

Evolving better brains: a need for neurotrophins?

Hanna Jaaro, Gad Beck, Silvestro G. Conticello and Mike Fainzilber

The NGF family of neurotrophins has a crucial role in regulating neuron numbers during vertebrate development. Six years ago the prediction was made that invertebrates with simple nervous systems, such as *Caenorhabditis elegans*, would lack neurotrophins. Surprisingly, it now appears that not only *C. elegans* but also *Drosophila melanogaster*, lack homologs of the neurotrophins or their trk receptors. Furthermore, functional studies indicate that control of neuronal numbers in *Drosophila* is primarily dependent on steroids. By contrast, a recognizable trk homolog exists in molluscs, a phylum that includes species with the most complex nervous systems in the invertebrate kingdom. This suggests that neurotrophic signaling mechanisms might be one of the prerequisites for evolution of complex nervous systems. Expansion of the genome projects to other invertebrates, such as molluscs and coelenterates, should provide new insights on the molecular correlates of building complex brains.

What is complexity in the nervous system? Can one define molecular characteristics for 'complex' brains versus 'simple' nervous systems? The recent bounty of genome sequence data provides the first comprehensive opportunity for neuroscientists to identify complexity-determining genes; for example gene families that are found or expanded primarily in organisms with more complex nervous systems. In order to do this, the attributes that are useful for distinguishing a 'complex' from a 'simple' nervous system, at least at a first approximation, must be defined. Recent reviews on this issue do not provide any easy answers^{1,2}, and in the words of Koch and Laurent 'While everyone agrees... that Albert Einstein's brain was more complex than that of a housefly, nervous system complexity remains hard to define quantitatively or meaningfully'³. Paraphrasing recent work in information theory⁴, one might be able to correlate nervous system complexity with the amount of information a brain stores about its environment. Although intuitively satisfying, this criterion is not easily measured across a wide-range of organisms, and surrogate criteria must be sought. One option is to compare the number of functional interconnections within a nervous system, for example, neuronal number multiplied by functional synapses multiplied by the number of distinct molecular interactions available at each synapse. However, because even this measurement is non-trivial, for our current purposes we will simply point out that neuronal

numbers are only one component of brain complexity. It follows that mechanisms that control neuronal numbers must have influenced the capacity to evolve complex brains. It is therefore of interest to look at gene families that are known to be involved in regulating neuronal numbers, such as the neurotrophins.

Neurotrophins and control of neuronal numbers

The NGF family of neurotrophins are protein growth factors with crucial roles in the determination of neuronal survival and regulation of neuron numbers throughout vertebrate development⁵. The classical neurotrophic hypothesis holds that neurotrophins are produced in limiting amounts and that survival of those innervating neurons is dependent on winning the competition for sufficient quantities of the factors⁶. The utility of this mechanism for regulating development of a large and complex CNS is readily apparent because it enables the system to 'build itself', while retaining a wide degree of inherent variability in its final size and connectivity. More recently there has been increasing appreciation of the importance of neurotrophins in plasticity and

...mechanisms that control neuronal numbers must have influenced the capacity to evolve complex brains.

learning processes in the adult CNS (Ref. 7) and in immune-nervous system interactions⁸. Thus, this gene family appears to regulate complexity of the nervous system on both the phenotypic and functional levels. Indeed, the pleiotropic roles of neurotrophins and other growth factors have generated much debate over the precise definition of the term 'neurotrophic'^{9,10}. In order to focus the forthcoming discussion, we will restrict our definition of neurotrophic mechanisms to its strictest sense – that is to regulation of neuronal numbers by a secreted growth factor.

Absence of neurotrophins outside the vertebrate lineage

Although neurotrophins have been characterized throughout the vertebrate lineage¹¹, to date there have been no reports of neurotrophin homologs in invertebrate phyla¹². Indeed Barde¹ made the prediction that short-lived, small-sized species would not require neurotrophic mechanisms for nervous system development or maintenance. The recent and imminent completion of genome sequencing for the NIH (Nematode, Insect, Human) pantheon of models^{13,14} provides a timely reference point to examine this hypothesis. We will start by reviewing work aimed at the discovery of neurotrophins and their receptors in invertebrates.

