Cell Death & Trophic Factors II

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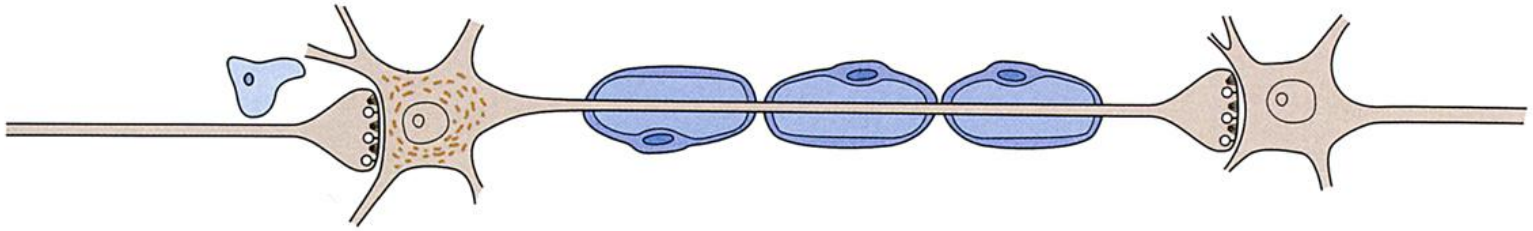
Course News

Graduate School Discussion

Wednesday, Nov 28
11:00am (right after lecture)
In Mayo 3-100

with Dr. Paul Mermelstein
(invite your friends)

Remember?



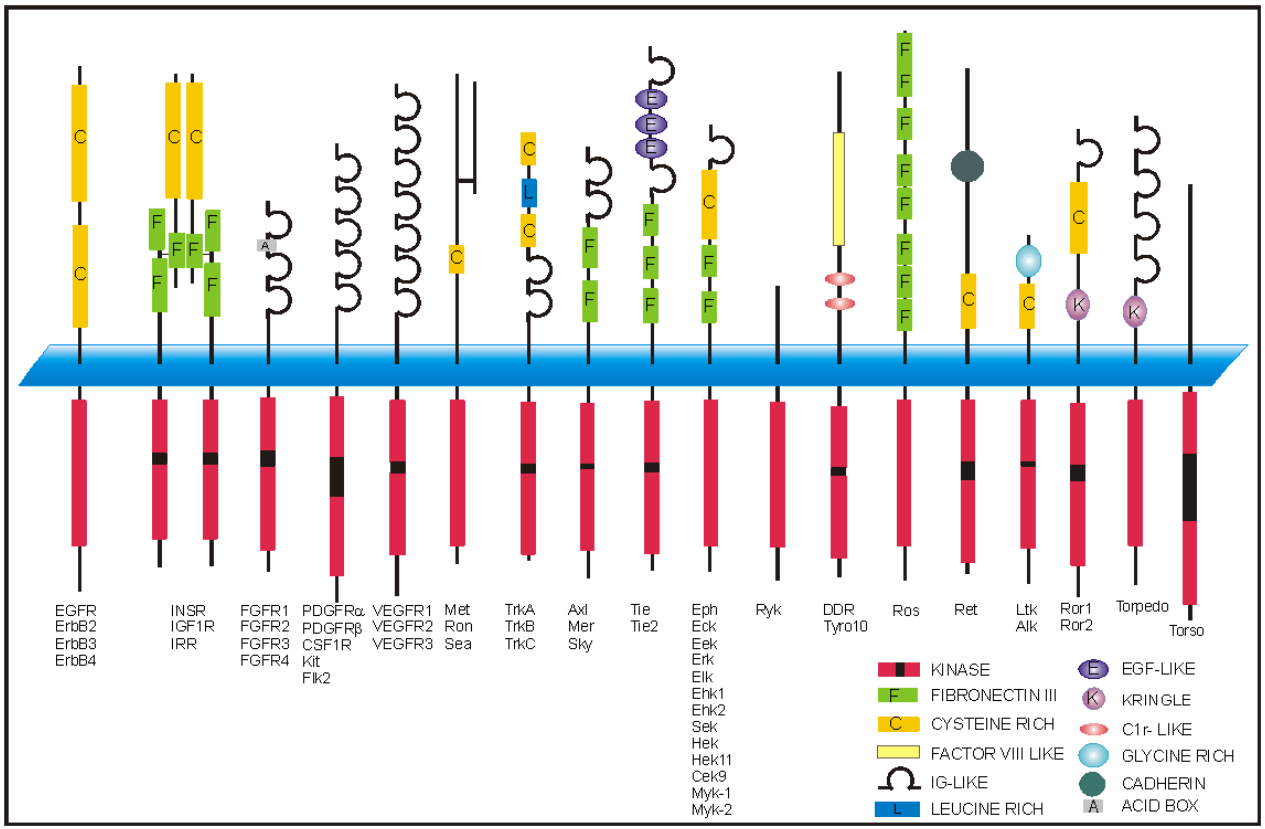
Neurotrophins are cell survival factors that neurons get from their target cells!

There is a family of neurotrophins, and different types of neurons are sensitive to different neurotrophins!

Cells that receive too little of the appropriate neurotrophin die!

High Affinity Neurotrophin Receptors

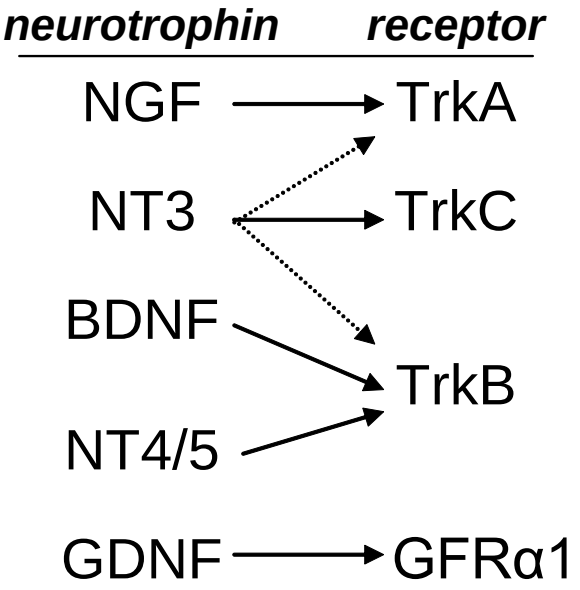
- A neurotrophin binds with high affinity and activates a specific cell surface receptor.
- TrkA is the high affinity NGF receptor and is expressed by cells that respond to NGF.
- Trk A is member of a family of neurotrophin receptors.



High Affinity Neurotrophin Receptors

- Each neurotrophin binds with high affinity to one receptor.

