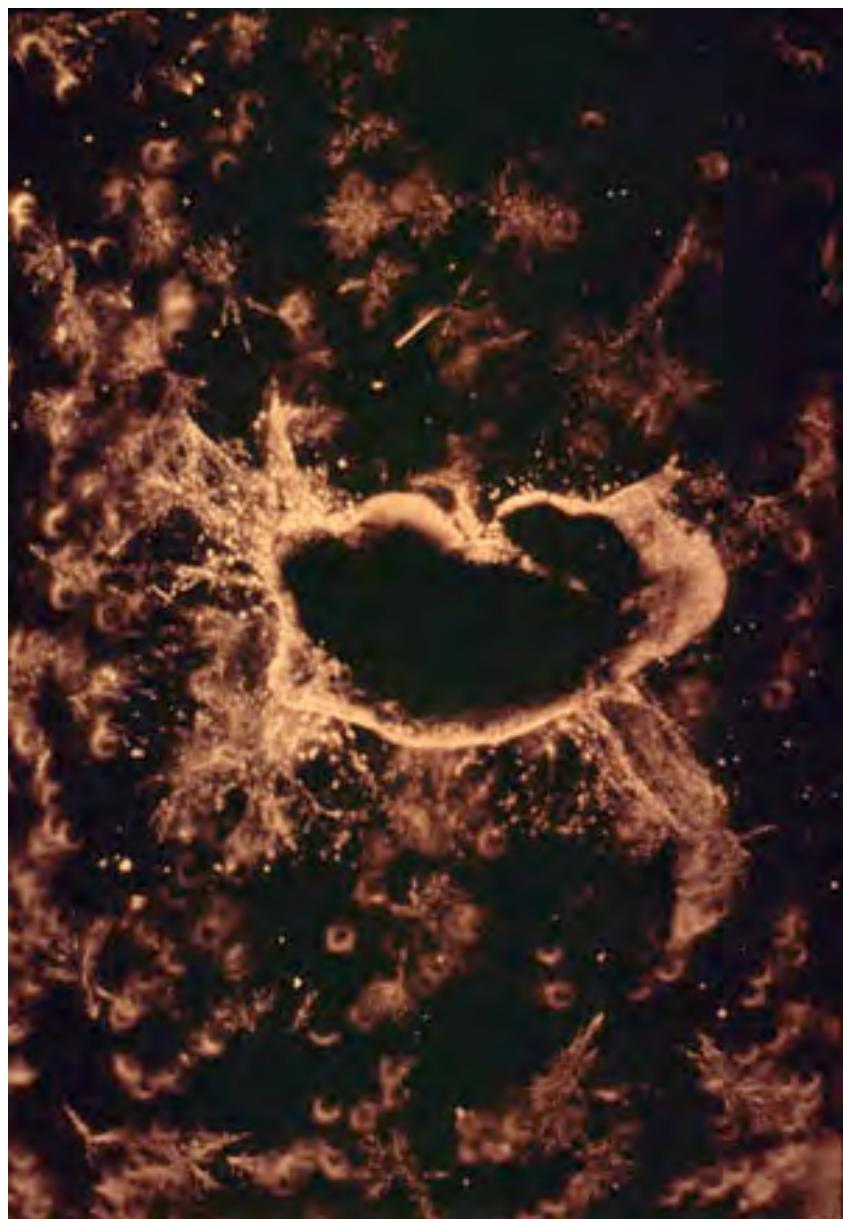
Stanley Cohen

Think biochemical purification!!!

What is the strategy to purify the survival factor? How do we proceed?

1. Abundant starting material – sarcoma 180
2. Fractionation procedures – how does this work?
3. A reliable, quick and at least semi-quantitative assay – the “halo assay”....

## Dorsal Root Ganglia Cell Culture Assay for Neuronal Cell Survival



## So, what happens next?

1. Use sarcoma cells as starting material; very hard work; not much survival activity is there
2. Got an “enriched” preparation of survival activity, but it contained both protein and nucleic acid....which is the real survival factor?
3. Phosphodiesterase story and Arthur Kornberg..... good thinking and great serendipity....Snake venom is a better starting material!
4. Mouse salivary gland is even better!
5. Purification of relatively small quantities of Nerve Growth Factor (NGF)

**BIG QUESTION:** Is this NGF, derived from a mouse salivary gland, based on work with tumor cells and snake venom, really of any physiological relevance....or have we gone on a crazy chase and lost our grip on reality?

### What is the effect of NGF in a real animal?

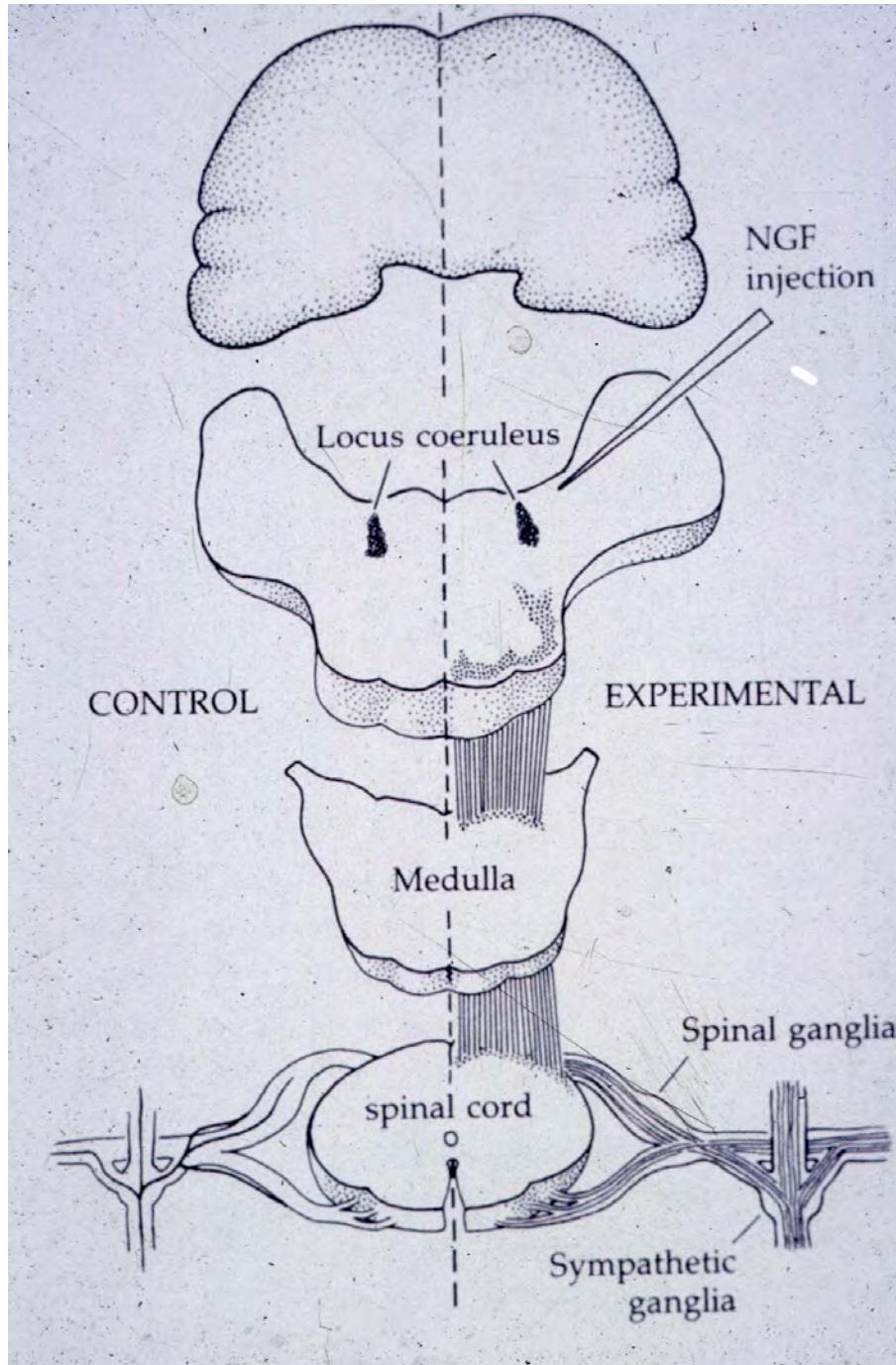
Four important predictions that must be fulfilled in order for NGF to be a real neuronal survival factor:

1. If we add extra NGF, neuronal cell death should decline and neuronal survival should increase;
2. If we remove endogenous NGF, neuronal cell death should increase and neuronal survival should decrease;
3. NGF must be synthesized in targets in proportion to levels of innervation;
4. NGF must be synthesized in targets at the right time in development;

# Prediction #1: How about adding extra NGF?

## Total Nerve Cell Numbers in Sympathetic Ganglia of Mice Injected with Salivary Gland Fractions

<u>Age</u> <u>(Con/Exp't)</u>	Ganglia	Control	Experimental	Ratio
12 days	thoracic N. 6	1365	3212	
	thoracic N. 7	1165	2731	
	thoracic N. 8	1155	4422	
	thoracic N. 9	1026	2849	
	thoracic N. 10	1465	2567	
	TOTAL	6276	15781	2.5
19 days	thoracic N. 2	14,400	30,800	
	thoracic N. 3	1644	3684	
	thoracic N. 4	1770	2133	
	thoracic N. 5	1094	3144	
	thoracic N. 6	820	2328	
	thoracic N. 7	1362	2758	
	thoracic N. 8	1392	4162	
	TOTAL	22,482	49,009	2.18



## Observations:

1. Increased neuronal survival;
2. Chemotaxis – growth of axons to site of injection (just like the tumor);

So, prediction #1 is fulfilled

Prediction #2:  
How about eliminating NGF?....in the 1960's?

No knockouts, no anti-sense, no RNAi, no dominant negatives....no recombinant DNA...  
.....no monoclonal antibodies....but they  
were learning about antibodies....

“Immunosympathectomy”

First use of function blocking antibodies!!!

Use purified NGF to raise anti-NGF in rabbits, inject antisera into newborn mice/chicks....and hope (assume?) that there will be function blockers in the antibody preparation!

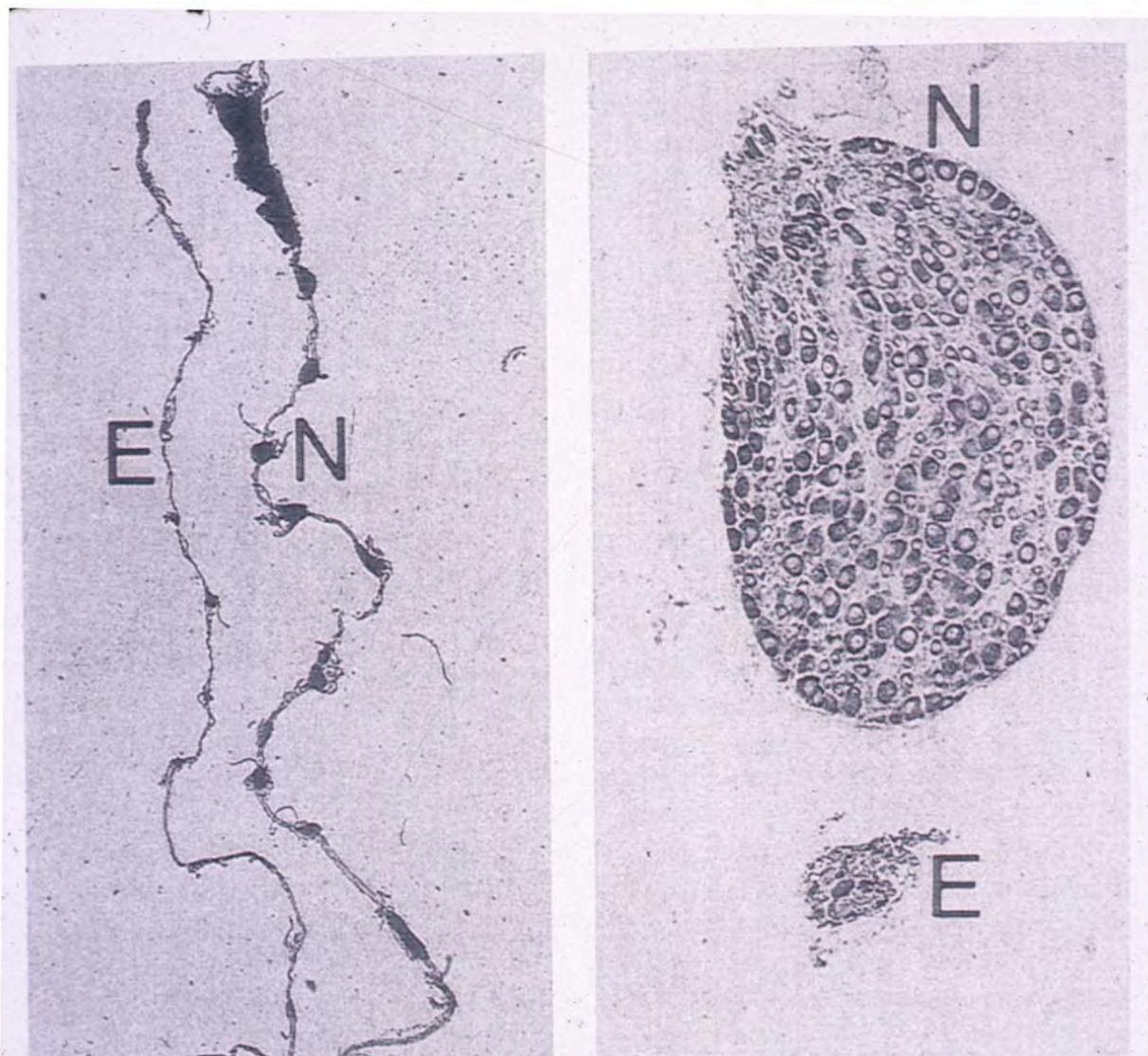


FIGURE 21.2 Immunosympathectomy. Antibodies that selectively block NGF activity were administered to newborn mice to deprive the developing animals of endogenous factor. Sympathetic ganglia were examined several weeks after the treatment (N, normal ganglia; E, ganglia deprived of NGF for 3–5 days). Note the almost complete disappearance of the sympathetic chain ganglia (left) and the loss of neurons in individual ganglia (right).

