National Research Council of Italy

Nerve Growth Factor: the physiology of a many-faced protein

Laboratory of Integrative NeuroPharmacology
Dott Luigi Manni

NGF: a many-faced protein

Once upon a time…

One upon a time…	the Nobel experiments
	Neurotrophic Factors
Biology of NGF	cells and tissues
	nervous system
NGF gene	regulation
	transcription
proNGF protein	conformational structure
	structure
	cleavage sites
	trafficking dynamics
	maturation
	glycosilation
	release and extracellular processing
proNGF/mNGF receptors	challenge
	dissociation constant
	trkA/p75 interaction
	signaling
NGF retrograde transport	signaling endosome hypotesis
	proNGF?
mNGF/proNGF ratio	

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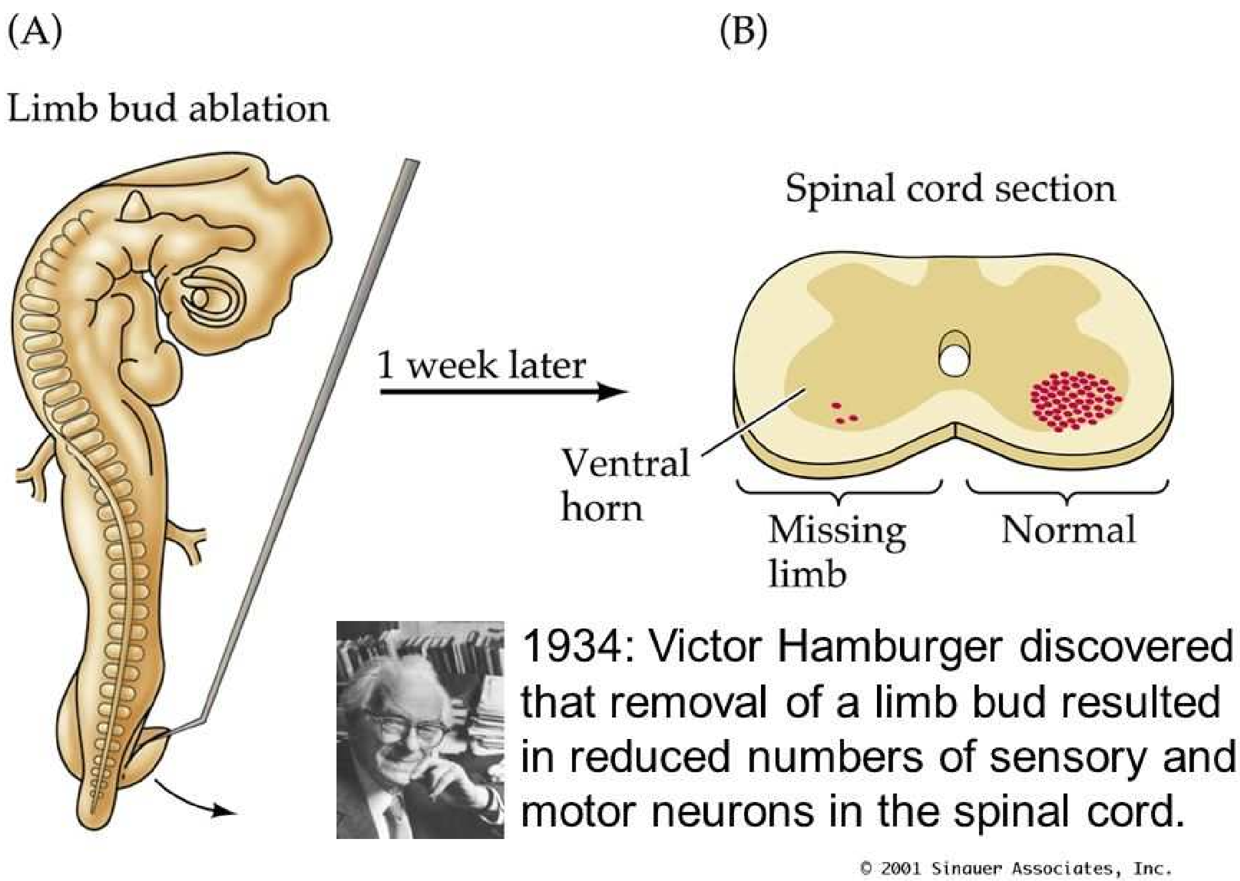
Once upon a time...



Wrong Hypothesis

Hamburger hypothesized that the targets of innervating neurons (the limb bud) provide signals that drive the differentiation of spinal cells into neuronal phenotype.

Once upon a time…

In 1942, Levi-Montalcini and Levi proposed that target derived signals maintain survival of differentiating neurons. In 1949, Hamburger and Levi-Montalcini repeated the limb bud experiments and found that their results supported the neurotrophic hypothesis.

LEVI-MONTALCINI R, HAMBURGER *V. J Exp Zool. 1951 Mar;116(2):321-61.*

COHEN S, LEVI-MONTALCINI R. *Cancer Res. 1957 Jan;17(1):15-2.*

LEVI-MONTALCINI R, COHEN S. *Ann N Y Acad Sci. 1960 Mar 29;85:324-41.*

Once upon a time: the Nobel experiments

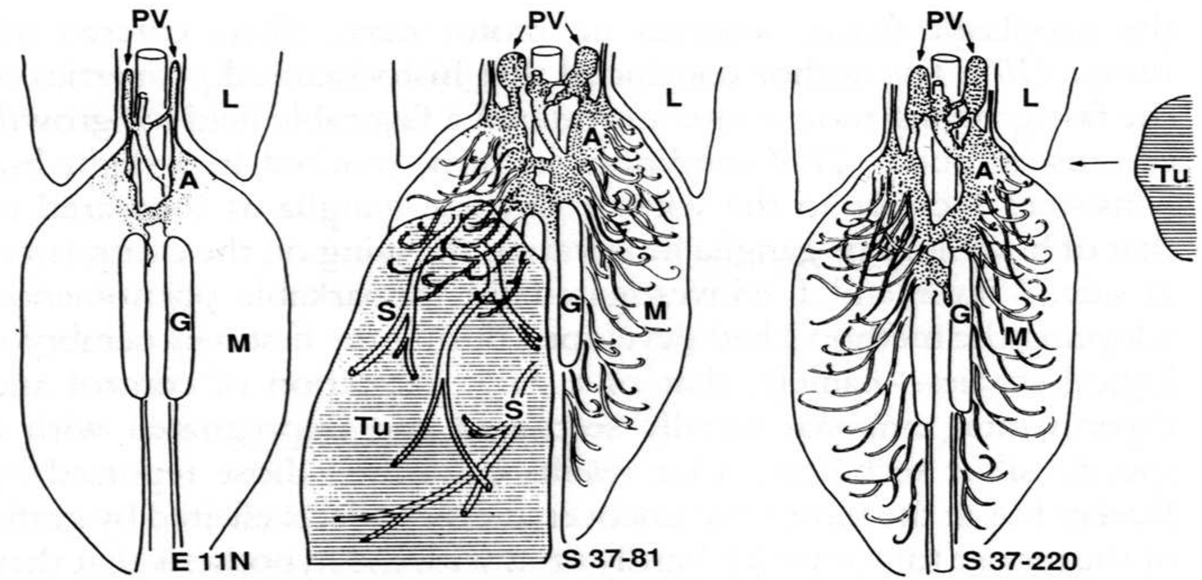


Fig. 2. Semidiagrammatic reconstruction of a normal 11-day chick embryo (E 11N), of an 11-day embryo carrying an intra-embryonic transplant of mouse sarcoma (S 37-81), and of an 11-day embryo with a transplant of sarcoma 37 on the chorioallantoic membrane (S 37-220). Note the hyperplastic growth of the prevertebral ganglia in embryos carrying tumor transplants. Visceral nerve fibers from these ganglia invade the nearby mesonephroi. The abbreviations are A, adrenal; G, gonad; L, lung; M, mesonephros; PV, prevertebral ganglia; S, sensory nerves; and Tu, tumor [from (*12*)].

Once upon a time: the Nobel experiments

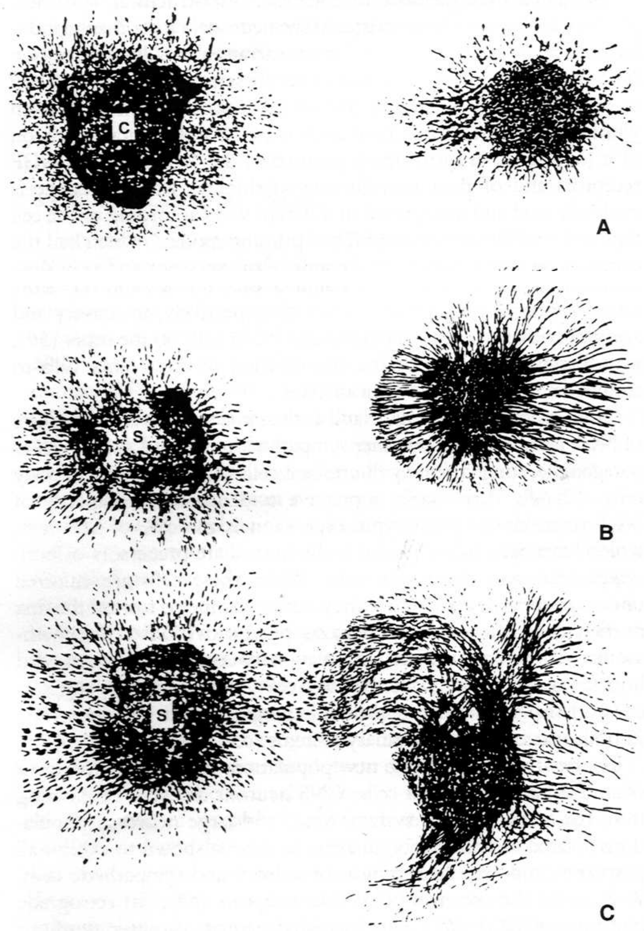
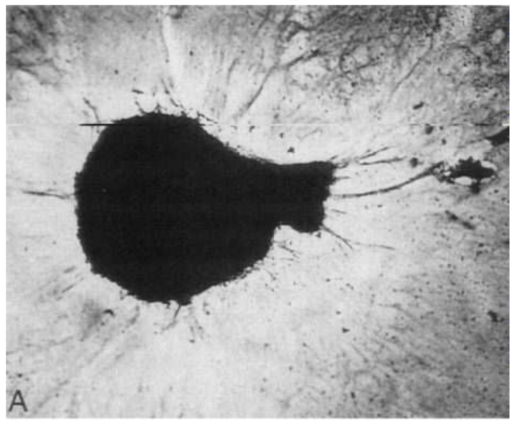
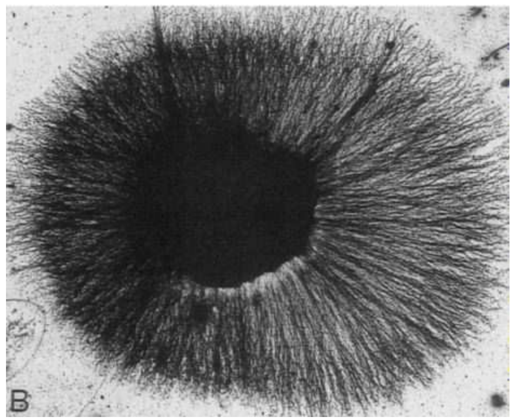
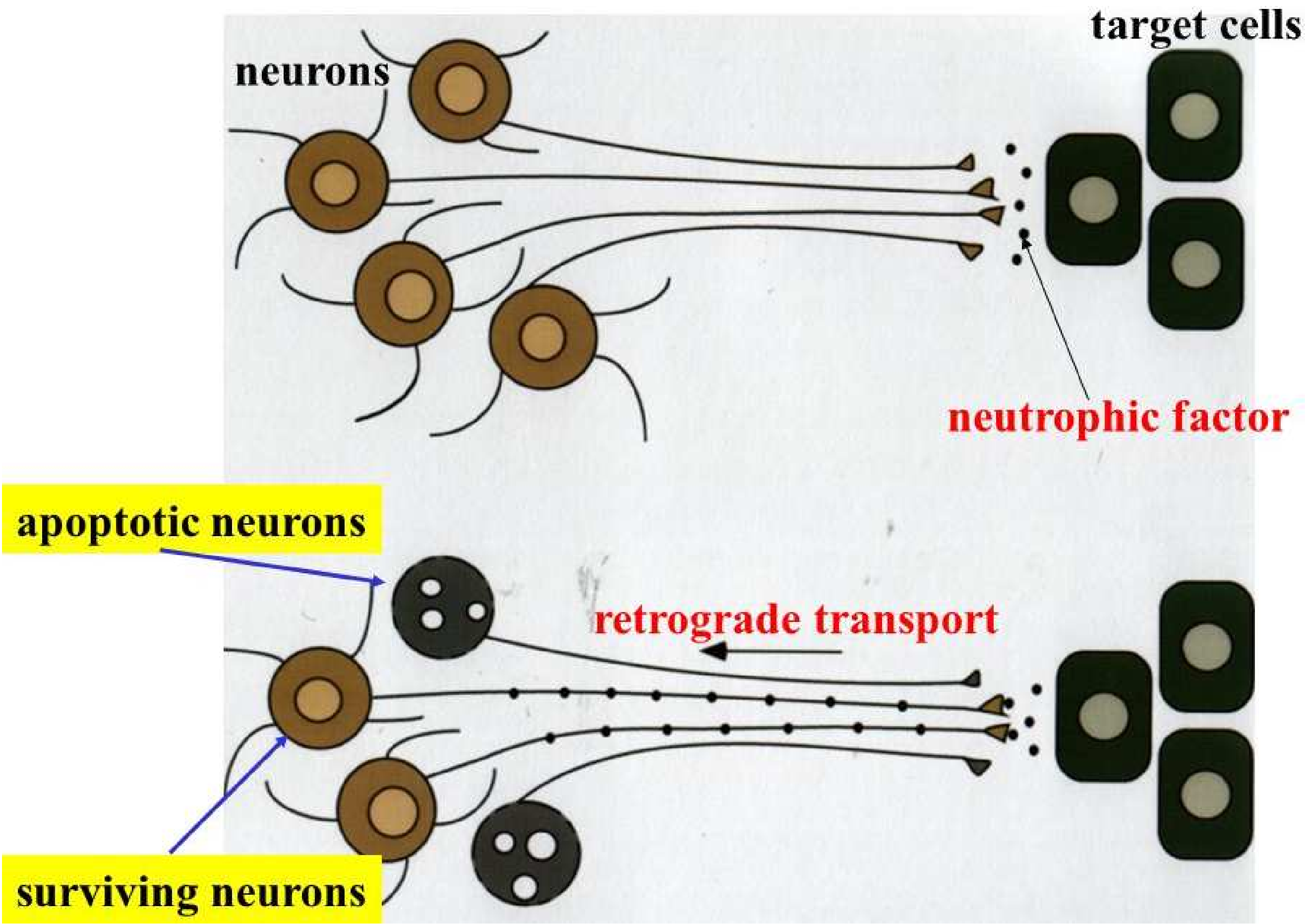


Fig. 3. Drawings illustrating the in vitro "halo" effect on 8-day chick embryo sensory ganglia cultured in the presence of fragments of mouse sarcoma 180 for 24 hours (**B**) or 48 hours (**C**). In (**A**), the ganglion, which faces a fragment of chick embryonic tissue (c) shows fibroblasts but few nerve fibers. In (B) and (C), the ganglia, facing fragments of sarcoma 180 (s) show the typical "halo" effect elicited by the growth factor released from the sarcoma. Note in (C) the first evidence of a neurotropic effect of the growth factor.