- The ability of a neuron to respond to a neurotrophin depends on the receptor it expresses.



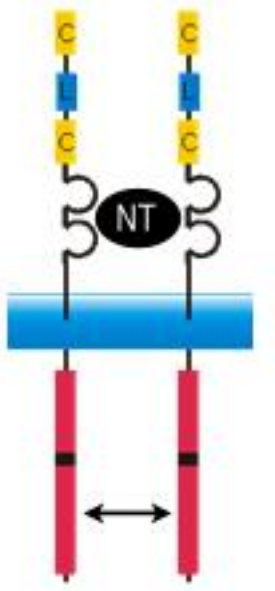
High Affinity Neurotrophin Receptors

- Different neurons express different receptors and respond to different neurotrophins.

receptor	ligand	DRG neuron type
TrkA	NGF	nociceptive neurons
TrkB	BDNF, NT3/4	mechanoreceptor neurons
TrkC	NT3	proprioceptor neurons

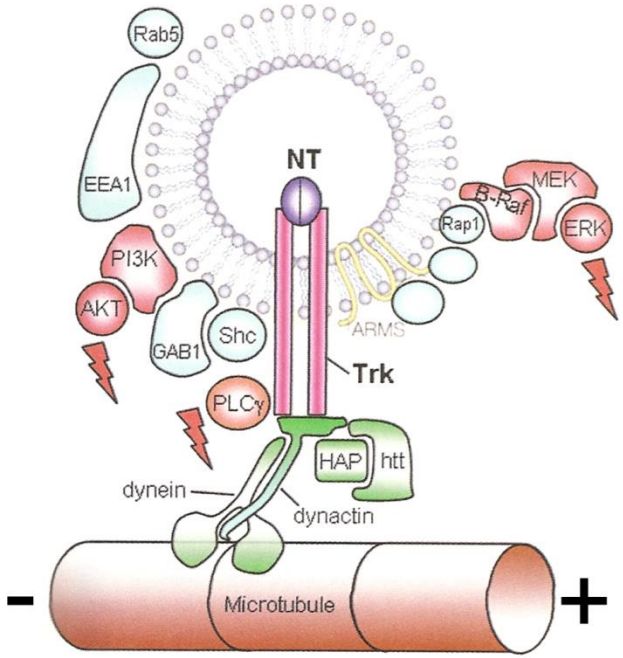
High Affinity Neurotrophin Receptors

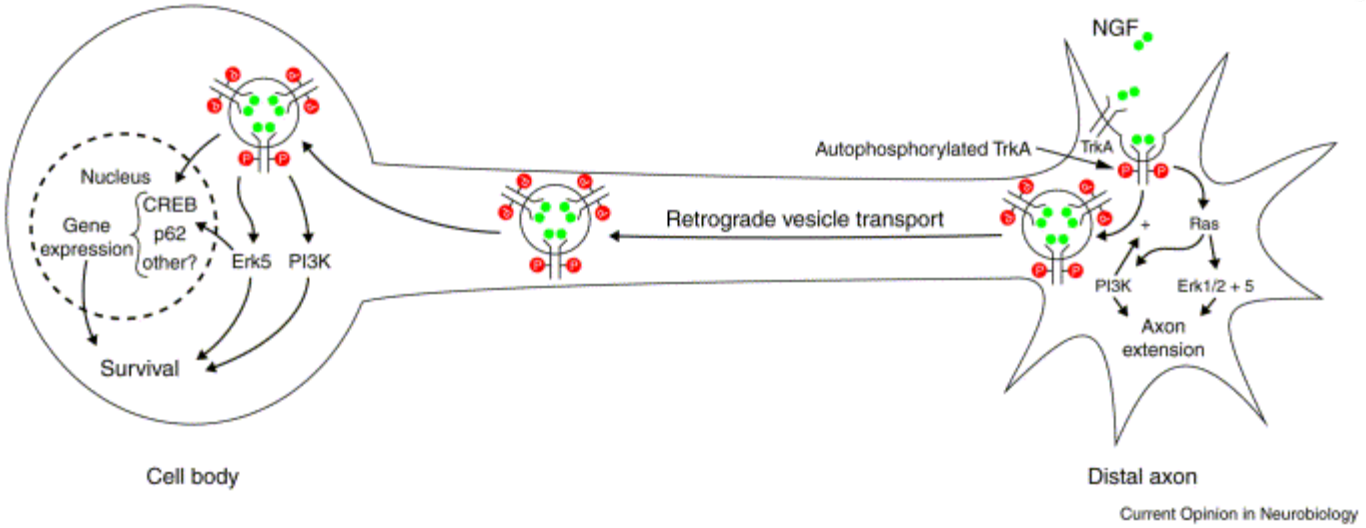
- Trk's are receptor tyrosine kinases.
- Neurotrophin dimerize the Trk's.
- Trk dimmers autophosphorylate their cytoplasmic domains, which activates the complex.



High Affinity Neurotrophin Receptors

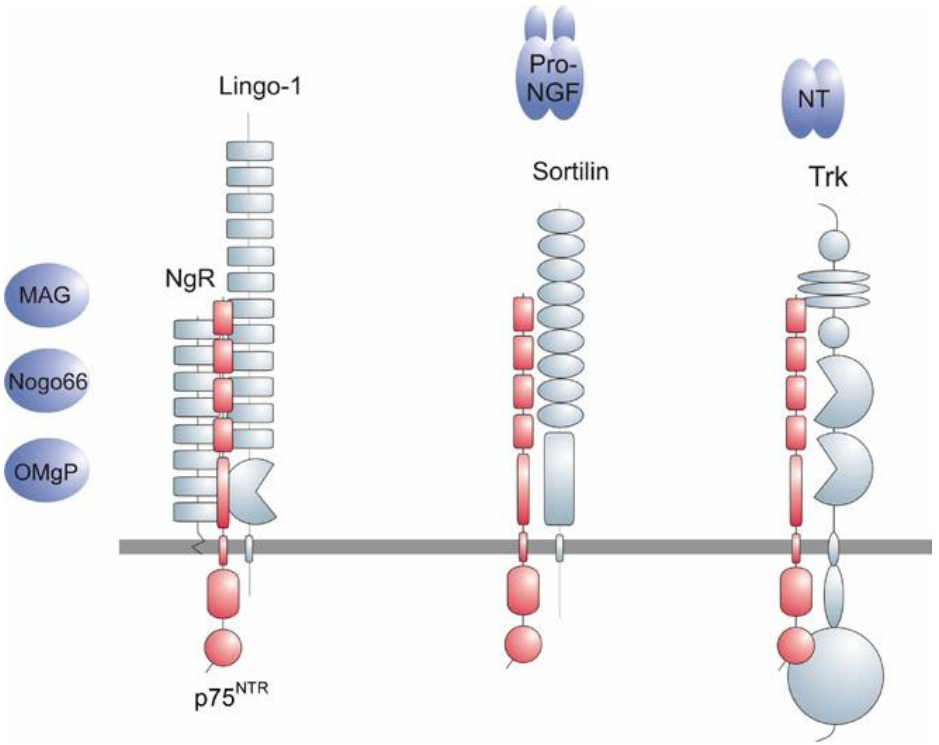
- The activated receptors with their ligand are internalized in an endosome and transported to the soma.
- Endosomes are transported retrogradely using the motor protein, dynein, in association with microtubules.
- Activated Trk's phosphorylate numerous target proteins in the soma.