Hanna Jaaro
Gad Beck
Silvestro G. Conticello
Mike Fainzilber*
Laboratory of Molecular
Neurobiology, Dept of
Biological Chemistry,
Weizmann Institute of
Science, 76100 Rehovot,
Israel.
*e-mail: mike.fainzilber@
weizmann.ac.il

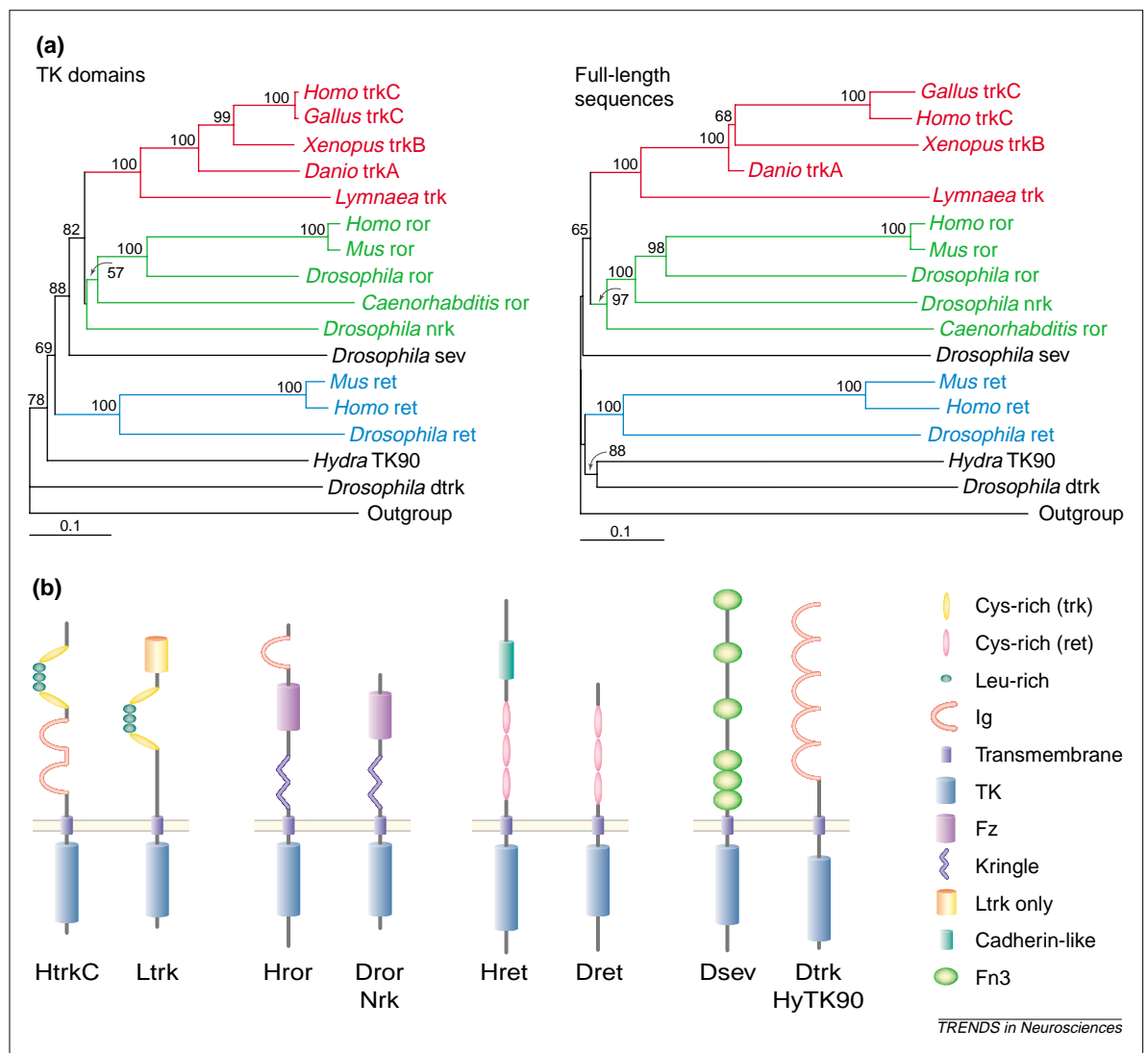


Fig. 1. Candidate neurotrophic receptor tyrosine kinases in invertebrates (a) Phylogenetic trees of 'neurotrophic' receptor tyrosine kinases (TK). *Drosophila* representatives in the trees are those for which e-values $< e^{-53}$ were obtained in Blast searches of the fly genome with the full-length open reading frame of human trkC; except for Dret and 'Dtrk', which were added to the alignments separately. In all cases the original published sequence was used for the tree except for Dret and 'Dtrk', for which sequences based on genomic clones were more complete (accession numbers CAB96180 and AAF58596, respectively). The outgroup is composed of receptor tyrosine kinases more distantly related to trkC than 'Dtrk'. The trees were generated by the neighbor-joining method after alignment of the sequences using the default parameters of CLUSTALX. (b) Schematic representations of the receptor tyrosine kinases present in the phylogenetic trees. Domains were defined according to the published descriptions of each receptor, updated and expanded by scanning the sequences through the InterPro database at EMBL (<http://www.ebi.ac.uk/interpro/scan.html>).

Early optimism tempered by 'empty' genomes