Once upon a time: neurotrophic factors



Once upon a time: neurotrophic factors

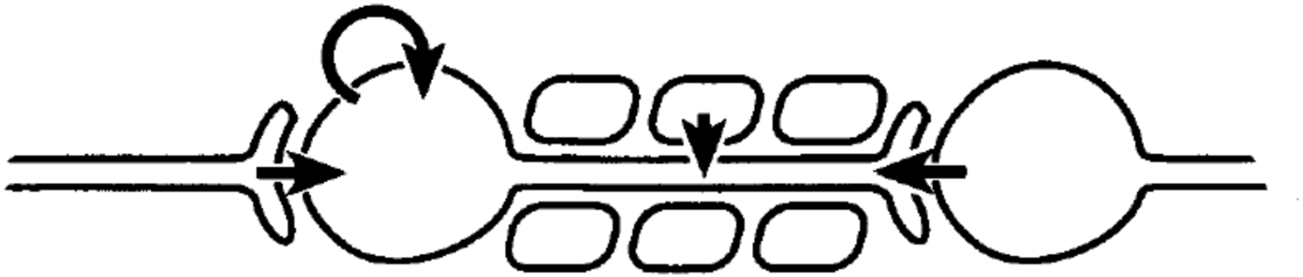
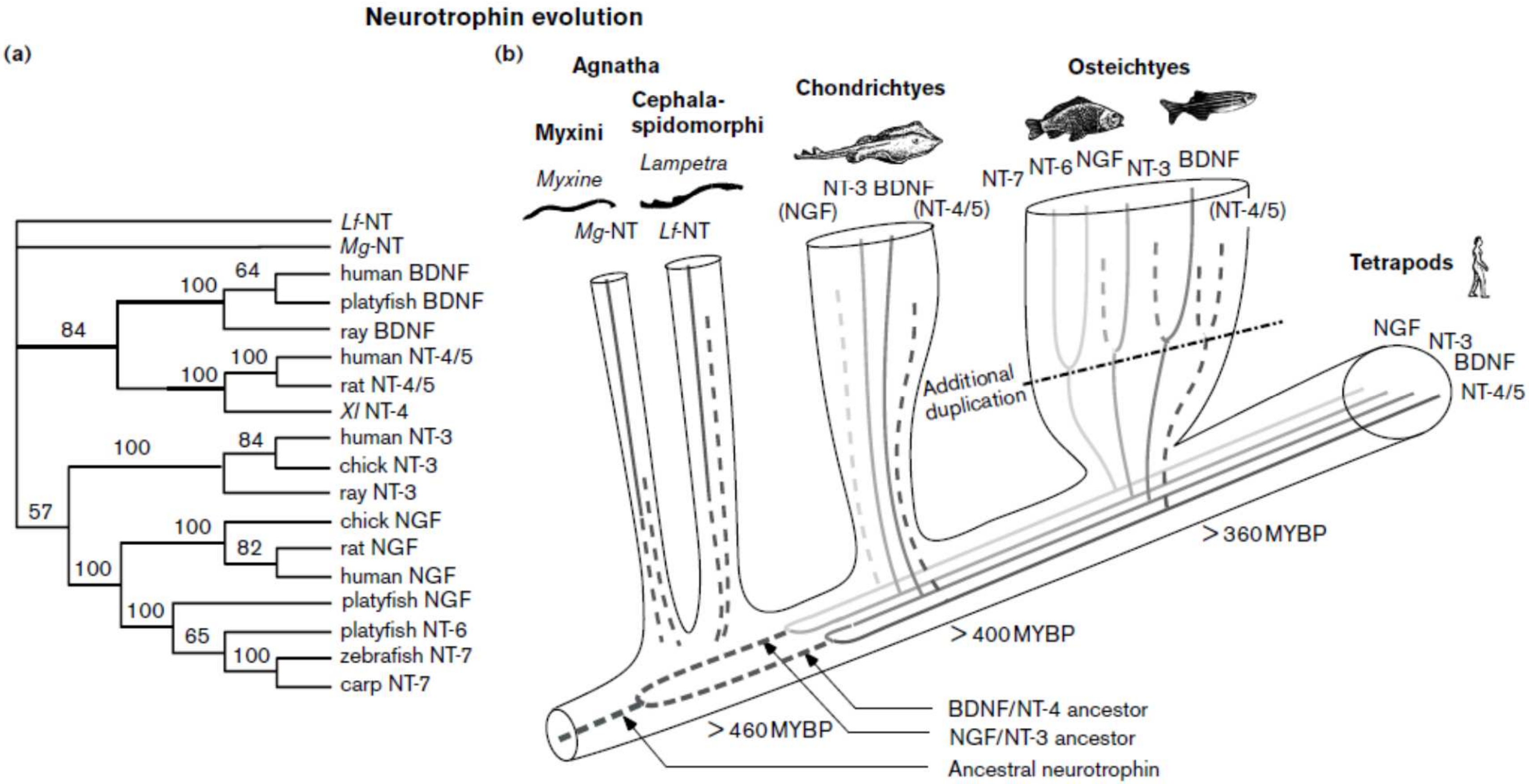


Figure 2. Schematic representation of possible sources for trophic support. The *center* neuron is drawn as member of a neuronal chain, with glial cells ensheathing its axon. The neuron might obtain trophic substances via anterograde transport from the afferent neuron, by means of an autocrine loop, from ensheathing glia cells, or by retrograde axonal transport from the neuron it innervates (classic notion). Trophic influence is shown by *arrows*.

Korsching S. 1993. The neurotrophic factor concept: a reexamination. J Neurosci 13:2739-48.

Once upon a time: neurotrophins



Hallbook, F., *Evolution of the vertebrate neurotrophin and Trk receptor gene families. Curr Opin Neurobiol, 1999.* ***9(5): p. 616-21.***

Biology of NGF

- One upon a time…
  - the Nobel experiments
  - Neurotrophic Factors
- Biology of NGF
  - cells and tissues
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- NGF gene
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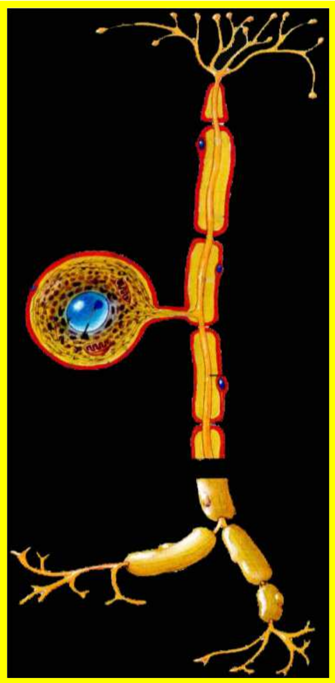
Biology of NGF

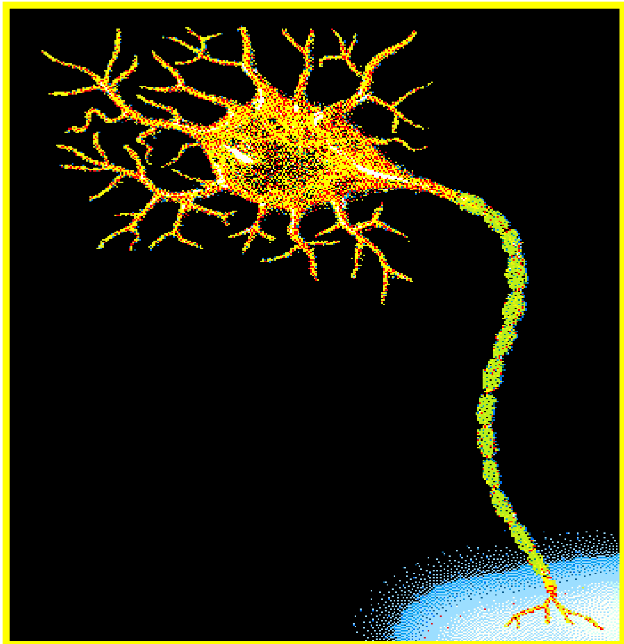
Durante lo sviluppo

•proliferazione dei precursori neuronali
•sopravvivenza
•differenziamento

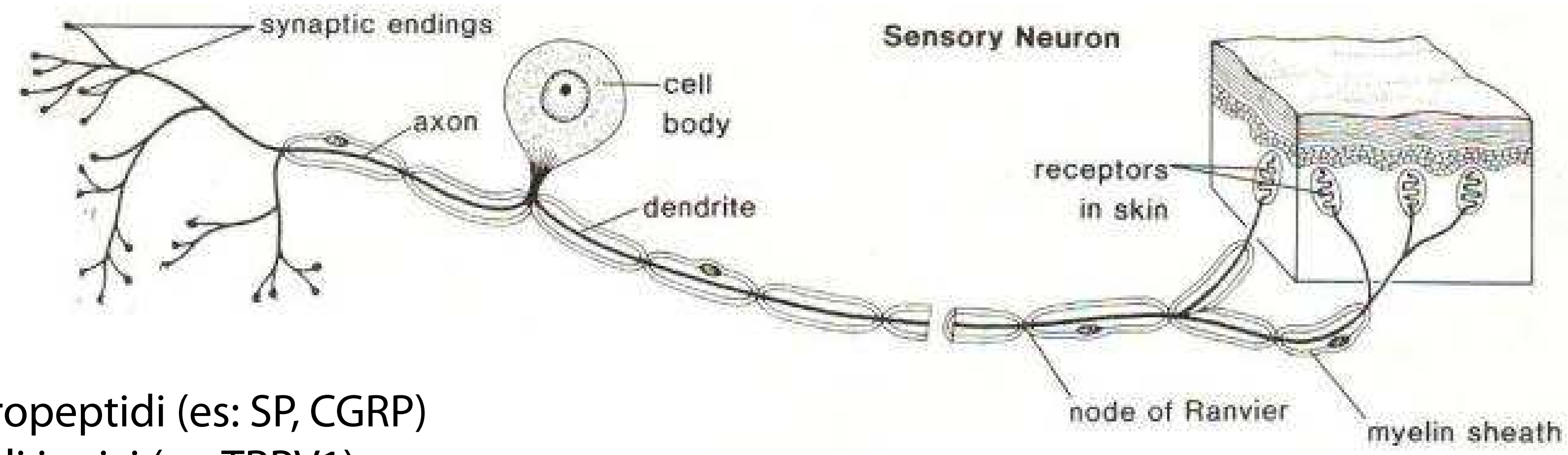


Durante la vita adulta

•biosintesi dei neurotrasmettitori e neuropeptidi
•plasticità sinaptica
•innervazione organi bersaglio
•organizzazione strutturale del neurone

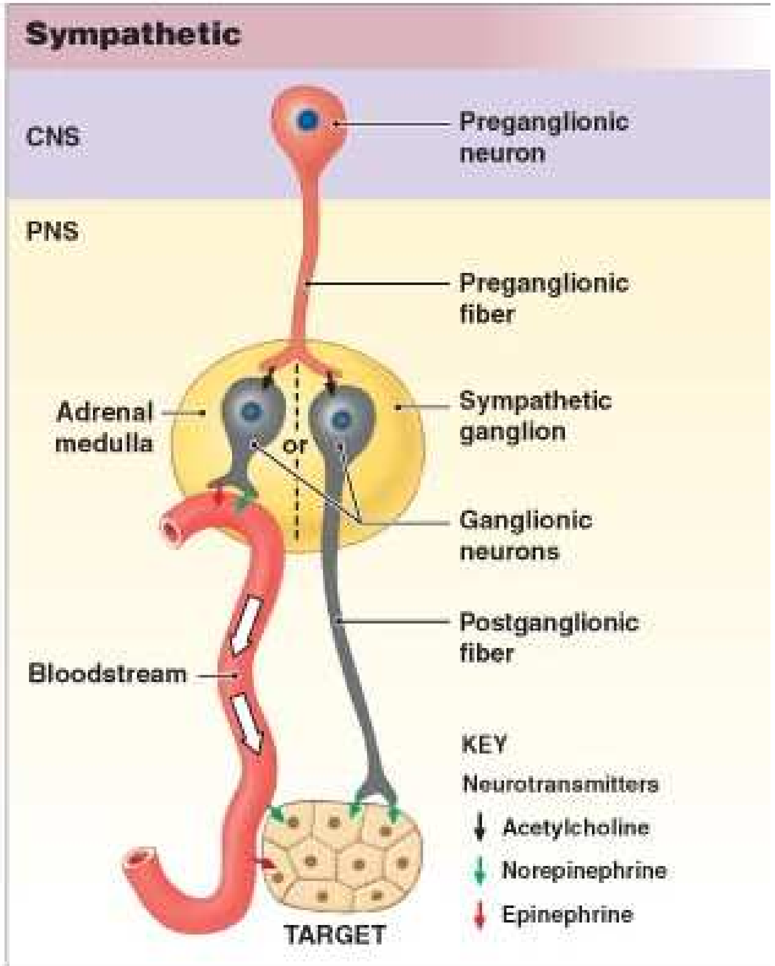


Biology of NGF



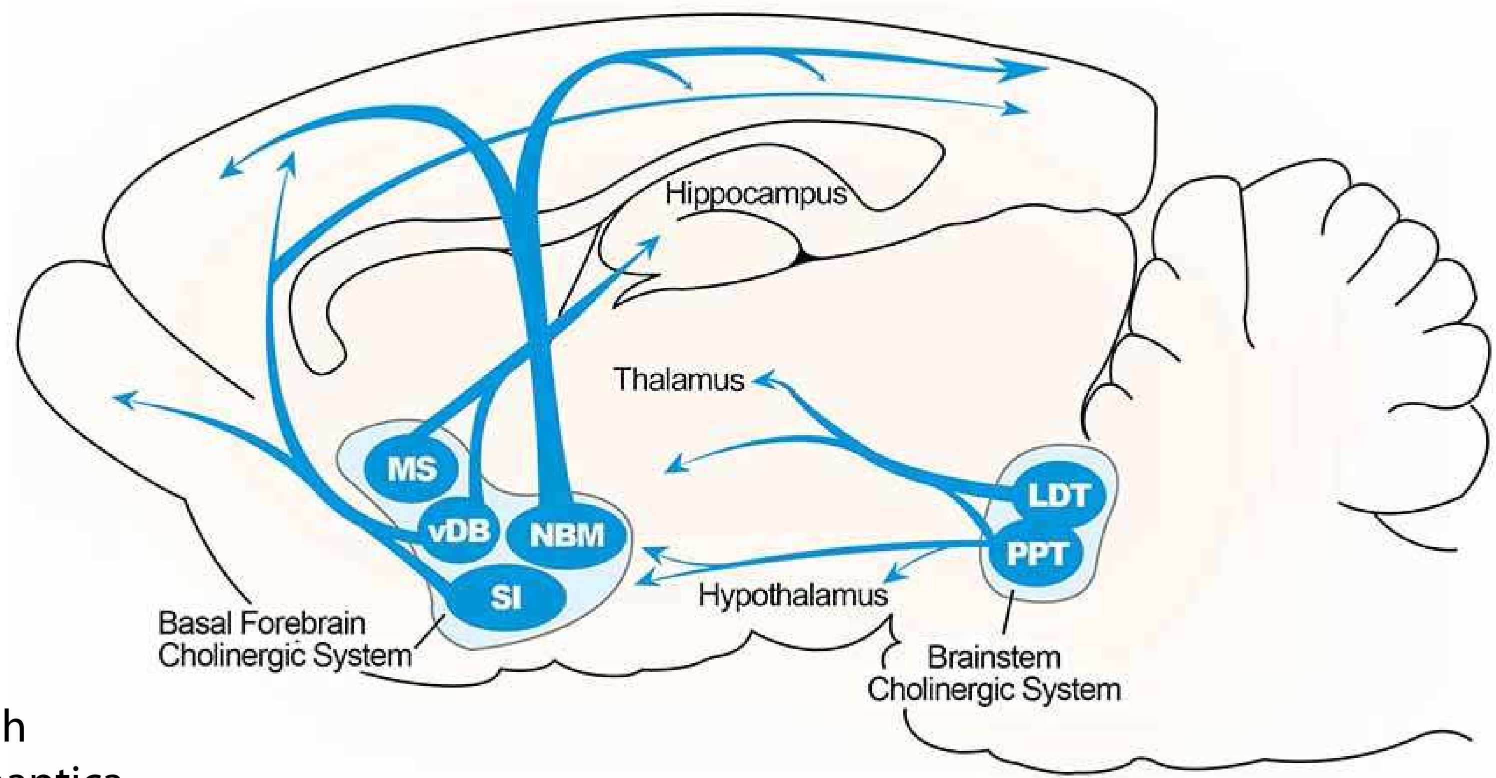
Sintesi di neuropeptidi (es: SP, CGRP)
Sintesi di canali ionici (es: TRPV1)

Biology of NGF



Sopravvivenza
Sintesi di neurotrasmettitori
Sintesi di neuropeptidi (es: NPY)

Biology of NGF



Sintesi di Ach
Plasticità sinaptica
Neurogenesi

NGF gene

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Physiological factors influencing NGF gene expression

Enhancers:

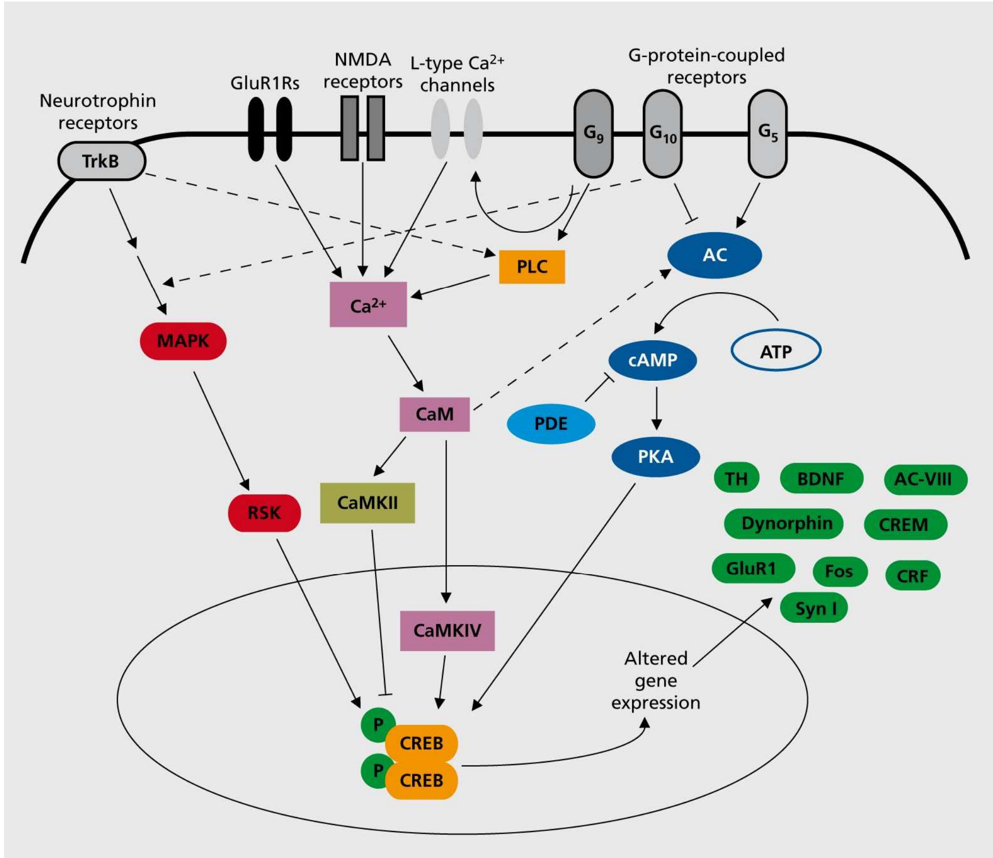
Glutammate
Norepinephrine
Acetylcholine
Dopamine
Corticosteroids
Testosterone/Estrogens
Cytokines
1,25-dihydroxyvitamin D3

Inhibitors:

GABA
3,5,3'-triiodothyronine (T3)
Cytokines

NGF gene: regulation

Different signal transduction pathways converge on the same transcription factors activation, resulting in neurotrophin gene-expression modulation.



