# The evolutionary origins of antagonistic neurotrophin signaling

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A competitive balance between constructive and destructive developmental cues governs both the form and function of the vertebrate nervous system. In this issue, Foldi et al. (2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201607098) explore the evolutionary origins of these cues and report that in *Drosophila melanogaster* pro- and mature neurotrophins are capable of inducing death and survival pathways, respectively, by binding Toll receptor family members, which then recruit distinct sets of effector proteins.

Seminal work from Viktor Hamburger and Rita Levi-Montalcini established a major tenant of our modern understanding of nervous system development: the neurotrophic factor hypothesis (Hamburger and Levi-Montalcini, 1949). The findings and implications of this hypothesis include the following. (a) Neurons are produced in excess, and a percentage are eliminated over development. We now know that this overproduction and refinement is a common design principle of many nervous system components, including synapses and axons. (b) Neurotrophic (neuron nourishing) factors emanate from targets like the muscle and skin, which are required for neuron survival. If a target is removed or added, excess death or survival is observed, respectively. Stanley Cohen worked with Levi-Montalcini to purify NGF, the first of four neurotrophin family ligands discovered in vertebrates (Cohen, 1960). (c) Implicit within these findings is the notion of competition for construction/destruction decisions (e.g., survival/death, axon growth/degeneration, and synapse stabilization/restriction). We now appreciate that the decision to survive or die can often times be represented by two antagonistic signaling pathways. For example, in vertebrate sympathetic neurons, target-derived NGF binds an axonal receptor tyrosine kinase, TrkA, to mediate constructive events, whereas the tumor necrosis factor receptor (TNFR) family member $p75^{NTR}$ mediates destructive events. Importantly, $p75^{NTR}$ promiscuously binds all neurotrophins or can bind proneurotrophins using VPS10 family members like sortilin or Sorcs2 as coreceptors (Lee et al., 2001; Majdan et al., 2001; Nykjaer et al., 2004).

Although the molecular logic for how antagonistic constructive and destructive signaling pathways tune the number of axons, synapses, and neurons is becoming established for vertebrates, the evolutionary history of these pathways is less clear. A combination of bioinformatic and experimental analyses revealed a single Trk receptor and neurotrophin ligand in both the lancelet *Amphioxus* and the snail *Aplasia* (Benito-Gutiérrez et al., 2005; Kassabov et al., 2013). In 2008, Zhu et al. (2008) suggested that *Drosophila melanogaster* also encodes the neurotrophin ligands *Drosophila* neurotrophin 1 (DNT1), DNT2, and Spätzle (Spz). As might be expected, these are more distantly related to vertebrate neurotrophins than those identified in *Amphioxus* or *Aplasia*, but they retain a cystine knot that is characteristic of the neurotrophins, as evidenced further in this issue by Foldi et al. with new structural modeling. Moreover, these putative *Drosophila* neurotrophins are required for motor neuron survival and axon growth (Zhu et al., 2008). Importantly, there are no Trk receptor homologues in *Drosophila*, which has led some to argue that DNT1, DNT2, and Spz are not bona fide neurotrophin orthologues (Kassabov et al., 2013). If not Trk, what are the receptors that these DNTs use to promote the apparent neurotrophin-like activity? A follow-up study by McIlroy et al. (2013) elegantly showed that instead of Trk receptors, DNT1 and 2 display physical and genetic interactions between the Toll-6 and -7 to promote cell survival. Notably, although Toll receptors are widely regarded as being involved in innate immunity, the first Toll phenotype discovered was developmental. Indeed, Toll mutant *Drosophila* larvae produced an underdeveloped ventral portion so profound that when discovered, Christiane Nüsslein-Volhard exclaimed, "Das war ja toll!" (meaning, "That was great!"), leading to the receptor's naming.