## Effects of Antiserum on Cell Number in Mammalian Sympathetic Ganglia

Animal	Age (days)	#injections	Ganglion	Number of Cells		Ratio (Con/Exp't)
				Control	Expt'l	
mouse	20	20	Super. Cerv.	13,300	91	0.7:100
mouse	25	25	Super. Cerv.	16,415	279	1.7:100
mouse	20	8	Super. Cerv.	16,447	421	2.6:100
mouse	120	8	Super. Cerv.	14,800	140	0.9:100
mouse	120	20	Super. Cerv.	13,000	110	0.8:100
rat	7	7	Super. Cerv.	32,000	2310	7.0:100
rabbit	3	3	Super. Cerv.	66,300	6200	9.0:100
rabbit	5	5	Super. Cerv.	67,000	9300	14:100
rabbit	7	7	Super. Cerv.	68,000	11,050	16:100
cat	7	7	Super. Cerv.	114,000	8600	7.7:100

## “Immunosympathectomy”

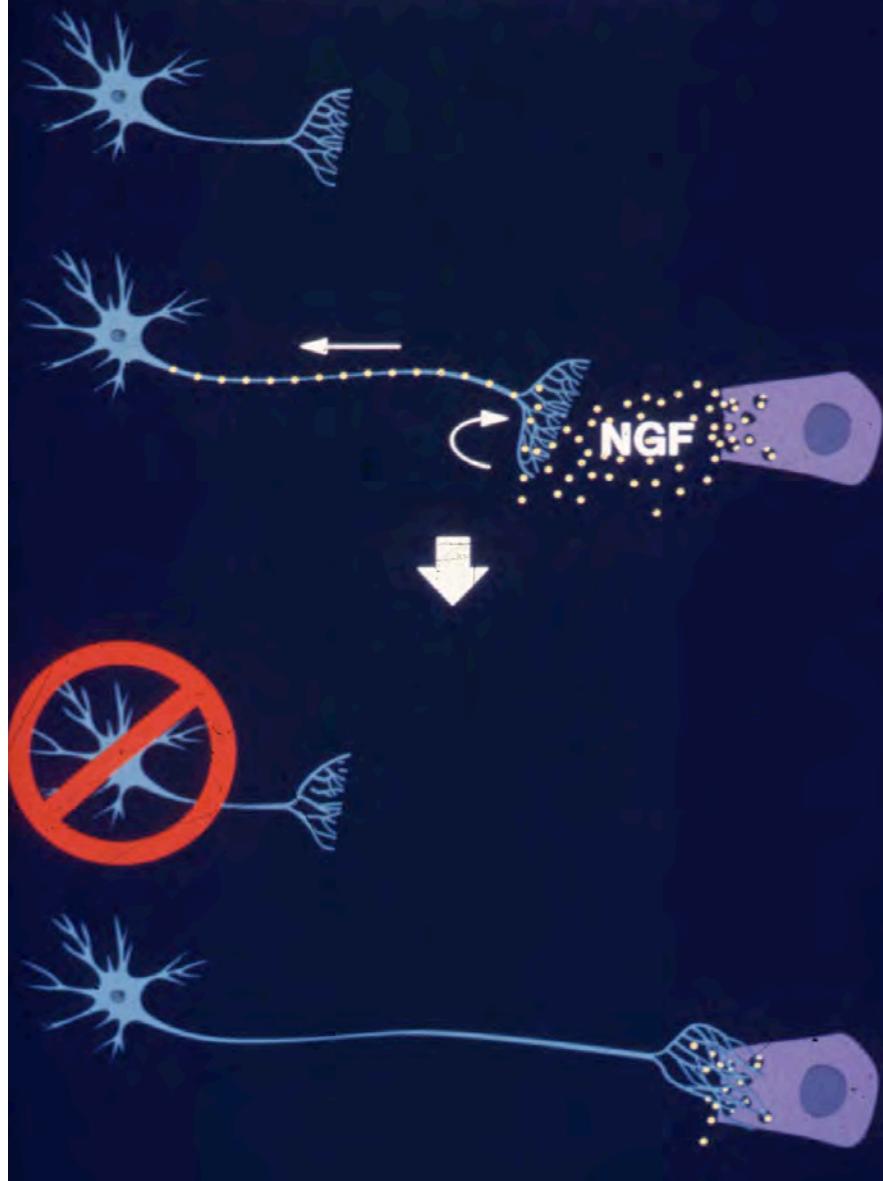
Complete destruction of sympathetic nervous system;  
Partial destruction of sensory nervous system;  
No effect on motor neurons or brain

Same general effects observed when tested in adult animals....but somewhat less extensive

This experiment provides physiological relevance to NGF!

Conclusion: NGF is essential for the proper development and maintenance of the sympathetic and sensory nervous systems.

## NGF IS A TARGET-DERIVED TROPHIC FACTOR



People are very comfortable with this model in the 1970's, even though:

1. Prediction #3 has not been addressed (technology was not yet available to address it);
2. Prediction #4 has not been addressed (technology was not yet available to address it);
3. There is no good information regarding HOW NGF works;
4. How about the rest of the nervous system where NGF has no effect, like the CNS, which still undergoes neuronal cell death?

Basically....there is NO DIRECT DATA to support the model that NGF is a target derived neuronal survival factor.

Next phases of the work required cleaner and  
Much larger quantities of NGF....  
Purification of Experimentally Useful Quantities of NGF



Ralph Bradshaw  
Wash Univ. and UCI



Eric Shooter  
Stanford Univ.

....and the development of new assays that were much more sensitive, i.e. could detect and measure much lower quantities of NGF and NGF mRNA than could be detected with earlier assays

1. Isolation of NGF cDNA for use as a probe to detect NGF mRNA on RNA blots (i.e., “Northern blots”);



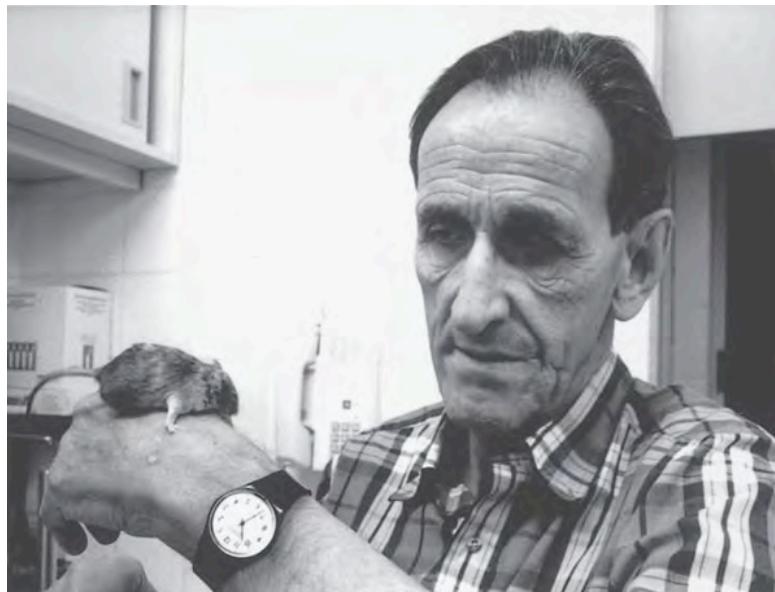
**Bill Rutter**  
UCSF



**Axel Ullrich**  
Genentech

....and the development of new assays that were much more sensitive, i.e. could detect and measure much lower quantities of NGF and NGF mRNA than could be detected with earlier assays

## 2. Improved immunological assays to detect NGF protein using monoclonal antibodies.



Hans Thoenen  
Max Planck Inst.



Sigrun Korschning  
Max Planck Inst.

## Prediction #3

NGF must be synthesized in targets in proportion to levels of innervation;



# Where is NGF protein synthesized? Does it correlate with symp/sensory innervation? (in adult rat tissues)



## Densely innervated targets

Salivary gland	0.5 ng NGF/gm wet weight
Atrium	1.0 ng NGF/gm wet weight
Iris	1.9 ng NGF/gm wet weight

## Poorly innervated targets

Ventricle	<0.3 ng NGF/gm wet weight
Skeletal muscle	<0.3 ng NGF/gm wet weight

“The correlation between NGF levels and density of innervation is consistent with the concept that the production of NGF in target organs determines the density of innervation by the sympathetic nervous system.”



Han Thoenen

## Where is NGF protein synthesized? Does it correlate with symp/sensory innervation? (in adult rat tissues)



Sigrun Korschning

In the same study....

Superior cervical ganglia	25 ng NGF/gm wet weight
Stellate ganglia	19 ng NGF/gm wet weight

Both of these ganglia innervate symp/sensory targets.

Despite the very high levels of NGF in these ganglia,  
there is NO NGF mRNA! How is this possible?

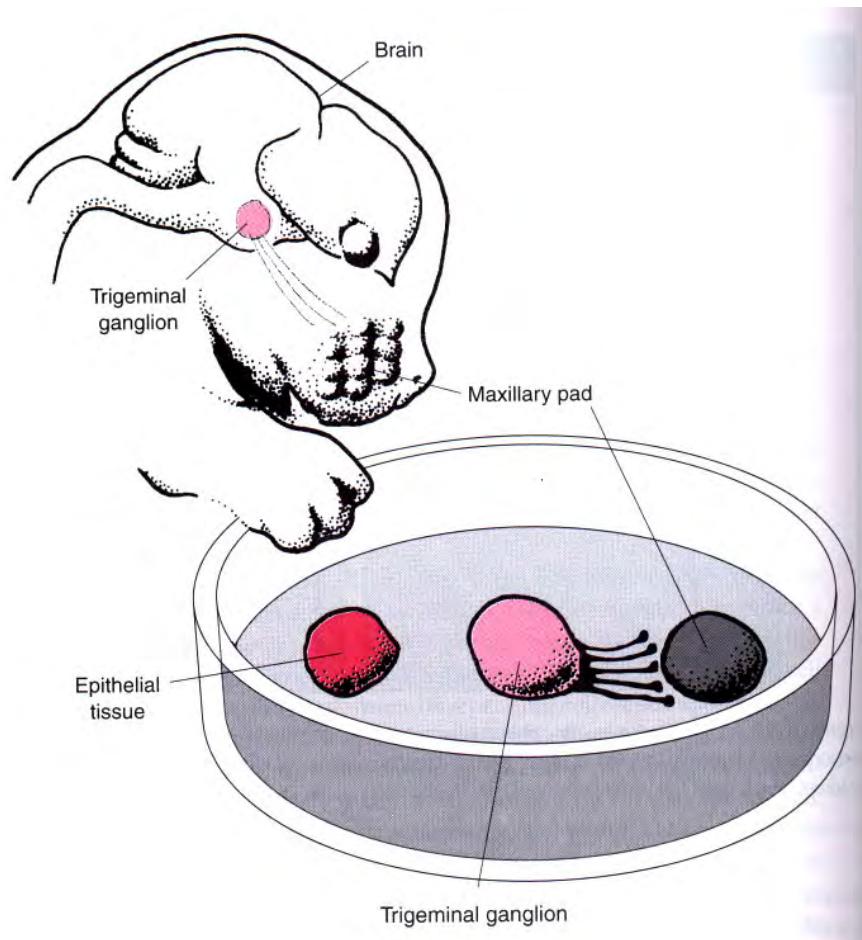


Lou  
Reichardt  
UCSF



1. Confirms Korschung/Thoenen work; good correlation between NGF levels and amount of symp/sensory innervation
2. Also shows a good NGF mRNA to NGF protein correlation;
3. Surprisingly, they found lots of NGF mRNA in a couple of regions of the brain, including the hippocampus and neocortex (cholinergic). This raises the question of why there were no effects on these regions in the immunosympathectomy expt?

Prediction #4: NGF must be synthesized in targets at the right time in development;



Alun Davies -1987

## Maxillary Pad Innervation Expt



8      9      10      11      12      13      14      15      16      17      18      19

Axons extending out

axon arrival

50% of neurons die

► NGF mRNA synthesis

► NGF protein synthesis

Good correlation between initiation of NGF synthesis and the arrival of the first axons at target.....NGF is synthesized in the right place and at the right time to be a target derived survival factor;

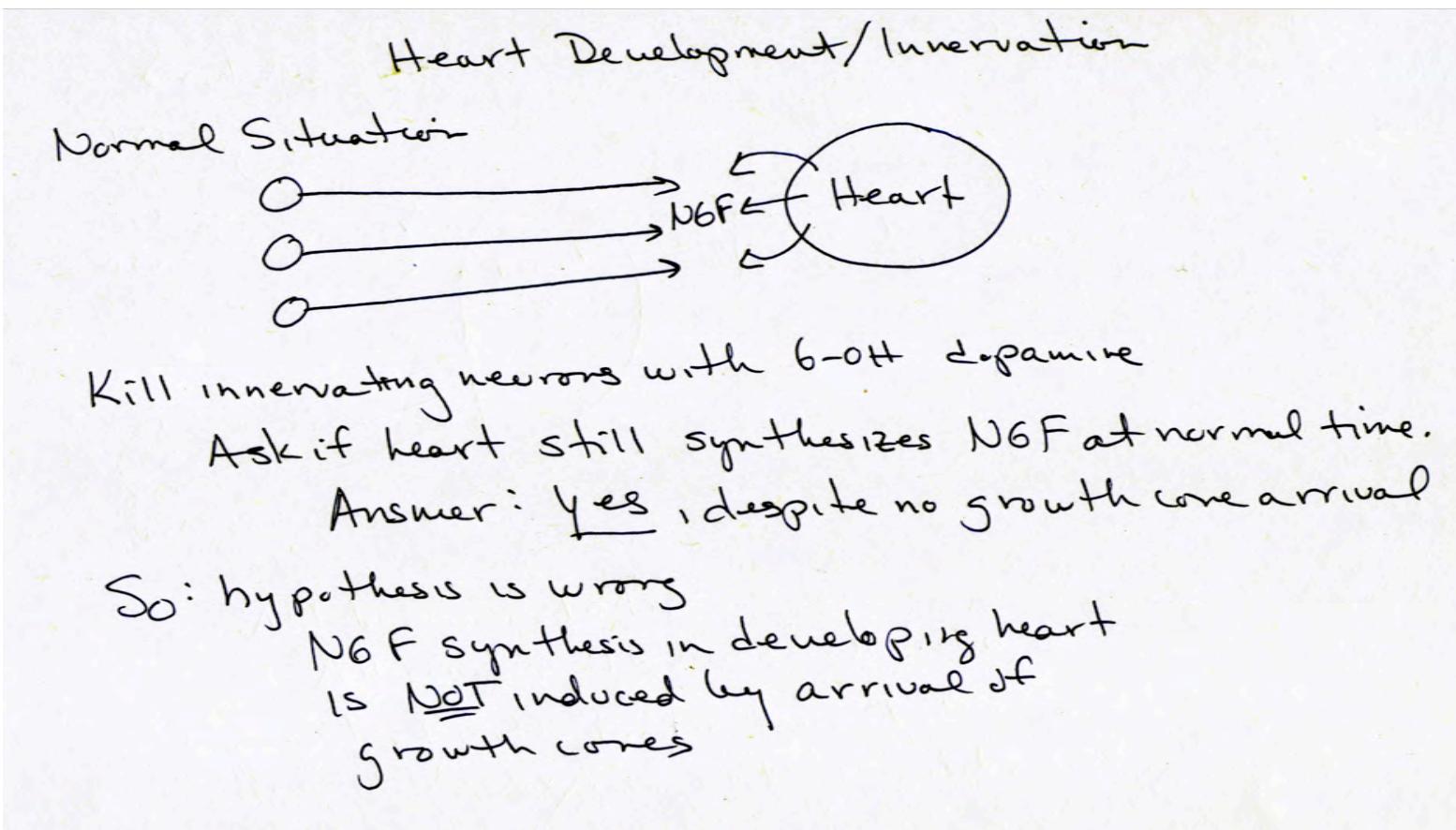
But, for pathfinding, chemotaxis by a gradient of NGF is unlikely in this system, unless there is NGF being synthesized by some other cells along the pathway, since NGF synthesis in the target is not present until the time of growth cone arrival.