NGF gene: regulation

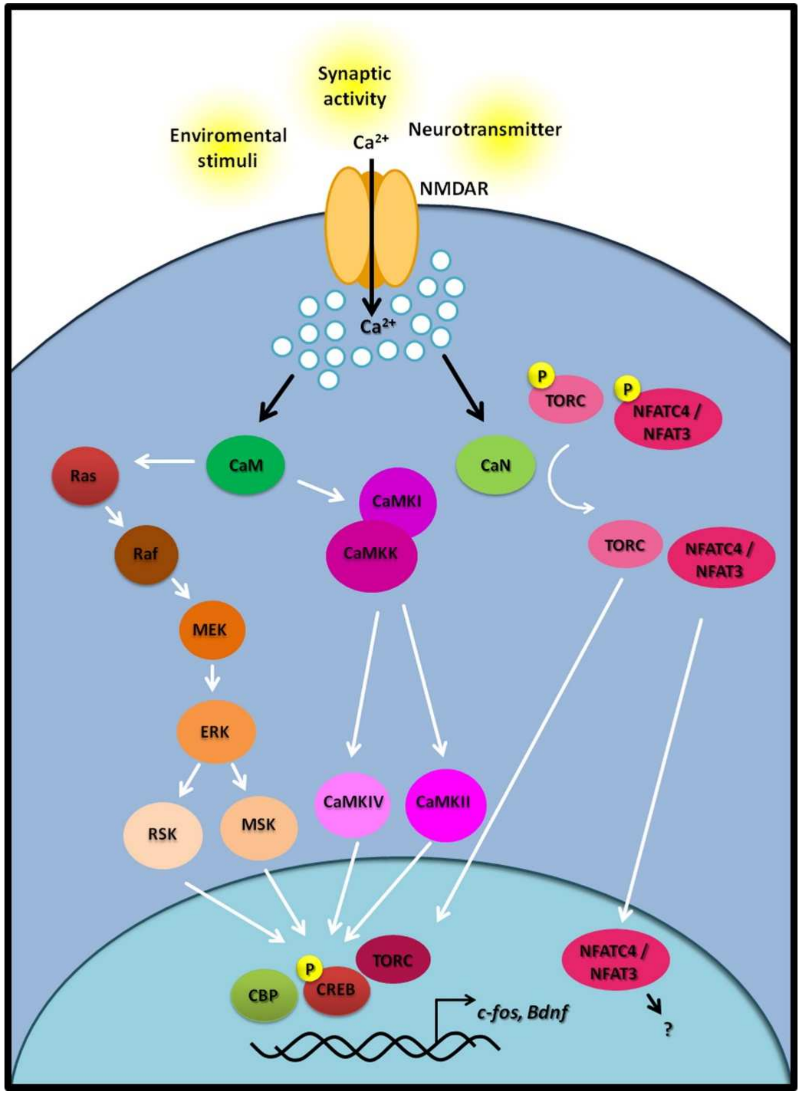
Activity-mediated transcription.

NMDA receptors (NMDAR) open, allowing the influx of Ca^{2+}

Calmodulin (CaM) transduces the elevation of calcium into the activation of CREB in the nucleus

Activation of immediate early genes, such as *c-fos.*

Calcineurin (CaN) leads to TORC and NFAT nuclear translocation and regulation of their target genes.



NGF gene: transcription

Two Promoters
Alternative splicing → Four different mRNA transcripts

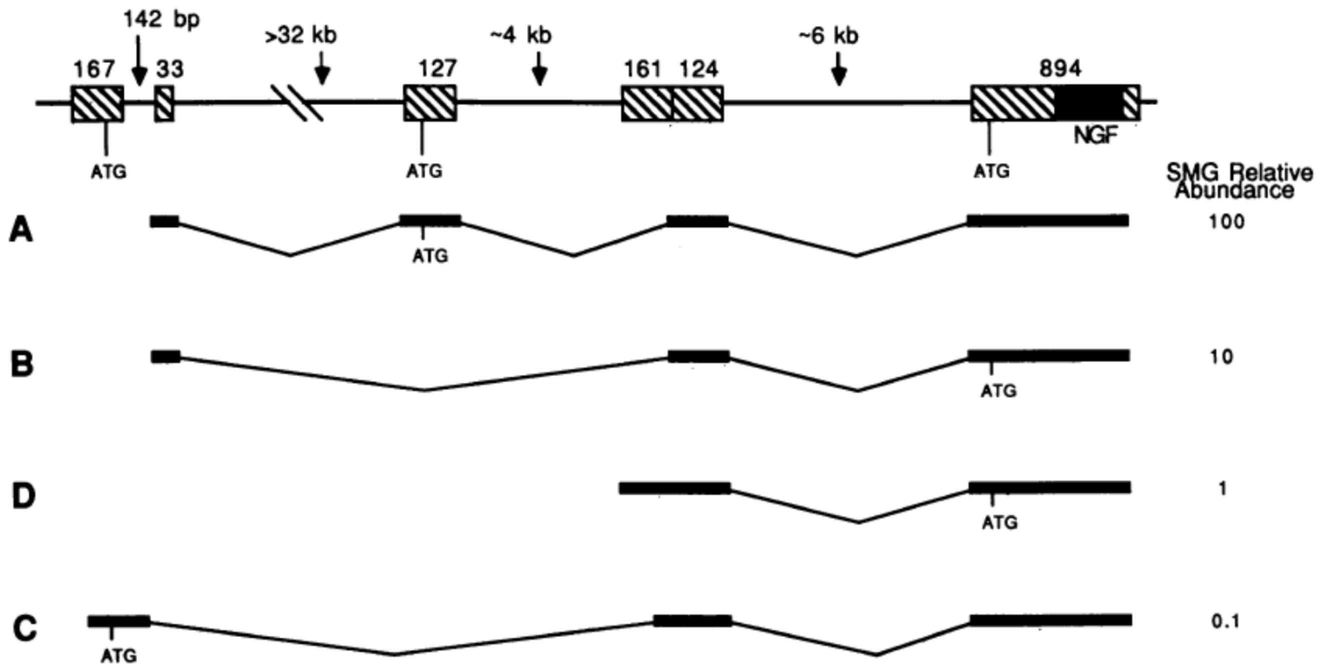


FIG. 4. Diagrammatic representation of the predicted NGF transcripts in relation to the gene. The gene is shown at the top with exons as boxes and introns as lines. The size of the exons (in base pairs) is shown above the boxes; the size of the introns is also shown; mature NGF is stippled. The four identified NGF transcripts are depicted below in order of decreasing submaxillary gland (SMG) abundance (A, B, D, and then C). The thick lines represent sequences formed in the mature RNA, and the thin lines represent regions that are removed by splicing of a primary transcript. The presumed sites for initiation of translation are indicated (ATG).

proNGF protein

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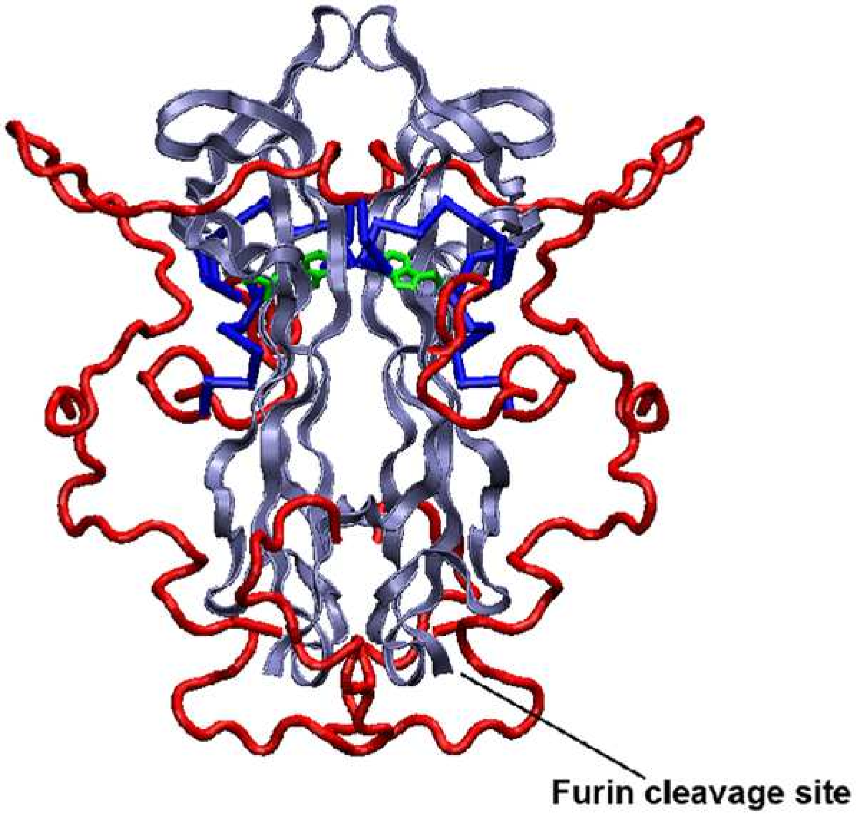
proNGF protein: conformational structure

Intrinsically unstructured proteins are characterized by a low content of bulky hydrophobic amino acids and a high proportion of polar and charged amino acids.

Thus disordered sequences cannot bury sufficient hydrophobic core to fold like stable globular proteins.

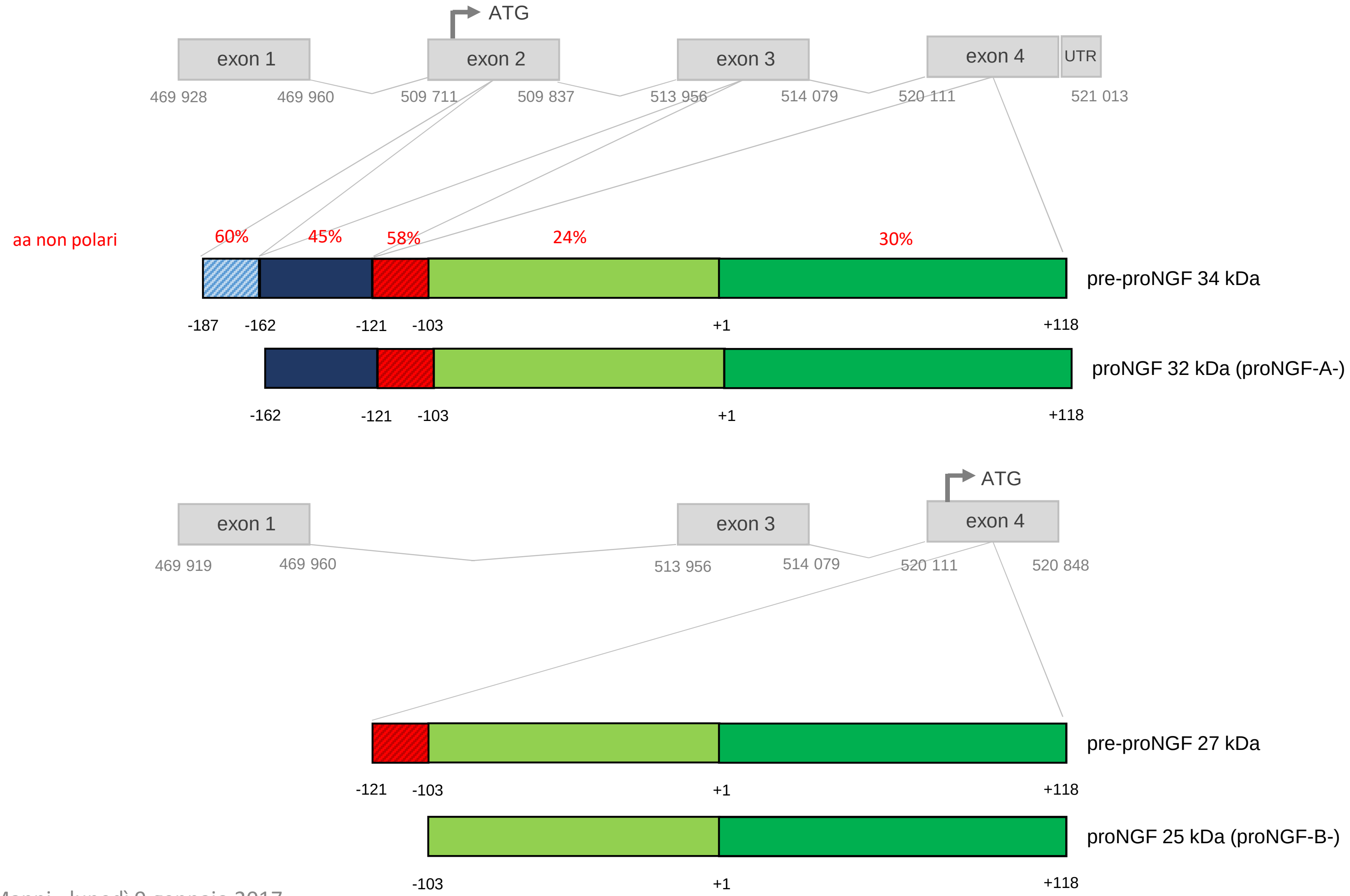
In some cases, hydrophobic clusters in disordered sequences provide the clues for identifying the regions that undergo coupled folding and binding.

Unfolded proteins also have exposed backbone peptide groups exposed to solvent, so that they are readily cleaved by proteases.



Paoletti F, et al. (2011) PLOS ONE 6(7): e22615. doi:10.1371/journal.pone.0022615

proNGF protein: structure



proNGF protein: structure

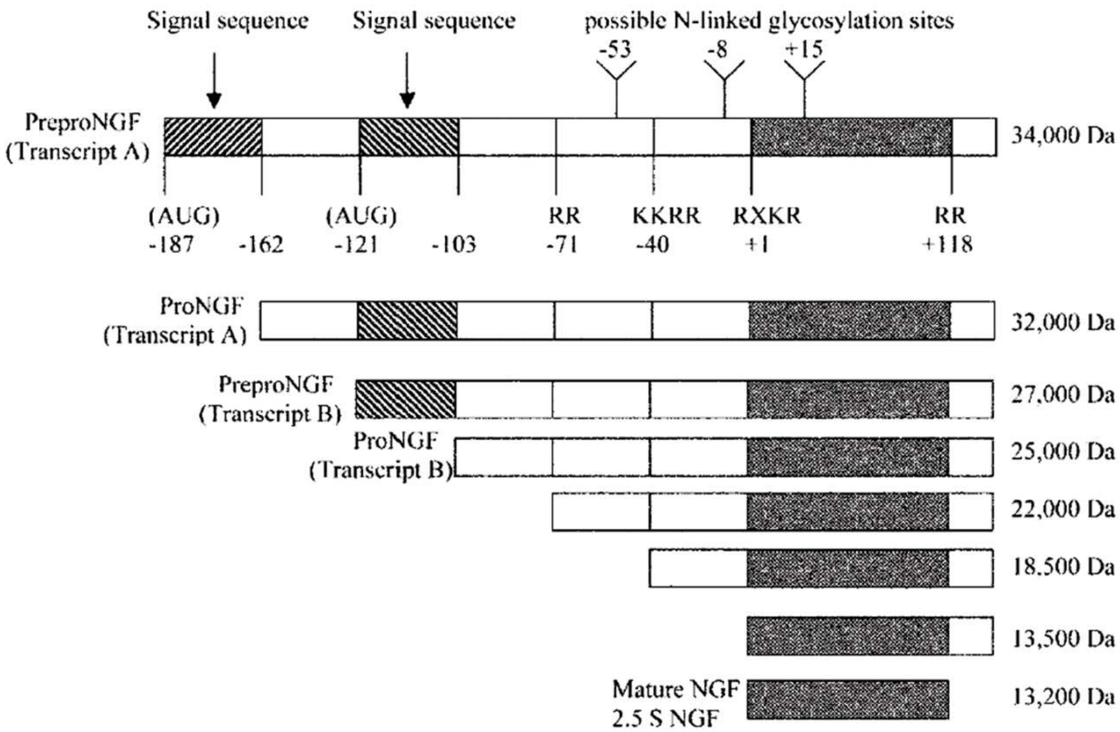
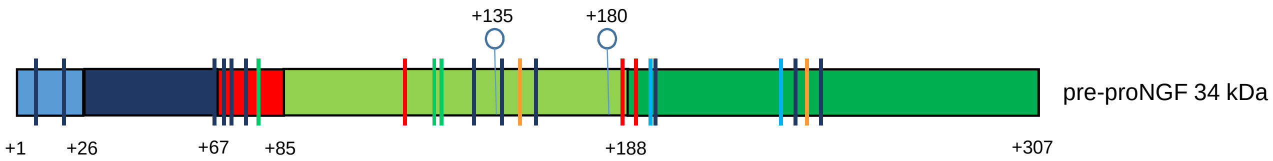
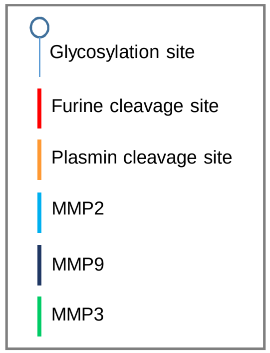


Fig. 1. Likely intermediates in NGF biosynthesis. Translation initiation sites at −187 and −121 are marked by vertical lines, as are potential cleavage sites at −71, −40 and +1. 'Y' represents potential glycosylation sites.