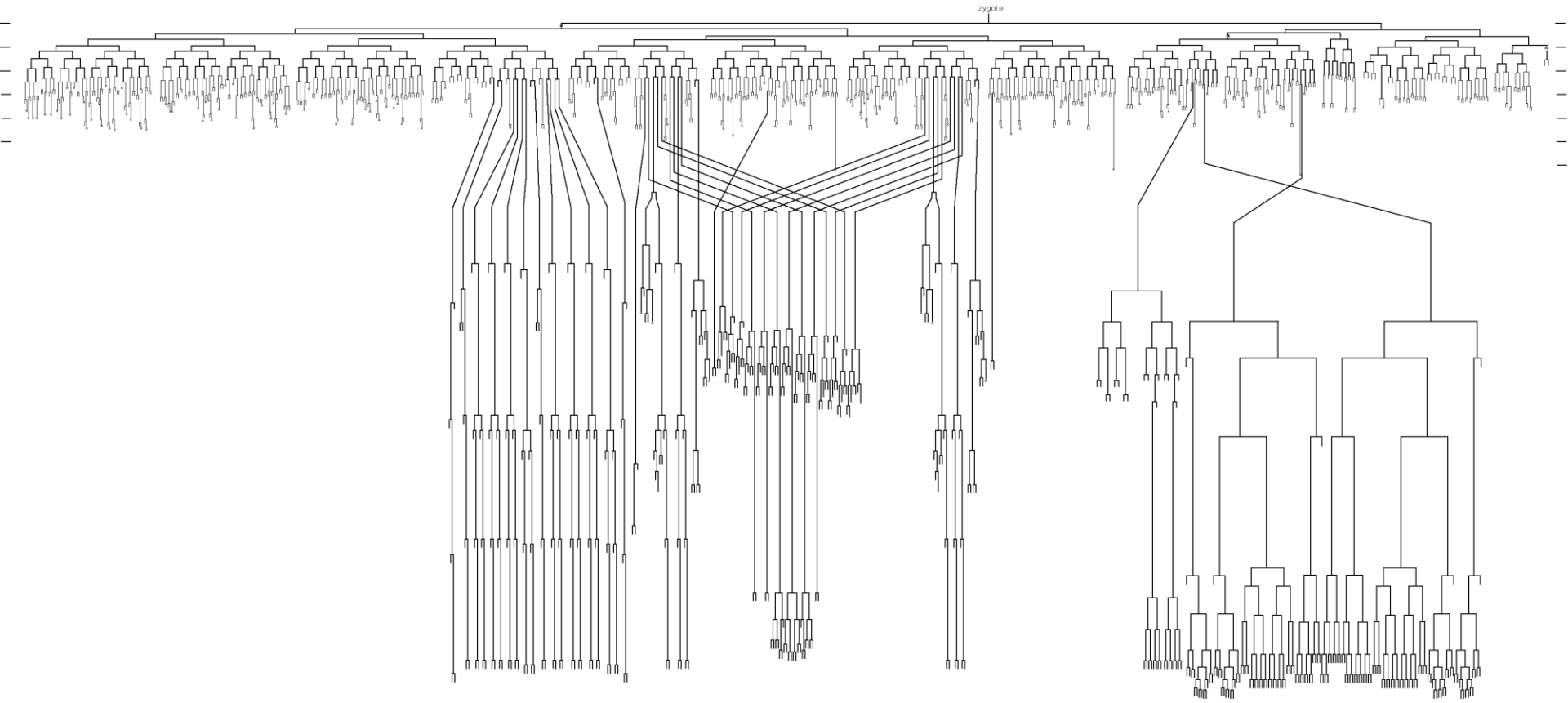
p75 Neurotrophin Receptor

- Neurons also express a low affinity neurotrophin receptor, $p75^{NTR}$.
- $p75^{NTR}$ amplifies the action of the Trk.
- $p75^{NTR}$ functions with other receptors.
- Binding of proneurotrophin to $p75^{NTR}$ in association with the sortilin receptor can result in cell death.



Molecular Regulation of Developmental Cell Death in C. elegans

- Of the 1090 somatic cells generated, 131 undergo precisely timed, stereotypic death.



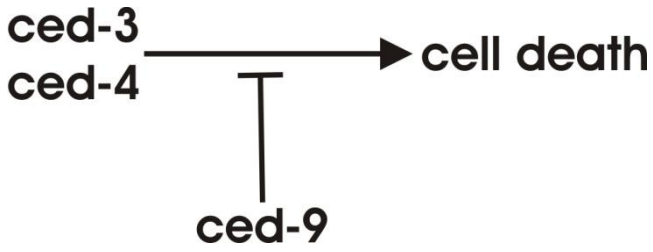
Molecular Regulation of Developmental Cell Death in C. elegans

H. Robert Horvitz looked for mutations that would alter cell death in C. elegans:

- Cell death requires expression of two genes, ced-3 and ced-4.
- In mosaic ced-3-null/wild type animal, only ced-3+ (i.e. wild type) cells die, suggesting that the gene acts cell autonomously.
- ced-3 protein is a protease.

Molecular Regulation of Developmental Cell Death in C. elegans

- ced-9 mutation is lethal with all cells dying, but only if ced-3 and ced-4 are active; therefore, ced-9 represses the function of ced-3 and ced-4 in cells that survive.



Caspase

- Vertebrates have at least 10 genes similar to ced-3, the caspase family.
- Caspases are cysteine-aspartic proteases.
- Caspases cleave very specific targets as apposed to nonspecific protein degradation by many proteases.
- Caspase-3 gene can replace ced-3 in worms.
- Caspase-3 knockouts have reduced cell death in developing brain, and the brain is approximately double in size. In many cells, caspase-3 appears to be obligatory for apoptosis.

