Neurotrophin and its biological function

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The Development of Nervous System

Initiation
- Neuron
- Axon
- Repellent
- Attractant
- Target

Repulsion
- Target

Attraction
- Target

Targeting
- Target

Development
The Discovery of Nerve Growth Factor (NGF)

• Rita Levi-Montalcini (1986, Nobel Prize for Physiology or Medicine) and Victor Hamburger

• NGF saga
  1934 wing bud extirpation on CNS development in chick embryos
  1939 limb transplantation on spinal cord and sensory ganglia
  1944 effects of peripheral factors on proliferation and differentiation of spinal cord
  1952 mouse sarcoma 180, 37 promote neural growth
  1954 golden halo- a bioassay for nerve-growth promoting factor
  1956-1960 with Stanley Cohen, isolation of NGF
Ablation experiments

Transplantation Experiments

Conclusion: **Targets of innervation secrete limited amounts of survival factors that ensure a balance between tissue size and innervation.**
Receptors for NGF

• Two receptors: TrkA and p75NTR
• TrkA: a single-pass transmembrane receptor tyrosine kinase (RTK)
• p75NTR: a transmembrane glycoprotein

Kinetic characterization of NGF interactions with TrkA and with p75NTR demonstrated that, although dissociation constants for each receptor are very similar, the kinetics are quite different. NGF associates with and dissociates from p75NTR much more rapidly than from TrkA, and the presence of p75NTR increases the rate of NGF association with TrkA.
NGF

- 7S NGF complex and 2.5S NGF

Nature 401: 184-189, 1999
Figure 2  Interactions of neurotrophins (NT) with Trk and p75<sup>NTR</sup> receptors.

All neurotrophins share 50% sequence identity by pair-wise comparison
TrkB has 88% conserved sequence for intracytoplasmic domain compared with TrkA
Furin cleavage in Golgi or convertase cleavage in secretory vesicles.
Upon ligand binding, Trk receptors undergo dimerization and autophosphorylation on several key tyrosine residues, which then serve as docking sites for a variety of effector proteins including Shc, PLC-γ, FRS-2 and APS.

Major signaling pathways initiated by activated Trks in neurons include the Raf-MAPK pathway and PI3-K/Akt pathway.
Effects and Functions of NGF

• Rationale: Both NGF and its receptors are produced during development, adult life, and aging by many cell types in the CNS, PNS, immune system and various tissues. Therefore, different functions might be played by NGF.

• So, how many functions? How to study it?
Effects and Functions of NGF
NGF function: (1) neuron survival

- Target-derived neurotrophic factor paradigm: NGF released by postsynaptic targets acts on presynaptic neurons to build or maintain functional contacts and enhance the function of well-defined neural circuits.
How retrograde signal is transduced over the long distance in neurons from distal axons to bodies?

- **Fact and Rationale:** Quantitative theoretical analysis reveals that under typical protein diffusion rates and cytoplasmic phosphatase activity levels, activated MAP or other tyrosine kinases should be dephosphorylated to basal levels within a few microns of the site of activation.

- **Four models:**
  1. Lateral wave propagation model (Domino model)
  2. Signaling endosome model
  3. Retrograde effector model
  4. Multivesicular body with packed phosphorylated kinase
Models for neurotrophin retrograde signaling
Following binding of NGF to its receptor tyrosine kinase TrkA, a variety of signaling molecules are recruited to the receptor complex. Likewise, elements of the clathrin-mediated endocytosis pathway are recruited to the activated receptor and serve to promote the formation of clathrin-coated vesicles containing NGF bound to activated TrkA associated with such signaling effectors as Ras and Erk. Following uncoating, early endosomes marked by EEA1 containing NGF bound to activated TrkA and associated with Ras and Erk, along with the motor protein dynein, are retrogradely trafficked along microtubules from the axon tip to the neuron cell body.
Models of organelles involved in retrograde axonal transport
Retrograde transport of NGF was first demonstrated in 1974 by injecting $^{125}$I NGF into the anterior chamber of the mouse eye. The radial fibers of iris is innervated by ipsilateral SCG.
How to study retrograde transport in neuron?

--- Compartmented cultures of neurons

**Figure 1** Compartmented cultures of rat sympathetic neurons. (A) Schematic diagram of a Teflon divider seated into a 35 mm tissue culture dish with silicone grease, dividing it into a center compartment, consisting of a slot communicating with the perimeter of the dish, and left and right distal compartments. The series of horizontal lines represents scratches made in the dried collagen.
(A) The dependence on NGF for cell survival suggest an action on the cell soma. When sympathetic neurons are grown in a three-compartment culture chamber, NGF is required in the central compartment where the cell bodies of the neurons are located.

