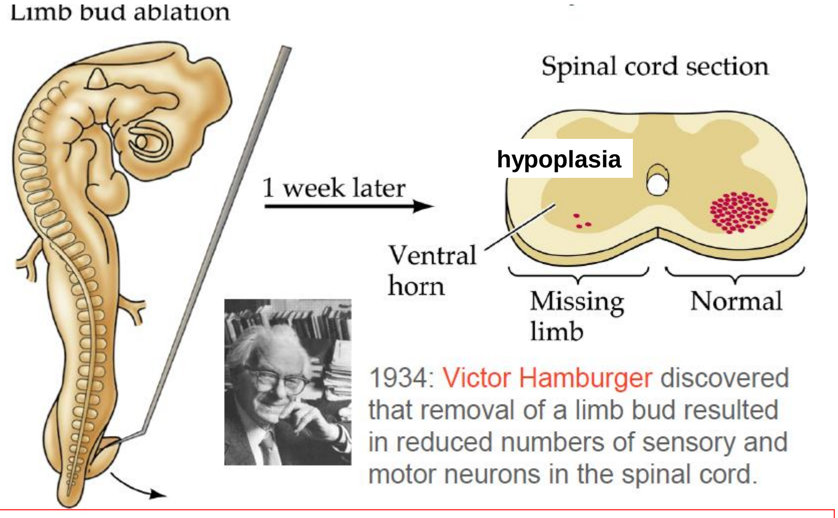
Lessons (16-17)

Neurotrophic Factors and their signaling

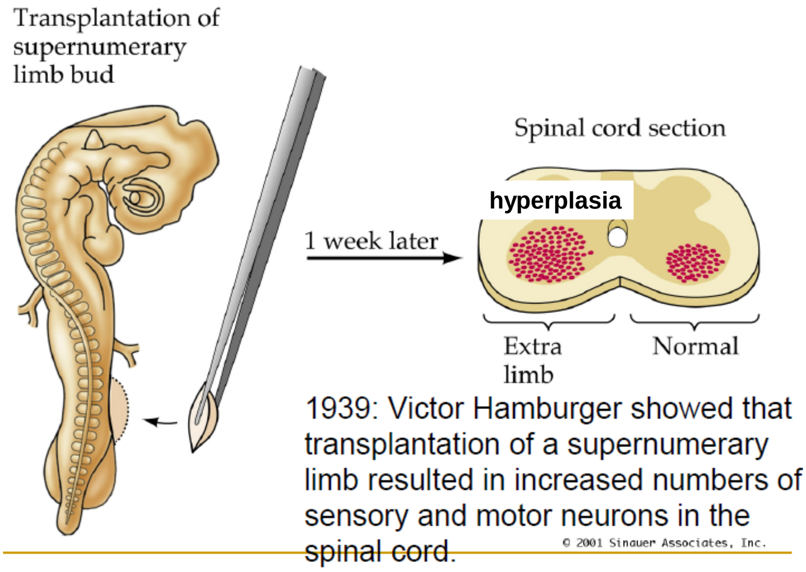
First experiment by Viktor Hamburger (1934)



1934: Victor Hamburger discovered that removal of a limb bud resulted in reduced numbers of sensory and motor neurons in the spinal cord.

[e]very structure within the growing limb, muscle as well as sensory organs, send[s] stimuli to the central nervous system. Each part of the peripheral field controls directly its own nervous center, i.e., the limb muscles affect the lateral motor centers, the sensory fields control the ganglia.

Second experiment by Viktor Hamburger (1939)



1939: Victor Hamburger showed that transplantation of a supernumerary limb resulted in increased numbers of sensory and motor neurons in the spinal cord.

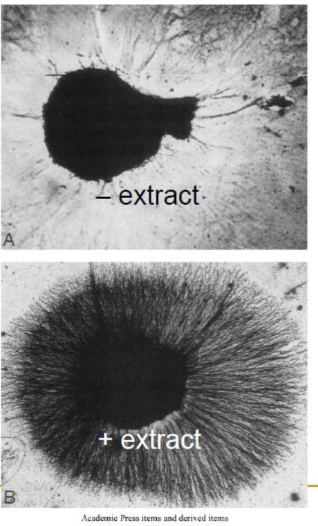
The Neurotrophic factor hypothesis

Based on his limb-bud experiments, V. Hamburger ***hypothesized*** *that the targets of innervating neurons provide signals that recruit undifferentiated cells to develop into sensory or motor neurons.*

(but he was wrong)

The discovery of Nerve Growth Factor

1954: neurite outgrowth assay



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1960: NGF purified

1969: NGF purified to homogeneity

Stanley Cohen   Rita Levi-Montalcini

1986: Levi-Montalcini and Cohen split the Nobel prize for Physiology or Medicine
"for their discovery of growth factors"

8

The Neurotrophin Family

NGF: sympathetic neurons and some sensory neurons + neurons of the basal ganglia (CNS)

BDNF: NGF-related factor purified in 1982 from pig brain (shares ~50% homolog with NGF)

NT-3 and NT-4/5: were obtained by PCR cloning

All these factors are synthesized as ~250 aa precursors that are processed into 120 aa proteins

Families of growth and trophic factors

Family	Specific examples
Neurotrophin	Nerve growth factor (NGF)
	Brain-derived ticurotrophie factor (BDNF)
	Neurotrophin 3 (NT-3)
EGF	Epidermal growth factor (EGF)
	Transforming growth factor alpha (TGFα)
	Vaccinia virus growth factor
	Amphiregulin (AR)
	Schwannoma-derived growth factor (SDGF)
FGF	Acidic fibroblast growth factor (aFGF)
	Basic fibrobiast growth factor (bFGF)
	INT-2, FGF-5, FGF-6, KGF, HST/KGF
Insulin-like	Insulin
	Insulin-like growth factors (somatomedins)
	Relaxin
Others	Growth hormone (GH)
	Platelet-derived growth factor (PDGF)
	Mast cell growth factor (MGS)
	Colony stimulating factors
	Ciliary neurotrophie factor (CNTF)
	Glial maturation factor
	Protease ne)án 1, II
	Sweat gland factor
	Cholinergic neuronal differentiation factor (CDF)
	Muscle-derived growth factors (MDGF)
	Striatal-derived neuronotrophic factor
	Transforming growth factor beta (TGFβ)/inhibirVactivin family
	Membrane-associated neurotransmitter stimulating factor (MANS)
	Thrombin
	Entactin
	Erythropoietin
	Neurite inducing factor
	Stem cell factor (SCF)
	Interleukin 1,3,6
	Glial-derived nexin
	Heparin-binding NF
Extracelluiar matrix/adhesion factors	Laminin
	Fibronectin
	Purpurin
	Apolipoproteins
	Gangliosides
Nonpeptide hormones	Steroid
	T3/T4

What is a growth factor ?
What is a trophic factor ?

DEFINITION

Growth factors include substances that stimulate cells to divide (hyperplasia) or increase in size (hypertrophy). Many growth factors are now known to exist

Trophic factors include those substances that have effects on cell differentiation, cell survival, expression of a specific cellular phenotype (e.g. a cell becomes an inhibitory or an excitatory neuron), cellular morphological plasticity, as well as cell hypertrophy including, for example, the induction of neurite extention (also considered a "trophic" action). Importantly, some growth factors may also act as trophic factors and viceversa and each growth/trophic factor may have a specific combination of cellular effects.

Neurotrophic factors

Neurotrophic factors are endogenous soluble proteins regulating survival, growth, morphological and synaptic* plasticity, or synthesis of proteins for differentiated functions of neurons or glial cells.
(* synaptic plasticity = regulation of the transmission activity at the level of the synapse)

List of characterized proteins exhibiting neurotrophic activities

Growth factor	References
Nerve growth factor (NGF)	Thoenen et al., 1987
	Whittemore and Sciger, 1987
	Hefti et al., 1989
Brain-derived neurotrophic factor (BDNF)	Barde et al., 1982
	Leibrock et al., 1989
Neurotrophin-3 (NT-3)	Ernfors et al., 1990
	Hohn et al., 1990
	Maisonpierre et al., 1990
	Rosenthal et al., 1990
Neurotrophin-4 (NT-4)	Hallbrook et al., 1991
Neurotrophin-5 (NT-S)	Berkerneier et al., 1991
Ciliary neurotrophic factor (CNTF)	Lin et al., 1989
	Stóckli et al., 1989
Heparin-binding neurotrophic factor (HBNF)	Kovesdi et al., 1990
Growth factors vith neurotrophic activity	
Basic fibroblast growth factor (bFGF)	Morrison et al., 1986
	Walicke, 1988
Acidic fibroblast growth factor (aFGF)	Walicke, 1988
Insulin-like growth factors (IGFs), insulin	Aizenman et al., 1986
	Baskin et al., 1987
Epidermal growth factor (EGF)	Fallon et al., 1984
	Morrison et al., 1987
Transforming growth factor cc (TGFcc)	Deryncl,, 1988
	Fallon et al., 1990
Interleukin 1	Spranger et al., 1990
Interleukin 3	Kamegai, 1990
Interleukin 6	Harna et al., 1989
Protease nexin 1 and Il	Monard, 1987
	Oltersdorf et al., 1989
	Whitson et al., 1989
Cholinergic neuronal differentiation factor	Yarnarnori et al., 1989

Which cell synthesizes the neurotrophic factors ?

