NGF and its receptor

Levi-Montalcini and Hamburger

- An excess of nerves, and death of the failures
- # dying neurons in DRG ~ 1/size of receptor field
  - More death if target tissue reduced
  - Less death if more target added, OR
  - If retrograde transport is blocked
- Nerve growth factor (salivary gland)
  - In pure form, decreases DRG death
  - Decreases death in other sensory neurons, but not all
  - Increases axonal/dendritic arborization

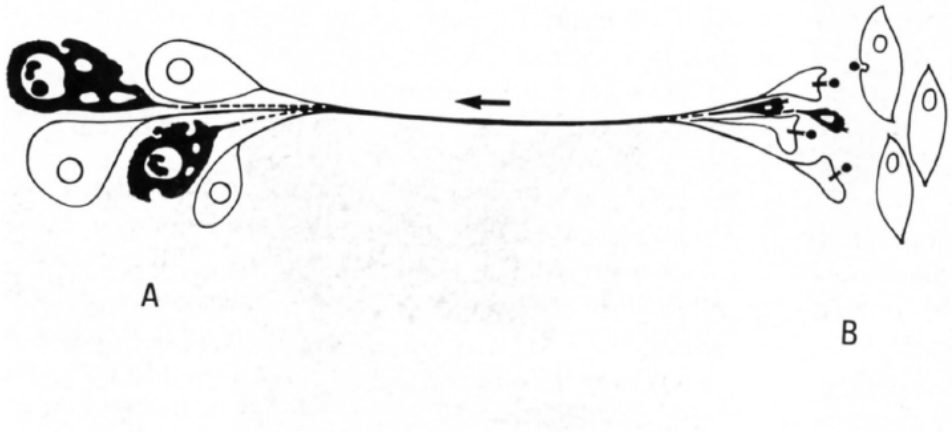
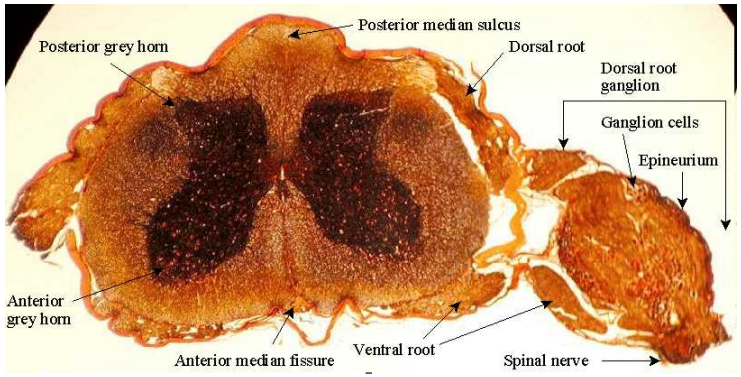


Figure 1. A Schematic View of the Target-derived, Trophic Support of Embryonic Neurons

(A) A group of neurons soon after their axons have reached their target cells (B). Note the two dark neuronal cell bodies degenerating together with their axons and terminals. (B) The target cells, site of synthesis of limiting amounts of a protein (●), necessary for the survival of the neurons. The arrow indicates the retrograde axonal transport of the protein released by the target cells, which is recognized and internalized by specific receptors (—) present on the nerve terminals.