Although DNTs acting through Toll receptors explain constructive neurotrophin signaling, an explanation for destructive pro- or mature neurotrophin signaling was unknown until now. In this issue, Foldi et al. (2017) suggest that like survival and axon growth signaling, death signaling is also mediated by DNTs and Toll receptors. They describe a three-tier hierarchy capable of interpreting survival versus death decisions through DNT–Toll signaling.

## Tier 1: Regulated processing

Vertebrate neurotrophins are synthesized in a pro- form that is incapable of binding to Trk family members but is capable of engaging a $p75^{NTR}$–sortilin family member complex to induce cell death and other destructive processes (Lee et al., 2001; Nykjaer et al., 2004). The conversion of proneurotrophin

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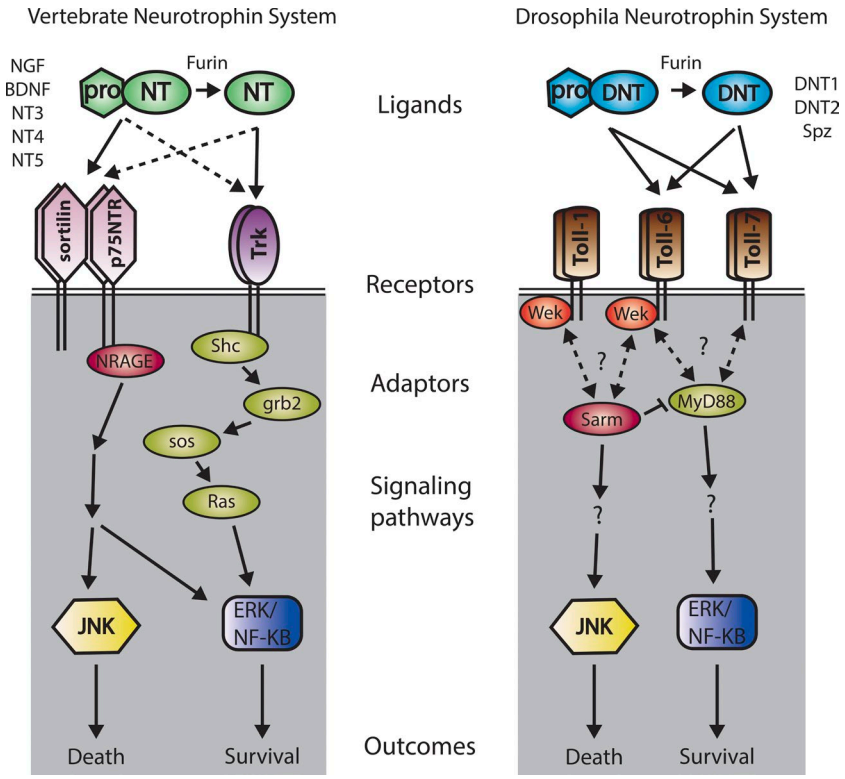


Figure 1. **Schematic comparing *Drosophila* and vertebrate neurotrophin signaling.** The work on the *Drosophila* neurotrophin system from the Hidalgo laboratory (Zhu et al., 2008; McIlroy et al., 2013; Foldi et al., 2017) shows dramatic similarities and parallels with the vertebrate neurotrophin system. Neurotrophic ligands exist in pro- and mature forms that enact destructive and constructive outcomes, respectively. These ligands bind with varying affinity to families of receptors: vertebrate sortilin–p75$^{NTR}$ and TrkA, TrkB, and TrkC and *Drosophila* Toll-1/-6 and Toll-6/-7 for cell death and cell survival, respectively. Depending on cell type, developmental stage, and potential coreceptors and interactors, these receptors signal differentially depending on adaptor proteins. Through these extrinsic and intrinsic features, a cell will either survive or undergo cell death. BDNF, brain-derived nerve factor; ERK, extracellular signal-related kinase; NFKB, nuclear factor κB; NT, neurotrophin; SOS, Son of sevenless homologue 1.

to mature neurotrophin is therefore a key regulatory step for construction/destruction decisions. Foldi et al. (2017) report that, similar to other species, pro-DNTs contain furin protease cleavage sites. Importantly, they observed secretion of both cleaved and uncleaved DNTs in S2 cells and also found that this processing occurred in vivo. Although DNT1 tended to be resistant to cleavage in vivo, DNT2 was processed efficiently. They found that pro-DNTs were capable of inducing death-associated signaling pathways like JNK, whereas mature DNTs were capable of activating survival-associated pathways like extracellular signal-related kinase.