Neurotrophic factors are produced by all cells of the nervous system, including neurons, glial cells, ependimal cells, blood vessels endothelial cells and cells from innervated tissues such as muscles, epidermis, etc…

How are neurotrophic factors released from cells ?

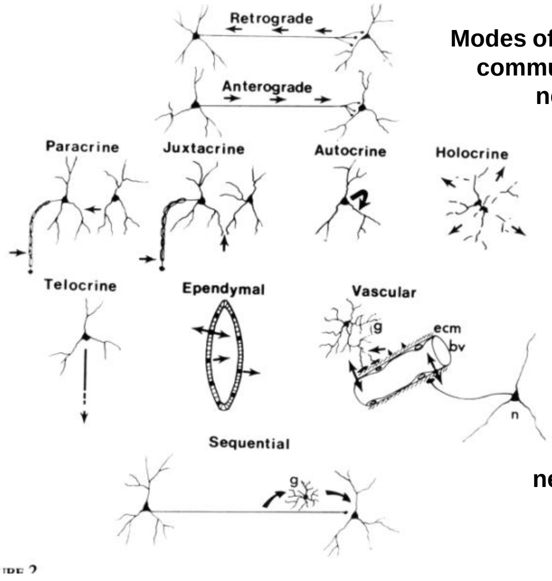


FIGURE 2

Modes of secretion and intercellular communication mechanisms of neurotrophic activity.

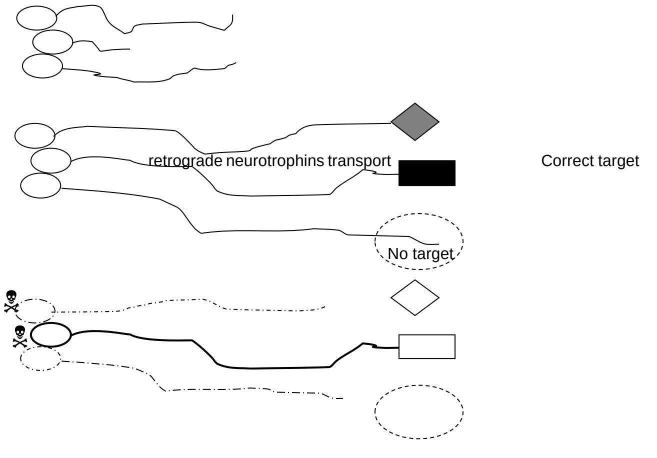
Abbreviations: g, glial; n, neuronal; bv, blood vessel; ecm, extracellular matrix.

What cellular actions have neurotrophins ?

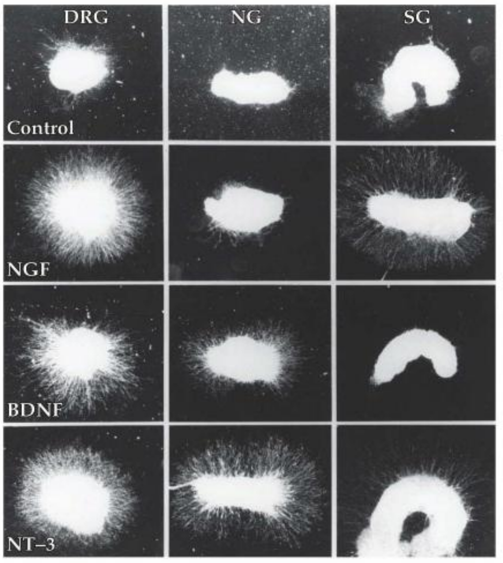
- Neurotrophins will be taken as example because they were the first neurotrophic factors to be discovered and because they have a large number of cellular effects. In particular neurotrophins have a:

- 1) Trophic action as cell survival factors
- 2) Trophic action by stimulating growth of cellular processes (axons and dendrites = neurites, i.e. they promote *neuritogenesis*)
- 3) Trophic action on the cellular phenotype
- 4) Trophic/growth action on cellular dimensions
- 5) Trophic action on morphological plasticity
- 6) Trophic action on synaptic plasticity
- 7) Trophic action on cellular differentiation

Neurotrophins were first described for their trophic role on cell survival.

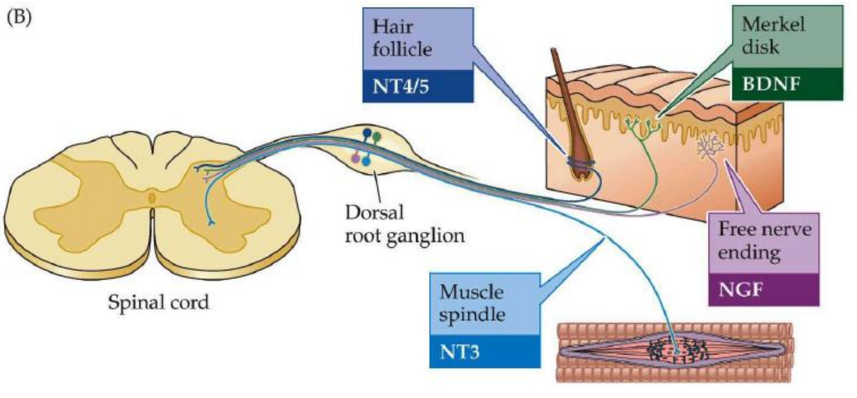


Different Neurons have Different Requirements for Neurotrophic Factors



DRG = Dorsal Root Granglion, NG = Nodose Ganglion, SG = Sympatethic ganglion

Knock-out mice have highlighted the role of neurotrophins in survival of different neuronal types



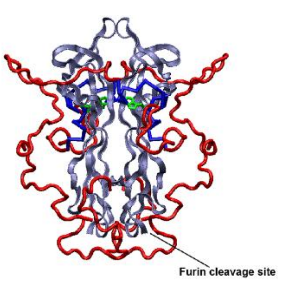
Neurotrophic Factors signalling

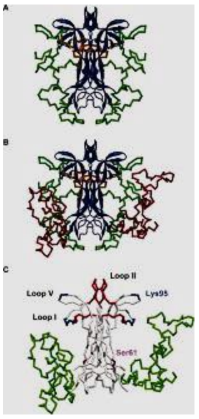
The neurotrophins and their receptors

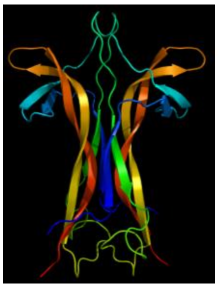
Pro-neurotrophins | Mature-neurotrophins