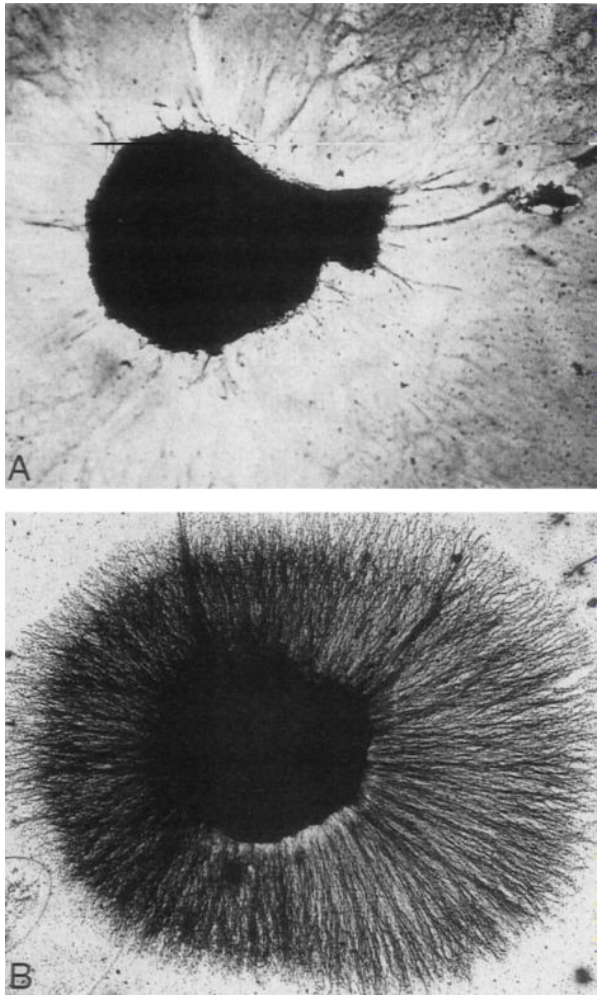
Levi-Montalcini and Hamburger

- Making a nervous system
  - Start with an excess of nerves
  - Death of the failures (and not failure to form)
- # dying neurons in Dorsal Root Ganglia
  - ~ 1/size of receptor field
  - More death if target tissue reduced
  - Less death if more target added, OR
  - If retrograde transport is blocked



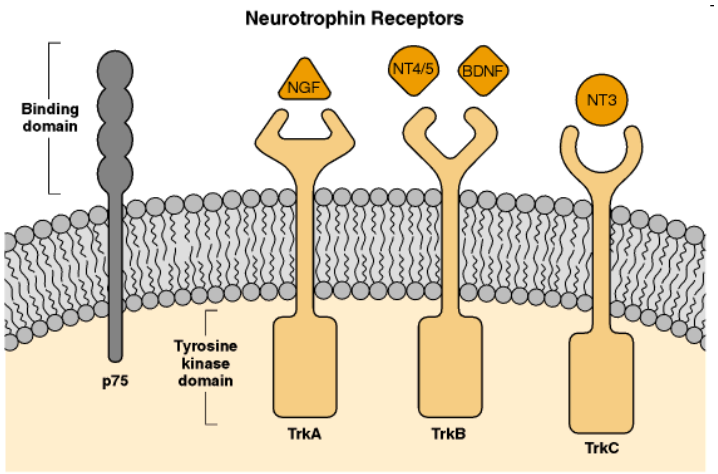
Protein properties

- NGF
  - Encourages neurite outgrowth in vitro
  - Larger precursor becomes smaller active product
  - Secreted (extracellular) protein
  - Made in target tissues, not in neurons



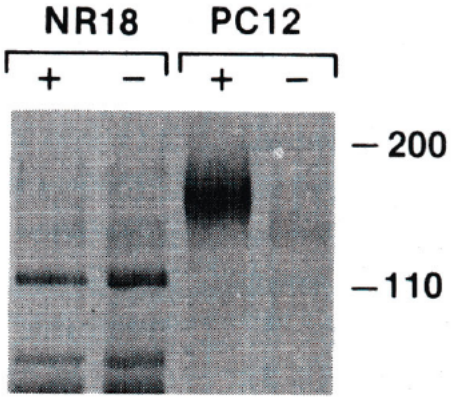
Protein properties

- Relatives
  - Brain derived Neurotropic Factor (BDNF)
    - Low concentrations
    - Complementary specificity
  - Others by molecular biology