proNGF protein: cleavage sites



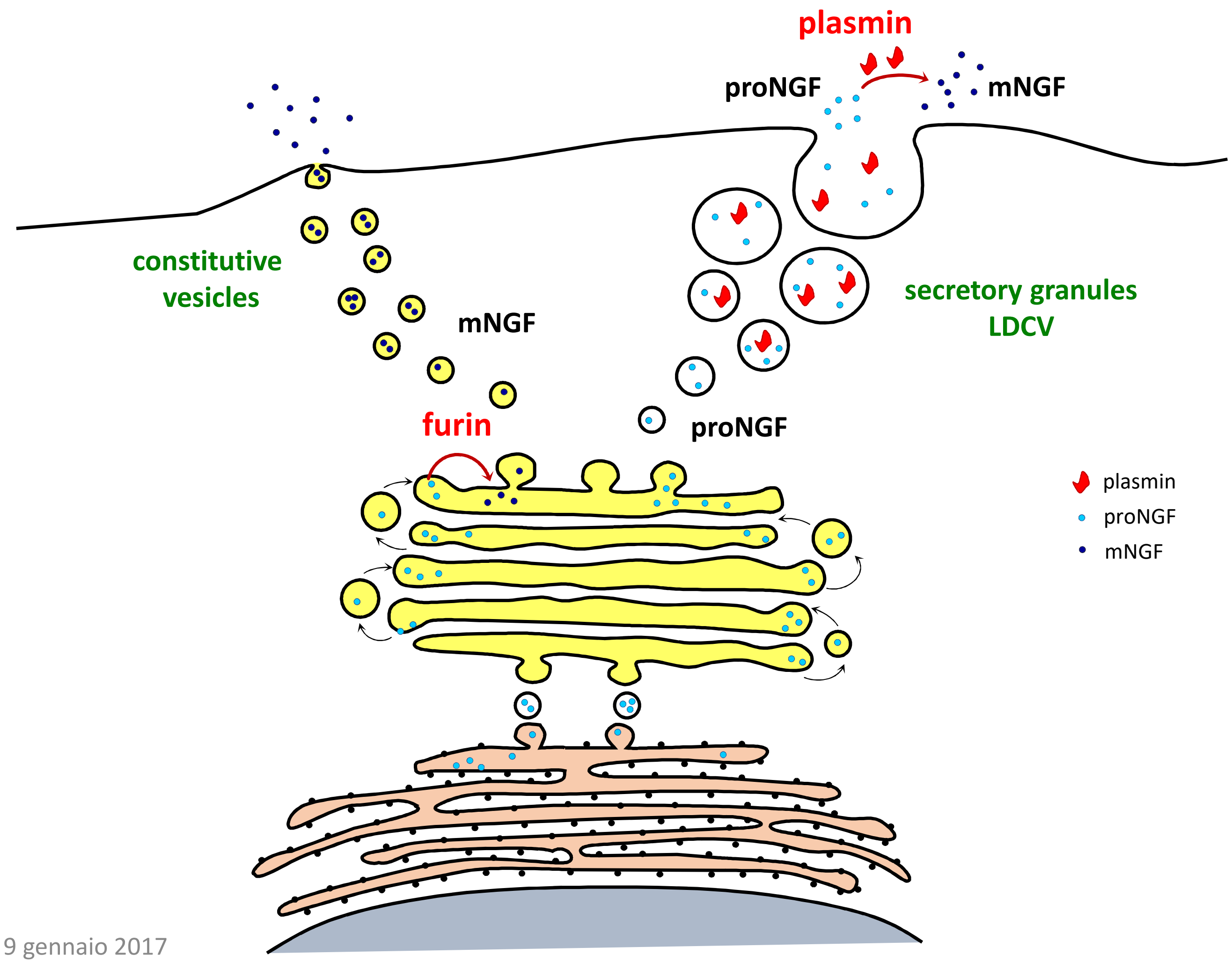

```
mouse MLCLKPVKLG SLEVGHGQHG GVLACGRAVQ GAGWHAGPKL TSVSGPNKGF AKDAAFYTGR 60
rat   ------RAPG AEGERIALLH RVLACGRAVQ GAGWHAGPKL TSVSGPNKGF AKDAAFYPGH 54
                 * :        .  ********** ********** ********** ****** .*:

mouse SEVHSVMSML FYTLITAFLI GVQAEPYTDS NVPEGDSVPE AHWTKLQHSL DTALRRARSA 120
rat   SEVHSVMSML FYTLITAFLI GVQAEPYTDS NVPEGDSVPE AHWTKLQHSL DTALRRARSA 114
      ********** ********** ********** ********** ********** **********

mouse PTAPIAARVT GQTRNITVDP RLFKKRRLHS PRVLFSTQPP PTSSDTLDLD FQAHGTIPFN 180
rat   PAEPIAARVT GQTRNITVDP KLFKKRRLRS PRVLFSTQPP PTSSDTLDLD FQAHGTISFN 174
      *:  ****** ********** :*******:* ********** ********** ******* .**

mouse RTHRSKRSST HPVFHMGEFS VCDSVSVWVG DKTTATDIKG KEVTVLAEVN INNSVFRQYF 240
rat   RTHRSKRSST HPVFHMGEFS VCDSVSVWVG DKTTATDIKG KEVTVLGEVN INNSVFKQYF 234
      ********** ********** ********** ********** ******.*** ******:***

mouse FETKCRASNP VESGCRGIDS KHWNSYCTTT HTFVKALTTD EKQAAWRFIR IDTACVCVLS 300
rat   FETKCRAPNP VESGCRGIDS KHWNSYCTTT HTFVKALTTD DKQAAWRFIR IDTACVCVLS 294
      *******.** ********** ********** ********** :********* **********

mouse RKATRRG 307
rat   RKAARRG 301
      ***:***
```



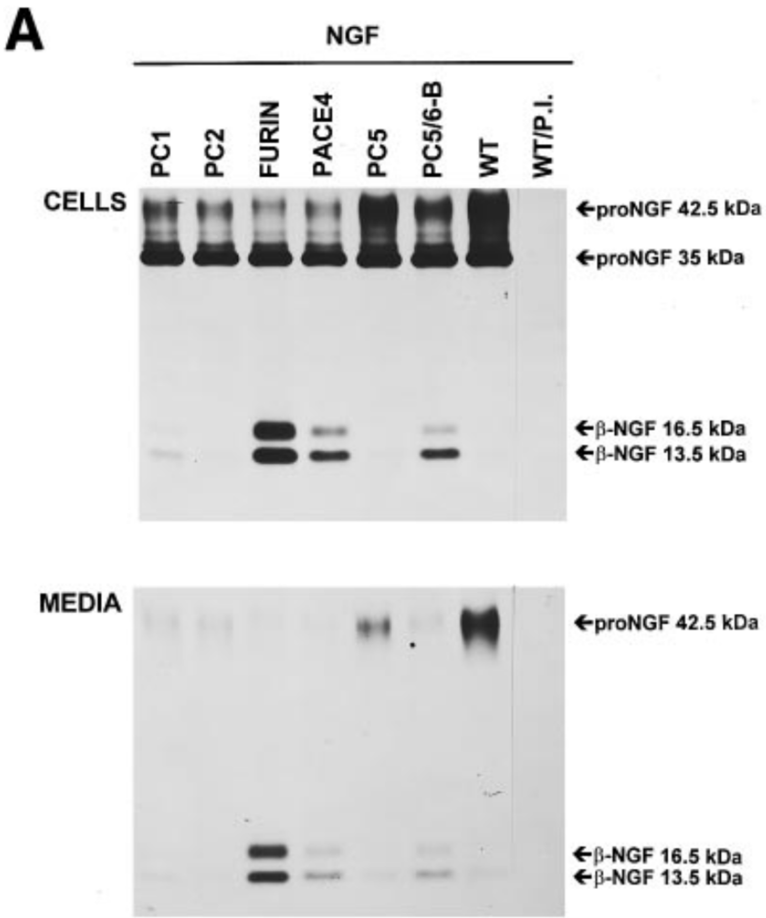
signal sequence I — pro-sequence I — signal sequence II — pro-sequence II — mNGF

***** single, fully conserved residue.
: conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix.
. conservation between groups of weakly similar properties - scoring =< 0.5 in the Gonnet PAM 250 matrix.

proNGF protein: trafficking dyinamics



proNGF protein: maturation

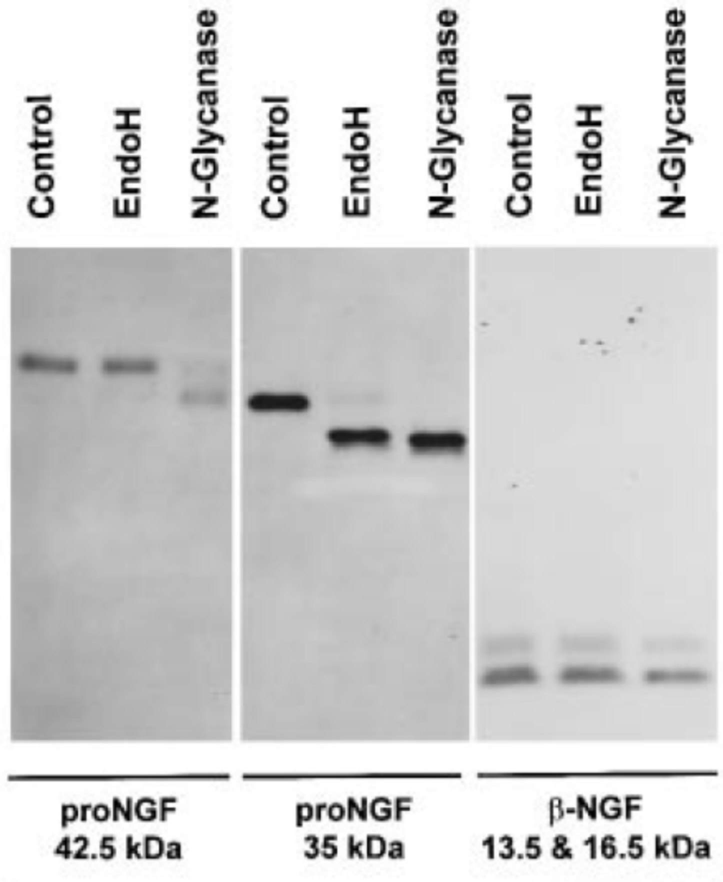


La furina e in misura minore altre due pro-convertasi, generano la produzione e la secrezione di NGF maturo

Seidah NG, et al. Biochem J 314 (Pt 3):951-60. 1996

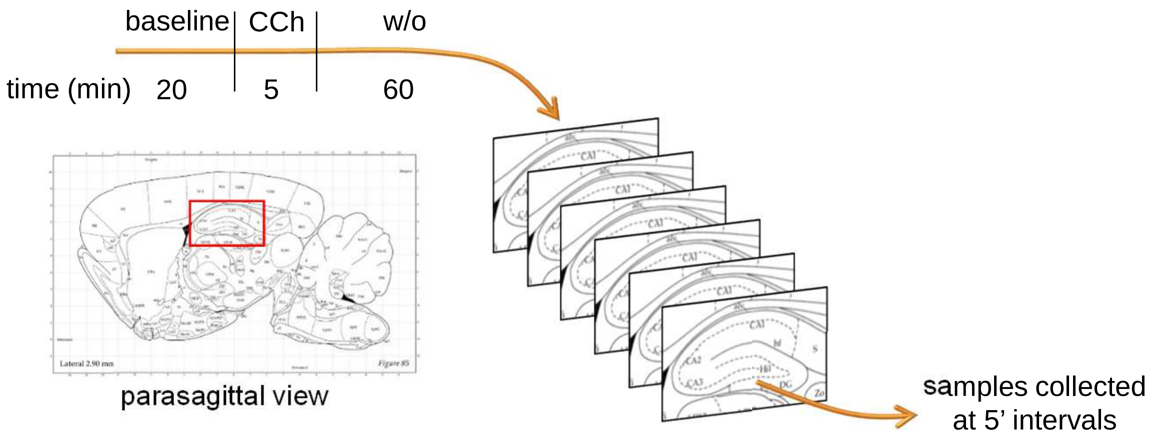
proNGF protein: glycosilation

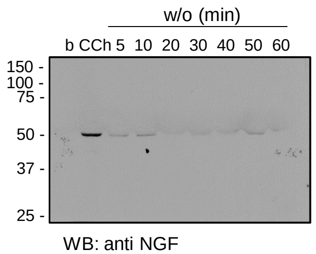
Le diverse forme di proNGF corrispondono a diversi gradi di glicosilazione della proteina.



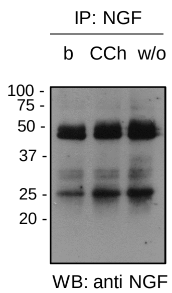
Seidah NG, et al. Biochem J 314 (Pt 3):951-60. 1996

proNGF protein: release

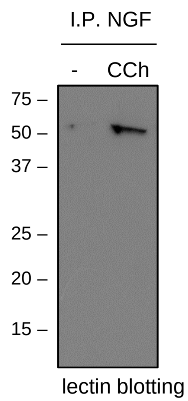




Released: 50 kDa proNGF



Extracellular space:
50, 32 and 25 kDa proNGF



Glycosylated 50 kDa proNGF

proNGF protein: release and extracellular processing

Activity-dependent release of precursor nerve growth factor, conversion to mature nerve growth factor, and its degradation by a protease cascade

PNAS | April 25, 2006 | vol. 103 | no. 17 | 6735–6740

Martin A. Bruno* and A. Claudio Cuello*†‡§

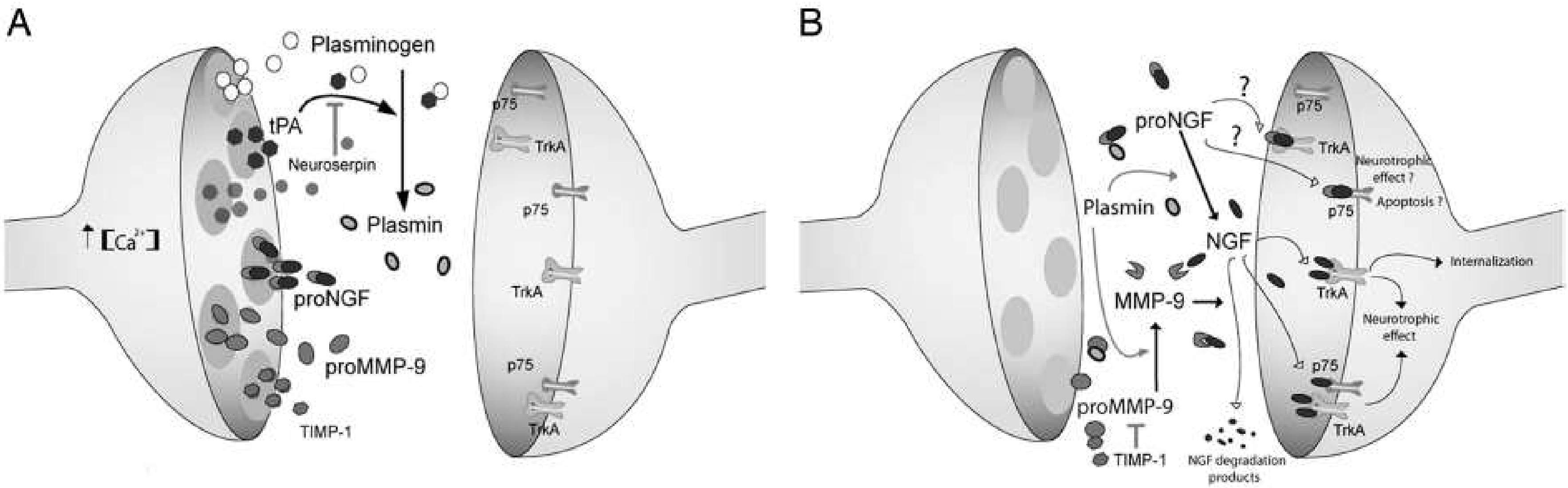
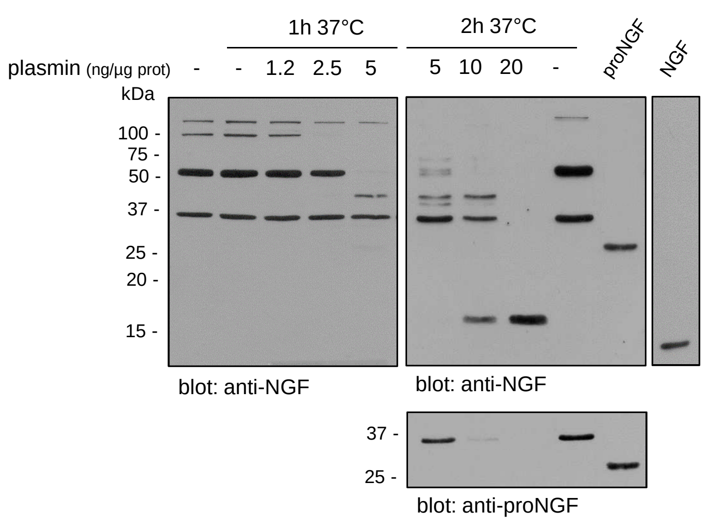
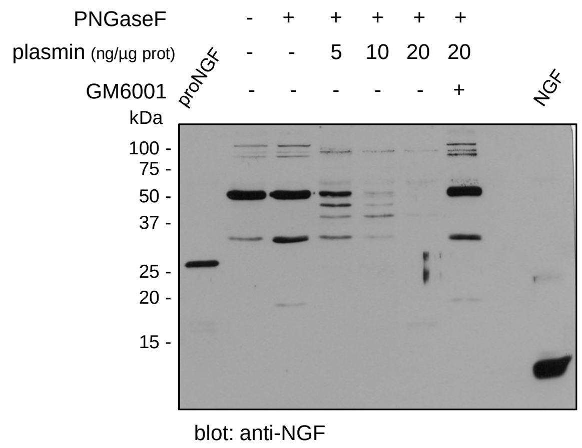
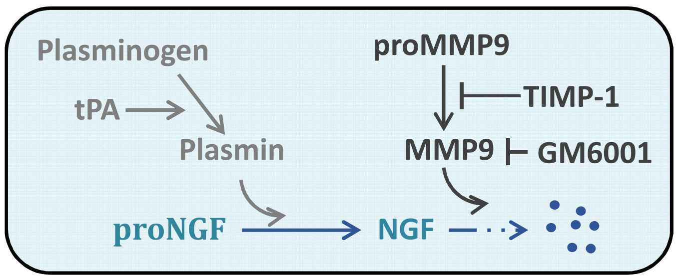