This raises an interesting possibility...might the arrival of the first growth cones be the signal to induce NGF synthesis in the target?



Lou Reichardt  
UCSF

Dennis Clegg  
UCSB



NGF promotes neuronal cell survival for sympathetic and sensory neurons (peripheral nervous system.....but naturally occurring cell death also occurs in the central nervous system.....but NGF doesn't affect CNS neurons.....maybe other target derived trophic factors acting in the CNS?

How would we go about finding these other putative factors?



Go back to the protein purification strategy.....  
with some modifications to adapt to the new  
question.....

- starting material – pig brain (CNS)
- new assay – ciliary ganglion (non-NGF  
responsive; parasympathetic nervous system)



Hans Thoenen  
Max Planck Inst.



Yves-Alain Barde  
University of Basel

Pig Brain--->fractionate--→1.5 µg BDNF  
(1.5 kg) and assay

BDNF=brain derived neurotrophic factor  
MW = 12.3 kDa  
pI = 10



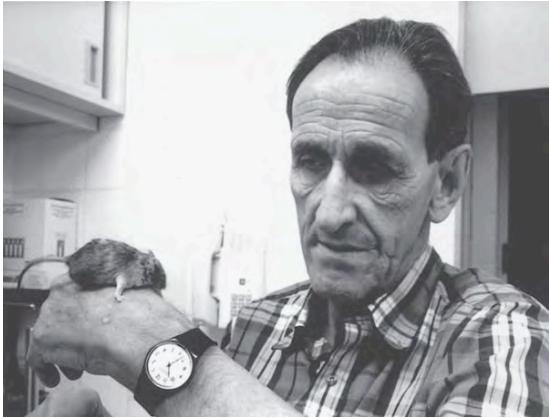
Hans Thoenen  
Max Planck Inst.



Yves-Alain Barde  
University of Basel

Using their precious 1.5 µg of BDNF, they chemically determined the amino acid sequence of a small region of BDNF and then used that information to isolate a BDNF cDNA, which in turn provided the entire amino acid sequence of BDNF.

And this revealed a 65% amino acid homology to NGF! related genes/proteins; a small “gene family” named the family “Neurotrophins”



Hans Thoenen  
Max Planck Inst.



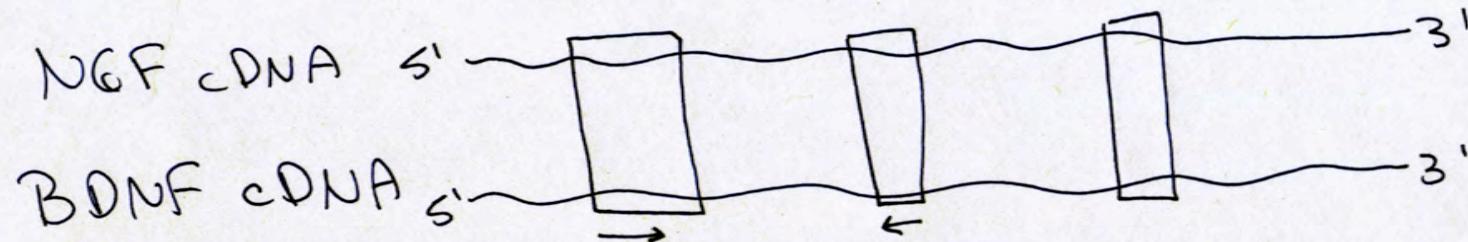
Yves-Alain Barde  
University of Basel

BDNF expression patterns:

very high levels in the CNS  
hippocampus (learning and memory)  
cortex (learning and memory)  
substantia nigra (relevant to Parkinson's)

expression increases as development proceeds;

# Are there more members of the Neurotrophin Family?

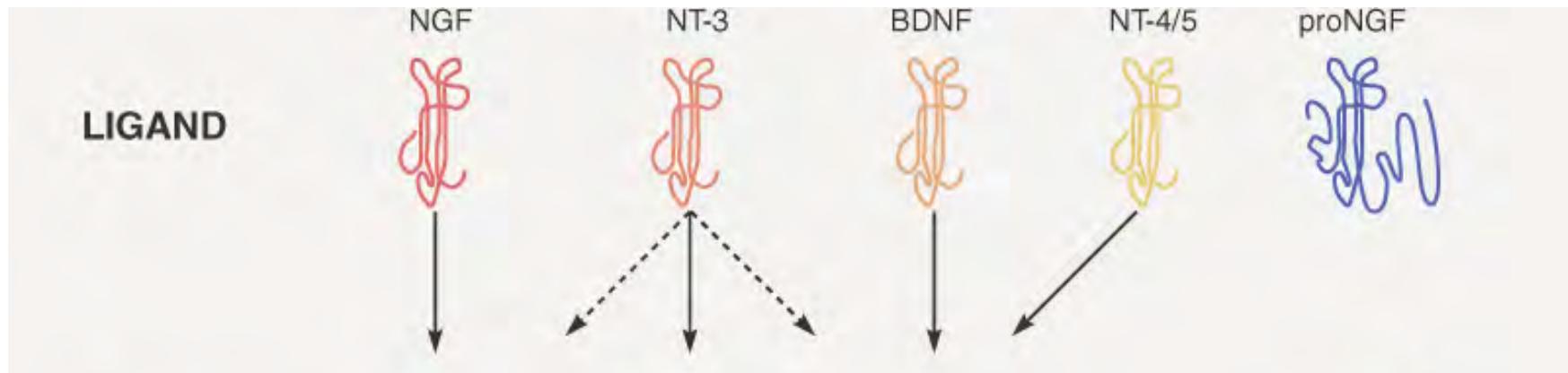


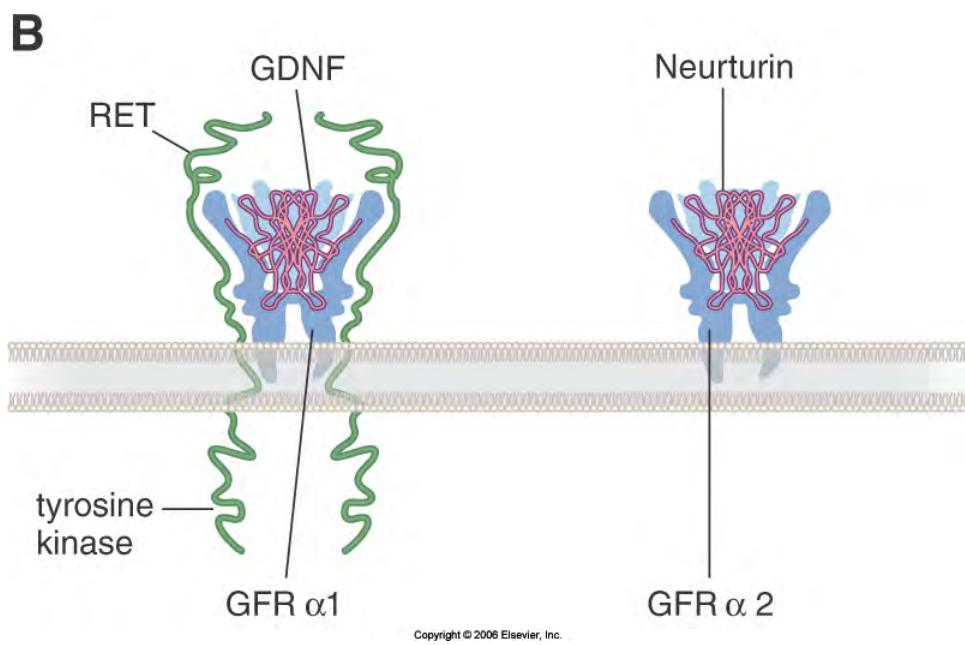
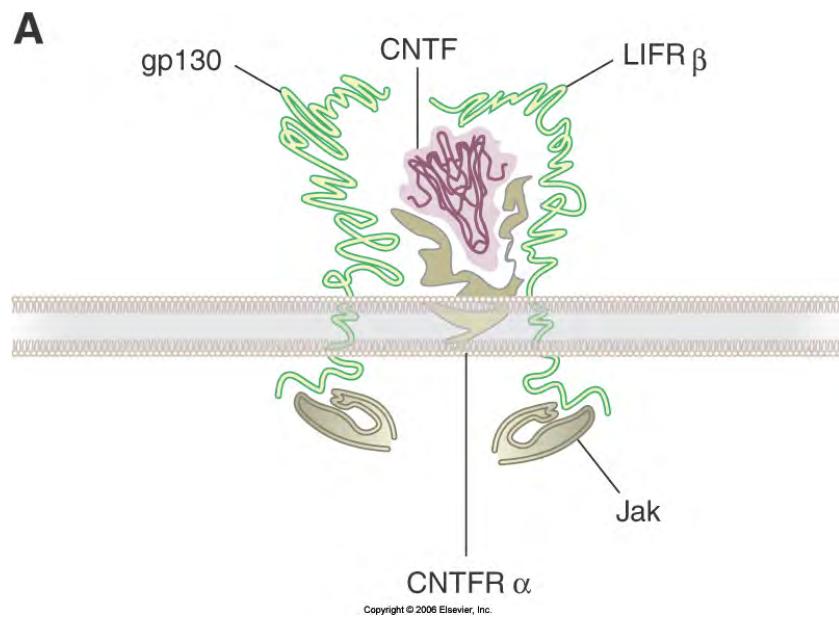
boxes = regions of high homology  
other regions show less homology

1. Synthesize oligonucleotide primers corresponding to homologous regions (arrows)
2. Use these primers to amplify a brain cDNA library
3. Sequence amplification products
  - Should get NGF PCR products
  - Should get BDNF PCR products
  - ANYTHING ELSE???