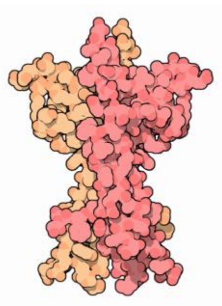
Protease

Mature-neurotrophins

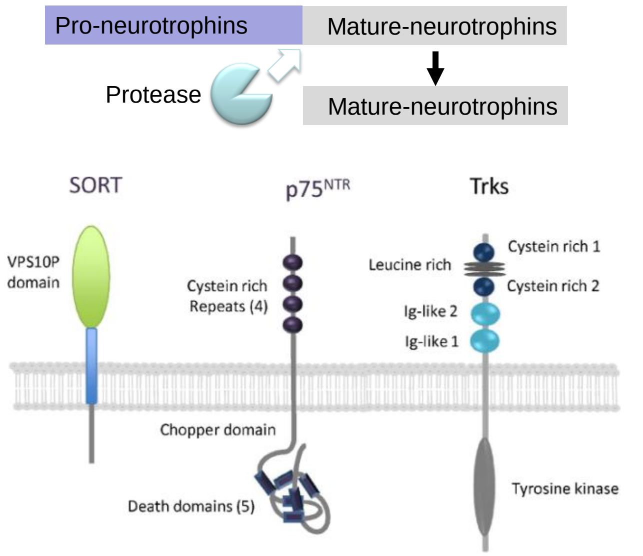




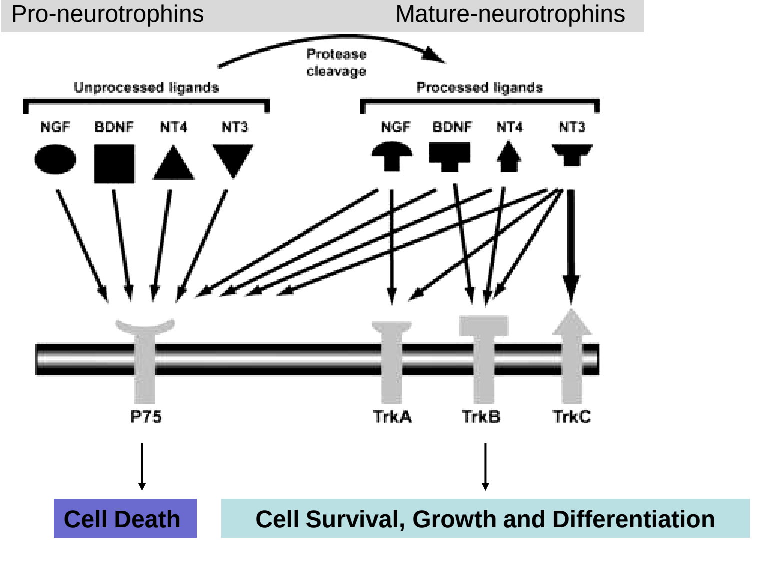




The neurotrophins and their receptors



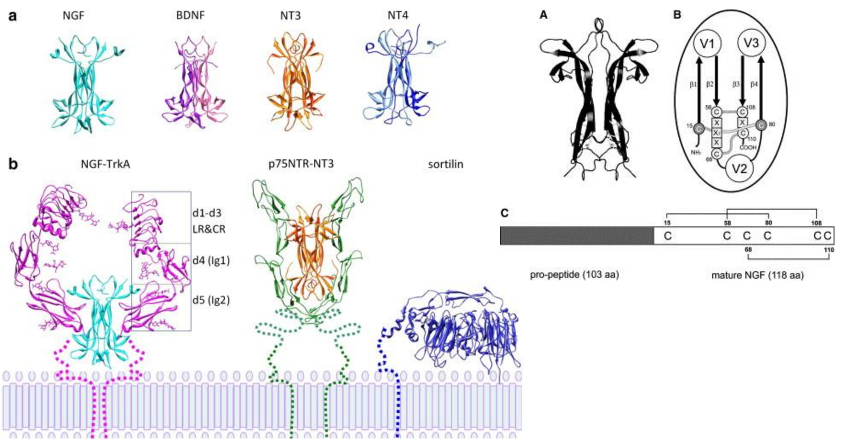
The neurotrophins and their receptors



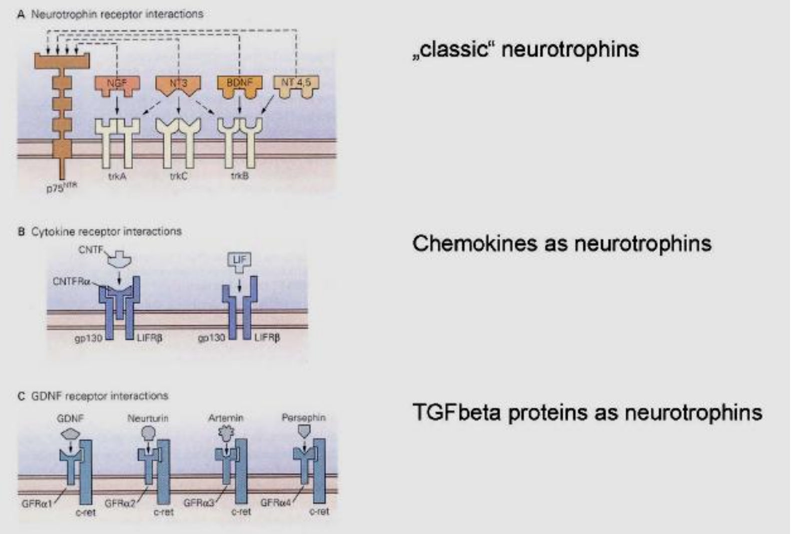
NT signalling through Trk and p75 receptors

Pro and mature-NT bind as dimers to p75- and Trk-receptors.

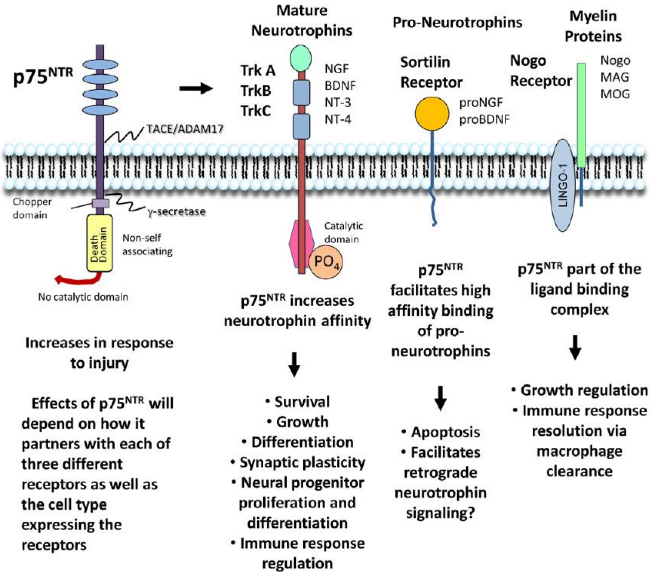
Trk receptor dimerization leads to trans-autophosphorylation and to the activation of intracellular signalling cascades mediating differentiation and survival.



The receptors of other neurotrophic factors can also dimerize

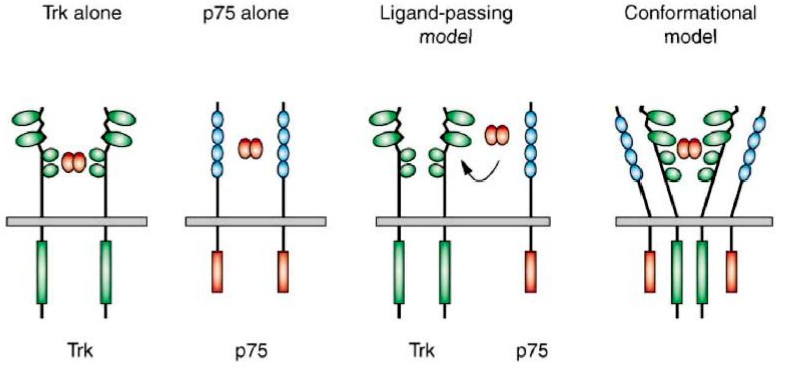


NT signalling transduction pathways and their functions



Models for Trk and p75^{NTR} interaction

The p75 receptor can bind to each NT, and also acts as a co-receptor for Trk receptors



Chao and Bothwell (2002) Neuron

Models for Trk and p75^{NTR} interaction: how were they discovered?