NGF Receptor

- Nerve cells bind NGF with low (10^{-9}M) and high (10^{-11} M) affinity
- TrK
  - Oncogene rearranged in a colon carcinoma
  - Only expressed in neural crest cells in vivo (but PC12 cells in vitro)
  - Tyrosine kinase
- NGF induces autophosphorylation
- Crosslinking experiment
  - Band created containing NGF and TrK
  - Another with p75 NGFR



NGF structure (1BET)

- Seven β strands with lots of twist and disulfide "knot"
- Sulfhydryls (extracellular protein)
- No visible N or C terminal
- Where does NGF bind to receptor?
  - Postulate variable regions (to explain specificity)

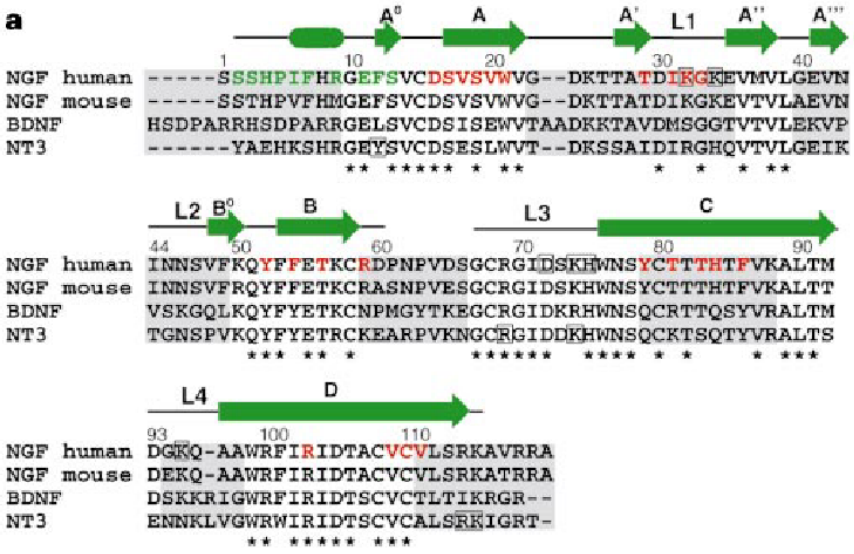


Figure 2 Sequence alignments. **a**, The neurotrophins. The numbering and secondary structure elements above the sequence refer to mature human NGF. Conserved residues

- Protein explorer (1BET)
  - (1BET) QuickView, Cartoon view
  - 1B8M (BDNF)
    - Note Dimer
    - Select :A, Display Only, Center/Cancel
- Variable regions
  - Advanced explorer/Seq3D/Display selected/Ball and Stick
  - Residues 29-35, 43-8, 92
- 1BET vs 1B8M
  - Spacefill; Color Polarity5

Ligand Receptor Interaction (1WWW)

- NGF interacting as dimer
- Dimer interface
  - Mix of hydrophobic and hydrophilic
  - Aromatic residue interactions

- Protein Explorer (1WWW)
  - Select :w,:v ; display only
  - W spacefill, Polarity 5; V cartoon
  - Reset; Hide X,Y; W, V Cartoon,
  - Select/Spacefill:
    - Phe49, Trp99
    - Trp21, Tyr52, Phe54; Phe86, Phe101
    - Phe12, Trp76
  - Edge/Pi interactions

- TrkA: Binding domain an IgG fold (β sandwich)
- Two binding surfaces
- Central (conserved) patch
  - Conserved Arg103
  - Phe327 stack, Carbonyl H-bond
- Red residues (conserved)

