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Commentary

Nerve growth factor (NGF) regulates many aspects of neuronal biology by retrogradely propagating signals along axons to the targets of those axons. How this occurs when axons contain a plethora of proteins that can silence those signals has long perplexed the neurotrophin field. In this issue of the *JCI*, Li et al. suggest an answer to this vexing problem, while exploring why the Elp1 gene that is mutated in familial dysautonomia (FD) causes peripheral neuropathy. They describe a distinctive function of Elp1 as a protein that is required to sustain NGF signaling by blocking the activity of its phosphatase that shuts off those signals. This finding helps explain the innervation deficits prominent in FD and reveals a unique role for Elp1 in the regulation of NGF-dependent TrkA activity.

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(H)Elping nerve growth factor: Elp1 inhibits TrkA's phosphatase to maintain retrograde signaling

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Nerve growth factor (NGF) regulates many aspects of neuronal biology by retrogradely propagating signals along axons to the targets of those axons. How this occurs when axons contain a plethora of proteins that can silence those signals has long perplexed the neurotrophin field. In this issue of the *JCI*, Li et al. suggest an answer to this vexing problem, while exploring why the Elp1 gene that is mutated in familial dysautonomia (FD) causes peripheral neuropathy. They describe a distinctive function of Elp1 as a protein that is required to sustain NGF signaling by blocking the activity of its phosphatase that shuts off those signals. This finding helps explain the innervation deficits prominent in FD and reveals a unique role for Elp1 in the regulation of NGF-dependent TrkA activity.

24 25 26 Innervation deficits in familial 27 dysautonomia

28 Familial dysautonomia (FD), also called
29 hereditary sensory and autonomic neuro-
30 pathy type III, is a genetic disorder that
31 affects the development and survival of
32 nerve growth factor-dependent (NGF-
33 dependent) sympathetic and sensory neu-
34 rons of the peripheral nervous system.
35 Individuals with FD exhibit a range of
36 perturbed autonomic (hence dysautono-
37 mia) and nociceptive phenotypes (1). FD
38 is caused by mutations in the Elp1 (IKB-
39 KAP) gene, best known as a scaffolding
40 component of the Elongator complex
41 that regulates tRNA modification and
42 therefore translation, and for its role in
43 normal transcriptional elongation (1–3).
44 Relevant to neuronal function, Elongator
45 is also required for appropriate neuronal
46 branching, organization of actin networks,
47 and acetylation of α -tubulin. Loss of Elp1
48 results in mitochondrial dysfunction (1–4).

50 ▶ Related Article: p. 2478

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55 and Koren-Tillery LLP, and has received or currently receives research support from QPharma and AC Immune. WCM holds
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A locus of action outside the nucleus is supported by evidence that Elp1 is readily detected in the cytosol. FD mouse models in which Elp1 is mutated or deleted show profound peripheral nervous system (PNS) and central nervous system (CNS) innervation deficits that recapitulate the FD human phenotype (5, 6). Given the many consequences of Elp1 mutation, it has been difficult to determine how and why the PNS is severely disrupted in FD.

The neurotrophic factor hypothesis is a classic concept in neurobiology first proposed by Levi-Montalcini and Hamburger (7) that states that developing neurons compete with each other for a limited supply of a neurotrophic factor provided by the target tissue. Successful competitors survive and innervate the target tissue, while unsuccessful neurons die. NGF is the prototypical target-derived factor, binding receptors at axon terminals innervating the target and transmitting its signals down the

axon toward the cell body to support the survival of neuronal cell bodies and locally to support sprouting. Similarly, adult sensory, sympathetic, and basal forebrain cholinergic neurons depend on target-derived NGF for axon and dendrite growth and the acquisition and lifelong maintenance of neuronal specification and neurotransmitter phenotypes. For decades, the neurotrophin field has focused on how retrograde signals are transmitted over remarkably long distances. For example, the cell bodies of sensory axons that innervate our fingertips and are responsible for thermo- and pain sensations are located near the spinal cord, up to a meter or more away (think of a giraffe). How does this extended signaling occur? NGF first binds to the TrkA receptor tyrosine kinase at nerve terminals, which changes the conformation of the TrkA dimer. This enables TrkA to transphosphorylate tyrosine residues on each monomer of the receptor, including on key tyrosine residues that function as recognition sites for intracellular signaling proteins that associate with and are phosphorylated and activated by TrkA (8, 9). The best known TrkA-bound protein is Shc, which activates the Ras-MAP kinase and Ras-PI3-kinase signaling pathways that mediate axonal and dendrite growth and cell survival (10). How then does target-derived NGF transmit its signals over long distances? Upon internalization, the NGF-TrkA complex is localized to membrane-bound organelles that are retrogradely transported toward the cell body by a microtubule-based motor system (11–13). TrkA is oriented with its ligand-binding domain inside, and its cytoplasmic kinase and substrate-bound signaling proteins outside of the organelle. In this manner, TrkA signals locally as it travels down the axon to stimulate axonal growth. Upon arriving at the cell body, the signaling endosome releases its contents to activate gene expression responsible for survival, growth, and neuronal specification. Dis-