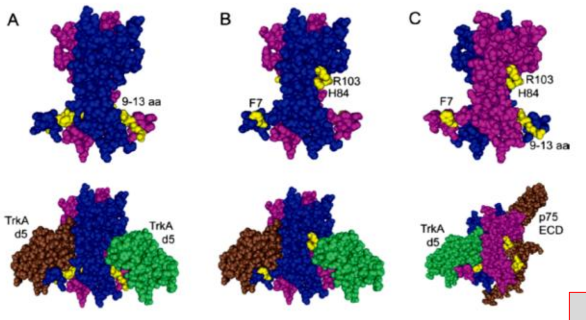


Fig. 1.
Design rationale for an NGF heteromutein capable of forming a TrkA-NGF-p75 complex but not a TrkA-NGF-TrkA complex. **A:** The 9–13 amino acids on the N-terminus of NGF are highlighted in yellow on each protomer (pink, blue) of NGF dimer (upper structure). These amino acids are deleted in the mutein Δ9/13 that is unable to bind TrkA. The lower structure shows the interaction of residues 9–13 with TrkA d5 domain (brown, green). **B:** The F7, H84, and R103 amino acids are highlighted in the NGF dimer (upper structure), and their interaction with TrkA d5 domains (brown, green) is shown in the lower structure. F7A/H84A/R103A (7-84-103) mutein is an NGF mutein binding weakly to TrkA. **C:** A dimer of NGF in which 9–13 amino acids are highlighted on one protomer (pink) and F7, H84, and R103 are highlighted on the other protomer (blue) of NGF shows that, if these highlighted residues are mutated or deleted, it would abolish TrkA binding on one side (upper structure). Therefore, a heterodimer of Δ9/13 mutein and the 7-84-103 mutein would lose TrkA binding on one side but still have the ability to form a heteroreceptor complex (p75 ECD, brown; TrkA d5, green; lower structure). PDB IDs for NGFTrkA(d5) complex and NGF-p75 complex are 1WWW and 1SG1, respectively.

Δ9/13 NGF mutein, a deletion of N-terminal residues 9–13 = **no TrkA binding or activity**

7-84-103 NGF mutein, mutated at residues F7, H84, and R103 to alanine, **has 200-fold reduced binding to TrkA and is signal selective for survival over neuritogenesis**

The two muteins form a heteromutein, which can bind TrkA on only one side at the NGF protomer–protomer interface. The heteromutein Htm1 has the ability to bind normally to p75, in the absence of TrkA homodimerization Htm1 induces cell survival at half-reduced efficiency than wt-NGF.

Mehta et al. 2012

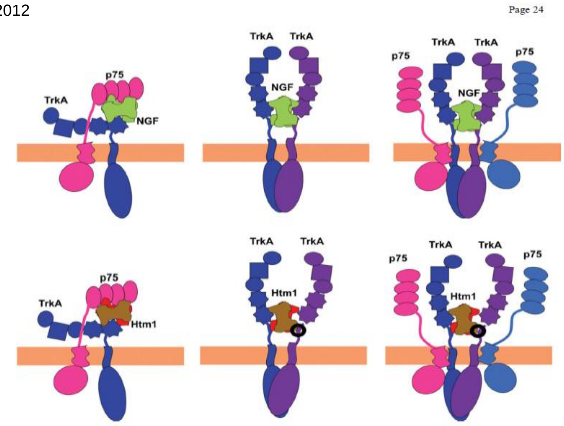
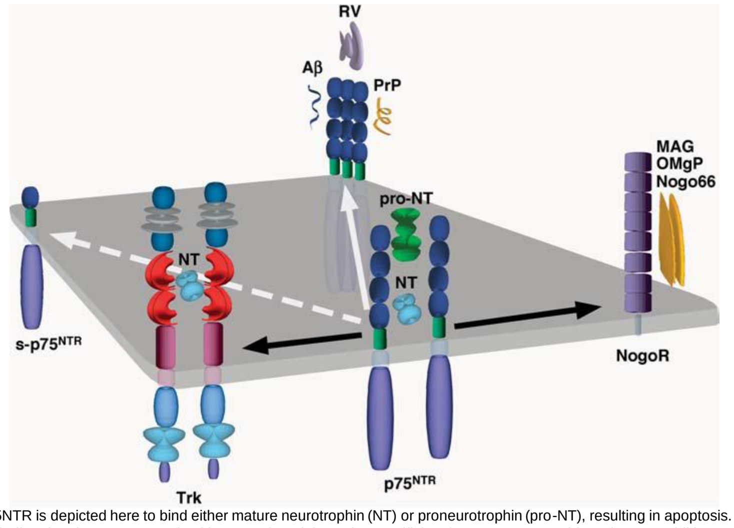


Fig. 10.
Model of ligand passing and TrkA presentation for NGF binding. Upper row: NGF. Lower row: Htm1. Left column: Putative complex of NGF or Htm1 with TrkA and p75 that represents an intermediate in the ligand-passing model. Note the antiparallel orientation of the two receptors resulting from the binding mode of each ligand relative to the membrane (Barker, 2007). The formation of this complex would be preceded by the binding of NGF or Htm1 to the p75 receptor. Middle column: NGF or Htm1 binding to a homodimeric TrkA receptor. The binding pathway for NGF or Htm1 to form this complex can occur by directly binding to TrkA or via the two-step ligand-passing process, which involves formation of the complex shown in the left column. Right column: Putative heteroreceptor complex of undefined stoichiometry in the Trk presentation model. This complex represents the TrkA presentation model. A pre-existing TrkA-p75 heteroreceptor complex can bind to NGF with greater affinity than TrkA alone. In the lower middle and lower right models, the area in the circle designates additional modes of interaction among Htm1 loop L-I, L-II, and L-IV residues and the extracellular, juxtamembrane region of TrkA to explain the cellular and signaling data (see Discussion).

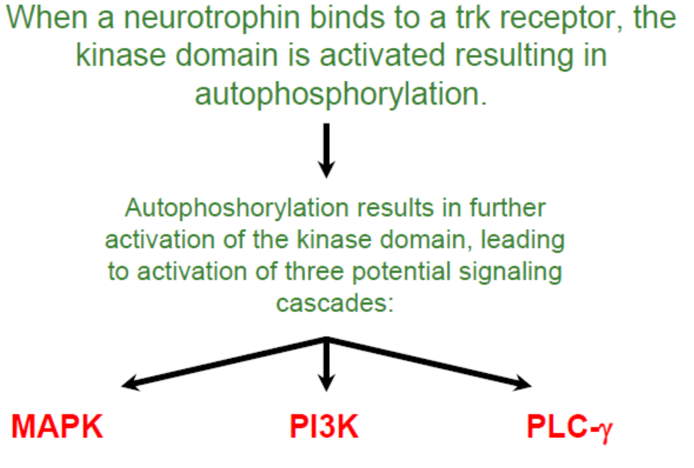
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3674885/pdf/nihms-463239.pdf

Distinct interactions of p75NTR with pro-, mature neurotrophins and other ligands.



Dimeric P75NTR is depicted here to bind either mature neurotrophin (NT) or proneurotrophin (pro-NT), resulting in apoptosis. It also interacts with dimeric Trk receptor tyrosine kinase and modulates the specificity of mature neurotrophin actions. In addition, p75NTR interacts with the Nogo receptor (NogoR) for axonal growth inhibition mediated by MAG, OMgp and Nogo66. The binding of rabies virus (RV), prion protein (PrP) or a *b-amyloid* to p75NTR appears to induce receptor trimerization. Finally, the potential interaction between full length p75NTR and its naturally occurring short isoform s-p75NTR is denoted by dashed arrow. The signaling mechanisms and biological actions of p75NTR activation were recently reviewed [20–22].

Trk receptor signaling



NT signalling transduction pathways mediated by trk receptors

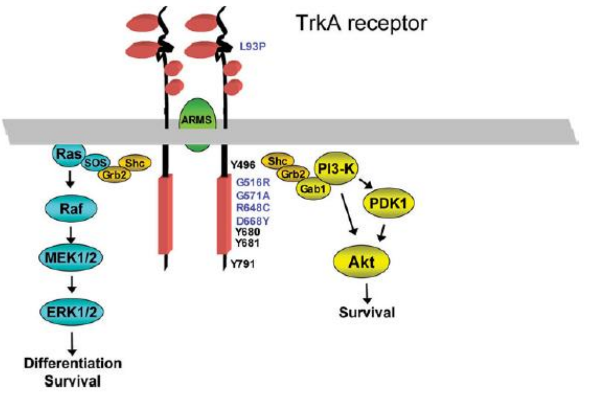
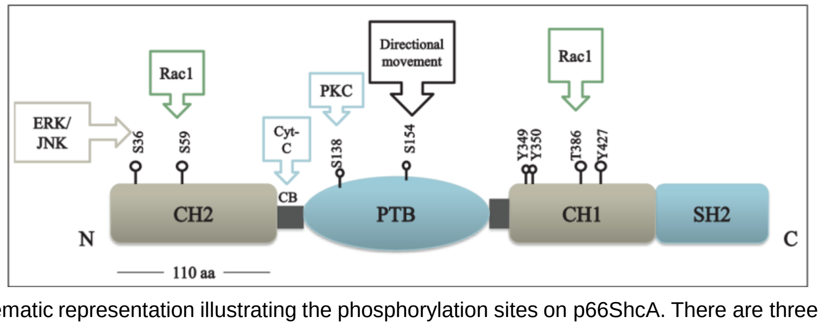


Figure 1 **TrkA receptors mediate differentiation and survival signalling through ERK, PI3K and PLC-γ pathways**
TrkA receptors recruit and increase the phosphorylation of PLC-γ and Shc, which leads to activation of PI3K and ERK. Highlighted residues (blue) are human mutations in TrkA that are associated with patients suffering from congenital insensitivity to pain [33–35]. Grb2, growth factor receptor-bound protein 2; Gab1, Grb2-associated binder-1; PDK1, phosphoinositide-dependent kinase 1; SH2B, Src homology 2-B; SOS, son of sevenless.