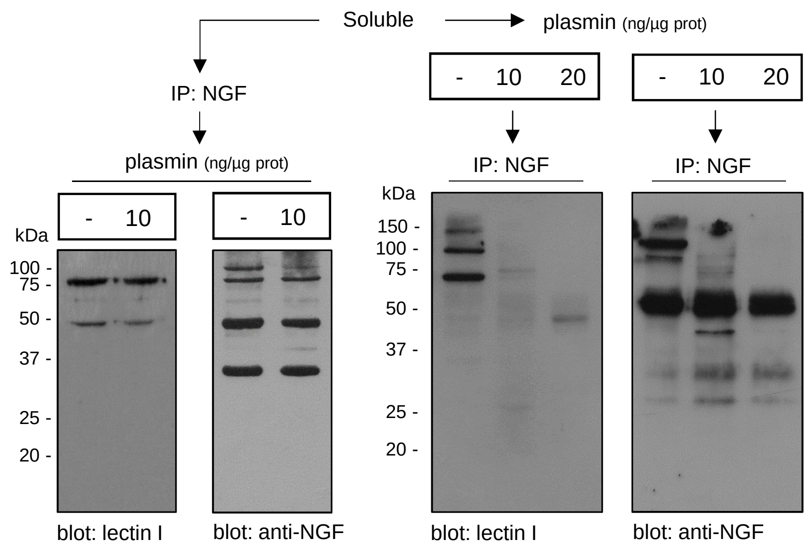
Fig. 7. Schematic representations of events leading to proNGF conversion into mNGF and its degradation. Neuronally stored proNGF, plasminogen, tPA, neuroserpin, proMMP-9, and TIMP-1 would be released into the extracellular space upon neuronal stimulation. Released tPA would induce the conversion of plasminogen to plasmin, where its activity is tightly regulated by secreted neuroserpin. The generated plasmin would convert proNGF into mature NGF and activate proMMP-9 into active MMP-9. Mature NGF would interact with its cognate receptors (TrkA and p75 neurotrophin receptor) or suffer degradation by activated MMP-9.

proNGF protein: release and extracellular processing

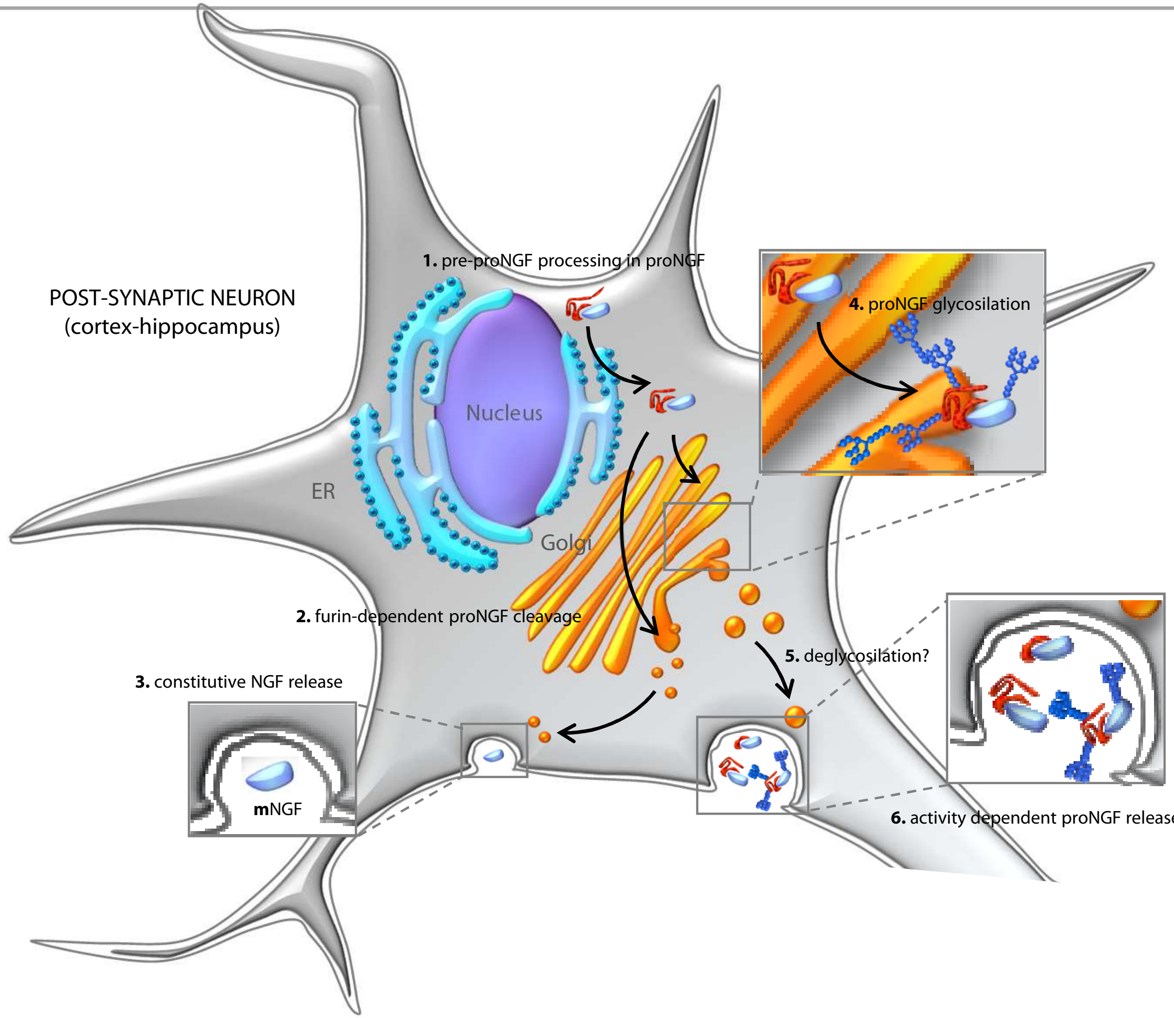


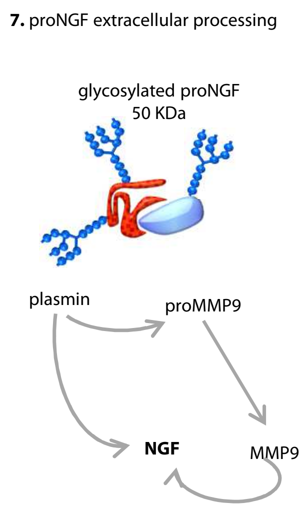






proNGF protein: release and extracellular processing





proNGF/mNGF receptors

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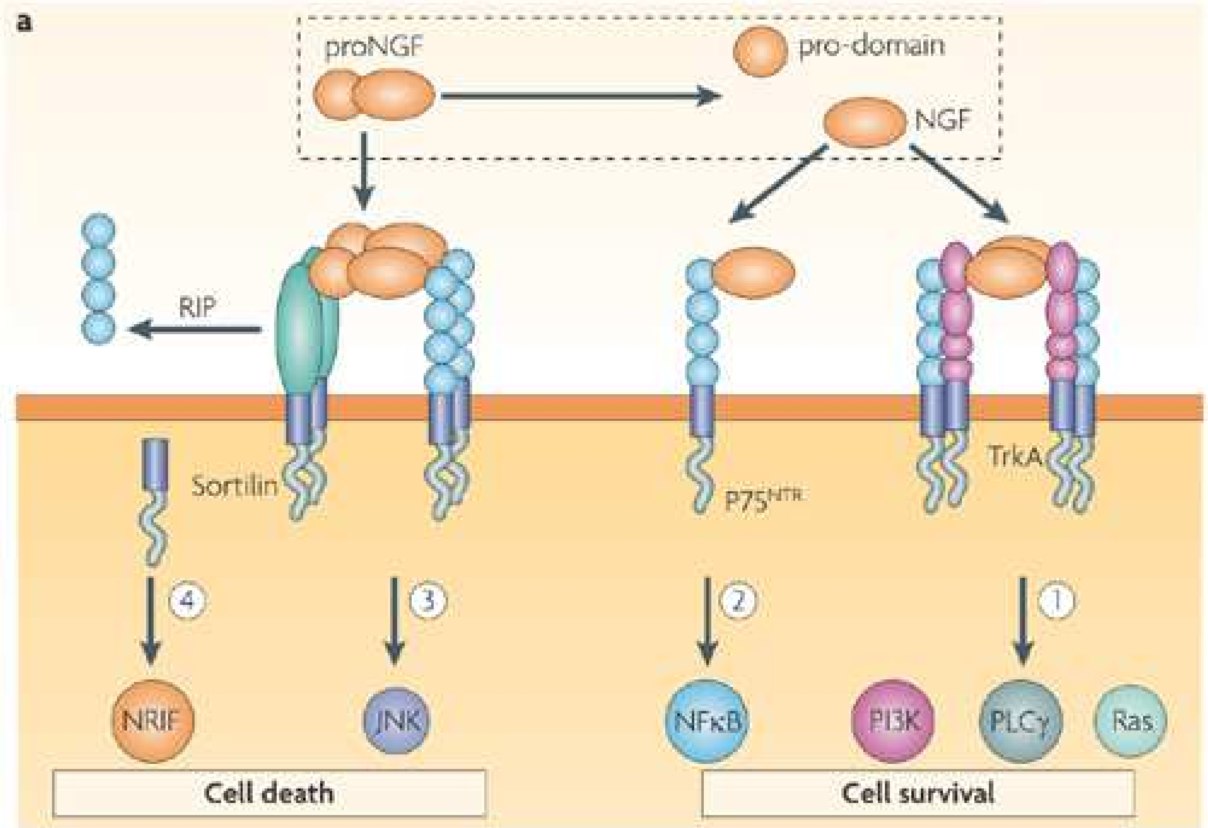
proNGF/mNGF receptors challenge



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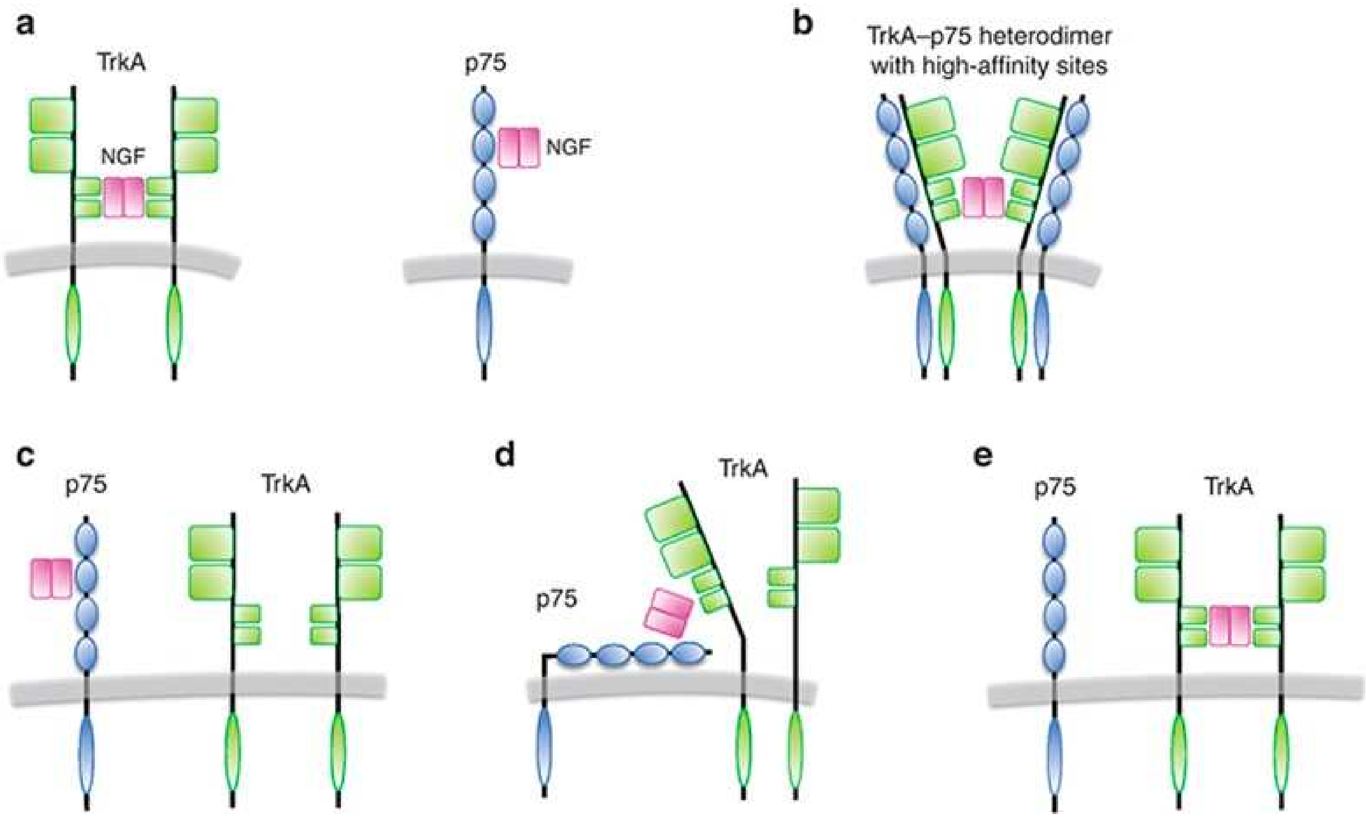
proNGF/mNGF receptors: dissociation constant

Affinità di legame = Kd

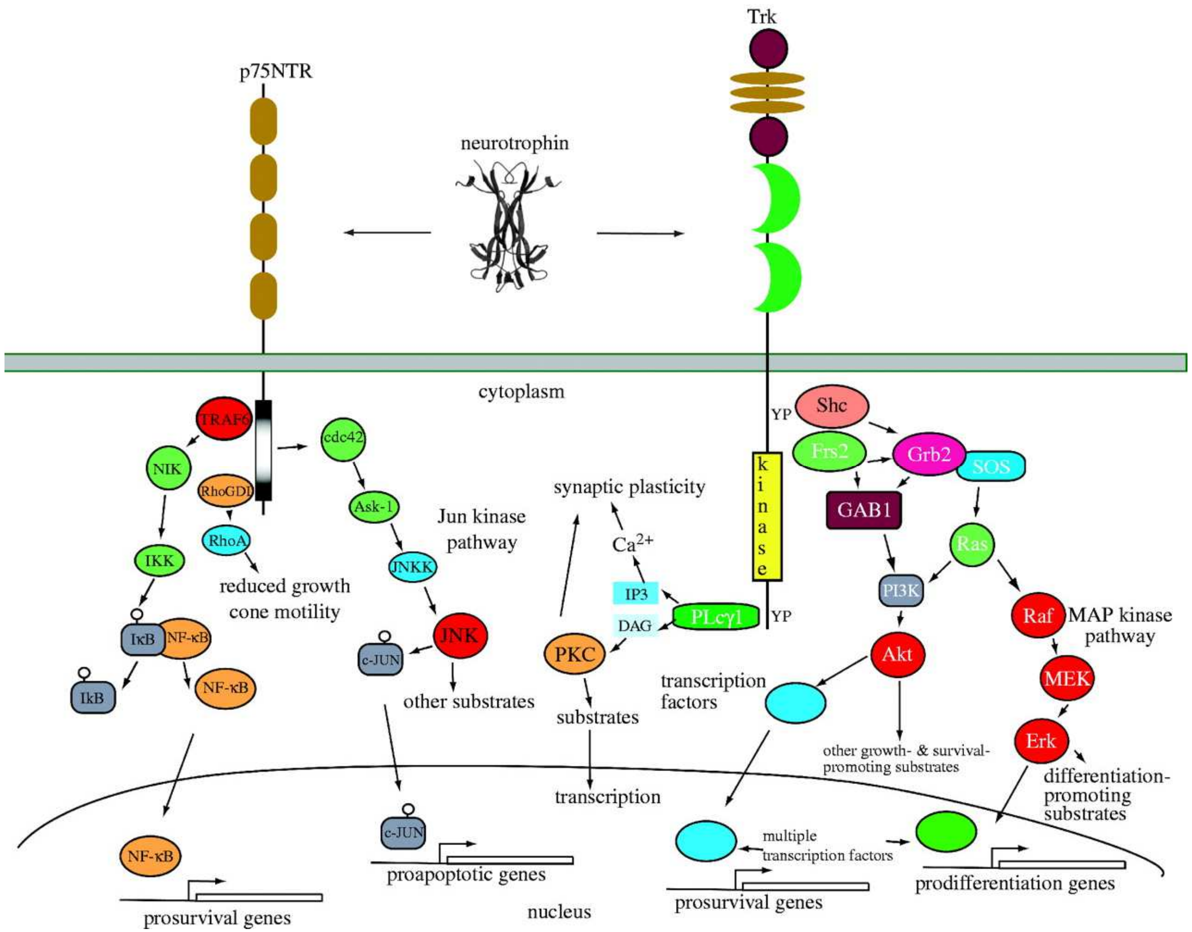
	TrkA	p75NTR	TrkA/p75NTR	p75NTR/sortilin
mNGF	1 nM	1 nM	0.03 nM	--------
proNGF	20 nM	15 nM	?	0.16 nM