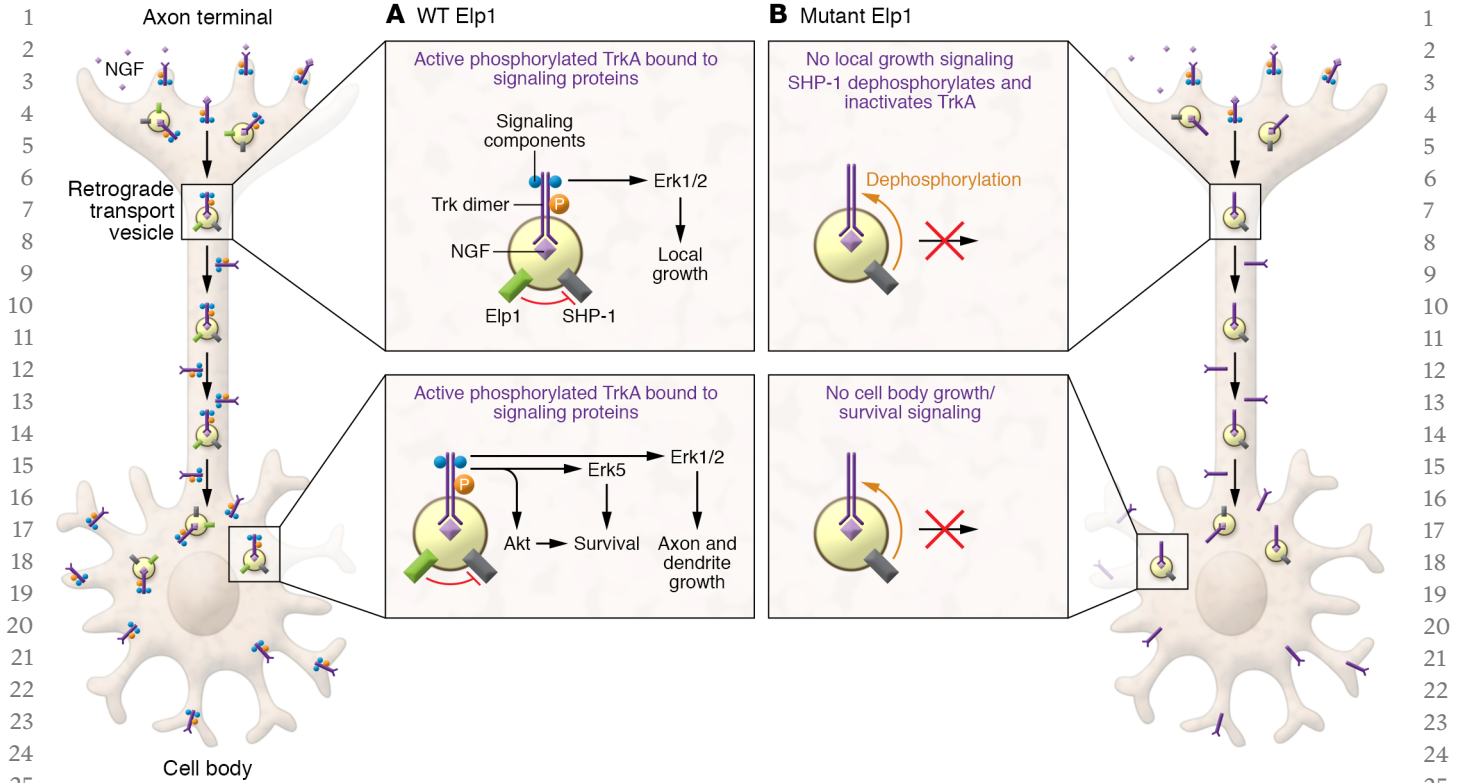


Figure 1. Elp1 in the regulation of NGF-dependent TrkA activity. (A) Retrograde signaling in WT axons. Elp1 suppresses SHP1 phosphatase activity and enables NGF-mediated neuronal innervation and survival. (B) In the absence of Elp1, SHP1 dephosphorylates retrogradely transported TrkA and inhibits NGF-mediated neuronal innervation and survival.