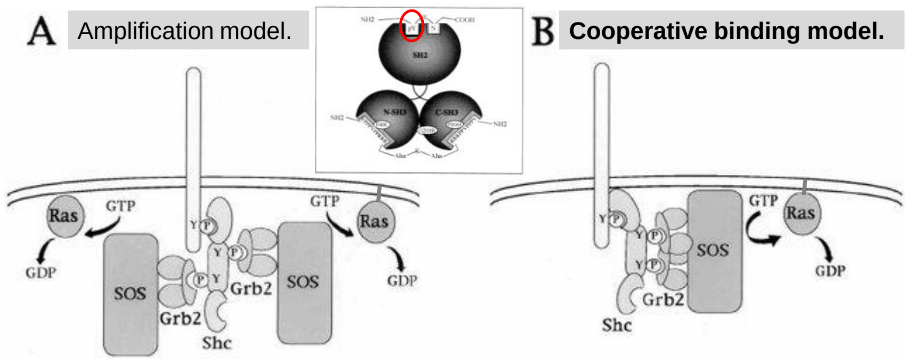
Shc is a multidomain protein able to interact with multiple partners



Schematic representation illustrating the phosphorylation sites on p66ShcA. There are three tyrosine phosphorylation sites and a threonine phosphorylation residue in the CH1 domain. There is one serine phosphorylation site on (Serine 138) the PTB domain as well as two serine phosphorylation sites in the amino terminal CH2 domain. p66ShcA has a unique cytochrome c binding region (CB). Ser36, Ser59, Ser138 and Thr386 are involved in the oxidative stress response, whereas S154 has a role in the directional movement of pancreatic cells. The phosphorylation of tyrosine residues in the CH1 domain are involved in MAPK activation (Adapted from Rajendran et al. 2010)

Published in Journal of molecular signaling 2017
Insights into the Shc Family of Adaptor Proteins
Samrein B. M. Ahmed, S. Prigent

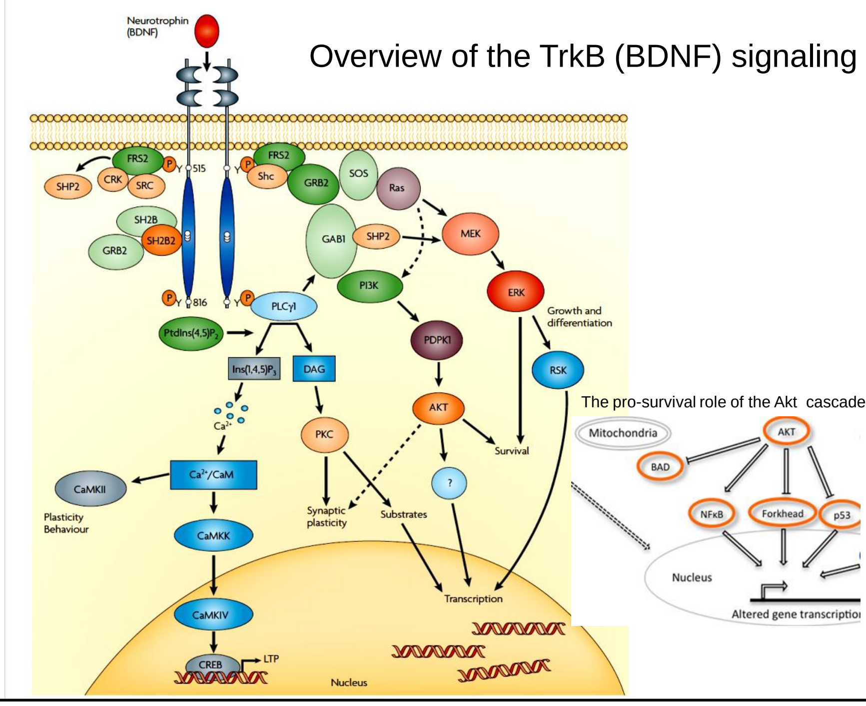
Two models describing Shc-Grb2-SOS interactions.



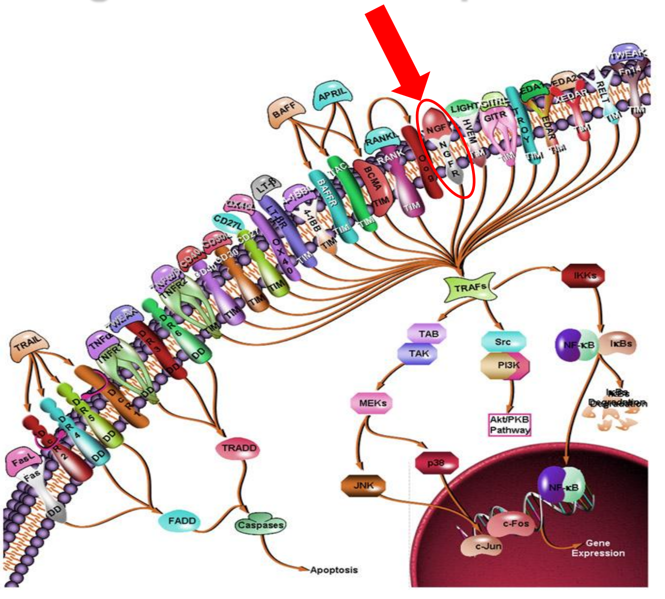
(A) Amplification model. The phosphorylation of two Grb2 binding sites on Shc may allow the recruitment of two SOS molecules, perhaps amplifying the signal and leading to more Ras activation.

B) **Cooperative binding model.** Shc may bind two Grb2 molecules that in turn bind to a single SOS molecule. The multiple protein-protein interactions afforded by such a four-way Shc-Grb2 2 -SOS complex might result in more stable binding than would be provided by the Shc-Grb2-SOS complex depicted in panel A and could explain the enhanced association between Grb2 and SOS that is observed upon BCR stimulation of cells. The identity of the transmembrane protein shown anchoring Shc at the membrane is unknown.