Nykjaer A, et al. Nature 427:843-8. 2004.
Barker PA. Neuron 53:1-4. 2007.

proNGF/mNGF receptors: trkA/p75 interaction

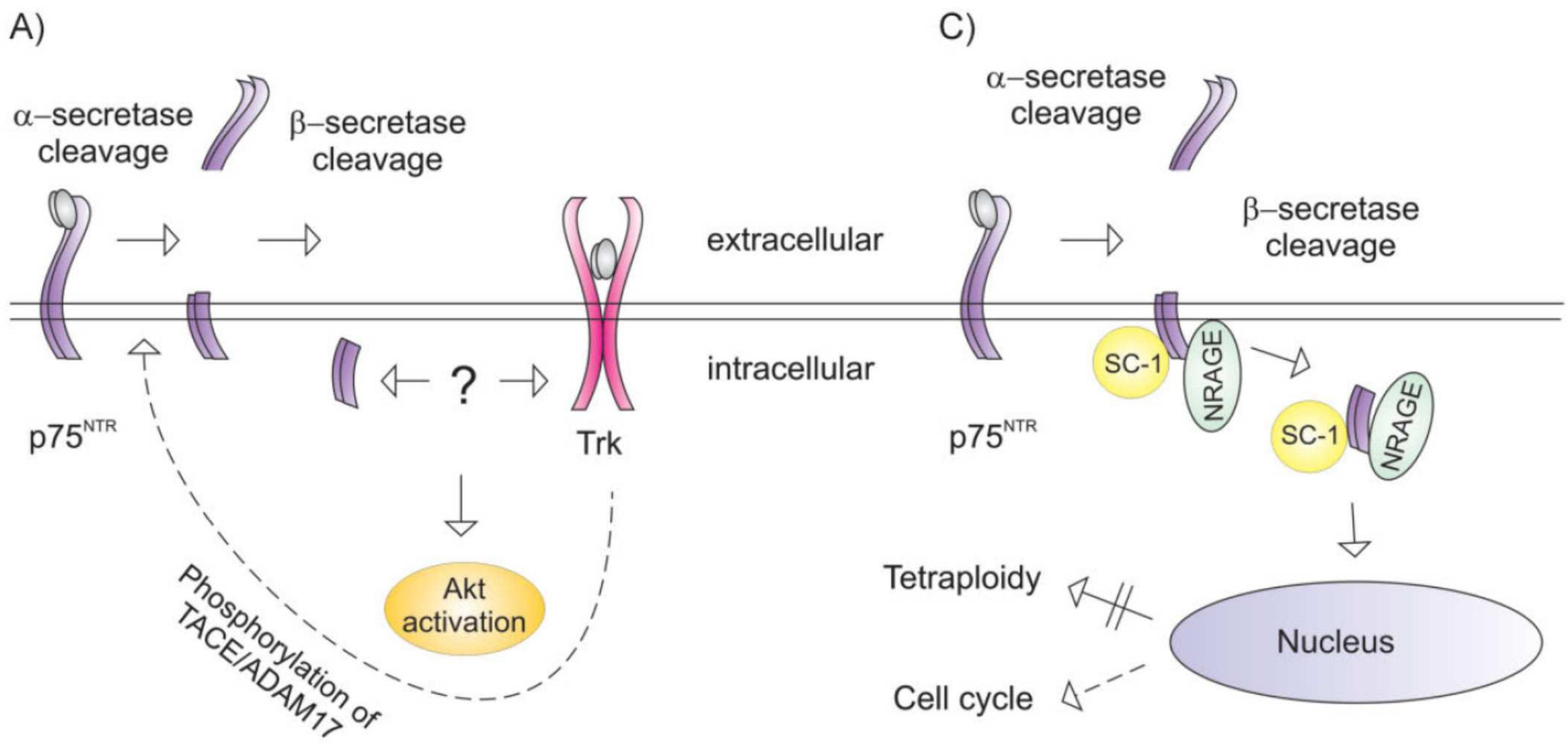


proNGF/mNGF receptors: signaling

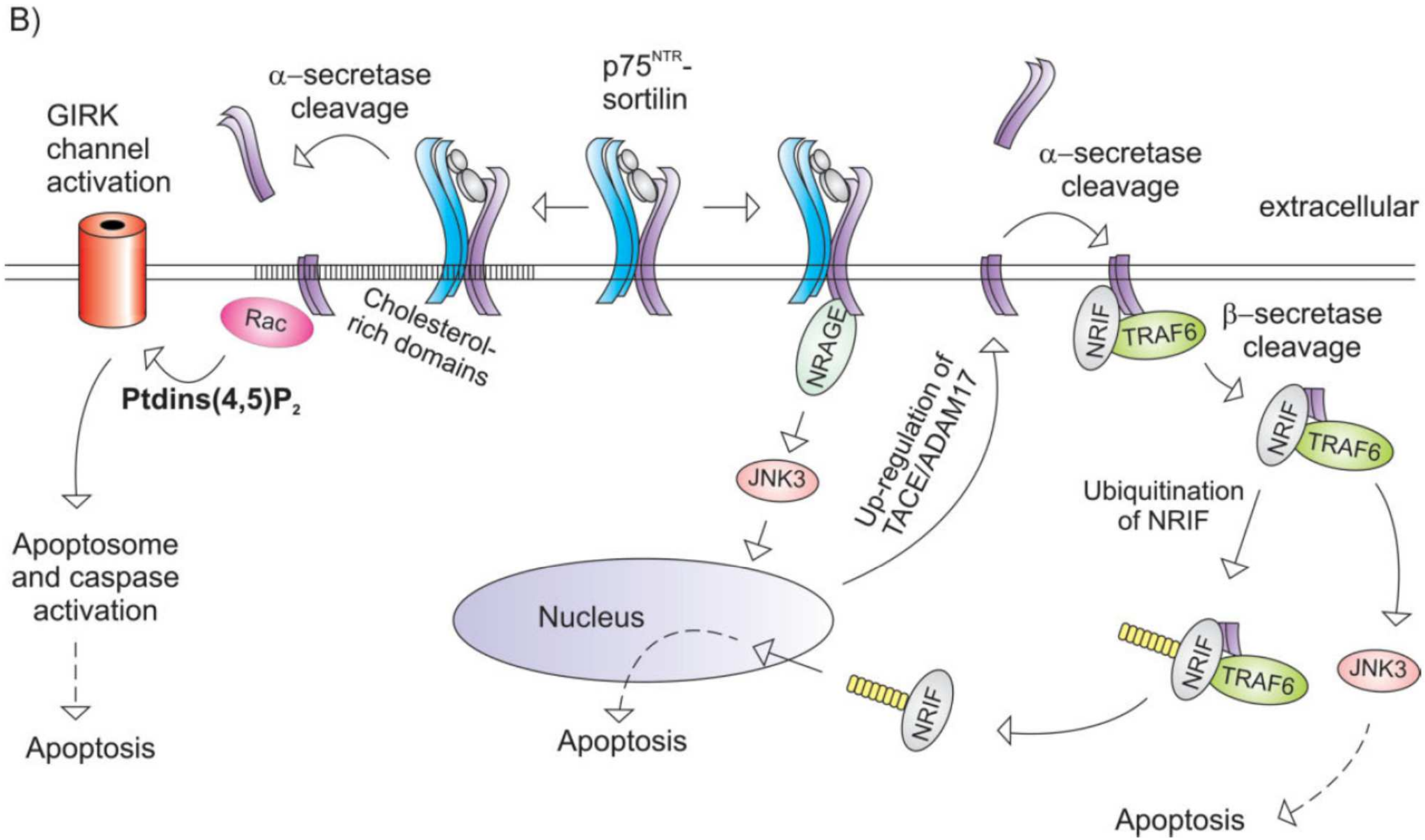


Louis F Reichardt Phil. Trans. R. Soc. B 2006;361:1545-1564

proNGF/mNGF receptors: signaling



proNGF/mNGF receptors: signaling



NGF retrograde transport

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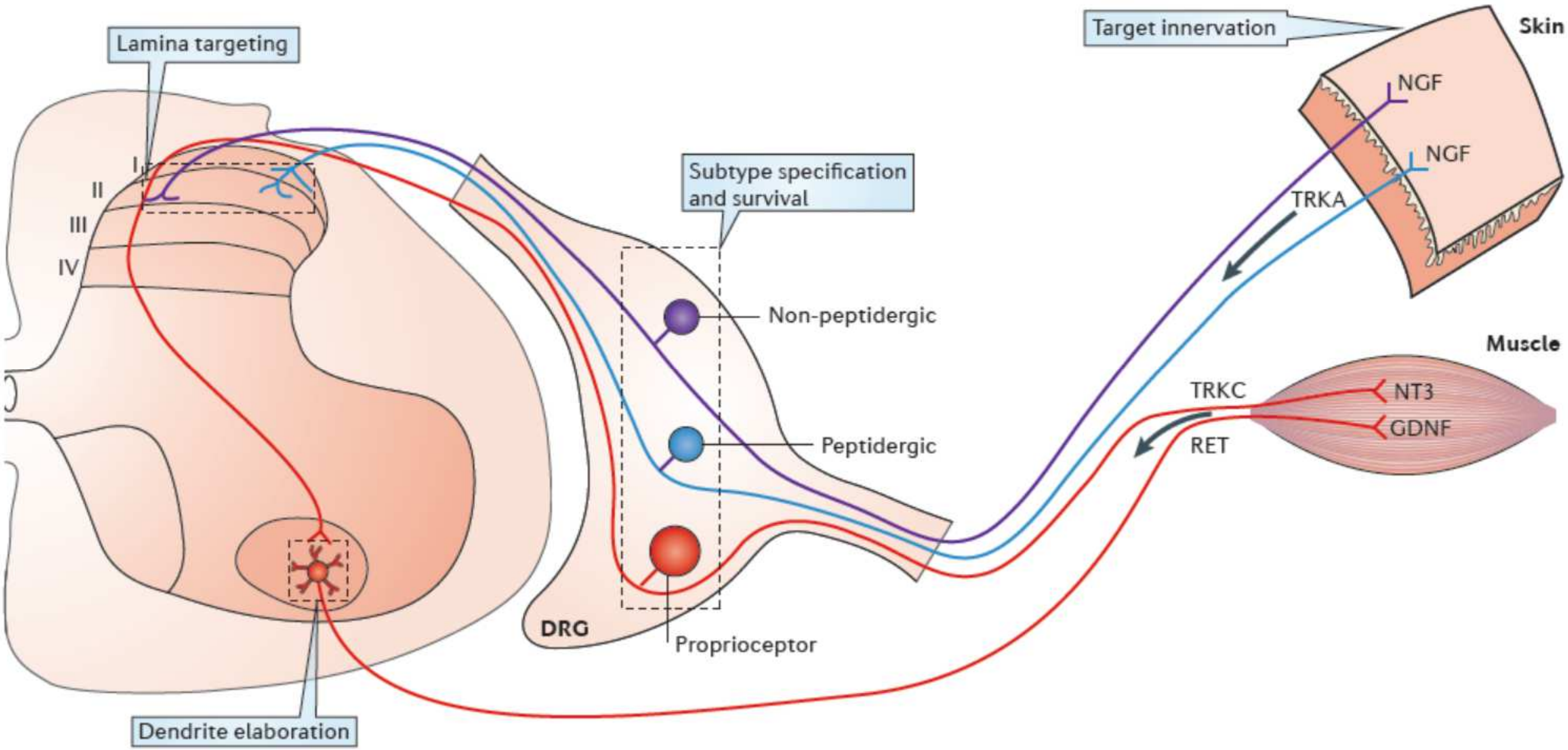
	trkA/p75 interaction
	signaling
NGF retrograde transport	signaling endosome hypotesis
	proNGF?
mNGF/proNGF ratio	

NGF retrograde transport



NGF retrograde transport

- ✓ NGF prodotto, rilasciato e captato a distanze considerevoli dal corpo cellulare.
- ✓ Assotomia, sia di neuroni simpatetici che sensoriali, provoca sofferenza e morte neuronale. Consistente con perdita di trasporto retrogrado.
- ✓ Somministrazione esogena di NGF può prevenire questo fenomeno.
- ✓ NGF radiomarcato iniettato nella camera anteriore dell'occhio viene ritrovato nel ganglio superior cervicale ((Hendry et al, 1974).

NGF retrograde transport

Pergamon

Progress in Neurobiology Vol. 57, pp. 451 to 484, 1999

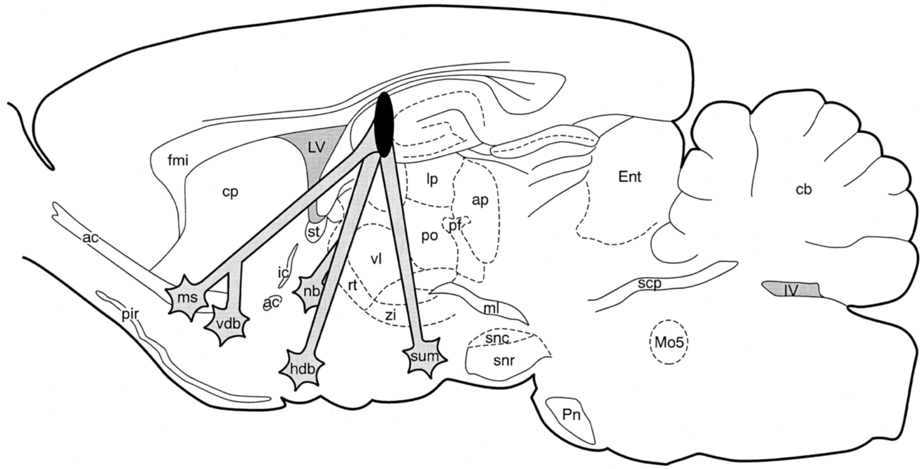
Printed in Great Britain
0301-0082/99/$ - see front matter

PII: S0301-0082(98)00059-8

DISTRIBUTION AND RETROGRADE TRANSPORT OF TROPHIC FACTORS IN THE CENTRAL NERVOUS SYSTEM: FUNCTIONAL IMPLICATIONS FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES

ELLIOTT J. MUFSON*‡, JEFFREY S. KROIN†, TIMOTHY J. SENDERA* and TERESA SOBREVIELA*

**Research Center For Brain Repair, Departments of Neurological Sciences and †Neurosurgery, Rush Alzheimer's Disease Center, Rush Presbyterian-Luke's Medical Center, 2242 W. Harrison Street, Suite 200, Chicago, IL 60612, U.S.A.*



Investigators employed radiolabeled tracing to develop a mechanism of action for NGF in the intact animal.

Iodinated NGF injected into the neocortex → retrogradely transported to the nucleus basalis of Meynert.

into the hippocampus → medial septal nucleus and vertical limb of the diagonal band of Broca

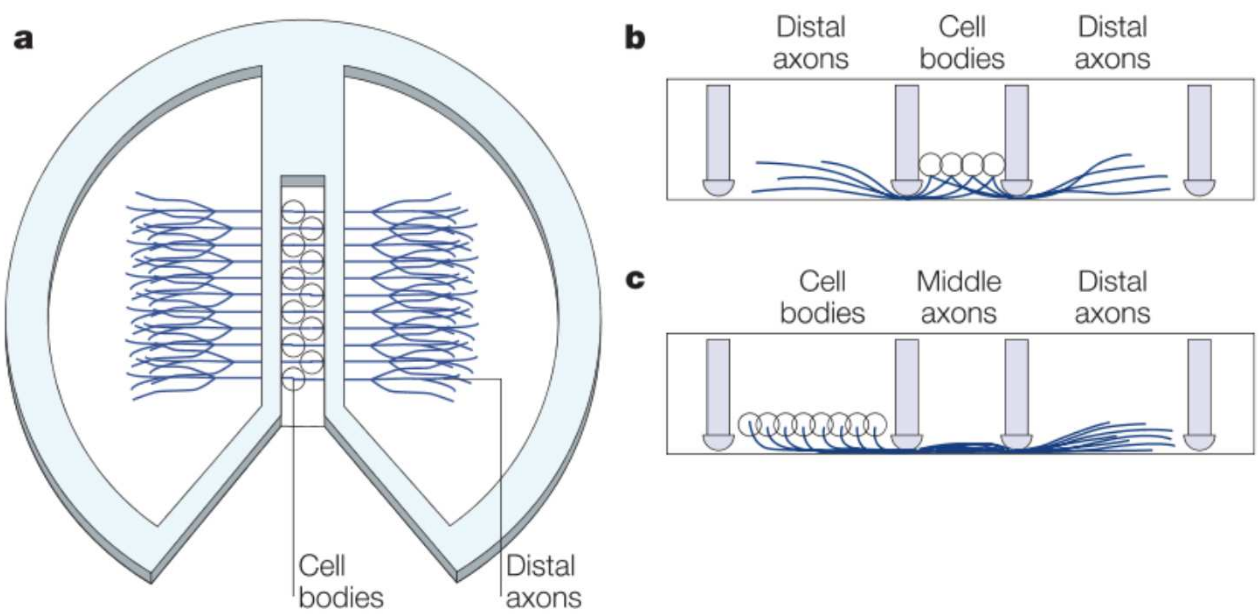
into the olfactory bulb → horizontal limb of the diagonal band.