31 ruption of activated TrkA endocytosis,
32 transport, or activity during the transport
33 process results in cell body apoptosis, indi-
34 cating the key role of retrograde signaling
35 endosomes — as essential carriers of NGF
36 signals. Notably, a portion of the activated
37 TrkA-containing endosomes traverses the
38 cell body and translocates to dendrites
39 where it mediates circuit formation and
40 synapse maintenance (14).

41
42 **Maintaining TrkA activity**
43 **through distance and time**

44 A puzzling question, however, is how TrkA
45 activity is maintained during endosomal
46 transport from nerve terminals to cell
47 bodies over long distances and in a pro-
48 cess whose duration can be many hours.
49 All signaling processes reversibly regu-
50 lated by phosphorylation require a kinase
51 that adds phosphates to proteins and a
52 corresponding phosphatase that removes
53 phosphates and inactivates the kinase-
54 activated signaling protein. Indeed, inhi-
55 bition of tyrosine phosphatase activity
56 elevates and sustains TrkA signaling both
57 in the presence and absence of NGF, indi-

cating that phosphatases normally keep
TrkA in the inactive state when not bound
to NGF and modulate and prevent TrkA
from overactivity following NGF binding (15). Overactive TrkA that induces
aberrant sprouting and nociceptive
responses is deleterious. TrkA possesses
several important tyrosine phosphorylation
sites, and at least eight different protein
tyrosine phosphatases are known to
dephosphorylate TrkA and suppress
signaling. Even more problematic is the
NGF-dependent association of TrkA
with SHP1 (also called PTPN6), a potent
phosphatase. SHP1 keeps TrkA in an off-
state in the absence of NGF and modu-
lates TrkA activity after activation by
NGF (15). One way to maintain TrkA
phosphorylation is to shift the balance of
the kinase-phosphatase relationship that
occurs when NGF binds and activates
TrkA kinase activity, overwhelming the
activity of nearby phosphatases. Howev-
er, since as few as one NGF molecule are
present in a signaling endosome that pre-
sumably contains many more activated
TrkA receptors (16), another mechanism

must suppress the activity of TrkA phos-
phatases during the retrograde transport
process.

The correspondence between the sensory
and sympathetic neurons affected in
FD patients and those neurons known to be
responsive to NGF produced in their targets
of innervation has long suggested a role for
NGF and NGF signaling in this disorder (1).
In this issue of the *JCI*, Li et al. (17) explored
the role of Elp1 in retrograde neuronal
signaling and survival. The researchers gener-
ated mice engineered to express the human
Elp1 mutation together with the WT Elp1
gene. The WT Elp1 gene was subjected to
conditional ablation by tamoxifen addition
to cultured sympathetic neurons from the
mice. Neurons only expressing the mutant
Elp1 failed to survive to the same extent as
WT neurons when NGF was added to axon
terminals in compartmented chambers,
and survival to WT levels by retrograde
NGF signaling was rescued when WT Elp1
was reexpressed in those neurons. While
NGF induced the same tyrosine transphos-
phorylation and internalization of TrkA at
axon terminals and TrkA retrograde endo-