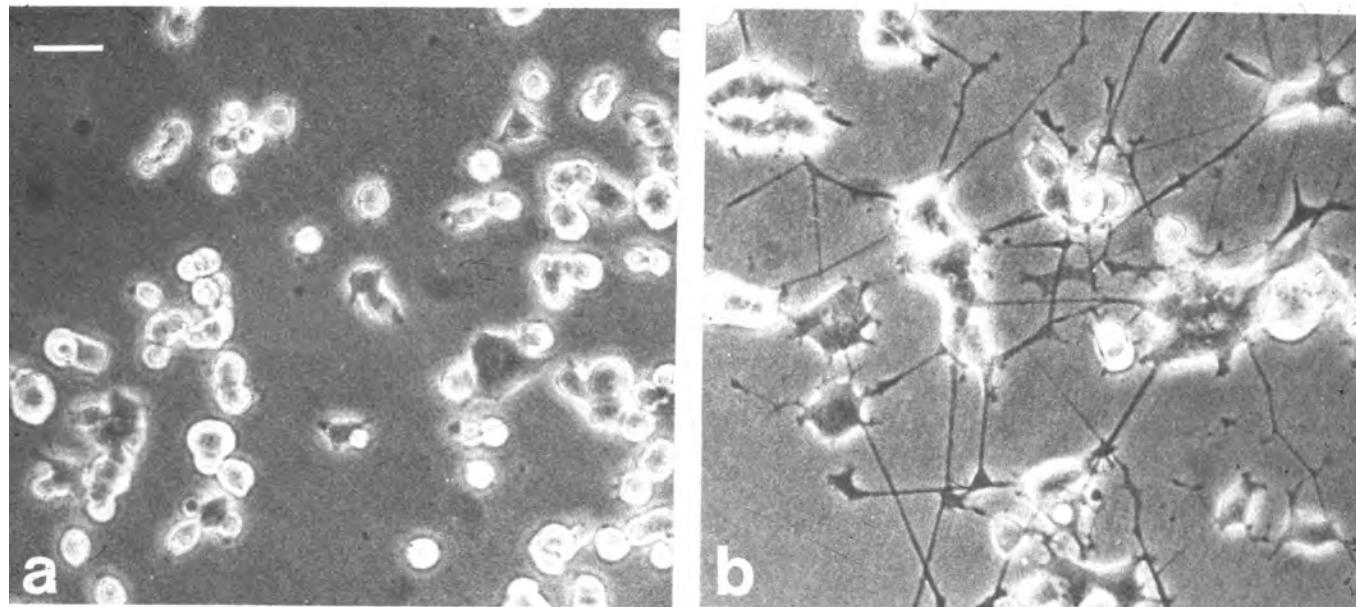
YES! NT3 and NT4

homologous to NGF and BDNF



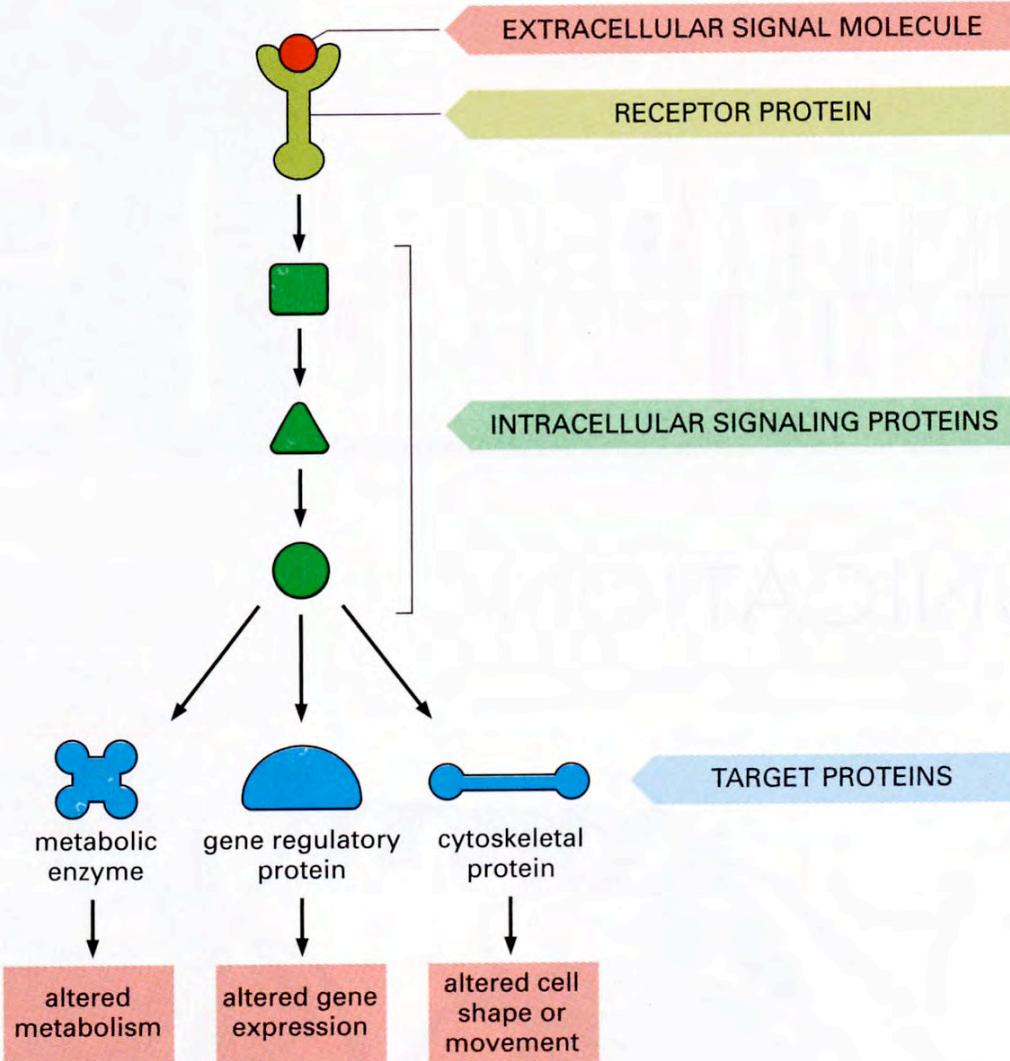


## How do NGF and the other neurotrophins work molecularly?



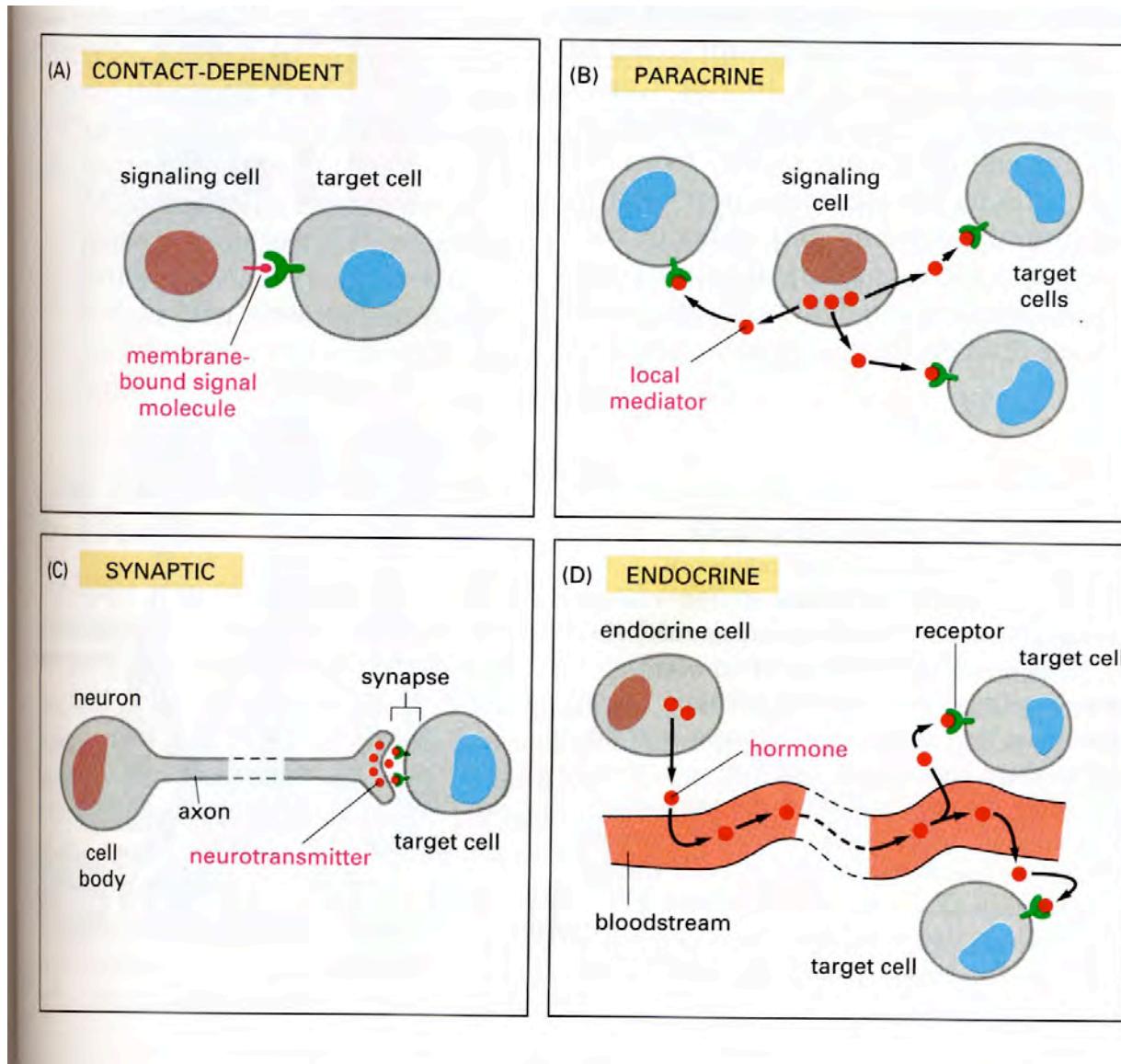
Need to start hunting for receptors

# Signal Transduction is Key to all of Development



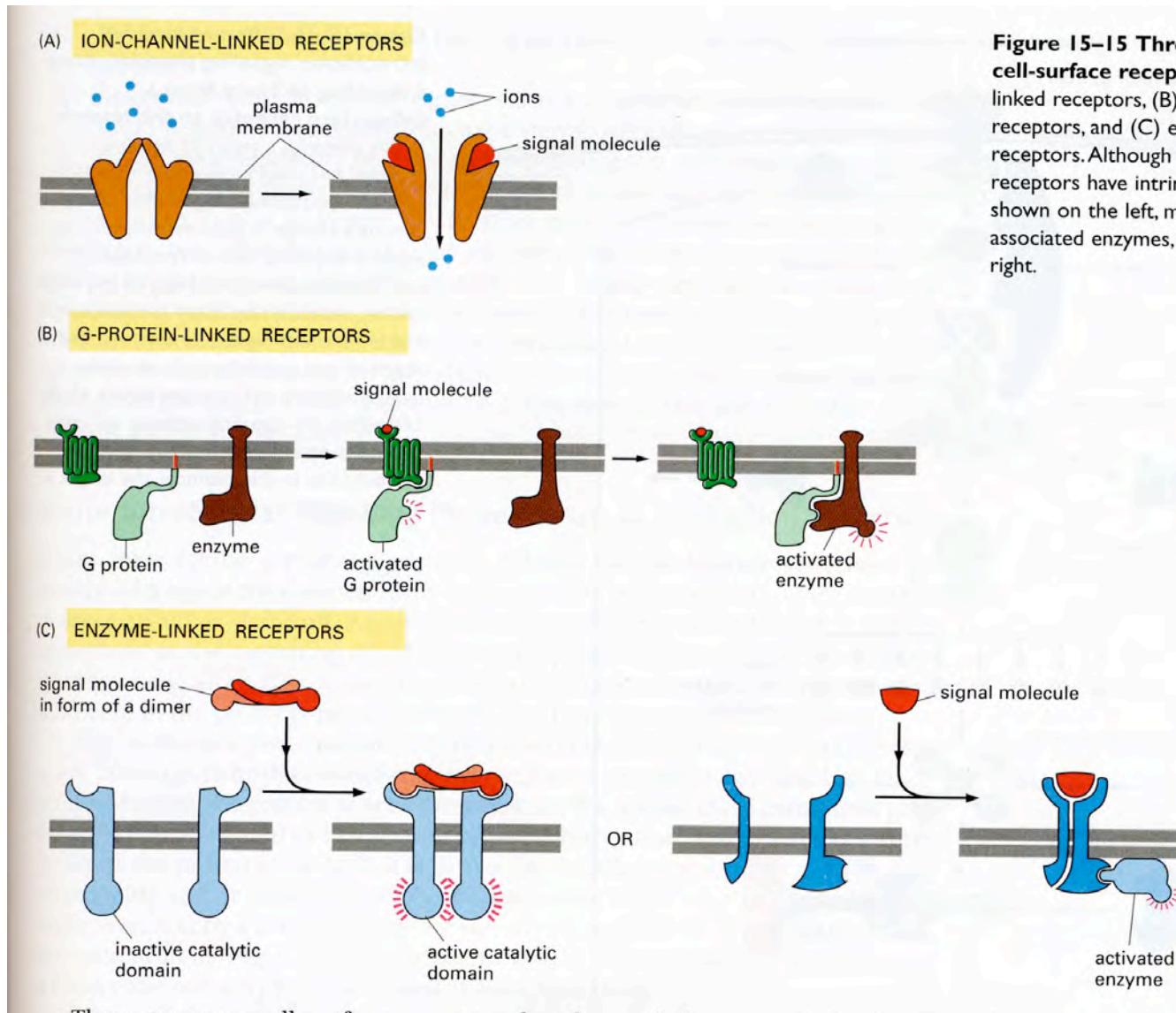
**Figure 15–1** A simple intracellular signaling pathway activated by an extracellular signal molecule. The signal molecule binds to a receptor protein (which is usually embedded in the plasma membrane), thereby activating an intracellular signaling pathway that is mediated by a series of signaling proteins. Finally, one or more of these intracellular signaling proteins interacts with a target protein, altering the target protein so that it helps to change the behavior of the cell.

# Four General Types of Intercellular Signalling



**Figure 15–4 Forms of intercellular signaling.** (A) Contact-dependent signaling requires cells to be in direct membrane–membrane contact. (B) Paracrine signaling depends on signals that are released into the extracellular space and act locally on neighboring cells. (C) Synaptic signaling is performed by neurons that transmit signals electrically along their axons and release neurotransmitters at synapses, which are often located far away from the cell body. (D) Endocrine signaling depends on endocrine cells, which secrete hormones into the bloodstream that are then distributed widely throughout the body. Many of the same types of signaling molecules are used in paracrine, synaptic, and endocrine signaling; the crucial differences lie in the speed and selectivity with which the signals are delivered to their targets.

# *Signaling Requires Receptors for Ligands: Three Classes of Cell Surface Receptors*



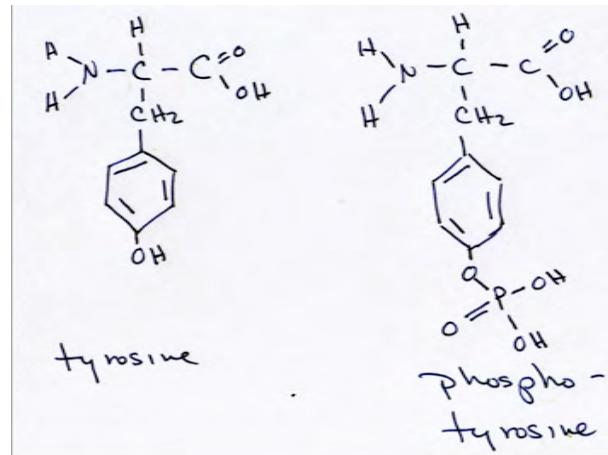
**Figure 15–15 Three classes of cell-surface receptors.** (A) Ion-channel-linked receptors, (B) G-protein-linked receptors, and (C) enzyme-linked receptors. Although many enzyme-linked receptors have intrinsic enzyme activity, as shown on the left, many others rely on associated enzymes, as shown on the right.



# The Hunt for the NGF Receptors

# Tyrosine Phosphorylation and Signal Transduction

## a very big deal in oncogenesis maybe involved in NGF action?



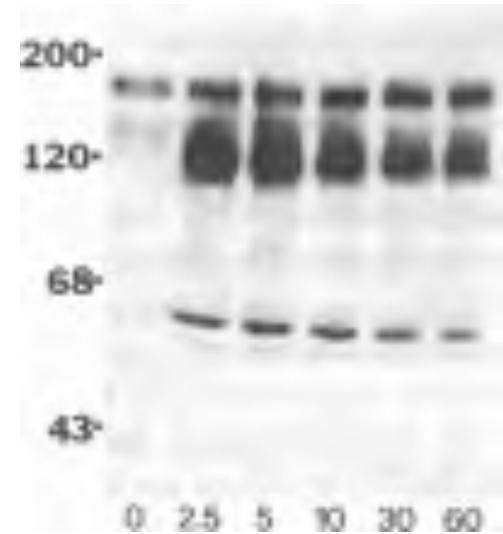
# Pamela Maher Salk Institute

PC12---->NGF for--->isolate total---> immunoblot  
 Cells    various           protein at           with anti-PY  
       times           various times    probe

## What does it mean?

- Big and rapid increase in tyrosine phosphorylation correlates with NGF treatment; maybe involved in NGF action? 

Time (min)	Relative Phosphorylation
0	0
2.5	0
5	~55
10	0
30	0
60	0
  - We don't know the identity of these proteins!!! Maybe NGF receptors???





Ralph Bradshaw



Eric Shooter

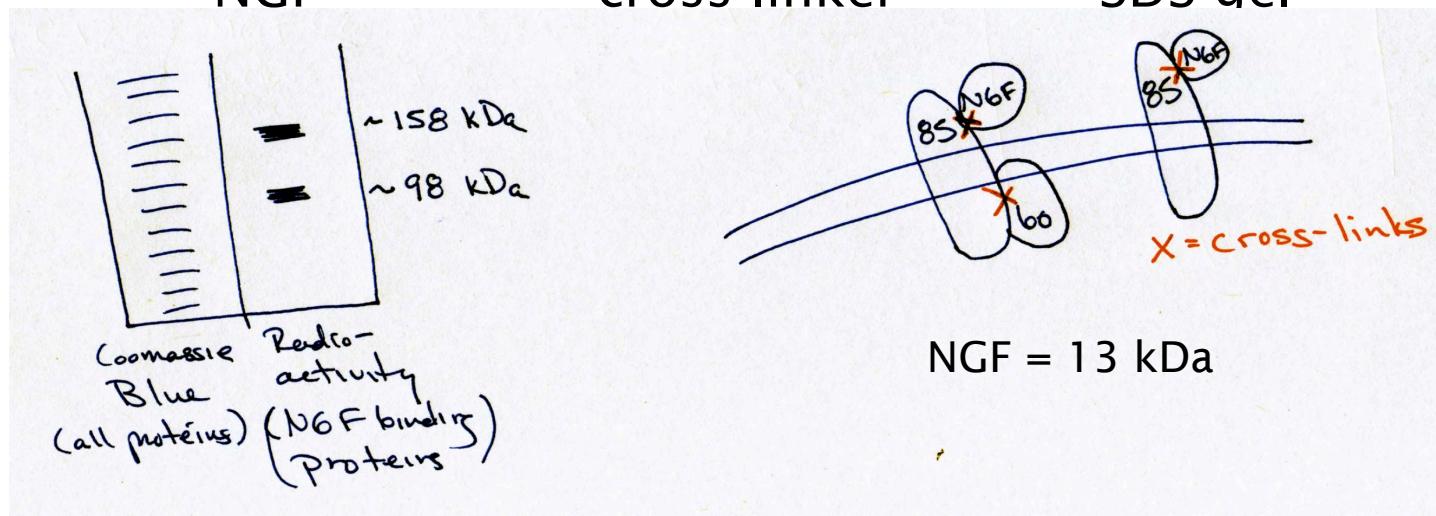


Lloyd Greene

## The Hunt for the NGF Receptors

Strategy: Ask what proteins NGF interacts with on plasma membrane.