NGF retrograde transport

FUNCTIONS AND MECHANISMS OF RETROGRADE NEUROTROPHIN SIGNALLING

Larry S. Zweifel, Rejji Kuruvilla‡ and David D. Ginty**

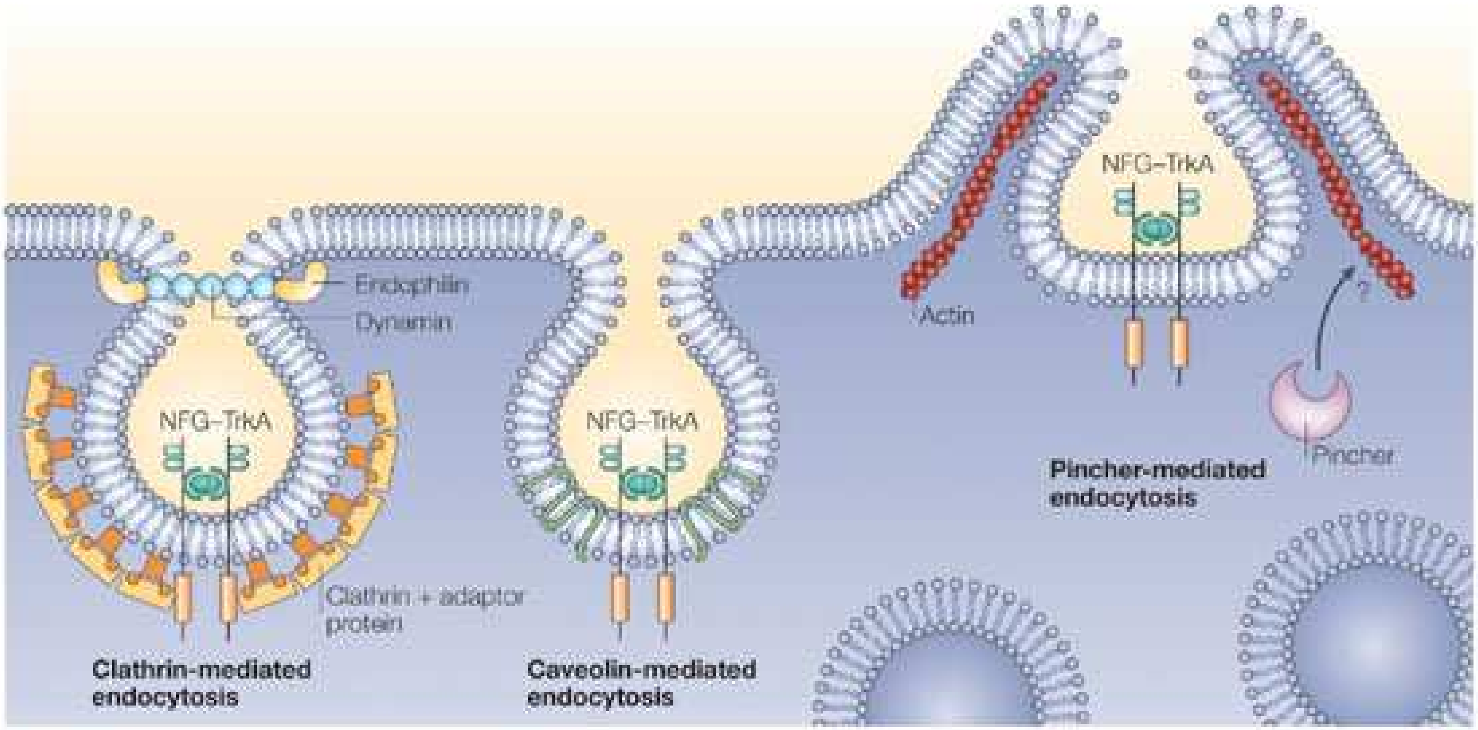
NATURE REVIEWS | NEUROSCIENCE VOLUME 6 | AUGUST 2005 | 615



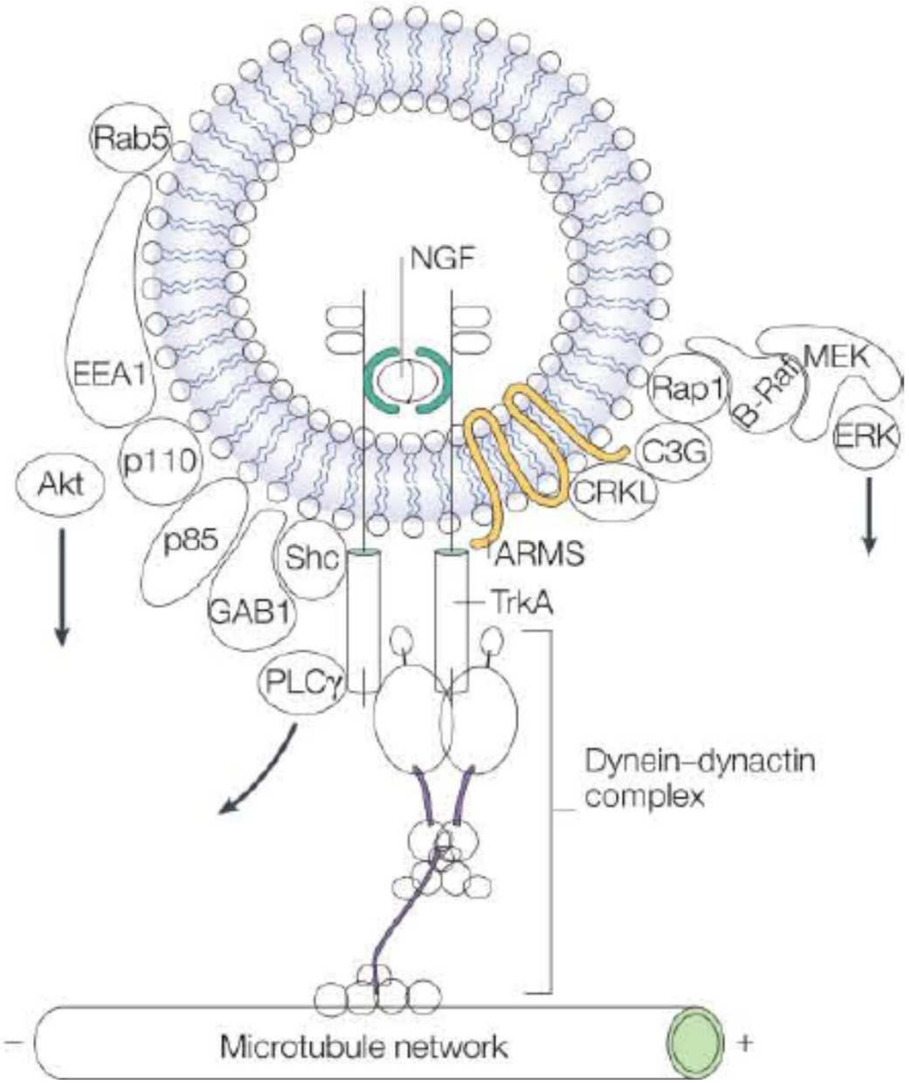
NGF retrograde signalling

- ✓ Signalling endosome
- ✓ Retrograde propagation of sigalling effectors
- ✓ Retrograde waves of Trk receprors activation along the plasma membrane
- ✓ Retrograde calcium waves from activated Trk receptors

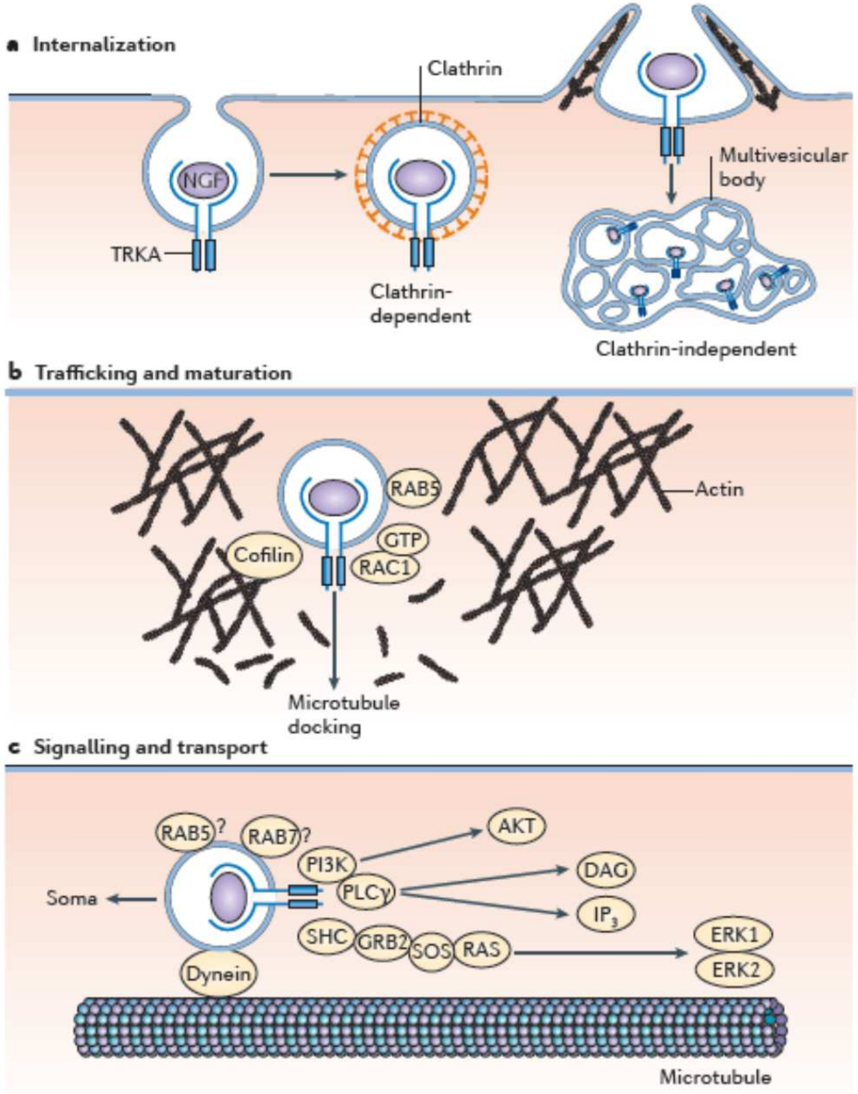
NGF retrograde transport: signaling endosome hypotesis



NGF retrograde transport: signaling endosome hypotesis

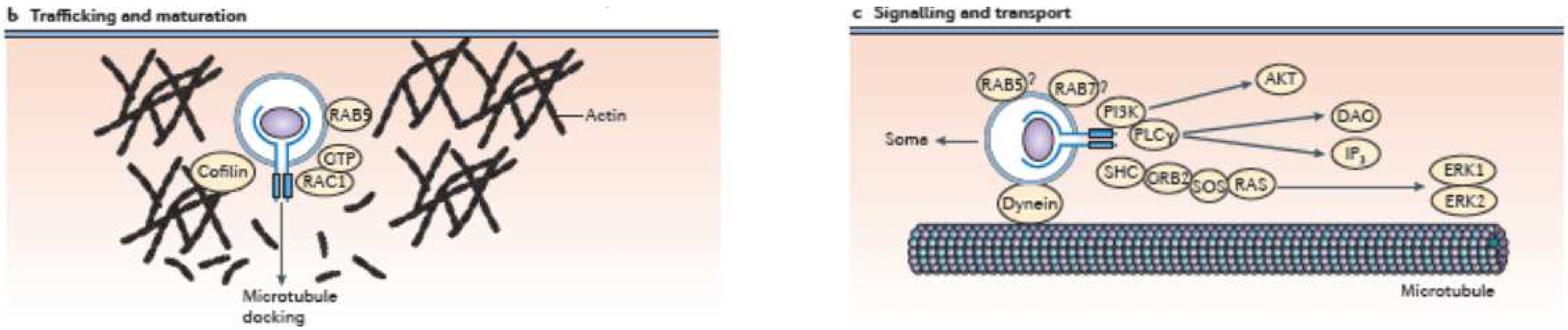


NGF retrograde transport: signaling endosome hypotesis



Harrington, A.W. and D.D. Ginty, *Nat Rev Neurosci, 2013*. ***14(3): p. 177-87.***

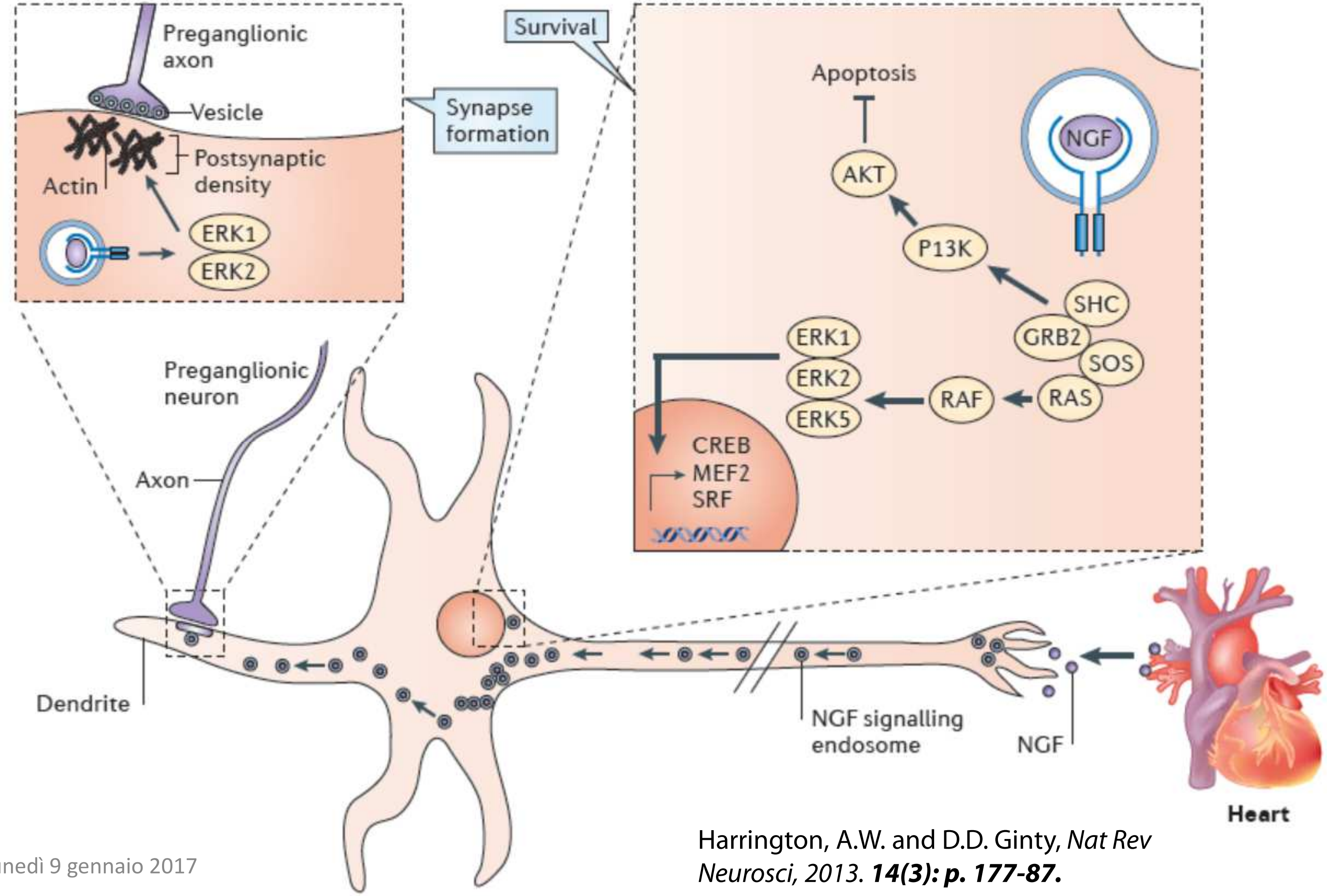
NGF retrograde transport: signaling endosome hypotesis



NGF-pTrkA complexes at the
surface are best able to activate PI3-K/Akt, whereas
NGF-pTrkA complexes internalized into signaling
endosomes
are best able to activate MEK/Erk

signaling endosome that travels
through the axon, potentially carrying activated signaling
molecules along, and activating others as it
passes through the axon

NGF retrograde transport: signaling endosome hypotesis



Harrington, A.W. and D.D. Ginty, *Nat Rev Neurosci, 2013.* ***14(3): p. 177-87.***

NGF retrograde transport: signaling endosome hypotesis

RESEARCH HIGHLIGHT

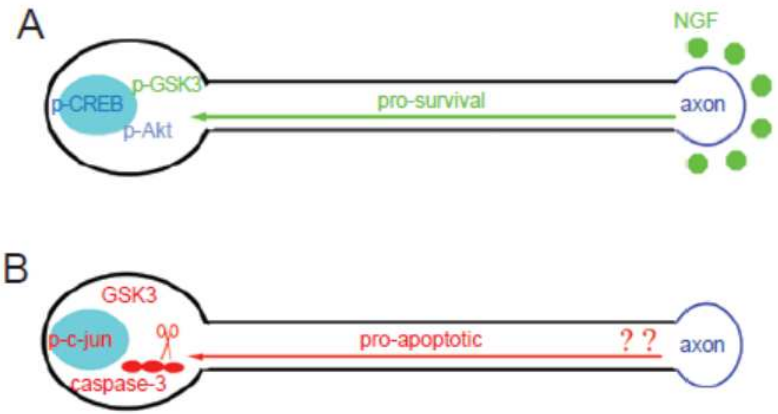
Cell Research (2009) 19:525-526.

www.nature.com/cr

NGF-dependent retrograde signaling: survival versus death

Yang Zhou[1], Ting-Jia Lu[1], Zhi-Qi Xiong[1]

[1]*Institute of Neuroscience and State Key Laboratory of Neuroscience, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yueyang Road Shanghai 200031, China*
Cell Research (2009) **19**:525-526. doi: 10.1038/cr.2009.47; published online 4 May 2009



NGF retrograde transport: proNGF?