1 somal transport in mutant and WT Elp1
2 neurons, TrkA was rapidly dephosphor-
3 ylated only in the Elp1 mutant neurons.
4 The appearance of specific phosphorylated
5 and activated signaling proteins in the cell
6 body, indicates retrograde NGF signaling.
7 However, none of these phosphorylated
8 proteins (Erk1/2, Erk5, Akt and CREB,
9 which are required for axonal growth or
10 survival) were observed in the Elp1 mutant
11 cell bodies following application of NGF to
12 the distal axons. In contrast, TrkA and TrkA
13 substrate phosphorylation was maintained
14 for up to five hours in WT neurons. Elp1 is
15 thus required to propagate NGF-dependent
16 retrograde signals and neuronal survival
17 (17). How then does Elp1 function to sus-
18 tain NGF signaling? The authors intuited
19 that Elp1 might suppress TrkA phosphatase
20 activity, and they focused on the previ-
21 ously identified TrkA-associated tyrosine
22 phosphatase SHP1 (15). NGF induced the
23 association of Elp1 and SHP1 with TrkA
24 and their recruitment to TrkA-containing
25 transport endosomes. Though SHP1 phos-
26 phatase was still recruited to the TrkA sig-
27 naling complex in Elp1 mutant neurons,
28 it was hyperactive; and inhibiting SHP1
29 activity or expression rescued the deficits
30 in retrograde NGF-mediated survival in
31 these neurons. Thus, at least in culture, the
32 consequence of the FD Elp1 mutation was
33 SHP1 hyperactivation that dephosphorylat-
34 ed retrogradely transported TrkA, resulting
35 in decreased NGF-mediated survival (ref.
36 17 and Figure 1).

37 A prominent human FD phenotype,
38 however, is deficits in sympathetic target
39 innervation. To begin to address whether
40 hyperactive SHP1 is responsible for this
41 phenotype, the authors examined neo-
42 natal mice with Elp1 specifically ablated
43 in sympathetic neurons. The neurons in
44 those Elp1-deficient mice showed dimin-
45 ished TrkA phosphorylation and inner-
46 vation defects. Remarkably, infusion of a
47 SHP1-selective inhibitor rescued the defi-
48 cits in TrkA phosphorylation and sympa-
49 thetic neuron innervation, consistent with
50 a unique and required role for Elp1 in reg-
51 ulating NGF-dependent TrkA activity and
52 neuronal innervation in FD.

53 Is the hyperactivation of SHP1 activ-
54 ity the major consequence of the FD Elp1
55 mutation on peripheral neuron innerva-
56 tion? A reduced velocity of retrogradely
57 transported NGF has been noted in sen-

sory neurons lacking Elp1, possibly due
to dysregulated Elongator activity that
can hyperacetylate α -tubulin and alter
protein translation (18). However, in the
present study, no deficits were observed
in the expression of TrkA and TrkA signal-
ing proteins, and there was no substantial
reduction in the amount of retrogradely
transported TrkA. The widespread CNS
phenotypes of patients with FD, including
seizures, visual and learning impairments,
heightened anxiety, and white and grey
matter structural deficits, suggest that the
Elp1 modulation of Elongator that medi-
ates many general cell functions (1, 3, 5) is
more likely to be mechanistically responsi-
ble for these perturbations.

Next steps

The next step is understanding how Elp1
maintains TrkA activity during retrograde
transport. For example, does Elp1 inhibit
SHP1 activity directly, outcompete SHP1
from binding to TrkA at its association site
(Y490), or sterically bind to and prevent
SHP1 from dephosphorylating TrkA? Ret-
rogradely transported TrkA is not only pro-
tected from SHP1 by Elp1, but from all of
the many TrkA phosphatases. This protec-
tion suggests that Elp1 has a more general
role as an inhibitor of TrkA dephosphor-
ylation. Whether Elp1 also inhibits TrkA
dephosphorylation by the SHP1 relative
and TrkA phosphatase SHP2 (19) would be
helpful to know. Other questions are: How
are Elp1 levels and association with TrkA
regulated by NGF? Does Elp1 in the ret-
rograde transport complex function as an
Elongator regulator when it arrives in the
TrkA signaling endosome at the cell body?
Finally, is Elp1 involved in determining the
levels and temporal activity of TrkA in other
neurons, including NGF-dependent basal
forebrain cholinergic neurons important for
attentive learning and memory or neurons
that utilize TrkB for synaptic plasticity? Elp1
emerges as a prime candidate to not only
preserve TrkA activity during long-distance
transport, but as a fine-tuner of Trk activity
by regulating Trk phosphatases. It will be
exciting to further explore the spectrum of
Elp1-interacting receptor tyrosine kinas-
es, and what other Elp1-like proteins are
recruited to modulate TrkA activation.

The present study adds important
insights into the extraordinary ability of neu-
ronal cell bodies to sustain innervation of

distant targets through formation and trans-
port of signaling endosomes. Especially giv-
en these new disease-relevant observations,
this study makes a strong case for continued
attention to further elucidating this funda-
mental aspect of neuronal function.

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