Caspase

- Caspases are divided into apoptotic, pro-inflammatory, and other subfamilies. (Caspase-2, -3, -6, -7, -8, -9 & -10 are apoptotic.)
- Apoptotic caspases are subgrouped as initiators (e.g. caspase-8, -9 & -10) or executioners (e.g. caspase-3, -6 & -7).
- Initiator caspases are activated by intrinsic mechanisms (cytochrome C) or extrinsic mechanisms (death receptors).
- Initiator caspases cleave and thus activate executioners
- Executioner caspases cleave proteins that result in cell death.

Caspase

- Caspases are normally present in cells in the inactive, procaspase form. Cleavage of a procaspase is required to activate the caspase.

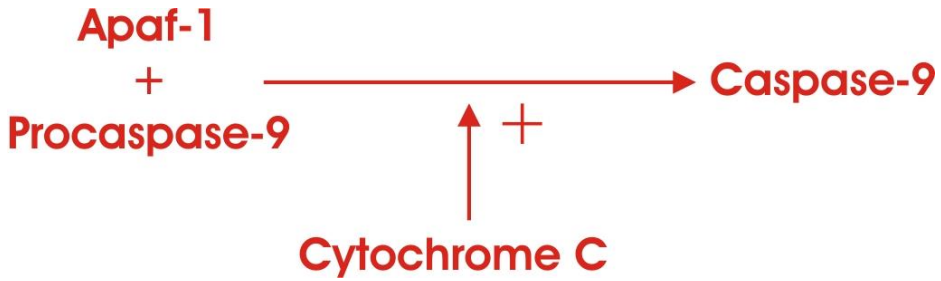
- Some cell types require different caspases for apoptosis. (i.e. Sympathetic neuron death following NGF-deprivation requires caspase-2 and not caspase-3.)

Caspase Activation

- Apoptosis protease-activating factor-1 (Apaf-1) is similar to ced-4 and is needed to activate certain initiator caspases.

- Apaf-1 binds procaspase-9 and cytochrome-C, which leads to active caspase-9.

- Cytochrome-C is released from mitochondria.

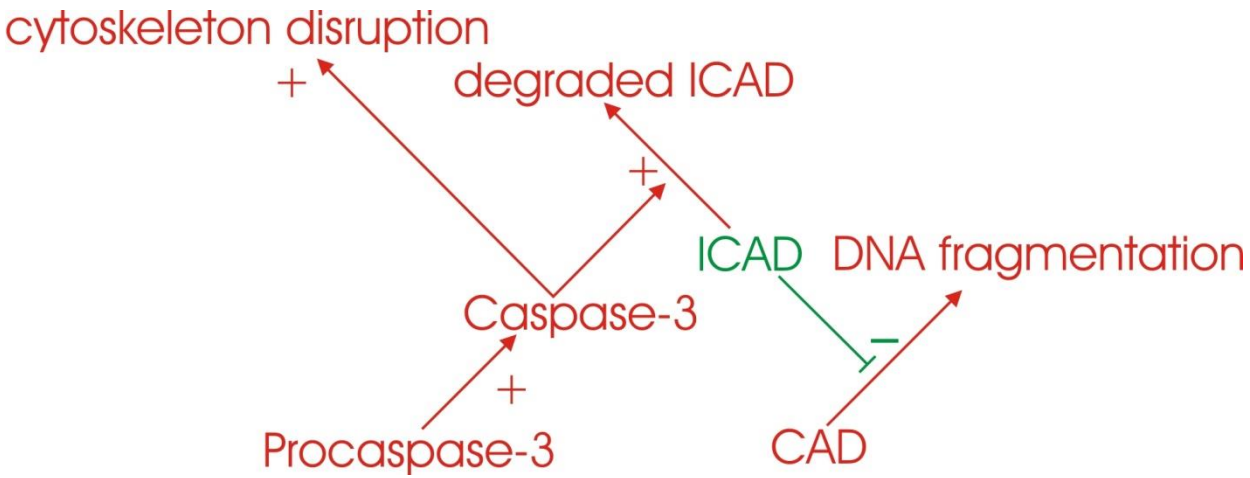


- Active caspase-9 cleaves procaspase-3 into active caspase-3. Once caspase-3 is active, cell death is inevitable.

CAD and ICAD

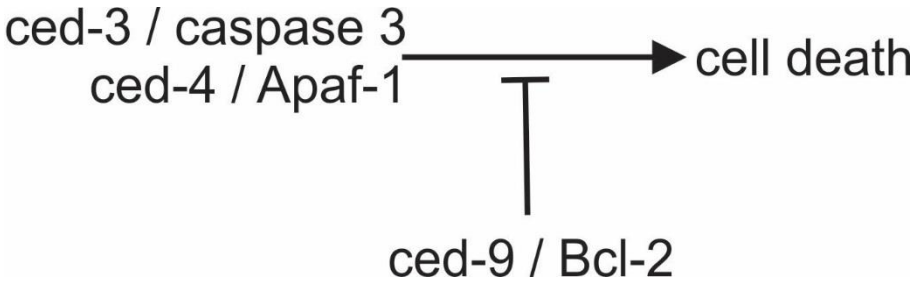
- Caspase-Activated-DNase (CAD) is normally inhibited in cells by CAD Inhibitor (ICAD).
- Caspase-3 degrades ICAD, which allows CAD to function.
- Active CAD enters the nucleus and cuts DNA into 180bp fragments.
- Mutation in the caspase-3 recognition site in ICAD prevents DNA fragmentation but not cell death.
  (i.e. Other targets of caspase-3 contribute to cell death.)

CAD and ICAD


cytoskeleton disruption
+
degraded ICAD
+
ICAD
DNA fragmentation
Caspase-3
–
+
Procaspase-3
CAD

Bcl-2

- Bcl-2 was first identified as a cancer causing viral gene.
- Bcl-2 is homologous to ced-9, and the Bcl-2 gene can substitute for ced-9 in worms.
  (i.e. Bcl-2 rescues cells from death in ced-9 mutants.)



Bcl-2

- Misexpression of Bcl-2 in cultured sympathetic neurons rescued cells from NGF deprivation. Misexpression of Bcl-2 also prevented death of all DRG neurons cultured without neurotrophin regardless of the trk expressed.

- Not all neurons are rescued by Bcl-2 overexpression (e.g. ciliary ganglion cells).

- Misexpression of Bcl-2 in transgenic mice (with the neuron specific enolase promoter) rescued some neurons from PCD.

- Bcl-2 knockout transgenic mice did not show altered cell death in nervous system but did show substantial cell loss in other tissues (e.g. kidney & thymus).