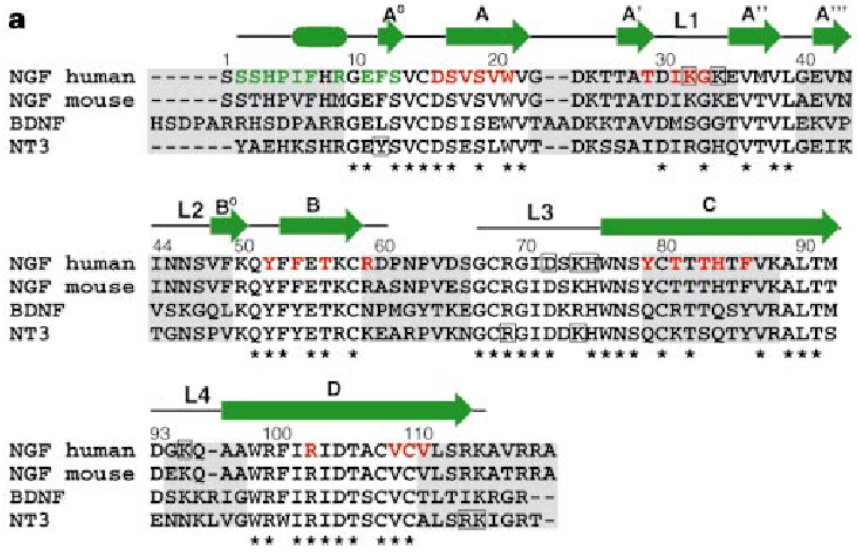
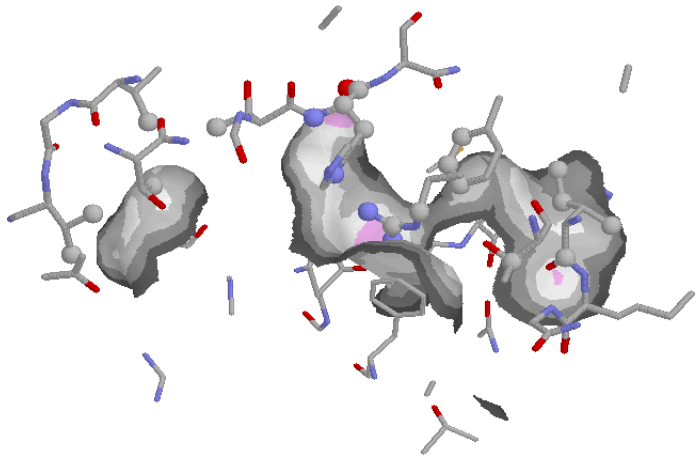


Figure 2 Sequence alignments. **a**, The neurotrophins. The numbering and secondary structure elements above the sequence refer to mature human NGF. Conserved residues

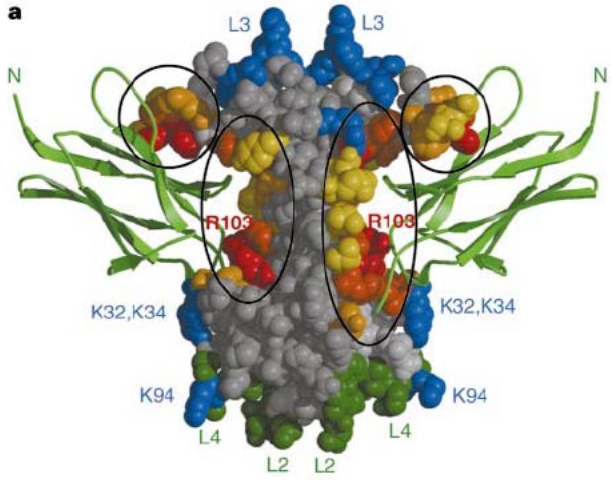
Ligand Receptor Interaction (1WWW)

- Protein explorer
- Quickviews (spin/water)
- Advanced explorer
- Contact surfaces
- :Y receptor; :W ligand
- Hide noncontact surface, show receptor atoms



Interaction cont'd

- N terminus of NGF visible!!
  - Only in complex
  - Short helix, well defined
- Buried in interface
- Both NGF and TrkA residues are not conserved
- Specificity surface



- Back/Reset View
  - Display/Cartoon; Color/N to C
  - Color/Temperature
- Advanced Explorer/Contact Surfaces
  - :Y Receptor
  - Ser2,Ser3,HIs4,Pro5,Ile6,Phe 7, Arg9 Ligand