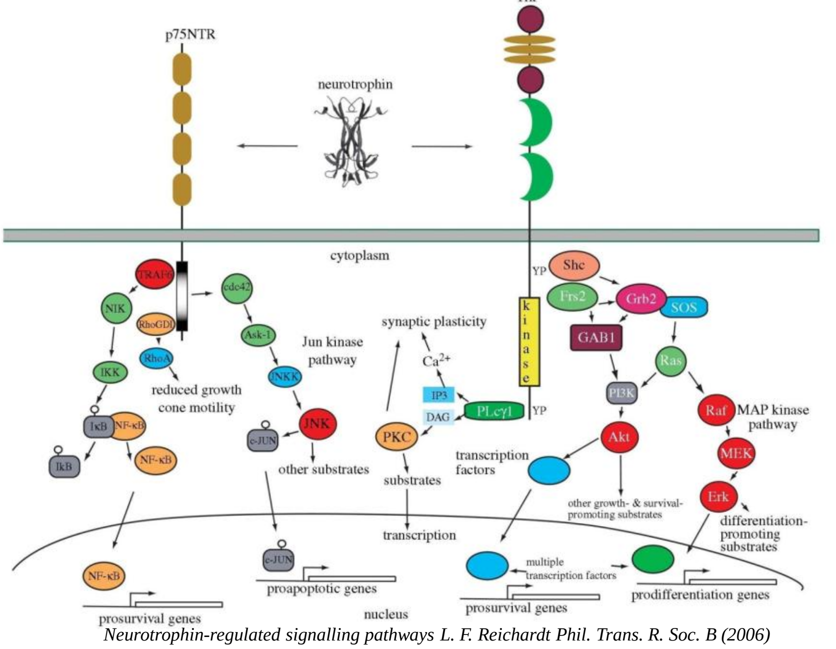
Overview of the TrkB (BDNF) signaling



p75 belongs to the TNF receptors family

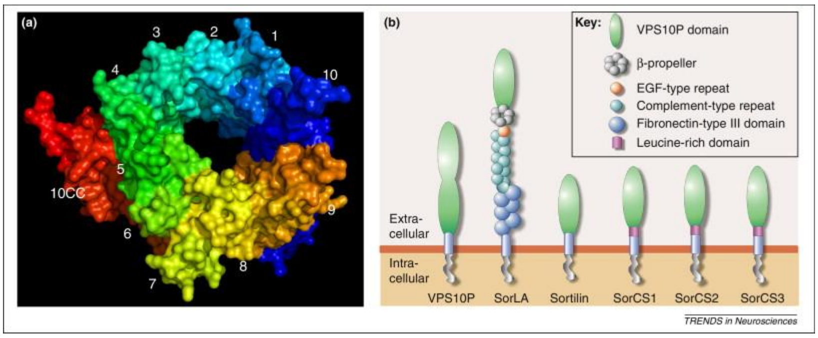


A comparison of p75 and Trk signaling



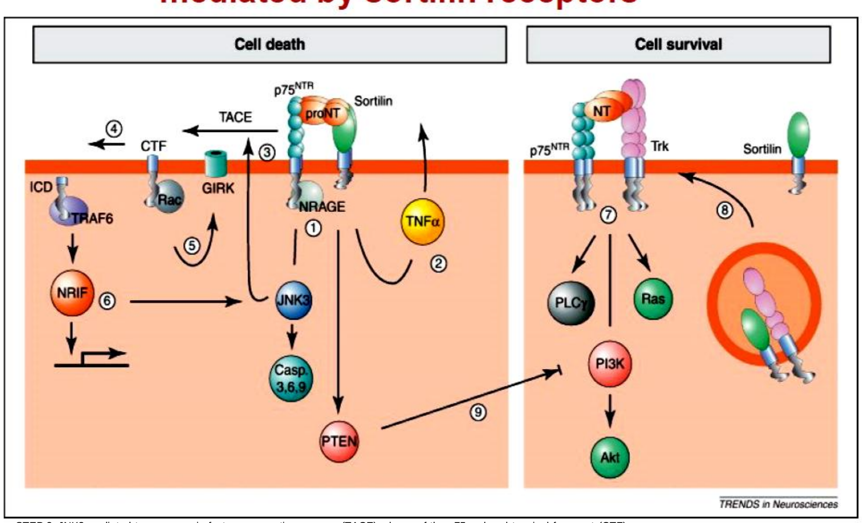
Neurotrophin-regulated signalling pathways L. F. Reichardt Phil. Trans. R. Soc. B (2006)

NT signalling transduction pathways mediated by sortilin receptors



The vacuolar protein sorting 10 protein (VPS10P) domain is a 700-amino-acid module that was first recognized in the *Saccharomyces cerevisiae* protein VPS10P, a sorting receptor that directs the trafficking of lysosomal enzymes from the Golgi to the vacuole

NT signalling transduction pathways mediated by sortilin receptors



STEP 3: JNK3-mediated tumor necrosis factor-a-converting enzyme (TACE) release of the p75 carboxyl terminal fragment (CTF)

STEP 4: gamma-secretase-mediated release of the soluble intracellular (ICD)

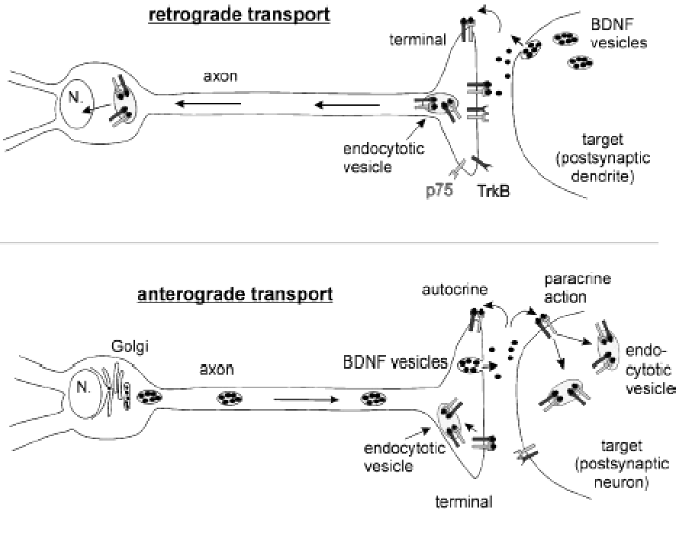
STEP 5: the CTF activates the G-protein-coupled inwardly rectifying potassium channel (GIRK) through Rac

STEP 6: The ICD forms a complex with neurotrophin receptor interacting factor (NRIF) and tumor necrosis factor receptor associated factor 6 (TRAF6) to promote ubiquitination of NRIF and nuclear translocation

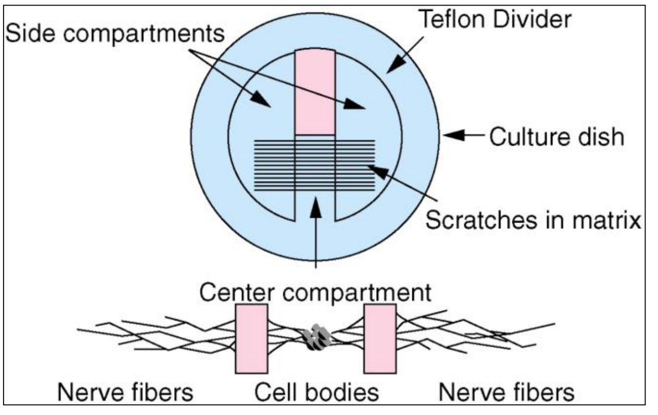
A spatial view of NT signalling pathways

Three are the neurotrophin signalling spatial ways in a neuron:
the signalling endosome
the signalling within the axons
the signalling at the cell body

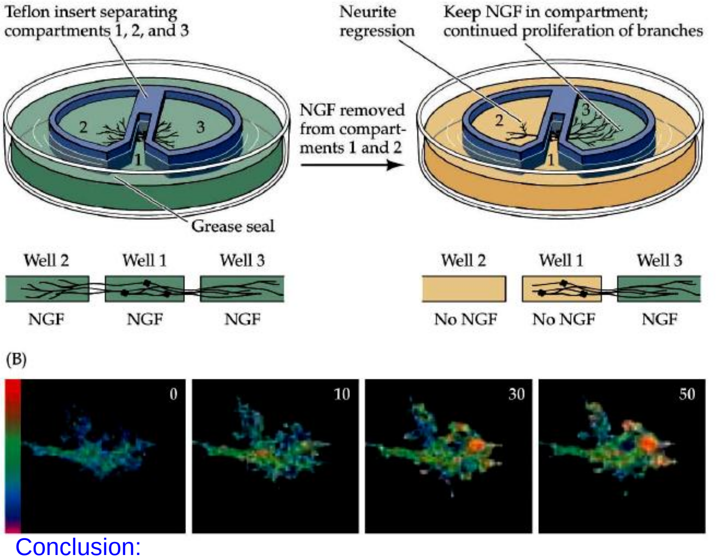
BDNF can be secreted from both axons and dendrites



The experiment of the Campenot's chamber



Neurotrophins influence neurite growth by local effects

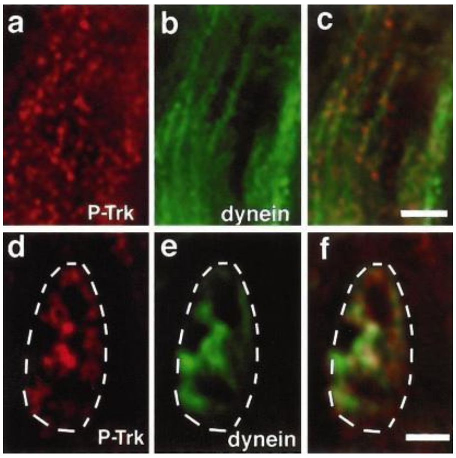


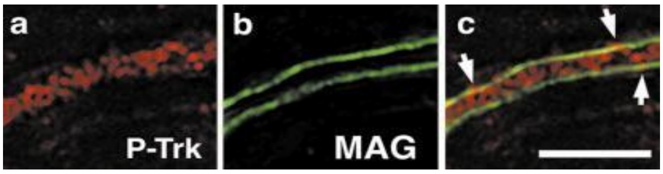
Conclusion:
NGF must be present at growth cones, not cell bodies.
NGF must be transported to the cell body by retrograde transport.