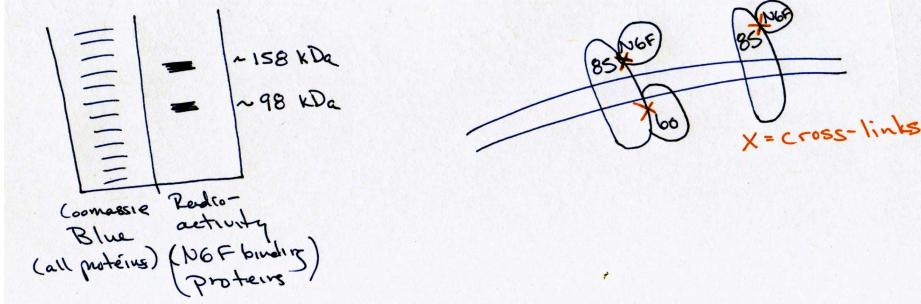
PC12 → add radioactive → add covalent → fractionate on → detect  
Cells            NGF            cross-linker            SDS gel            radioactivity





# Me (long ago)

# Is this 85 + 60 model correct?



Monte Radeke  
UCSB

PC12 → add non-→ add covalent→ purify all→ label all  
Cells radioactive cross-linker NGF containing complexes  
**(reversible)** protein complexes with radio-  
activity (anti-NGF column)

→ Reverse Cross-links fractionate on SDS gel

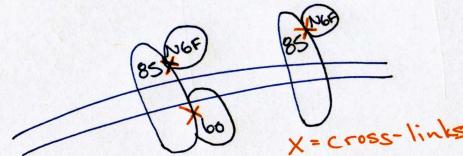
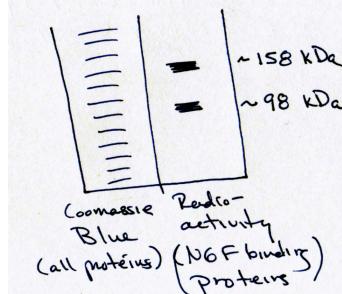
## Predictions:

- If model is right, see 85 and 60 kDa bands;
  - if model is wrong, see bands at other sizes



Me  
(long ago)

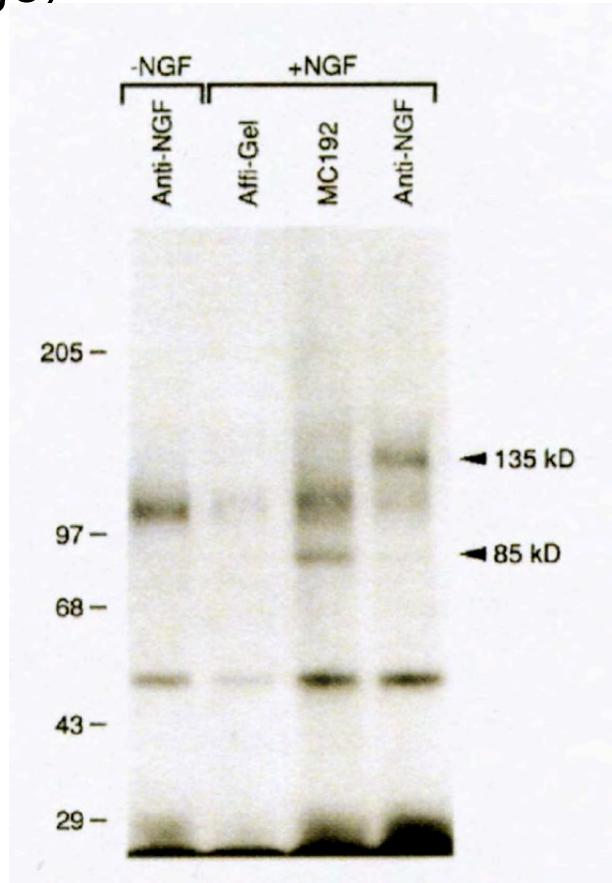
# Is this 85 + 60 model correct?



Monte Radeke  
UCSB

NO 60 kDa band exists!

There is a band at 135 kDa.....in rat cells (PC12 are of rat origin), the putative “NGF receptor” is ~148 kDa, not 158 kDa



Conclusion: the model is wrong; the 135 kDa protein is either the NGF receptor all by itself, or is part of some larger receptor complex..... but the identify of the 135 kDa band Is still unknown!

So, what is the ~135 kDa band?

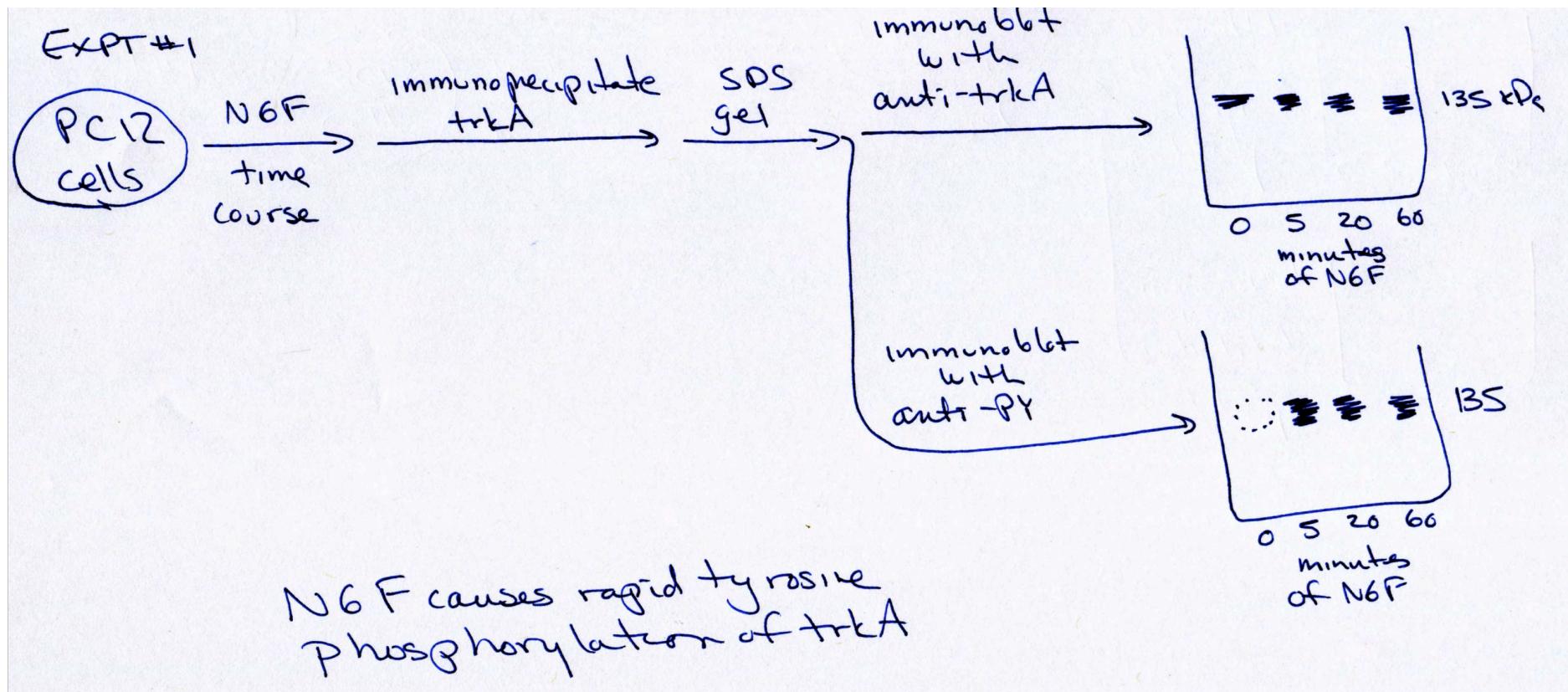
## Convergence of neurobiology with cancer biology

- “orphan receptors” – identified “receptors” with no known ligand
- called “receptors” because they look like receptors based upon
  - their known structural and/or cell biological properties

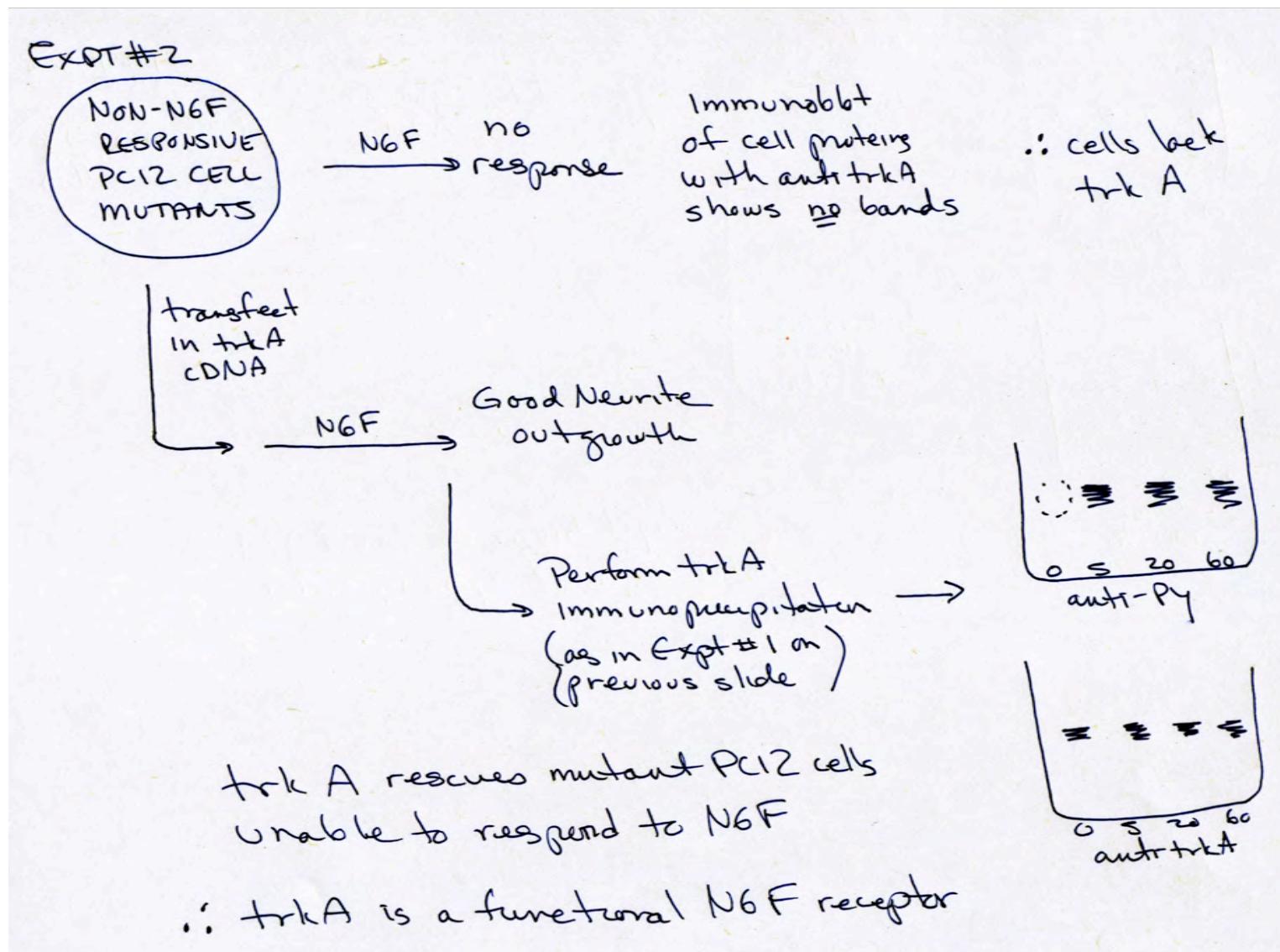
- TrkA –
- transmembrane protein; has intracellular tyr. kinase domain;
  - orphan receptor first discovered in 1981; had no known ligand;
  - discovered as an oncogene responsible for a colon carcinoma;
  - normal expression pattern is mostly in sympathetic and sensory neurons; some brain expression also;

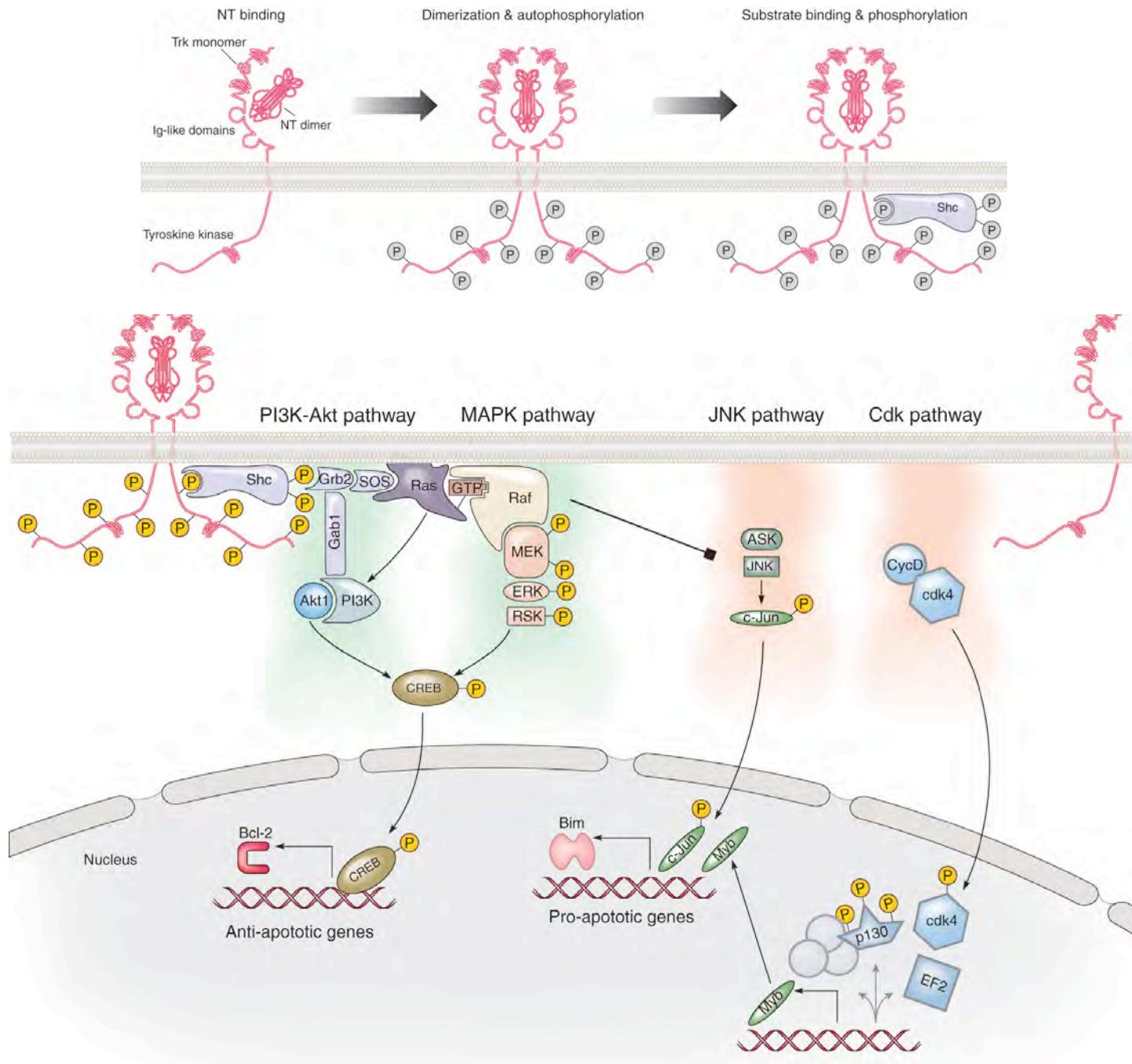
TrkA turns out to be the 135kDa NGF receptor