Initial studies on both insects and molluscs suggested the presence of endogenous neurotrophins or their receptors, based on antibody cross-reactivity, low-stringency hybridizations, or biological activities elicited in the test systems by murine NGF (Refs 15,16). In spite of this initial optimism and subsequent efforts by several groups to purify or clone invertebrate neurotrophins, no bona fide NGF

homolog was found. Misgivings about some of the early results were reinforced after invertebrate lectins were suggested as a probable source for false cross-reactivity of growth factor antibodies¹⁷. Completion of the *C. elegans* genome sequence confirmed the prediction that the 'hard-wired' nematode nervous system does not require neurotrophins or their receptors¹⁸. However, because the fly nervous system contains three orders of magnitude more neurons than that of *C. elegans*, neurotrophin homologs were still expected to emerge from the *Drosophila* genome project. Surprisingly, the recent 'essentially completed' *Drosophila* sequence¹⁹ seems to be devoid of these genes. This has been determined by searching the annotated genes in the fly database and by extensive Blast searches on the draft genome. By contrast, distant fly homologs of other growth factors can readily be found with similar Blast searches [e.g. dpp using human transforming growth factor β (TGF β); spitz and argos using mouse epidermal growth factor (EGF)]. None of the death-domain annotated fly genes are homologous to the p75 neurotrophin receptor. Finally, although several *Drosophila* receptor tyrosine kinases were identified