Bcl-2 Family

- Bcl-2 is one member of a family of molecules in vertebrates (Bcl-2 family).

- Some Bcl-2 family members promote cell survival, while others promote cell death.

- Increased Bax expression increased death of facial motor neurons (and others). Bax knockouts have reduced neuron PCD, and neurons without Bax do not die following target deprivation.

- Neurons from $Bax^{-/-}$ mouse embryos survive in culture without trophic factor, but require trophic factor to fully differentiate.

- Bax homo-dimers form pores in mitochondrial membranes. Pores allow cytochrome-C into the cytoplasm, thus leading to activation of caspase-9.

Bcl-2 Family

- Bcl-X_L is widely expressed in the nervous system (more than Bcl-2). Bcl-X_L overexpression protects many neurons from death in many situations. Bcl-X_L knockouts show massive death of postmitotic neurons.

- Family members form homo- and heterodimers. Some function as homodimers. Some inhibit the function of others by forming heterodimers.

- Cell death and cell survival depend on the relative levels of life and death promoting Bcl-2 family members.

Bcl-2 Family

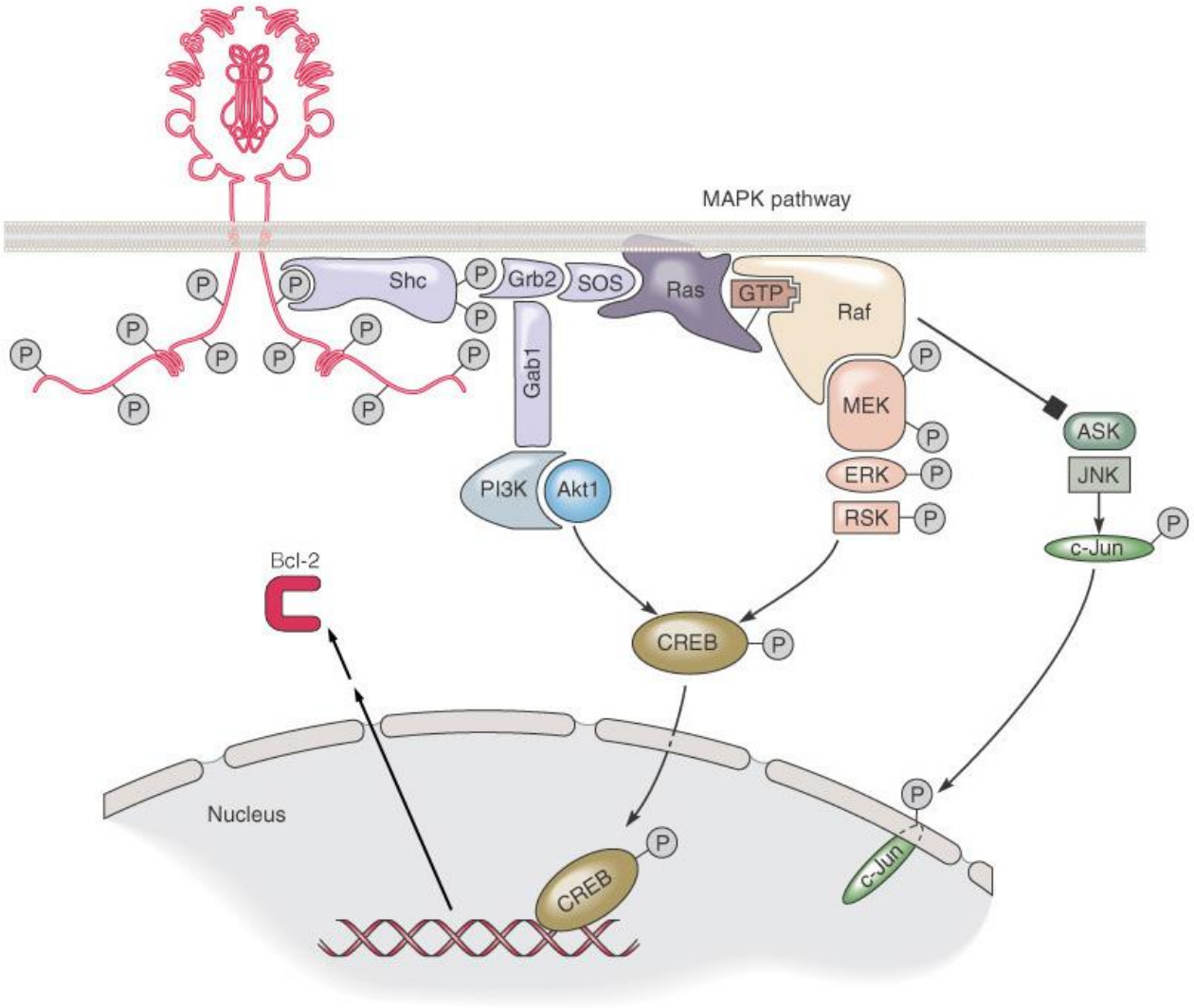
- Bcl2 family members:

antiapoptotic	proapoptotic
Bcl-2	Bax
Bcl-X_L	Bax-like
Bcl-W	Bik
Mcl-1	Bad
A1	Bid
	Bak
	Bok
	Egl1

Mechanisms of Neurotrophin Action

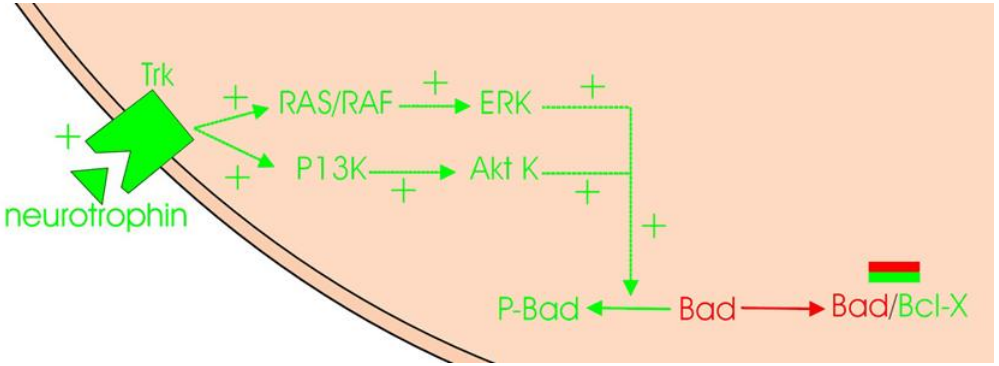
- Activation of neurotrophin receptors (Trk's) promotes cell survival.
- Trk's are tyrosine kinases, which activate several pathways.
- Phosphoinositide-3-kinase (PI3K) is activated by Trk's. PI3K activates serine-threonine protein kinase (Akt), and Akt phosphorylates CREB.
- PI3K activation can keep sympathetic neurons alive in culture in the absence of NGF.
- RAS/RAF pathway is also activated by Trk's. Activated RAF activates the MAPK pathway (MEK/ERK), which also activates CREB.
- CREB activation increases expression of antiapoptotic bcl-2 family members.