Co-localization of Dynein with p-Trk

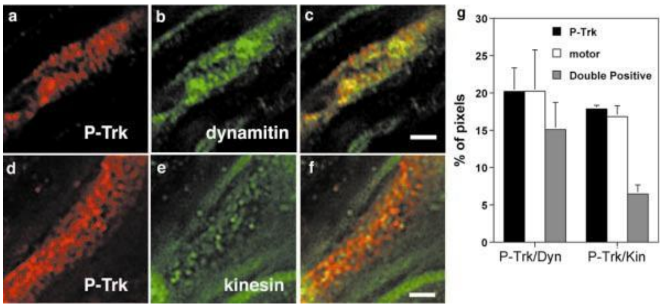
Longitudinal Section

Deconvoluted Cross Section – dashed line is the axon membrane

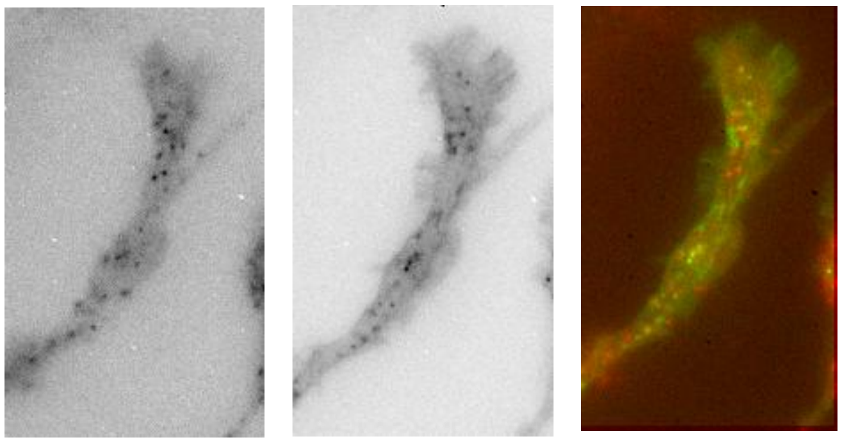




Staining of individual axons of the sciatic nerve
P-Trk (activated Trk) MAG myelin associated glycoprotein)

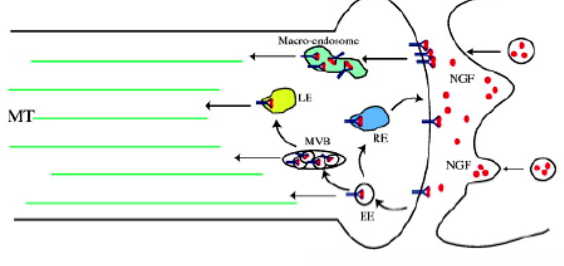


Correlation of Trk with Dynein/ Dynactin not with Kinesin in sciatic nerves
Dynamitin is the p50 subunit of dynactin (a dynein associated protein)



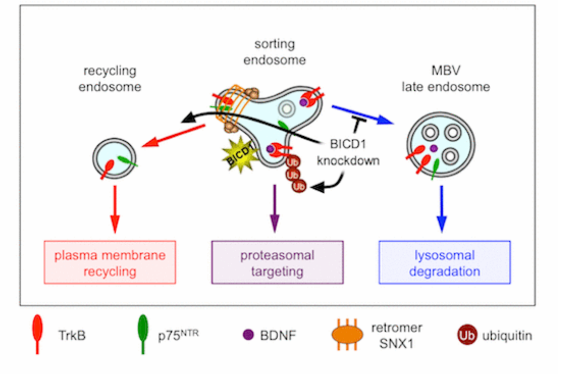
Dynein TrkB Both

B: Different types of NGF signaling endosomes

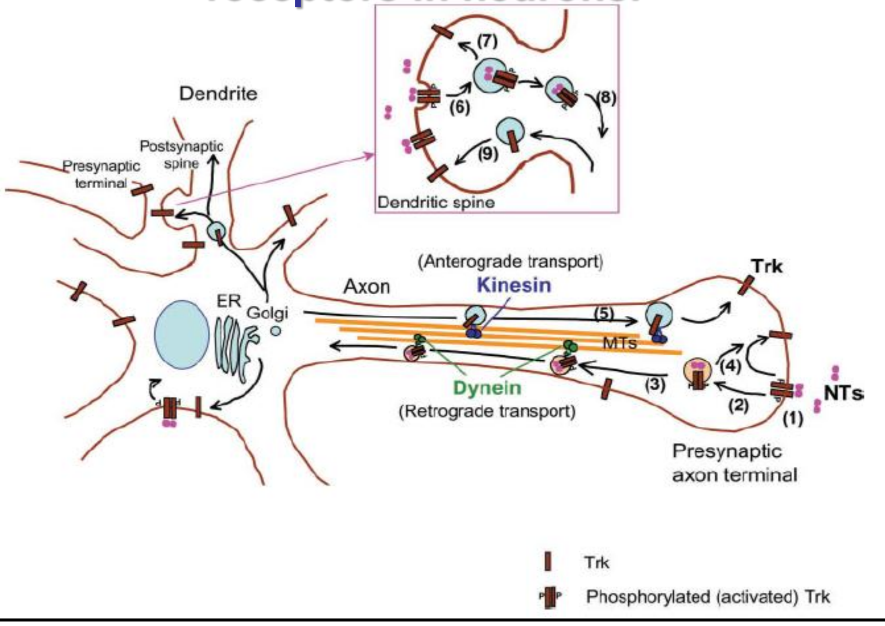


EE, early endosomes
RE, recycling endosomes
LE, late endosomes
MVB, multivesicular bodies

J Proteomics 72, 46-55 (2009)

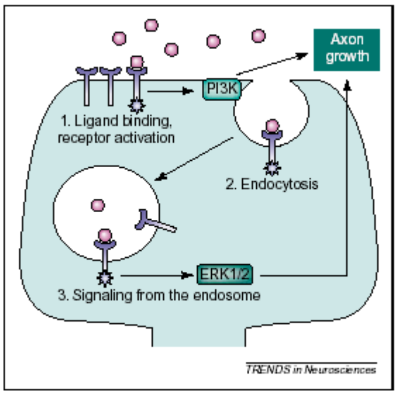


Trafficking and distribution of Trk receptors in neurons.

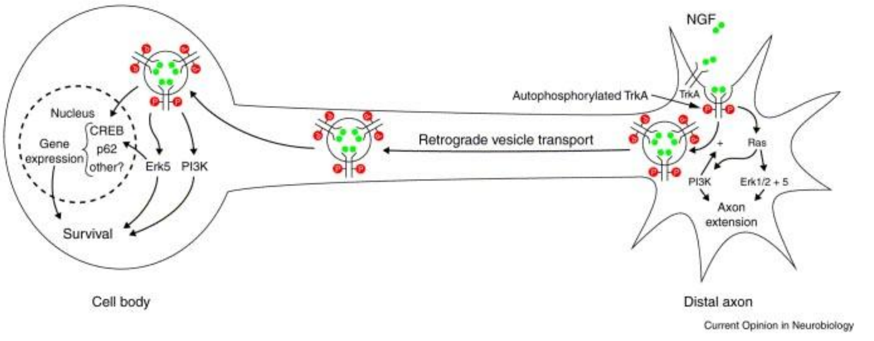


Activated Trk can signal locally and retrogradely using different signalling pathways

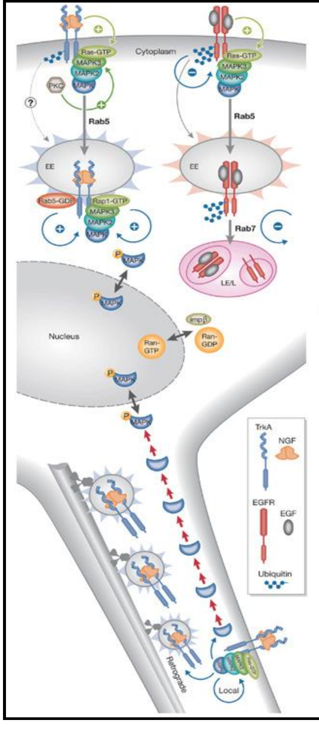
NT (purple spheres) binds to Trks (blue) at the nerve terminal. Location of the PI3 kinase cascade promotes both axon outgrowth and receptor endocytosis. Activated endosomal Trks within the terminal and axon might be primarily responsible for activating the erk172 pathway (from[Heerssen, 2002 #93]).