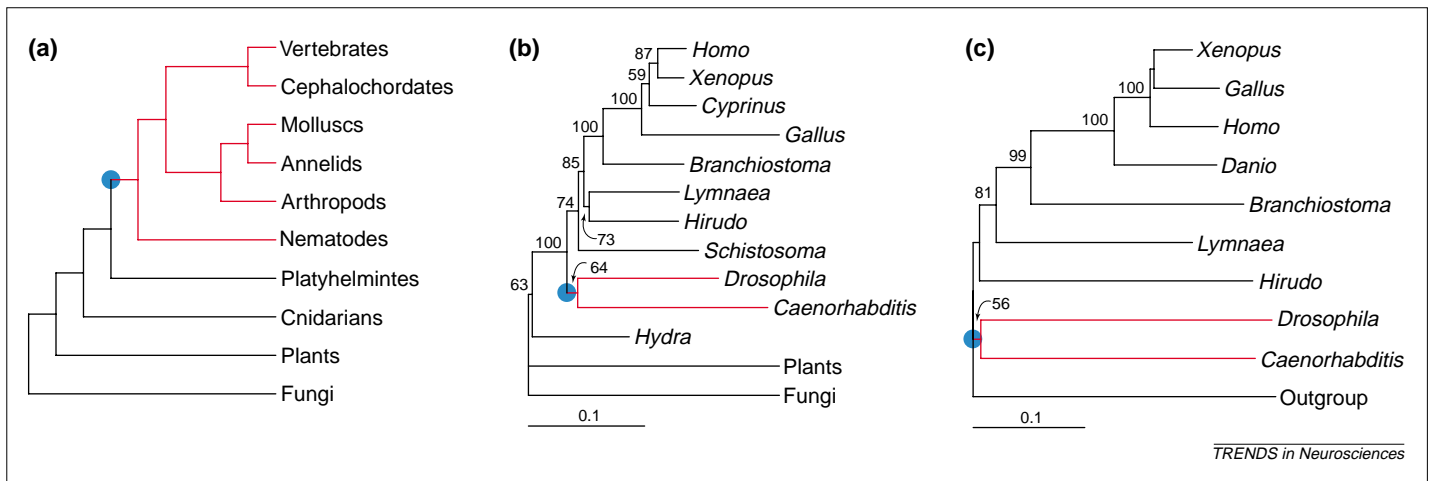


Fig. 2. Phylogenetic relationships of invertebrate model organisms. Comparison of 'classical' phylogeny (morphology-based) shown in (a) with molecular based phylogenies, rRNA tree in (b) and netrin family tree in (c). The rRNA tree was built from an alignment of small subunit ribosomal RNA (<http://rrna.uia.ac.be/>). The netrin family tree was built from an alignment of published netrin amino acid sequences together with a homolog from *Lymnaea stagnalis* (S.G. Conticello, and M. Fainzilber, unpublished data). The blue spots indicate the last common ancestor for the species joined by red branches. The netrin tree was generated by the neighbor-joining method after alignment of the sequences using the default parameters of CLUSTALX.

in Blast searches with vertebrate trks, none of them are clearly assignable to the trk family in phylogenetic trees, and there is no conserved extracellular domain structure characteristic for the trk family (Fig. 1). The fly receptor tyrosine kinase, originally called 'Dtrk' (Ref. 20) clearly belongs to a distinct family of cell adhesion kinases, together with a *Hydra* receptor called HTK90 (Fig. 1). This absence of trk receptors in *Drosophila* is in stark contrast to the conservation of many other ligands and receptors that are important in neuronal development²¹.

Neurotrophin concealed in the sequence gaps?

A caveat that should be noted is that the draft *Drosophila* sequence contains short (1–2 kb) gaps estimated to total 1–2% of the euchromatin portion of the genome¹⁹. Thus, there is a remote possibility that genes encoding neurotrophins and neurotrophin-receptor homologs might yet remain to be discovered in those gaps. However, this possibility is highly improbable, given the short lengths of these regions, and the assumption that at least one ligand and two receptor (*p75* and *trk*) genes are all included in these missing sequences. Furthermore, our group and others have expended much fruitless effort in a search for a bona fide *Drosophila* trk by homology-cloning techniques based on the highly conserved trk kinase domain. Some of these efforts resulted in the cloning of non-trk family receptor tyrosine kinases^{20,22,23} (Fig. 1). Thus, evidence for lack of a true *Drosophila* trk seems irrefutable. Nevertheless, evidence for the occurrence of trk receptors in other invertebrate phyla has been presented, mainly based on cross-reactivity of anti-trk antibodies^{24,25}. What then is the evidence for an invertebrate branch of the trk gene family?

The trk receptor family is evolutionarily ancient

The lack of neurotrophins and their receptors in both nematodes and insects might be attributable to loss of these genes during evolution, or conversely might be an indication that these genes were an innovation of the vertebrate lineage. Two independent lines of evidence suggest that the former is the case at least for the trk receptor family. First, a molluscan trk family member has been described from the snail *Lymnaea stagnalis*²⁶. This molecule, Ltrk, conserves intracellular signaling modules that are trk-specific and the characteristic array of cysteine-rich domains and leucine repeats in the extracellular domain (Fig. 1). By contrast, the IgG-like domains of vertebrate trks are not conserved in Ltrk, although some residues in the corresponding region of the Ltrk sequence might represent relics of an ancestral IgG domain²⁶. This suggests that the trk family originated before the separation of molluscan and vertebrate lineages, ~600 million years ago. Second, although classical morphology-based phylogeny would require independent trk gene loss in nematodes and insects, molecular phylogenies²⁷ place both these phyla on the same branch of the evolutionary tree (Fig. 2). Thus, the ancestral trk that must have existed before the branch point between mammals and molluscs, was probably lost in the common ancestor of nematodes and flies. Identification of a trk homolog in coelenterates would provide definitive confirmation of this hypothesis, and such efforts are currently underway in *Hydra* (A. Schaubmar, G. Dechant and C.N. David, pers. commun.). Indeed specific gene losses are not unprecedented in metazoan evolution, for example the lack of a voltage-dependent sodium channel and of Syk family kinases has been shown in *C. elegans*^{13,28}.

Because Ltrk and probably other trk family members are present in invertebrates, the question arises of the identity of the ligands for these receptors and whether such ligands might be neurotrophin homologs?