Mechanisms of Neurotrophin Action



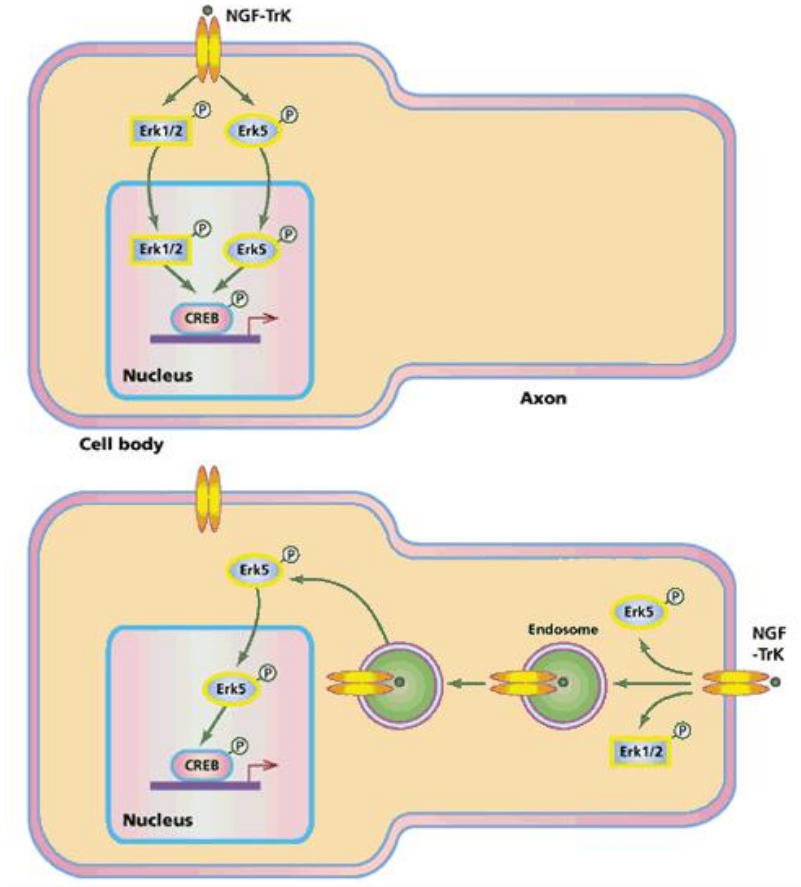
Mechanisms of Neurotrophin Action

- Bad (another Bcl-2 family member) is phosphorylated (deactivated) by Akt and ERK; dephosphorylated Bad can bind Bcl-X, which blocks Bcl-X's survival promoting effect.