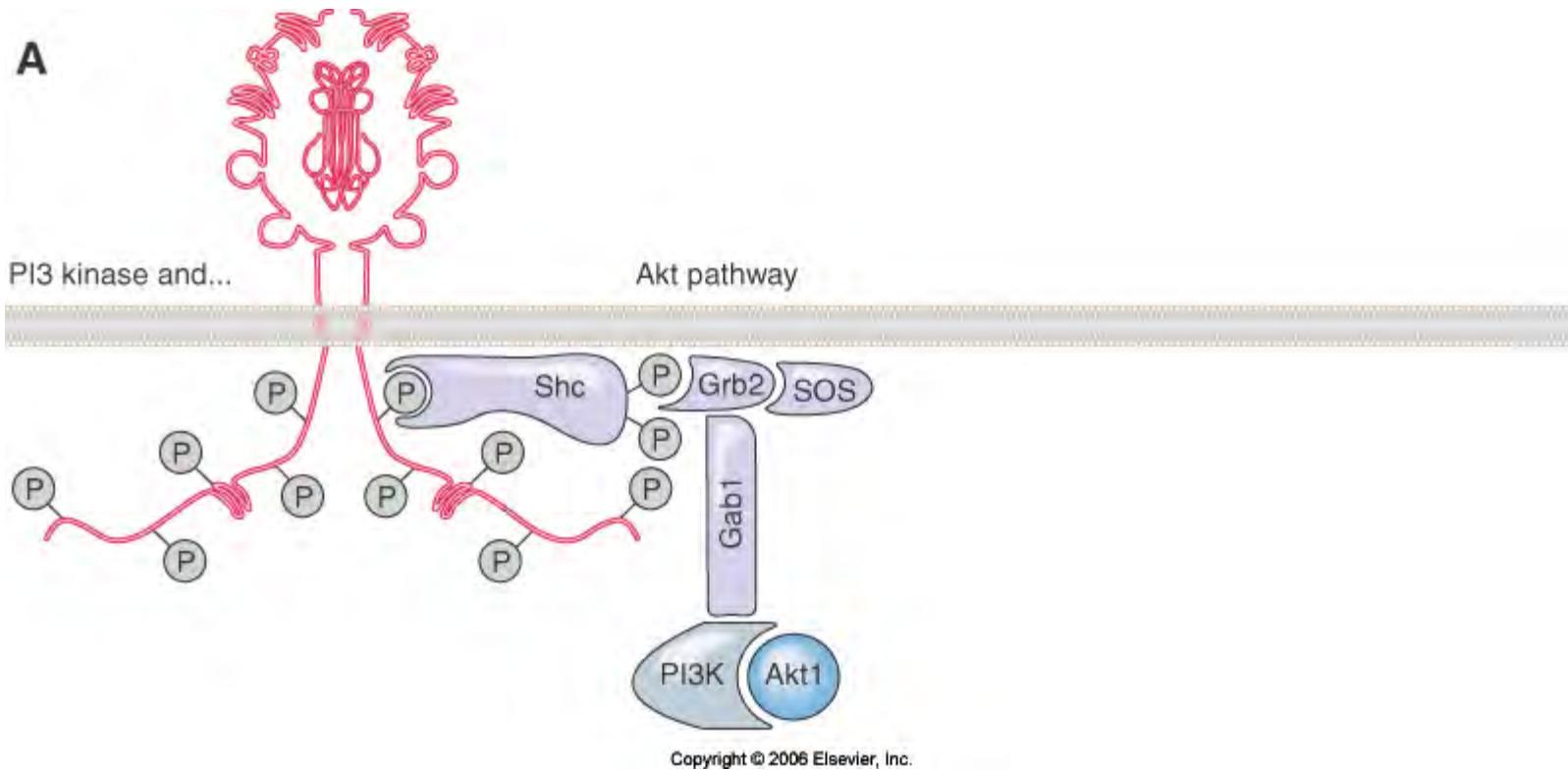
# Experiments supporting the conclusion that TrkA is the 135kDa NGF receptor

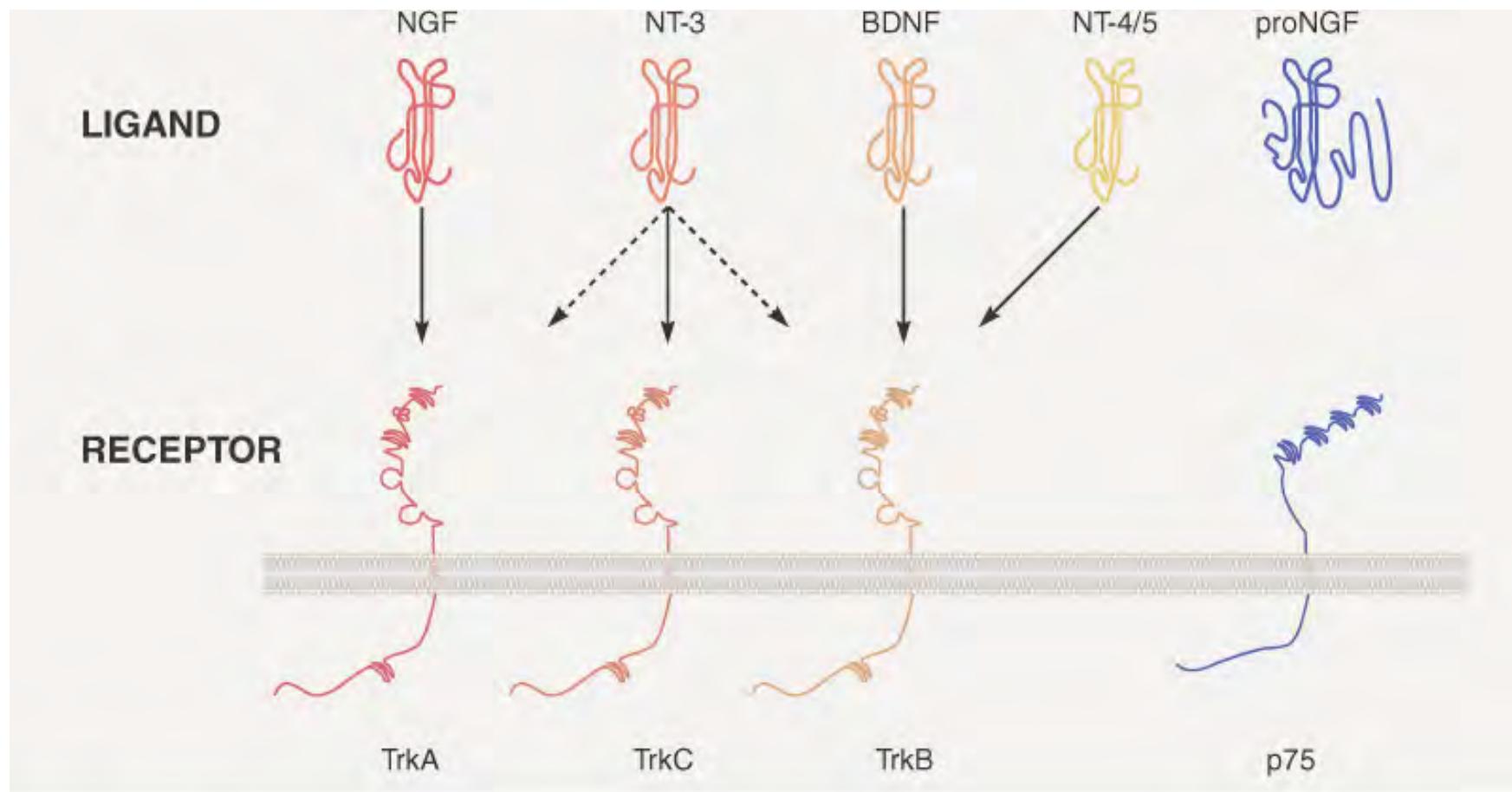


# Experiments supporting the conclusion that TrkA is the 135kDa NGF receptor







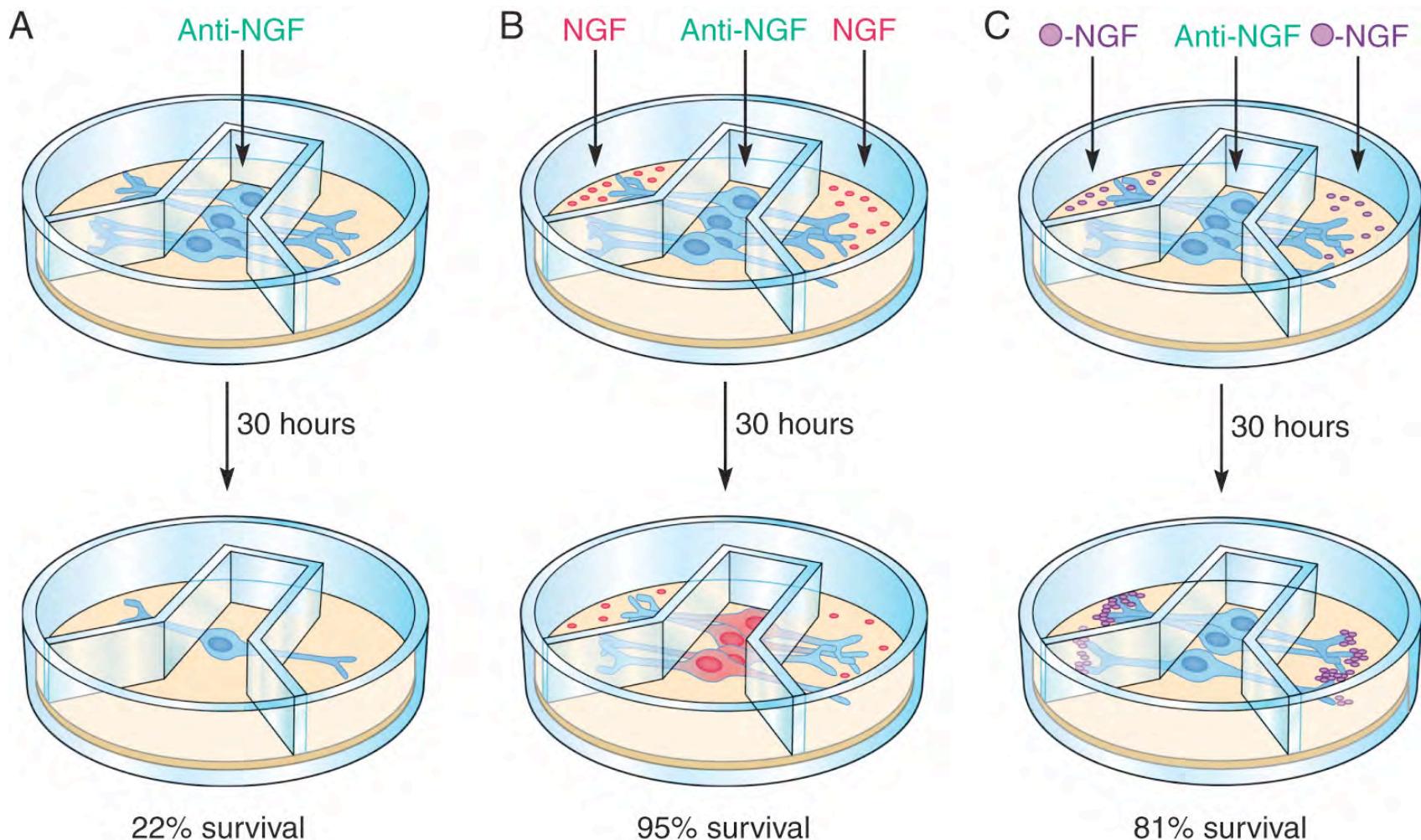


**CELL DEATH  
FOLLOWING  
RECEPTOR  
ELIMINATION**

70% DRG	20% DRG	30% DRG	50% DRG
95% SCG	0% SCG	-	-
70% trigeminal	20% trigeminal	60% trigeminal	-
0% cochlear	50% cochlear	15% cochlear	-
0% vestibular	15% vestibular	60% vestibular	-
basal forebrain atrophy	-	90% nodose	-
	-	basal forebrain cerebellum	-