Could invertebrate trks bind ligands unrelated to NGF? Functional protein homology can be present at the level of three-dimensional structure in the absence of

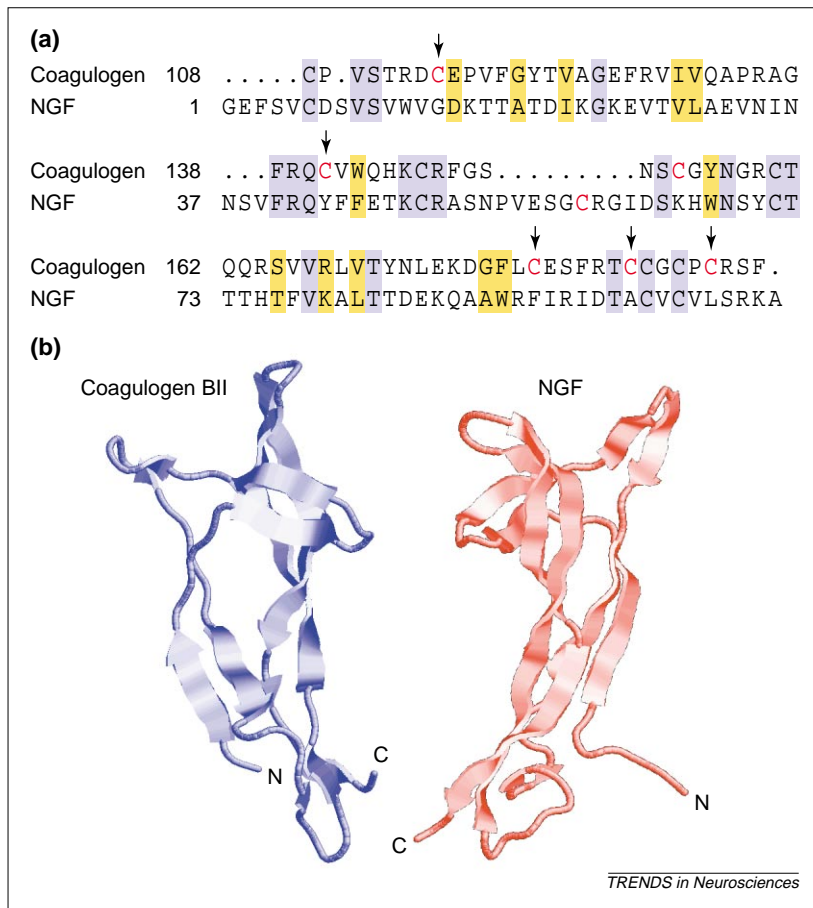


Fig. 3. A 'structural homolog' of NGF in invertebrates. Comparison of the BII domain of horseshoe crab coagulogen with mouse NGF. (a) shows amino acid sequence alignment based on sequence alone, without insights from the crystal structures. Blue indicates identity, yellow indicates similarity, and the arrows indicate non-alignable cysteine residues (red). (b) Ribbon representation of the crystal structures of the two monomers, juxtaposed to face each other. Note the same combination of secondary structure elements, and similar sizes and shapes of the loop regions, in spite of the lack of significant primary sequence homology.

obvious amino acid sequence homology. A striking example is the clotting protein coagulogen from horseshoe crab (*Limulus*), which contains a C-terminal domain with clear structural similarity to the NGF monomer, in spite of a lack of primary sequence homology²⁹ (Fig. 3). Thus, functional neurotrophin mimics could exist that are unrecognizable using homology-based methods. However, it should be noted that biological function does not necessarily follow structural similarity. The neurotrophins belong to a structural superfamily defined by a cystine knot and beta strands topology, which also includes the TGF β , platelet-derived growth factor (PDGF)-BB, and gonadotropin families³⁰. The structural fold shared by these distinct molecules acts as a scaffold for elaboration of distinct surface loops that provide the specificity for their diverse receptors and functions. Individual bonds of the cystine knot in gonadotropin have been suggested to be important for biogenesis and secretion, but not for bioactivity³¹. Therefore, an invertebrate molecule with a cystine knot motif should not automatically be considered as a

candidate neurotrophin. Indeed, in the case of coagulogen, the surface loops of the NGF-homologous domain are masked by the rest of the protein²⁹, apparently precluding a physiological role as a trk ligand.