(B) After neurites have grown into side compartments, NGF can be removed from the central compartment and the neurons are still alive, provided that side compartments contain NGF. These results demonstrate that NGF can be up-taken by axons and transported back to the soma.
Experimental results in support of signaling endosome model

1. NGF itself is retrogradely transported.
2. Phosphorylated trkA is retrogradely transported dependent on endogenous target-derived NGF.
3. Application of NGF in distal axons of compartmentalized neuron results in accumulation of P-trkA in soma.
4. Co-IP showed retrograde co-transportation of P-trkA and NGF.
5. Inhibition of ligand-receptor internalization by NGF-cross-linked to beads or dominant-negative dynamin prevented the appearance of P-CREB and P-ERK5 in soma.
Inhibition of TrkA kinase activity by K252a (100 nM) either in cell bodies, in distal axons, or in both leads to apoptotic cell death of sympathetic neurons supported by NGF (10 ng/ml) applied on distal axons.
Figure 5. Blocking Internalization and Retrograde Transport of NGF by K44A Dynamin Results in Increased Apoptotic Cell Death in Sympathetic Neurons Supported by NGF Added only to Distal Axons
NGF function: (2) Local axonal growth

Axon growth requires interplay of many factors.
1. Produce cytoplasmic membrane and proteins in proper proportion
2. Correct vesicle and protein targeting
3. Proper dynamic response of growth cone to environmental cues

Campenot Chamber
Dissect molecules acting on axons from acting on cell body

Genes and Development, 17: 941, 2003
CNTF + BDNF

P8 RCG infected with adeno-Bcl2

Neuron 33, 689-702
Function 3: Neurotrophins and synaptic plasticity

- **Synaptic plasticity and memory hypothesis**: Activity-dependent synaptic plasticity is induced at appropriate synapse during memory formation, and is both necessary and sufficient for the information storage underlying the type of memory mediated by the brain area in which the plasticity is observed.

- **MOLECULES responsible for synaptic plasticity**

  **Three criteria:**
  
  (1) express in the right place and at the right time for the form of synaptic plasticity being considered.
  
  (2) Expression must be activity-dependent.
  
  (3) It must regulate aspects of neuronal function that change activity in neural circuits, including synaptic function, membrane excitability, and neuronal morphology and connectivity.
Function 3: Neurotrophins and synaptic plasticity

- Hebb Hypothesis: coincident activity in the pre- and postsynaptic neurons of a synapse underlies the regulation of synaptic transmission.----Use dependent synaptic plasticity

- Shatz: During development, patterns and levels of activity forms the basis of competition between different axons for postsynaptic targets by stabilizing and elaborating coincident input and weakening and removing noncoincident inputs.
• Kandel: particular patterns of coincident activity in the pre- and post-synaptic cells induce both short-term and long-term synaptic changes, providing potential mechanisms for learning and memory.

• MOLECULES responsible for synaptic plasticity
  Three criteria: (1) express in the right place and at the right time for the form of synaptic plasticity being considered. (2) Expression must be activity-dependent. (3) It must regulate aspects of neuronal function that change activity in neural circuits, including synaptic function, membrane excitability, and neuronal morphology and connectivity.
Neurotrophins serve as modulators of synaptic plasticity

(1) Neurotrophins are expressed in cortex, hippocampus, and cerebellum

(2) Neuronal activity regulates neurotrophin mRNA level.
   In dissociated hippocampal neuronal culture, depolarization by glutamate receptor agonists or high potassium increases the level of mRNA encoding BDNF and NGF. Conversely, BDNF and NGF levels are downregulated in the presence of GABA through activation of GABA$_A$ receptors.

(3) Neurotrophins in plastic change:
   a. Acute effect on synaptic transmission
   b. Permissive role of BDNF for hippocampal LTP
   c. Instructive effects on electric excitibility by regulating the expression levels of voltage-gated ion channels.
   d. Neurotrophin influence the complexity of axons arbor and the form of dendritic tree.
   e. Synapse numbers, size, and maturity
Opposing Roles for Endogenous BDNF and NT-3 in Regulating Cortical Dendritic Growth

Cortical pyramidal neurons layer 4 in visual cortex
Neurotrophins and their receptors

All neurotrophins are capable of binding to p75NR, and each also binds to a specific Trk receptor: NGF binds to TrkA, BDNF and NT-4/5 to TrkB, and NT-3 to TrkC.
How the various biological effect of neurotrophin is cell-type-specifically regulated?