SCIENTIFIC REPORTS

OPEN

Precursor and mature NGF live tracking: one *versus* many at a time in the axons

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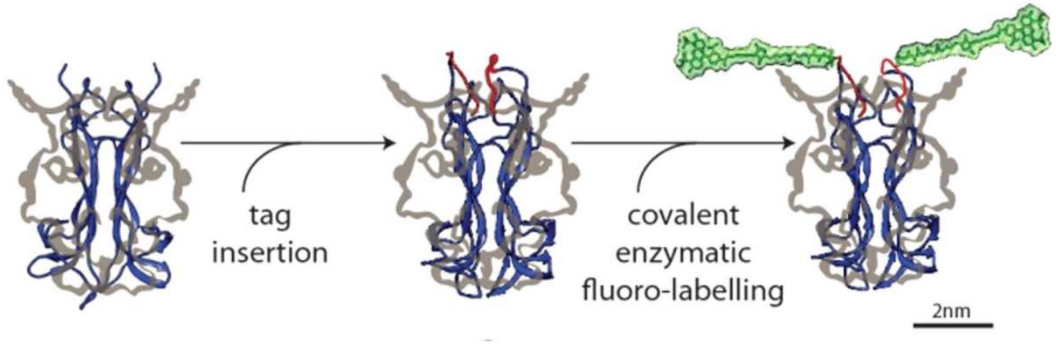
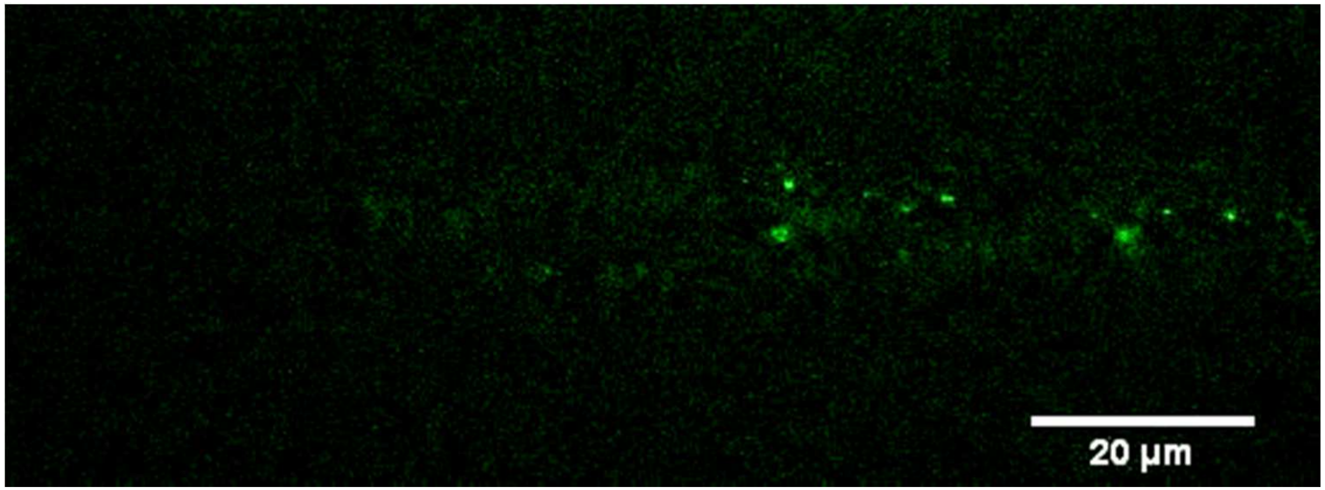
Teresa De Nadai[1], Laura Marchetti[1,2], Carmine Di Rienzo[2,3], Mariantonietta Calvello[1], Giovanni Signore[3], Pierluigi Di Matteo[1], Francesco Gobbo[1], Sabrina Turturro[4], Sandro Meucci[2,3], Alessandro Viegi[1], Fabio Beltram[2,3], Stefano Luin[2] & Antonino Cattaneo[1]



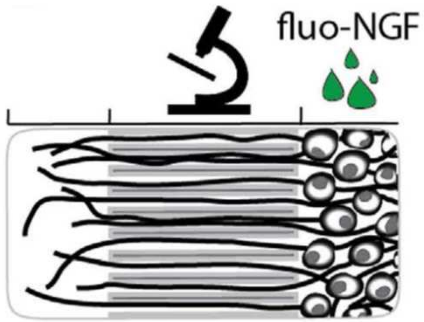


NGF retrograde transport: proNGF?

ANTEROGRADE

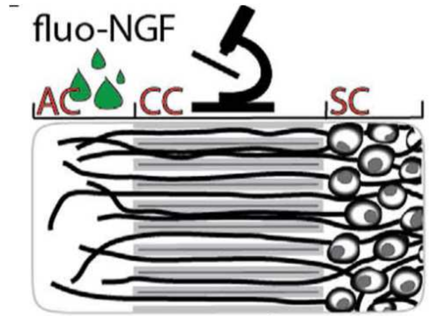
RETROGRADE

NGF
TrkA mediated



20’ after NGF administration

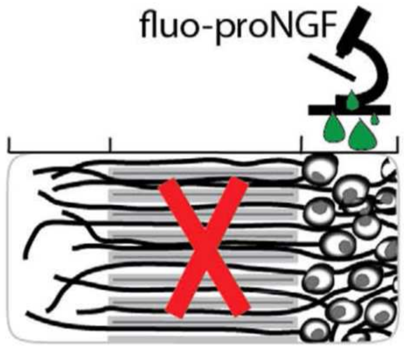
20’ after the first anterogradely moving

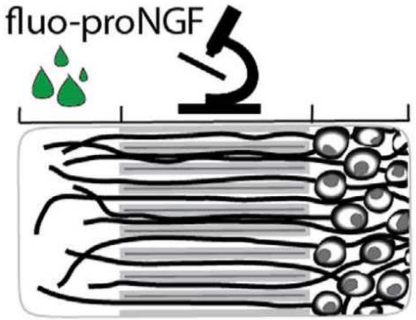


20’ after NGF administration

10’ after the first retrogradely moving

proNGF
TrkA/p75 mediated





35’ after proNGF administration

mNGF/proNGF ratio

mNGF/proNGF ratio

Affinità di legame = Kd

	TrkA	p75NTR	TrkA/p75NTR	p75NTR/sortilin
mNGF	1 nM	1 nM	0.03 nM	--------
proNGF	20 nM	15 nM	?	0.16 nM

Nykjaer A, et al. Nature 427:843-8. 2004.
Barker PA. Neuron 53:1-4. 2007.

mNGF/proNGF ratio

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Neuroscience Letters 380 (2005) 133–137
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'Mature' nerve growth factor is a minor species in most peripheral tissues

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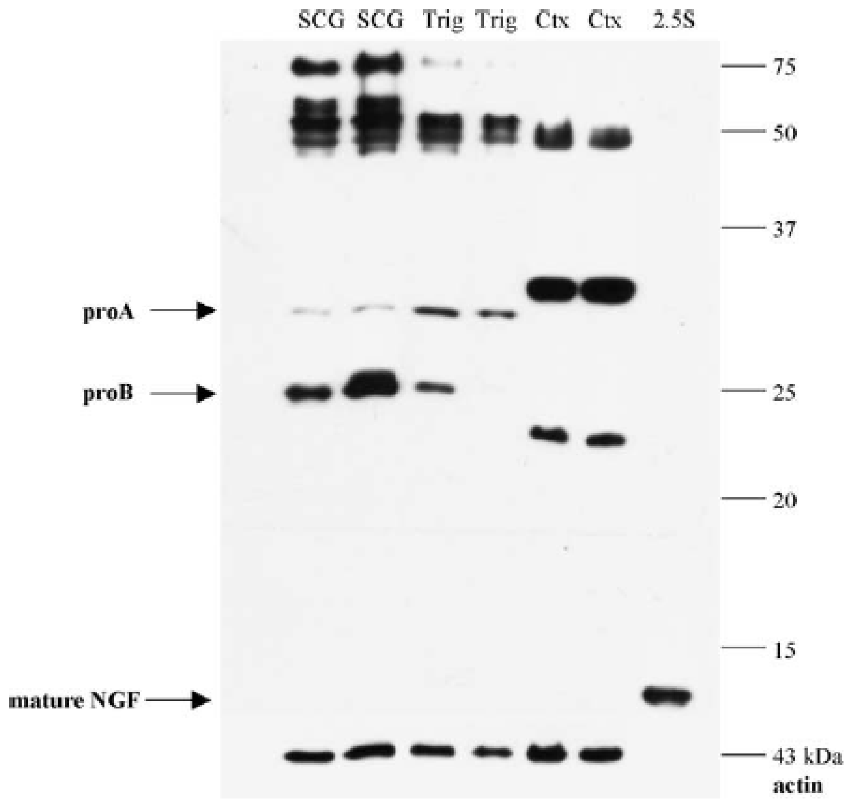

Fig. 2. Qualitative Western analysis of the SCG, trigeminal ganglion and frontal cortex of the rat showing characteristic pattern of NGF expression. Expression in the central nervous system (cortex) is included for comparison with peripheral ganglia. These blots represent characteristic staining patterns for three to five animals per tissue. For each tissue, blots from two *different* animals are shown. Blots were reprobed for actin as a loading control for samples of the same tissue type. SCG: superior cervical ganglion; Trig: trigeminal ganglion; Ctx: frontal cortex; 40 µg protein loaded for tissues; 2.5S = 15 ng NGF protein (Harlan).

mNGF/proNGF ratio

MCN

Molecular and Cellular Neuroscience **18**, 210–220 (2001)
doi:10.1006/mcne.2001.1016, *available online at http://www.*

The Precursor Pro-Nerve Growth Factor Is the Predominant Form of Nerve Growth Factor in Brain and Is Increased in Alzheimer's Disease

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[*]*Department of Pyschiatry and Behavioural Neurosciences,* [†]*Department of Biology, and* [‡]*Department of Psychology, McMaster University, Hamilton, Ontario, Canada L8N 3Z5*

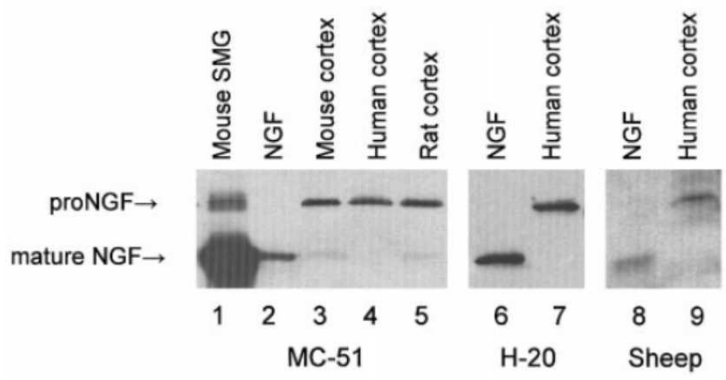

FIG. 1. Comigration of NGF-immunoreactive bands from mouse, rat, and human brain with proNGF from mouse SMG. Lane 1: 2 μg mouse submandibular gland homogenate; lane 2: 0.1 ng purified 2.5S NGF protein; lane 3: 80 μg mouse cortex and hippocampus homogenate; lane 4: 80 μg human cortex homogenate; lane 5: 80 μg rat cortex homogenate; lane 6: 7 ng purified 2.5S NGF protein; lane 7: 80 μg human cortex homogenate; lane 8: 0.2 ng purified 2.5S NGF protein; lane 9: 80 μg human cortex homogenate. Lanes 1–5: These samples were homogenized in RIPA buffer; all other samples in standard homogenization buffer. Primary antibody MC-51 was incubated at 1:2000 dilution overnight at 4°C and then with HRP-conjugated donkey anti-rabbit secondary antibody at 1:6600 for 1 h at room temperature. Lanes 6 and 7: Primary antibody H-20 was incubated at 1:2000 dilution overnight at 4°C and then with HRP-conjugated donkey anti-rabbit secondary antibody at 1:6600 for 1 h at room temperature. Lanes 8 and 9: Primary sheep antibody was incubated at 1:1000 dilution overnight at 4°C and then with HRP-conjugated donkey anti-sheep secondary antibody at 1:5000 for 1 h at room temperature.

mNGF/proNGF ratio

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**Department of Pyschiatry and Behavioural Neurosciences, †Department of Biology, and ‡Department of Psychology, McMaster University, Hamilton, Ontario, Canada L8N 3Z5*

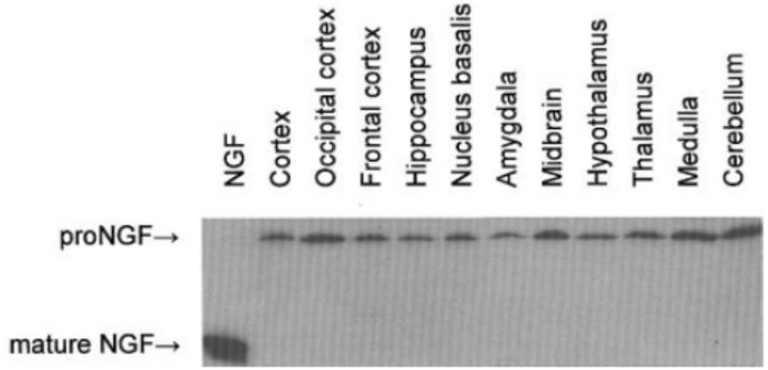
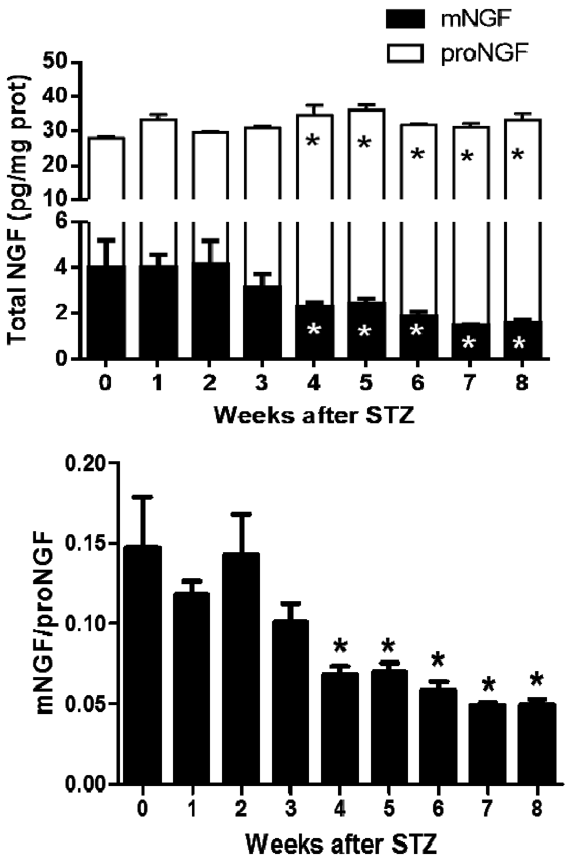


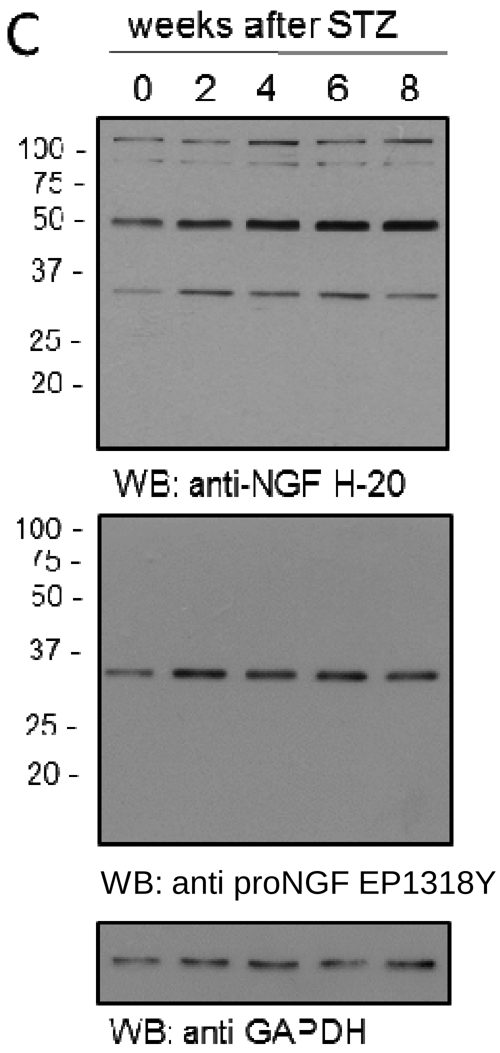
FIG. 5. ProNGF, but not NGF, is present in human brain regions. Lane 1: 6 ng purified murine 2.5S NGF. Lanes 2–12: 80 μg protein from indicated regions of human brain. The blot was developed with antibody H-20 as described in the legend to Fig. 1.

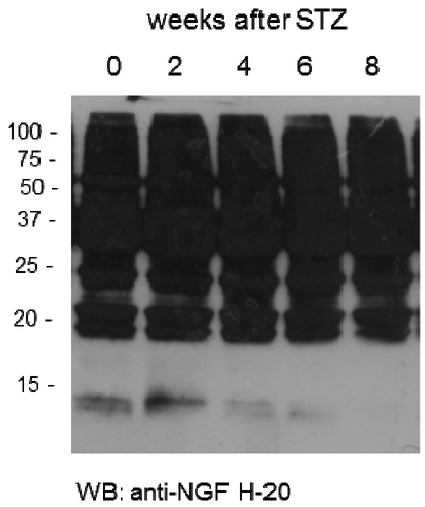
mNGF/proNGF ratio



Probabilmente il rapporto è sovrastimato a causa delle diverse *immunoreattività* dei saggi enzimatici.

mNGF/proNGF ratio





2,30	2,79	0,72	0,46	0,07	mNGF/proNGF (%)

mNGF/proNGF ratio

Progress in Brain Research, Vol. 146
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CHAPTER 7

ProNGF: a neurotrophic or an apoptotic molecule?

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