Another gene family that has been suggested to be structurally analogous to the neurotrophins are the spatzie and trunk ligands in *Drosophila*. Molecular modeling and biochemical analyses of spatzie showed that a proteolytically processed 12 kDa C-terminal fragment could adopt a cystine knot structure close to that of coagulogen and NGF (Refs 32,33). However, the toll family of receptors for these ligands are not receptor tyrosine kinases, instead they belong to a different category of signaling molecules, and have primary roles in embryonic dorsoventral patterning and in innate immunity in the adult. Although defects in muscle development, motoneuron positioning or differentiation and targeting and synapse formation have been reported for various mutants in the spatzie-toll signaling pathway^{34,35}, these findings are not consistent with a role for spatzie in control of neuron numbers or other neurotrophic activities. Instead, this signaling system in *Drosophila* appears to act as part of a network of guidance and targeting cues in the development of motoneurons, muscle, and their connections³⁵.

'Missing' extracellular domains in invertebrate receptors

As noted above, the extracellular portion of Ltrk does not conserve all the domains that are characteristic of vertebrate trks. Several groups have localized the specific neurotrophin-binding sites on trk receptors to the second IgG domain³⁶, that is, precisely the region that is not conserved in Ltrk (Ref. 26). Although low-affinity neurotrophin binding has been shown for constructs of leucine repeat modules alone³⁷, and mammalian NT3 can bind molluscan Ltrk (Ref. 26), resolution of this issue must await identification of bona fide trk ligands in invertebrates. Nevertheless, it is conceivable that invertebrate and vertebrate ligands for the same receptor family might have diverged so as to become unrecognizable as homologs. Such a situation might also be the case for the ret tyrosine kinase in *Drosophila*. Mammalian ret is the shared signaling receptor for the glial cell line-derived neurotrophic factor (GDNF) family of ligands⁵, however the fly homolog³⁸ lacks a cadherin motif characteristic of vertebrate ret (Fig. 1). A clear GDNF sequence homolog is not apparent in the *Drosophila* genome, however recent experiments suggest that a fly protein not obviously homologous to GDNF might act as a ret ligand (H. Sariola, pers. commun.).

Having considered the possibility that an invertebrate receptor tyrosine kinase might 'dispense' altogether with its conventional ligand, one might further ask if there is any evidence for neurotrophic activities of completely different ligand-receptor pairs in invertebrates.

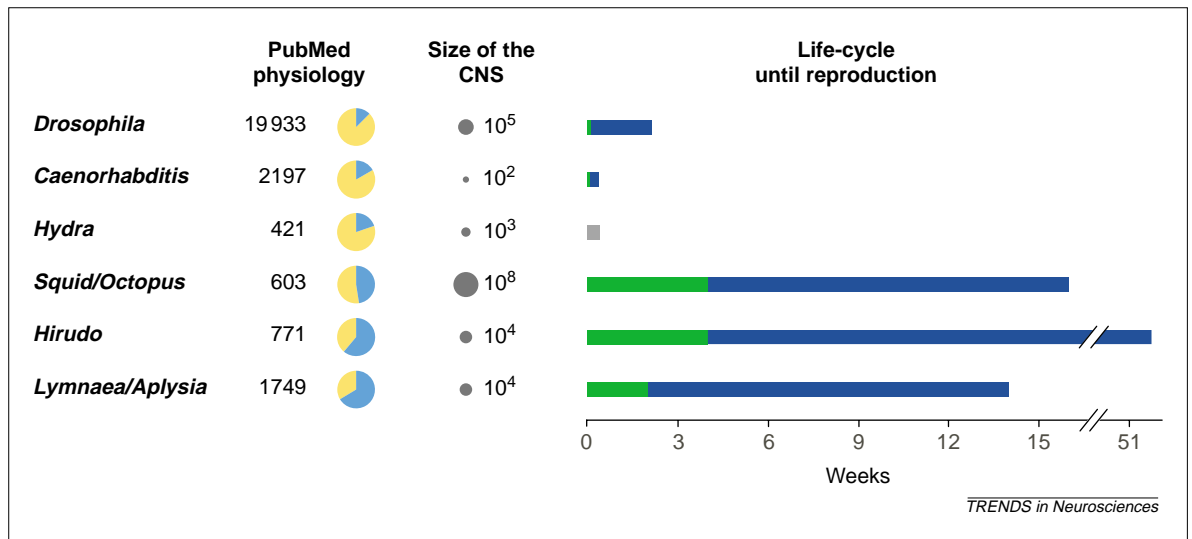


Fig. 4. Research focus, CNS size and life-cycle of invertebrate model organisms. Several commonly used invertebrate models were ranked according to the relative proportion of publications on the nervous system from the total publications indexed in PubMed under the MeSH term physiology (pie charts). We included only those models for which the total number of papers indexed under this MeSH term exceed 300 (numbers left of pie charts). A rough approximation of the size of the nervous system for each organism is depicted by a circle whose radius is proportional to the exponent of the number of neurons in central ganglia (for *C. elegans* and *Hydra*, the total number of neuronal cells in the animal). On the right side of the figure life-cycle lengths are depicted for each organism, subdivided into embryonic (green) and post-embryonic stages (blue). This distinction is not made for *Hydra*, which typically reproduces by budding and has been termed 'an endless embryo'.