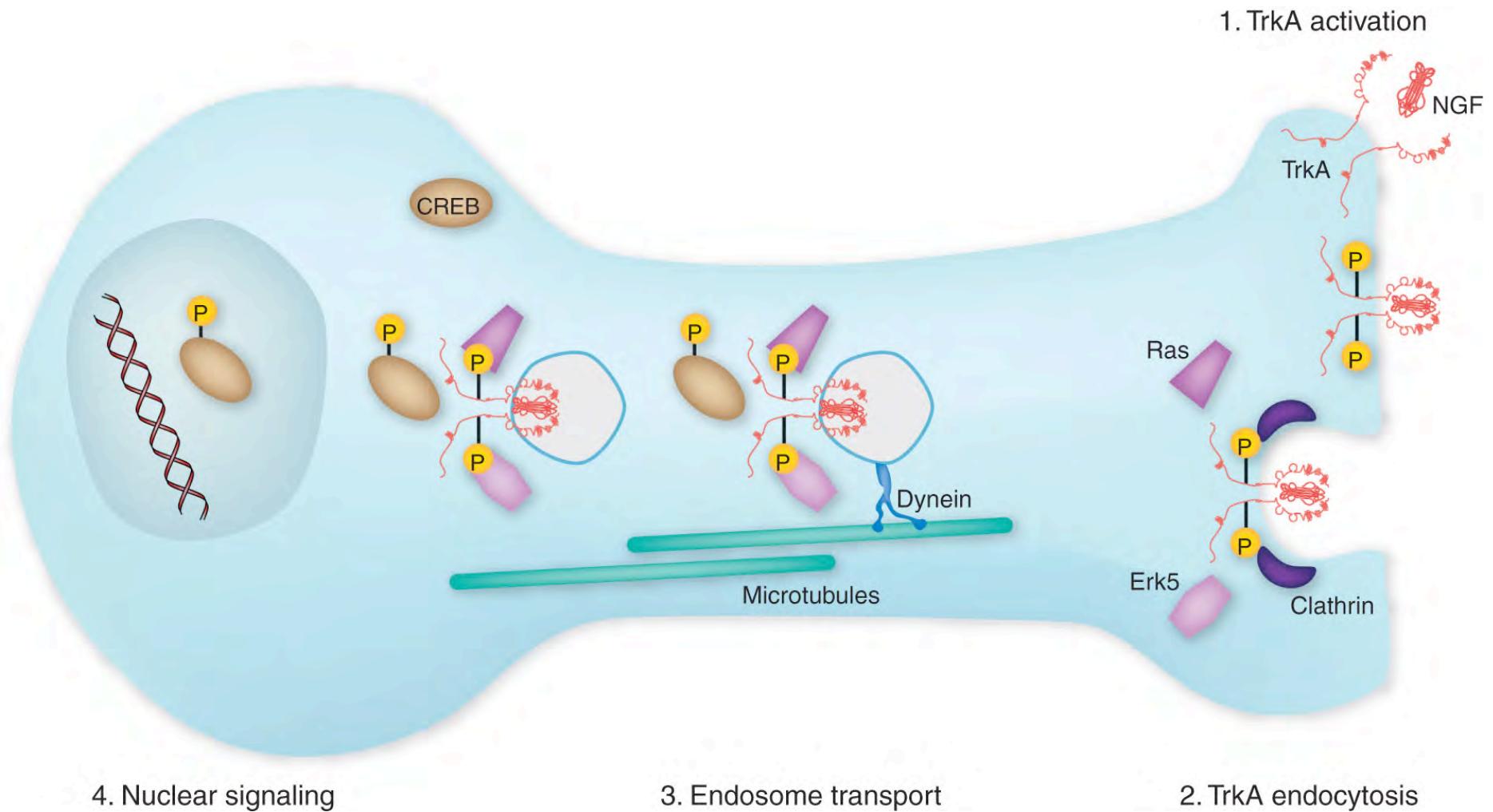
How does the neurotrophin  
signal reach the soma?

# The NGF signal can be acquired at the tips of growing neuronal processes to promote neuronal cell survival



# How is the survival signal relayed back to the cell body?

## The signaling endosome hypothesis



## 5. Target Mediated Regulation of Apoptosis

## 2 Kinds of Cell Death

### Necrosis:

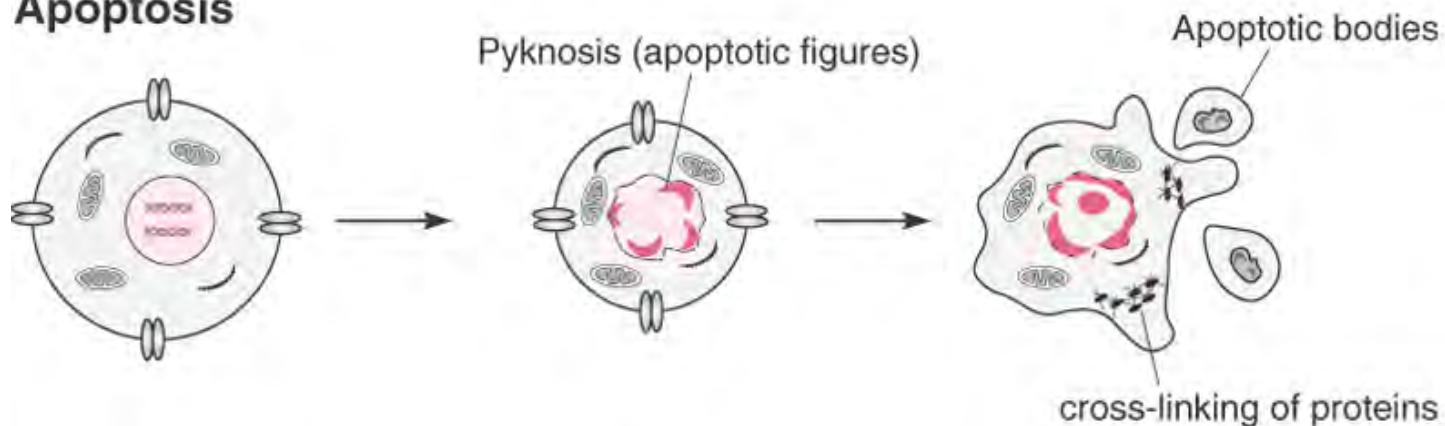
- initial swelling of the cell
- minimal effects on chromatin
- organelle breakdown
- cell membrane rupture
- major inflammatory response
  - macrophages invade; phagocytosis of debris

### Apoptosis:

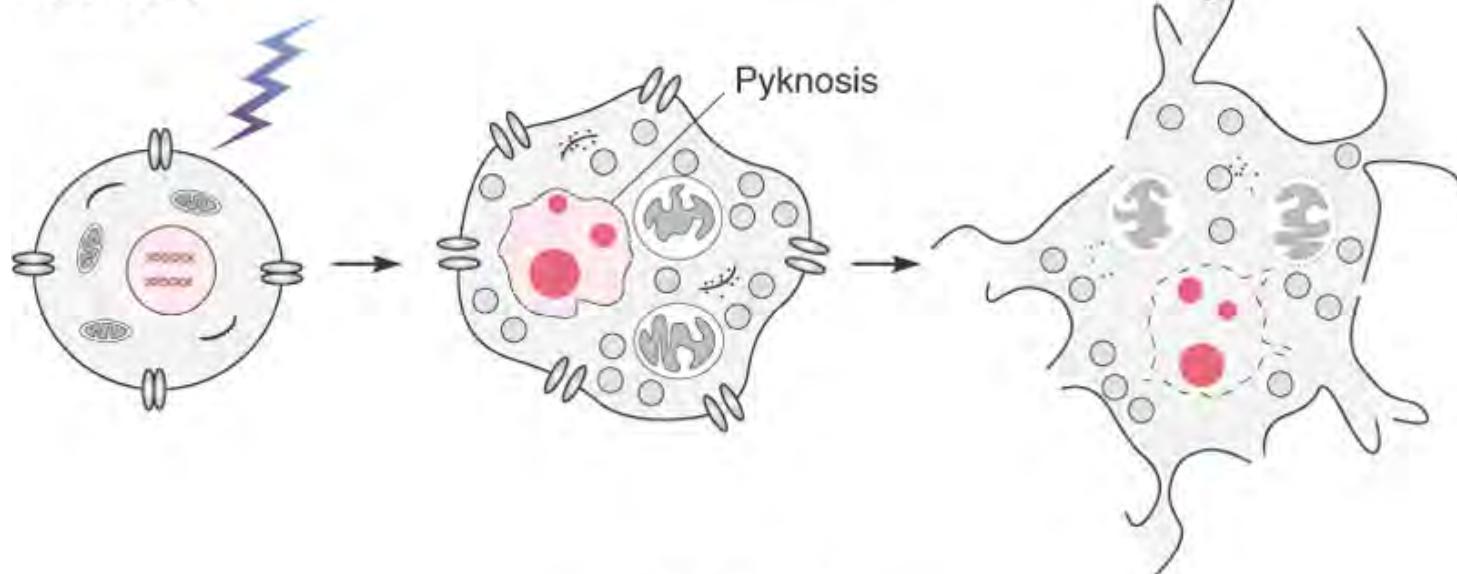
- metabolically active suicide process
- cells shrink in size
- chromatin condenses against nuclear envelop
- nuclear DNA fragmentation
- cyto and nucleus break up into small bodies
  - can be phagocytosed by adjacent cells

# Cell Death: Apoptosis versus Necrosis

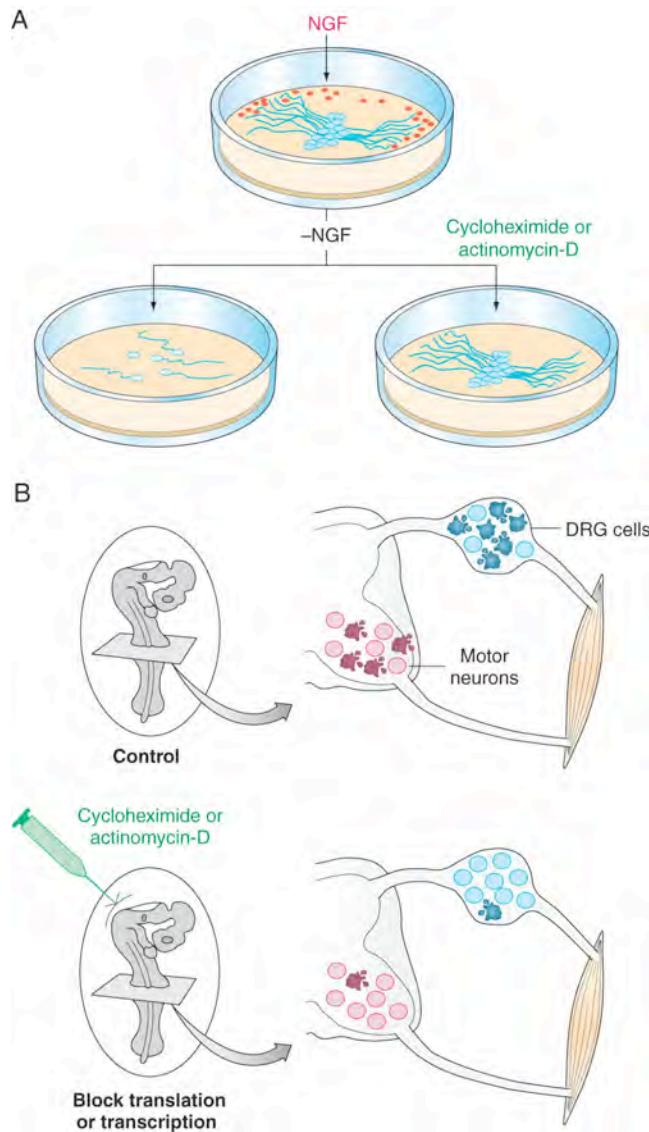
## Apoptosis



## Necrosis

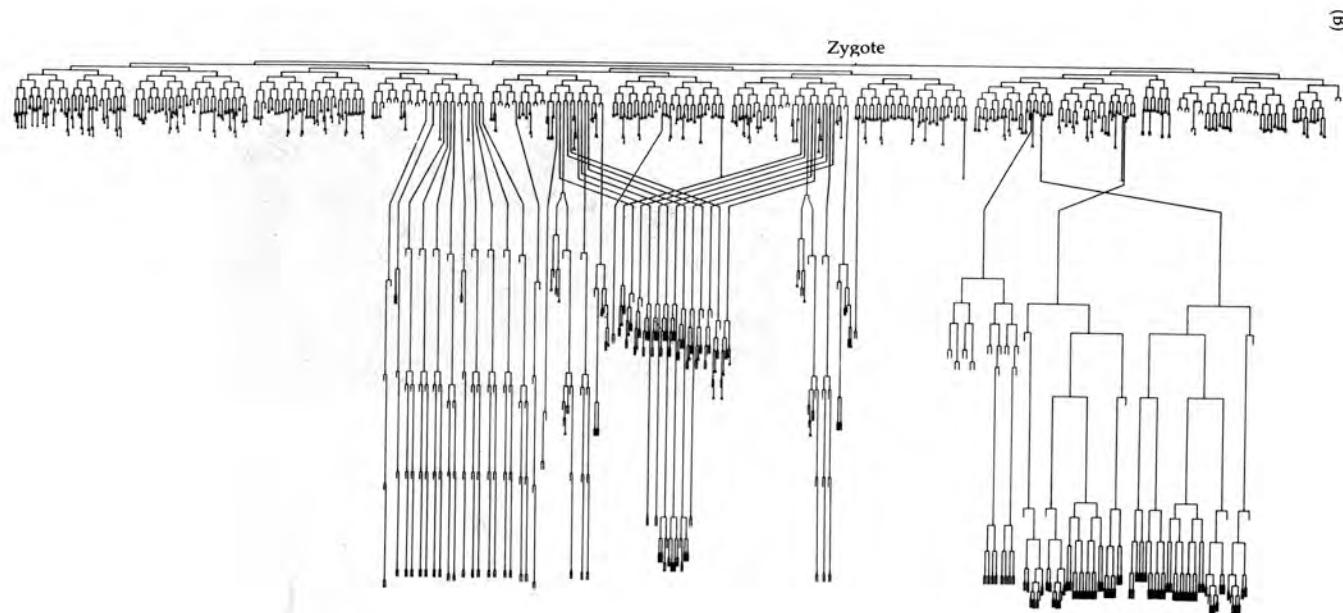
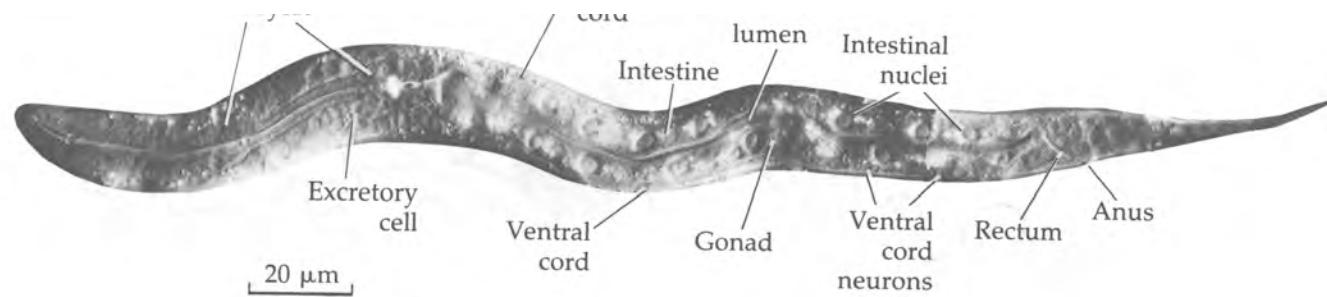


# Neuronal cell death can be delayed by blocking protein synthesis



Cell death is an active process; NGF is repressing a death program, i.e., it prevents cell suicide.

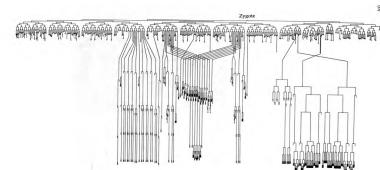
# *C. elegans* cell lineage



131 cells always die...always the same ones!