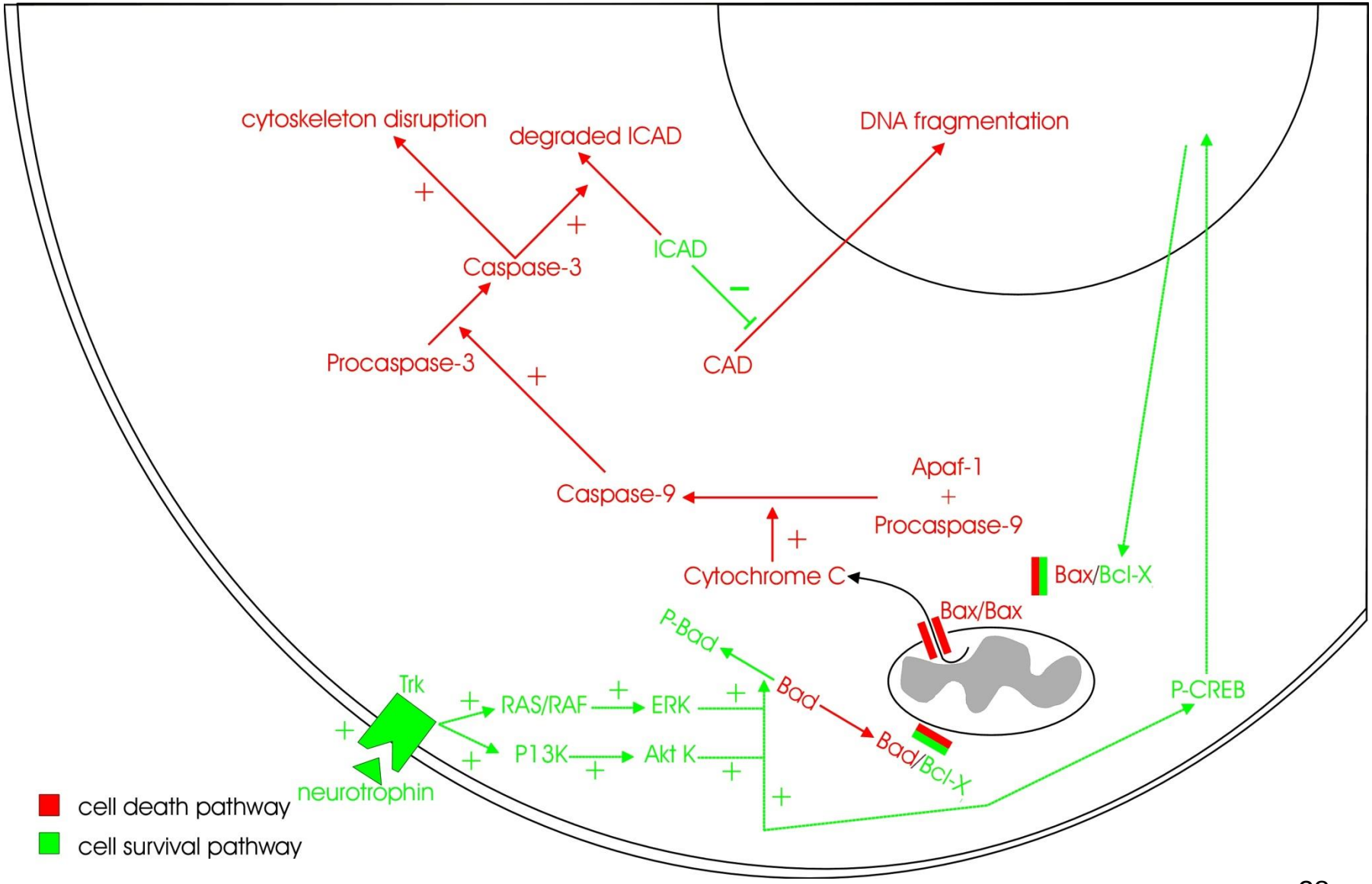


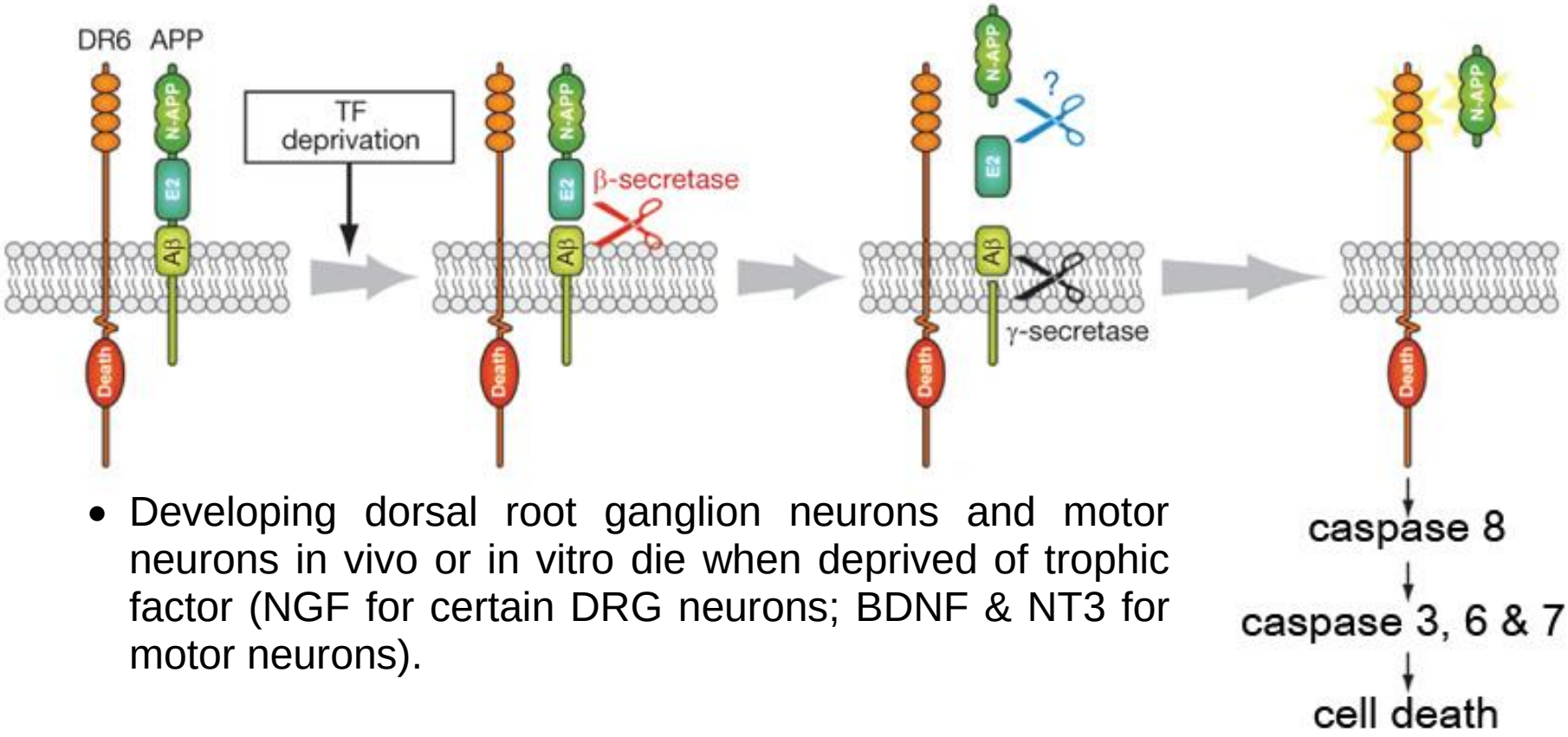
Mechanisms of Neurotrophin Action

- Anterograde and retrograde neurotrophin activate different signaling cascades, which may explain why a neuron requires presynaptic and postsynaptic connections to survive.



Life and Death Pathways





- Developing dorsal root ganglion neurons and motor neurons in vivo or in vitro die when deprived of trophic factor (NGF for certain DRG neurons; BDNF & NT3 for motor neurons).

- These neurons express the cell surface tumor-necrosis factor receptor, death receptor 6 (DR6). With the knockout of DR6, cell death is delayed for days following neurotrophin deprivation.

- DR6 is activated by a cleaved extracellular domain of amyloid-β precursor protein (APP) by secretases.

- Activated DR6 activates the caspase cascade leading to cell death.

Neurotrophins in the Adult Nervous System

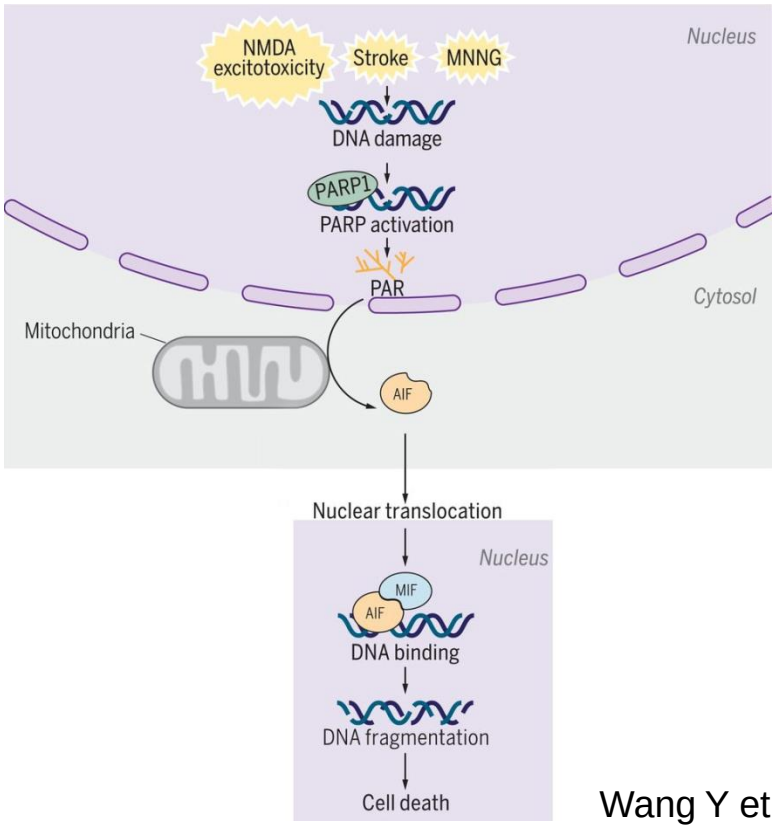
- Most neurons lose dependence on neurotrophin for survival as they mature.
- Adult neurons still express neurotrophin and neurotrophin receptors, and the intracellular signaling cascades are still activated in response to neurotrophin.
- Neurotrophins may mediate synaptic plasticity in the mature nervous system.
- Neurotrophins offer a potential therapy for rescuing neurons from neurodegenerative diseases.