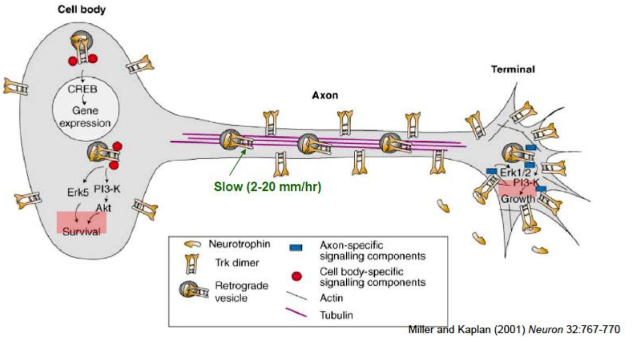
Transport of vesicle with NGF bound to TrkA (nerve growth factor receptor)



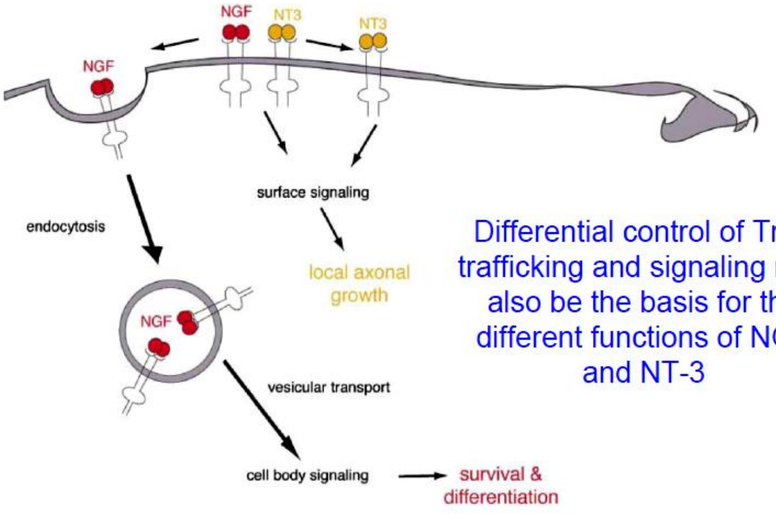
From Rosalind Segal



Activated Trk can signal locally and retrogradely using different signalling pathways



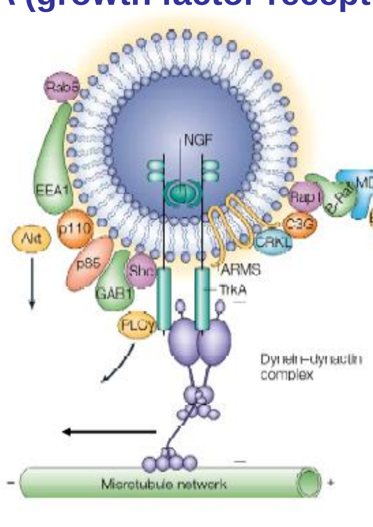
Miller and Kaplan (2001) *Neuron* 32:767-770



Differential control of TrkA trafficking and signaling may also be the basis for the different functions of NGF and NT-3

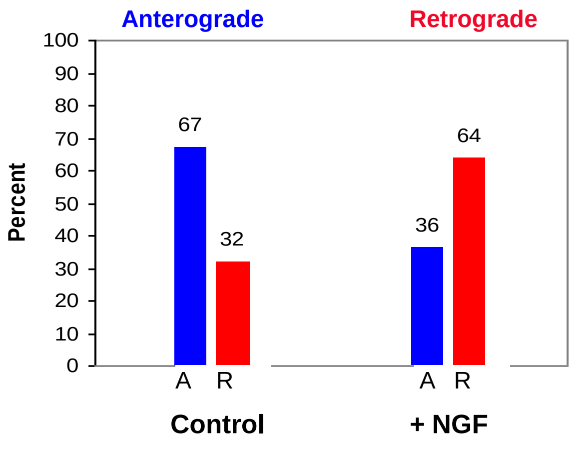
Kuruvilla et al. (2004) *Cell* 118: 243-255

Transport of a vesicle with NGF bound to TrkA (growth factor receptor)



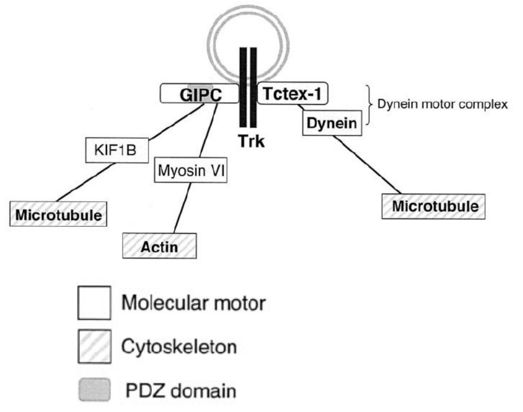
Nature Reviews Neuroscience 6, 615-625 (2005)

PC12 System (GFP-Dynein)
Regulation of Dynein Direction by NGF

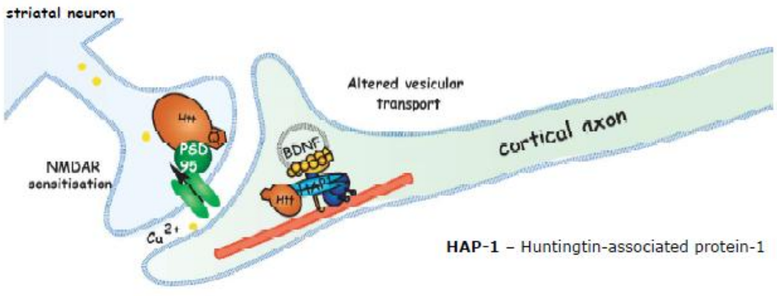


Model linking Trk-bearing vesicles to motors.

GIPC and Tctex-1 are Trk-interacting proteins that may be involved in Trk trafficking. The interaction of these proteins with Trk has been found by yeast two-hybrid system and coimmunoprecipitation (Lou et al., 2001; Yano et al., 2001). GIPC: a PDZ domain-containing protein; Tctex-1: a dynein light chain subunit. KIF1B and myosin VI were found to bind to GIPC by yeast two-hybrid screen (Bunn et al., 1999). A functional ternary complex of Trk-GIPC-KIF1B (or myosin VI) remains to be shown. The Trk-Tctex-1-dynein motor complex was detected by immunoprecipitation from brain lysate (Yano et al., 2001).



CHANGES IN THE TRANSPORT OF BDNF IN HUNTINGTON'S DISEASE

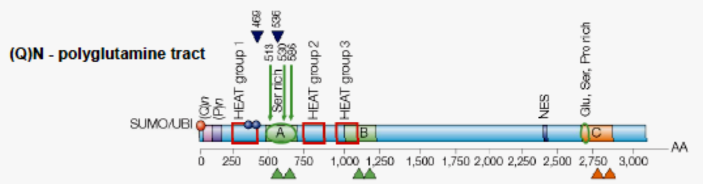


HAP-1 – Huntingtin-associated protein-1

Cell. Mol. Life Sci 63, 1642-2660 (2006)

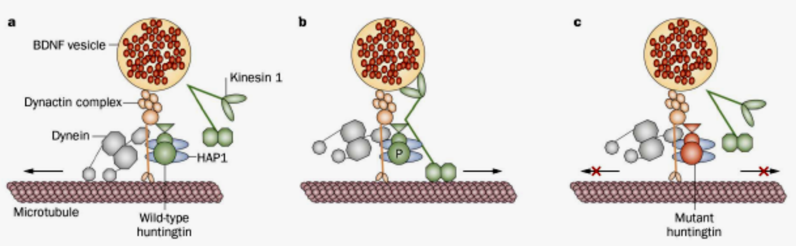
CHANGES IN THE TRANSPORT OF BDNF IN HUNTINGTON'S DISEASE

(Q)N - polyglutamine tract



Schematic diagram of huntingtin amino acid sequence.

Nature Rev Neurosci 6, 919-930 [2005]



Nature Rev Neurol 5, 311-322 [2009]