## Genetic screens for mutations in *C. elegans* cell death pathway



Screen for mutant animals with extra cells (less cell death) and mutant animals with less cells (more cell death)

ced-3 and ced-4 – mutant animals with extra cells (so the normal function of these defective genes must be to promote cell death)

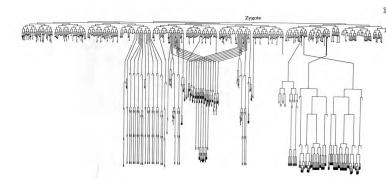
Ced-9 – mutant animals with fewer than normal number of cells (so the normal function of this defective gene must be to prevent cell death)

How is this going to tell us anything about mammalian cell death?  
(remember chordin and sog and dpp and BMP-4????)

What is known about these genes and what are their mammalian homologs?



## Ced-3 protein is a Cysteine Protease



Cysteine proteases have a cysteine at their proteolytic active site.  
Ced-3 is related to a mammalian protein known as “ICE”  
(30% homology)

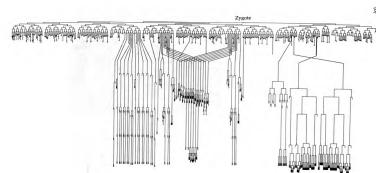
ICE = interleukin converting enzyme; part of immune system

ICE  
Inactive IL-1 $\beta$  -----> Active IL-1 $\beta$   
cytokine

ICE not previously thought to be involved in cell death decisions!



## Ced-3/ICE mediate cell death in worms and mammalian cells



Fibroblasts  
in  
Culture

<u>no manipulations</u>	~1% death
<u>vector alone</u>	1.4% death
<u>mouse ICE cDNA</u>	93% death
<u>worm Ced-3 cDNA</u>	93% death
<u>point mutated</u> active site	3.7% death

- ∴ strongly suggests that ced-3/ ICE can mediate cell death via its protease function in mammalian cells
- ∴ cell death machinery is in place and just need a trigger or switch to be activated

## How about ICE in neurons?

Sympathetic  
Neurons  
in  
Culture

- $\xrightarrow{\text{NGF}}$  no cell death
- $\xrightarrow{\text{No NGF}}$  massive cell death
- $\xrightarrow{\text{No NGF} + \text{cycloheximide}}$  no cell death  
(for a while)
- $\xrightarrow{\text{No NGF} + \text{crm A}}$   
(an ICE inhibitor)

$\therefore$  ICE is important for mammalian neurons  
and the cell death decision

## What next? How about ced-9?

- Very similar to a mammalian protein bcl-2 (23% identical)
- Originally identified as a cancer causing oncogene
- Normally, increased bcl-2 levels prevent lymphocytes from undergoing apoptosis

This is making some sense....ced-9 and bcl-2 are anti-death genes  
but is it real?

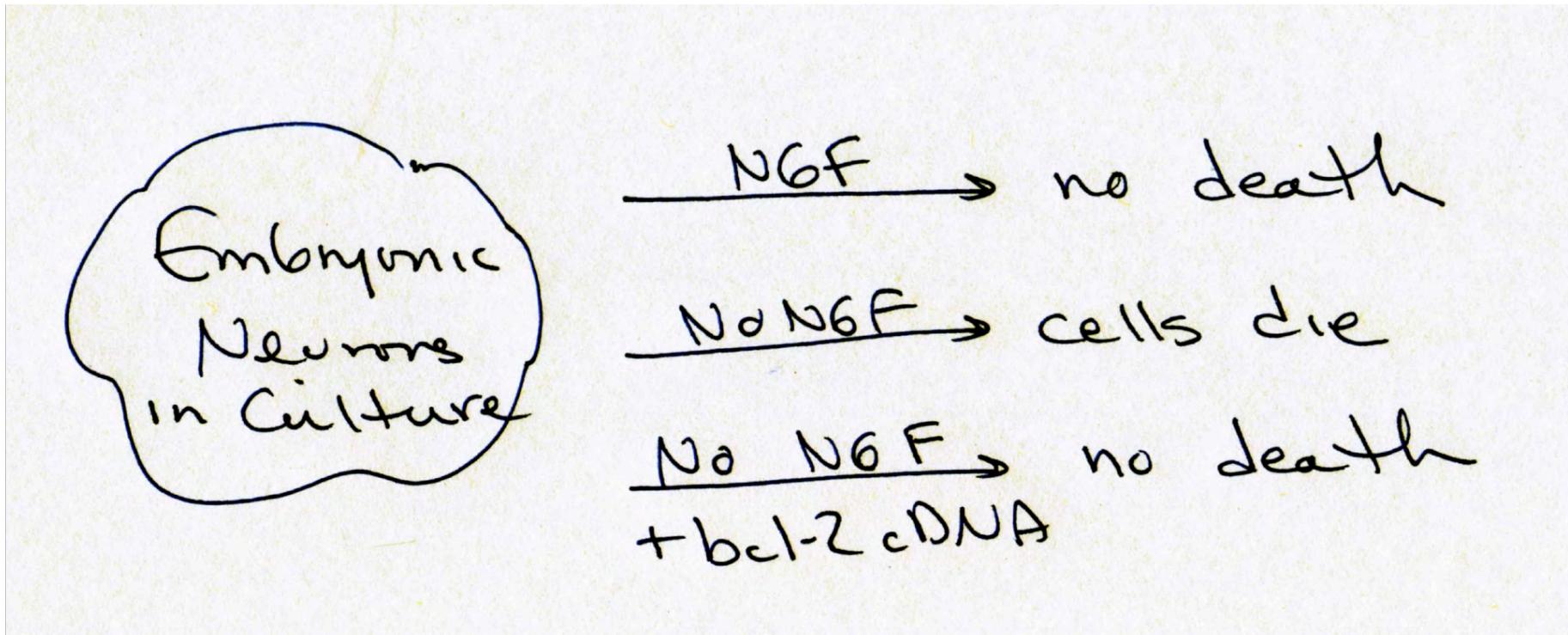
Ced-9 worms -----→ lots of extra cell death

transfect in mouse bcl-2 cDNA

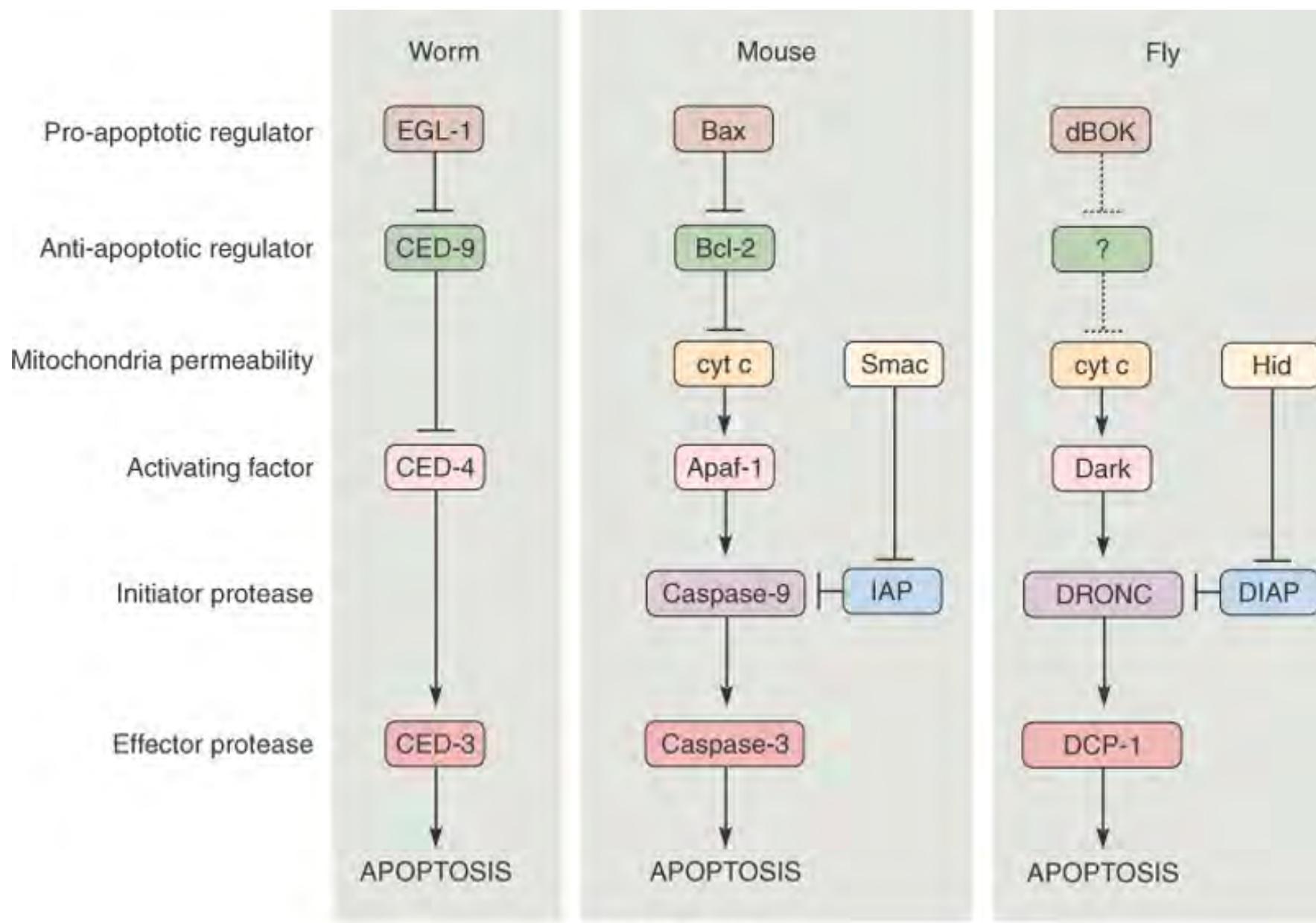
Ced-9 worms-----→normal levels of cell death

Remarkable evolutionary conservation!

## Will bcl-2 rescue mammalian cells?



bcl-2 can prevent cell death in mammalian neurons



# It is all about the bcl-2:bax ratio

Dimers that can form:

bcl-2:bcl-2

bcl-2:bax

bax:bax

In order to repress the cell death pathway, cells need lots of bcl-2:bcl-2 dimers to shut down the downstream part of the pathway

So...if the [bcl-2] is greater than the [bax], then there will be lots of bcl-2:bcl-2 dimers, so no significant cell death, but....

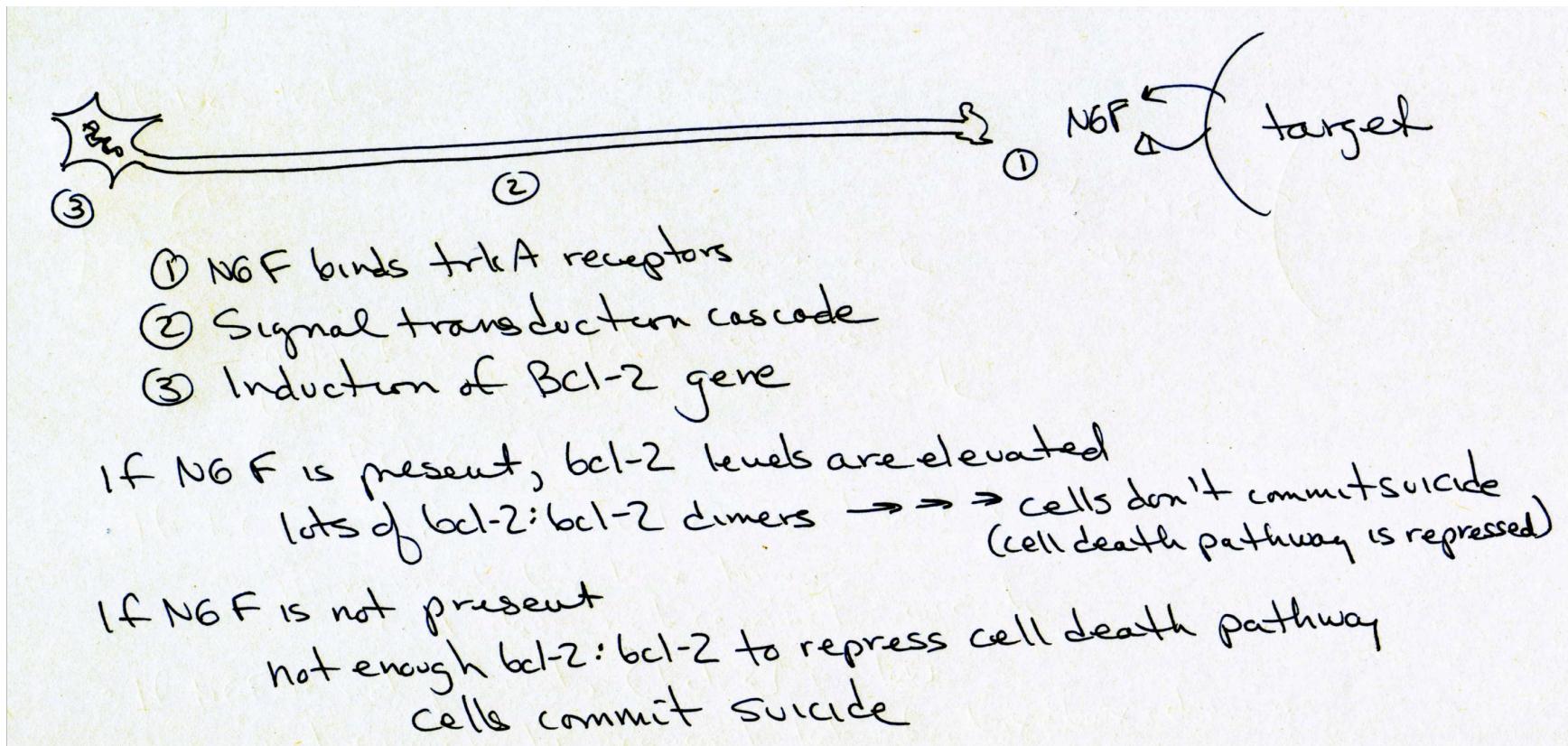
if the [bcl-2] is less than the [bax], then there will not be many bcl-2:bcl-2 dimers, so the cell death pathway will be active and there will be significant cell death

How might NGF and other survival factors regulate this system?

# How might NGF and other survival factors regulate this system?

NGF promotes cell survival, i.e., represses the suicide pathway.

Perhaps NGF induces transcription of the bcl-2 gene (and therefore the synthesis of bcl-2 protein), which in turn promotes assembly of bcl-2:bcl-2 dimers because of its increased concentration.



Does NGF signalling really induce bcl-2 transcription?

Riccio et al., Science 1999 (discussed in section)

CREB is a transcription factor that is necessary for NGF signaling

CREB is a transcription factor that is necessary and sufficient to promote survival, even in the absence of NGF;  
makes sense since it acts downstream of NGF-trkA

NGF mediated induction of Bcl-2 gene transcription requires CREB

Conclusion: NGF signalling activates CREB, which in turn induces bcl-2 gene transcription, leading to the repression of the cell death pathway.