### Tier 2: Preferential binding

Vertebrate pro- and mature neurotrophins bind to p75$^{NTR}$–sortilin or Trk receptors, respectively, to mediate distinct developmental outcomes. Do *Drosophila* pro- and mature neurotrophins also switch their preferences? Instead of binding to different sets of receptors, Foldi et al. (2017) demonstrate that pro- and mature DNT1 and 2 bind promiscuously to Toll-6 and -7, resulting in either cell death or survival. They leave open the possibility for a coreceptor mediating pro-DNT death signaling. However, Spz exhibited a preference for Toll-1, and this interaction primarily promoted death signaling. Nevertheless, indiscriminate binding between pro- and mature DNTs and Toll-6/-7 creates a question about how these ligands mediate survival and death pathways. The authors suggest a "DNT–Toll code," whereby survival depends on the composite expression (and possible heterodimerization) of the different Toll receptors, interactions with coreceptors that alter outcome, and the antagonistic effect of pro-/mature DNT binding. Future studies exploring this possibility will be informative for neurotrophin signaling across species.

### Tier 3: Differential recruitment of adaptor proteins

If a DNT–Toll code does exist, how are antagonistic downstream signals transduced in response to pro- and mature DNTs? Foldi et al. (2017) present evidence that recruitment of MyD88 to activated Toll receptors mediates survival, whereas recruitment of Sarm is required for death. One of the critical open questions for vertebrate neurotrophin signaling is the mechanism of cross talk between mutually antagonistic survival and death pathways. In their study, Foldi et al. (2017) demonstrate an elegant mechanism of cross talk whereby a scaffold protein, Wek, is recruited to Toll-6 receptors in particular developmental contexts. Importantly, although Wek can recruit both MyD88 and Sarm, in the "tug of war" for pathway dominance, Sarm seems to repress MyD88 to allow death signaling to "win."

It is tempting to speculate that Toll receptors may also play a role in vertebrate neurotrophic factor signaling. Classic evolutionary models of gene duplication and subfunctionalization would predict that vertebrate Toll-like receptors (TLRs) may have retained some neurotrophic signaling capacity. The argument against this is that TLRs largely expanded independently in *Drosophila*, with most of the nine *Drosophila* TLRs not having direct mouse or human orthologues. Despite this, these receptors may retain neurotrophic activity. Indeed, in a provocative supplemental experiment, Foldi et al. (2017) found that the neurotrophins NGF and brain-derived growth factor can stimulate nuclear factor κB transcriptional activity in HEK293T cells via vertebrate TLR4, which is normally expressed in the brain. This suggests that evolutionarily, neurotrophins did not simply swap Toll receptors for Trk and p75$^{NTR}$. Rather, they may have retained their ancient neurotrophic activity. This warrants further examination of Toll signaling in vertebrate development.

It is remarkable to consider that neurotrophic pathways could be conserved from insects to vertebrates using entirely different receptor systems but retaining similar ligands and downstream survival or death pathways (Fig. 1). As with the best studies, Foldi et al. (2017) bring about at least as many questions as they answer. Are classic Toll adaptor proteins co-opted by receptor tyrosine kinases or TNFR family members? Does a scaffold like Wek mediate survival/death cross talk? What is the role of Toll family members like TLR4 in nervous system development? This will be a fertile area of study for many years to come. Moreover, describing neurotrophin pathways in multiple model systems across disparate phyla will serve to speed up our molecular understanding of neural development within and across species.


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