- How a very large number of distinct biological events are triggered by limited repertoires of growth factors, receptors, and signaling pathway???
  - type of receptors
  - differential splicing
  - the presence or absence of pan-neurotrphin receptors-p75NTR
  - The differential endocytosis
  - Tempo of stimulation
  - Location and direction of signaling
The N termini of neurotrophins are important in controlling specificity, and the structure of this region is reorganized upon binding to a Trk receptor. Interactions with Trk receptors also alter neurotrophin structures in other regions. This deformability appears important for permitting some neurotrophins to activate more than one type of Trk receptor.

Each of the extracellular domains of Trk receptors helps to modulate ligand binding, either by directly interacting with neurotrophins or by modulating conformational changes in the ligand-binding Ig-2 domains of these receptors. Each of the extracellular subdomains also modulates receptor dimerization through interactions that are poorly understood.
All Trk family members have highly homologous intracellular domains. The sites of phosphorylation, and the nature of the pathways activated are similar.

Differences exists for signaling initiated by distinct Trk receptors. Example: proprioceptive neurons in DRG: TrkC/NT3--- Akt --- for branching and increased axonal caliber

nociceptive neurons in DRG: TrkA/NGF--- Raf--- for axonal elongation

Neuron 35:65-76, 2002
Insert presence: increase NT3 binding to TrkA, absent, only NGF.
Insert + : NT4, NT3, BDNF to TrkB
Truncated trk: trans expressed, enhance endogenous activity; cis expressed, dominant negative effect.
TrkC kinase insert: change substrate specificity.

**Figure 2** Interactions of neurotrophins (NT) with Trk and p75\textsuperscript{NTR} receptors.
Trk receptor specificities are altered by the p75 NTR

BDNF, NT-3 and NT-4/5 can each bind to the TrkB receptor, but in the presence of p75 only BDNF provides a functional response. Likewise, NGF and NT-3 both can bind to TrkA, but p75 restricts signaling of TrkA to NGF and not to NT-3.
The presence of p75NTR favors a conformation of TrkA that has a high-affinity binding site for NGF

1. mutations of the cytoplasmic or transmembrane domains of either TrkA or p75NTR prevent the formation of high-affinity binding sites on TrkA
2. An intact ligand-binding site in p75NTR is not essential for it to promote high affinity binding.
3. In the presence but not in the absence of p75NTR, the presence of only the first cysteine-rich domain of TrkA in an TrkA-TrkB chimera is able to mediate NGF-dependent receptor activation, which also supports an allosteric model.
Variation II: Ligand specifies the response

Examples:

NPXY motif of TrkB: Y>F mutation
   no response to NT4 induced survival
   little effect on BDNF survival effect
      (Erk1,2 activated)

The experiment suggests that NT4 dependent signaling has a greater reliance on molecules such as SI

Proneurotrophins promote apoptosis
Summary

• In summary, most survival-promoting and differentiation-promoting responses to neurotrophins require the presence of a Trk receptor on a neuron, but the competence of a Trk receptor to convey appropriate signals to the interior of the cell is regulated by additional factors, which include the proportions of truncated or insert-containing receptors produced by differential splicing, the presence or absence of p75NTR, and second messengers that promote vesicle-mediated receptor insertion into the plasma membrane.
Variation III: Tempo of Stimulation

Example:
Pulse of NGF ---maximal activation of PLC --- increased transcription of sodium channel PN1--- increased synaptic electrical activity--- synaptic plasticity
Variation IV: Location and Direction of Signaling

Figure 3  Location selectivity in Ras/MAPK pathways. Trk's are able to activate
Both NGF and NT3 signal through TrkA on the axons of developing sympathetic neurons. But NGF supports survival and differentiation, NT3 does not.

The explanation is that NT3 and NGF might signal at different location and developmental times.--- not proved.

Figure 1. Differences in NGF and NT-3 Signaling The figure illustrates initial binding of NGF (red) and NT-3 dimers (orange) to surface TrkA, resulting in surface signaling and axon growth promotion. NGF-TrkA dimers alone are endocytosed into signaling endosomes that are transported to the cell body where they signal to promote neuronal survival and differentiation.
Differential use of signaling components

Figure 3  Location selectivity in Ras/MAPK pathways. Trks are able to activate...
Figure 4  PI3 kinase pathways are critical for survival. Trk receptors recruit and stim-
Figure 5  PLC-γ at the synapse. Trk receptors may be present at both pre- and postsynaptic sites, where they can be activated by BDNF (filled circles), which is released in response to electrical activity. A pulse of BDNF preferentially activates
p75NTR Signaling Module

Neuron 42:529-533, 2004
Figure 2  Schematic diagram of p75NTR-mediated signal transduction pathways.