Table 1. Clinical trials with neurotrophins, CNTF and IGF.

	Disease	Type of trial	*n*	Application, dose	Result	Side effects
CNTF[a] ref. 39	ALS	Phase 1, placebo controlled, 2 weeks	57 patients plus 18 patients on placebo	Subcutaneous, 0.5–30 μg/kg, 3 injections per week	Safe, tolerated within acceptable limits, indications for efficacy	Fever, fatigue, cough, weight loss
CNTF[b]	ALS	Phase 1, placebo controlled, 4 weeks	43 patients in treatment and placebo groups	Subcutaneous, 2–200 μg/kg daily	Safe, tolerated within acceptable limits	Fever, HSV-1 stomatitis, diarrhea, fatigue, cough, weight loss
CNTF ref. 40	ALS	Phase 2–3, placebo controlled, 6 months	570 patients	Subcutaneous, 0.5–5 μg/kg daily	No beneficial effects, increased adverse events in the 5 μg/kg group, increased deaths	Injection site reactions, cough, asthenia, nausea, anorexia, weight loss, increased salivation
CNTF ref. 41	ALS	Phase 2–3, placebo controlled, 9 months	730 patients	Subcutaneous, 15–30 μg/kg, 3 times a week	No beneficial effects	anorexia, weight loss, cough
CNTF ref. 42	ALS	Phase 1, open label	6 patients	Cell capsules, intrathecal, approximately 0.5 μg/day	Safe, motor performance did not improve	Headache, radicular pain
CNTF ref. 43	ALS	Phase 1, open label, 48 h per week, 2-week cycles	4 patients	Intrathecal delivery with pumps, 0.4–8 μg/h	Tolerable side effects	Rise in lymphocyte numbers and protein levels in CSF, headache, radicular pain
CNTF ref. 44	M. Huntington	Phase 1	6 patients	Cell capsules implanted into the lateral ventricle	?	?
NGF ref. 45	M. Alzheimer	Phase 1, up to 3 months	3 patients	0.55–6.6 mg in total, infused into the lateral cerebral ventricle	No cognitive improvement, changes in EEG, increased nicotine binding in several brain areas	Back pain, weight loss
NGF ref. 46	Diabetic neuropathy	Phase 1– 2, placebo controlled, 6 months	250 patients	Subcutaneous, 0.3 μg/kg, 3 times a week	Preliminary evidence for efficacy, well tolerated	Injection site pain
NGF ref. 47	Diabetic neuropathy	Phase 3, placebo controlled, double blind, 48 weeks	505 patients NGF treated, 515 patients in placebo group	Subcutaneous 0.1 μg/kg, 3 times a week	No clinical benefit	Minor side effects, injection site pain
NGF ref. 48	HIV neuropathy	Phase 2, placebo controlled, 18 weeks	270 patients	Subcutaneous, 0.1–0.3 μg/kg, twice a week	Significant improvements in neuropathic pain	Injection site pain
NGF ref. 49	HIV neuropathy	Phase 2, open label follow-up study, 48 weeks	200 patients	Subcutaneous, 0.1–0.3 μg/kg, twice a week	Well tolerated, improvement in pain symptoms, no improvement of neuropathy severity	Injection site pain
BDNF[c]	ALS	Phase 1–2, 6 months	224 patients with BDNF, 59 patients with placebo	Subcutaneous, 10–300 μg/kg, daily	Safe, tolerable, less deterioration in forced vital capacity and walking speed	Injection site reactions, bowel urgency, diarrhea
BDNF ref. 50	ALS	Phase 2–3, placebo controlled, 9 months	748 patients with BDNF, 387 patients with placebo	Subcutaneous, 25–100 μg/kg	No significant effect, subgroup of patients with early respiratory impairment and those developing altered bowel function showed statistically significant benefit	Injection site reactions, diarrhea, bowel urgency, generally mild or moderate
BDNF ref. 51	ALS	Phase 1–2, placebo controlled, double blind, 12 weeks	25 patients	Intrathecal, continuous pump delivery, 25–1000 μg/day	Well tolerated at 150 μg/day or lower	Paraesthesias, sleep disturbance, dry mouth, agitation at higher doses
BDNF unpublished	ALS	Phase 2–3, placebo controlled, double blind	250 patients	Intrathecal	No clinical benefit	Paraesthesias, sleep disturbance
BDNF ref. 52	Diabetic neuropathy	Phase 1–2, placebo controlled, double blind, 3 months	21 patients with BDNF treatment, 9 patients with placebo	Subcutaneous, daily, 100 μg/kg	No measureable beneficial effect, safe, tolerable	Non painful injection-site reactions
NT-3 ref. 53	Healthy subjects, diabetic neuropathy, chemotherapy-induced neuropathy	Phase 1, placebo controlled, double blind, 7 days	49 healthy subjects treated with NT-3 and 21 with placebo, no published report on patient studies	Subcutaneous, daily, 3–500 μg/kg/day	Tolerable side effects, patient studies discontinued in 1997	Diarrhea, injection site pain, rise in SGOT and SGPT
IGF-1 ref. 54	ALS	Phase 2–3, placebo controlled, double blind, 9 months	176 patients with IGF-1, 90 patients with placebo	Subcutaneous, 50 or 100 μg/kg/day	Trend to functional improvement	Injection site pain, no major side effects
IGF-1 ref. 55	ALS	Phase 2–3, placebo controlled, double blind, 9 months	124 patients with IGF-I, 59 patients with placebo	Subcutaneous, 100 μg/kg/day	No significant clinical improvement	Injection site pain

Necrosis

- Stroke, excitotoxicity or oxidative stress activates a caspase-independent enzyme cascade that results in DNA fragmentation and cell death.



Wang Y et al. (2016) Science 354:82