Other ligand-receptor families in invertebrates

Do other families of receptor tyrosine kinases replace trophic roles in invertebrate CNS? Candidates for such roles include the EGF receptor³⁹, ret (Ref. 38) and ror (Ref. 23) receptor families (Fig. 1). Particularly interesting is the ror family because it is conserved throughout invertebrate phyla, including two distinct members with neuronal-restricted expression in *Drosophila*^{22,23}, although its ligand(s) are unknown. However, a detailed analysis of ror function in *C. elegans* highlights roles in processes that are not related to neuron survival⁴⁰. Ret is lacking from the *C. elegans* genome, and a study on the physiological role of *Drosophila* ret has yet to be carried out. Such work is eagerly awaited, especially because of the role of mammalian ret in supporting survival of dopaminergic neurons and motoneurons⁵, although a conserved ret ligand is not evident from the fly genome sequence (but see also above). Finally, the 'thousand and one roles of the fly EGF receptor'³⁹ do not yet include studies showing trophic support of neurons, although Ras signaling downstream of the EGF receptor has been shown to inhibit the apoptotic gene *hid* in *Drosophila* eyes^{41,42}. It will be of interest to see if the EGF receptor pathway can regulate neuronal survival in the fly. Furthermore, a recent study on molluscan EGF showed effects on neuronal growth *in vitro*⁴³. It should also be noted that a candidate molluscan trophic factor unrelated to the neurotrophins has been shown to bind to

mammalian p75 (Ref. 44), and that the major ligands affecting nerve cell differentiation in coelenterates are small neuropeptide-like sequences⁴⁵. Endogenous receptors for these candidate factors are yet to be identified, but evidence thus far is unavailable to suggest that these or any other peptide growth factors regulate neuronal numbers in the organisms in question. A further possibility is that regulators of cell numbers in invertebrate nervous systems might not be peptides at all.

Steroid hormones as regulators of neuronal populations in *Drosophila*

In nematodes, differentiation and cell fate is set by interactions between neuronal precursors or is solely dependent on cell lineage⁴⁶. Accordingly, the number of neurons (and their connections) in the nervous system of *C. elegans* is practically invariant, and it is possible to rationalize a complete lack of neurotrophic mechanisms⁴. By contrast, insects extensively remodel their nervous systems during metamorphosis, to accommodate the physical and behavioral differences between the larval and adult animal. This remodeling involves programmed cell death of entire neuron populations, and would appear to be the most relevant scenario for neurotrophic mechanisms in insects. However, a series of studies on motoneuron death in the moth *Manduca sexta* has shown that death is induced primarily by stereotyped changes in ecdysteroid hormone levels⁴⁷, which regulate most major aspects of insect metamorphosis. Similar results have been obtained on peptidergic neurons of *Drosophila*, wherein fluctuations in levels of 20-hydroxyecdysone regulate levels of apoptotic gene transcripts^{48,49}.

In addition to the above, two neuron populations in *Manduca* and *Drosophila* have been suggested to be dependent on target cells for survival at different stages of development^{50,51}. The molecular cues active in these interactions have not been identified, although probable candidates are contact-mediated effects via cell adhesion molecules. Nevertheless, the

emerging picture suggests that steroid hormones provide the principal signal for switching neuron populations in insects. In contrast to neurotrophin-mediated survival and death decisions in vertebrates, steroid regulation of insect neurons does not entail a competitive selection process, but instead a more drastic 'all-or-none' switching of neuron populations.

Searching for invertebrate neurotrophins

The data above suggests that the lack of neurotrophins in the completed invertebrate genomes (i.e. *Drosophila*, *C. elegans*) probably correlate with the fact that these represent the species whose short generation time and CNS developmental patterns minimize requirements for trophic mechanisms. This was predicted for the ~300 neuron network that comprises the simple nervous system of *C. elegans*. Surprisingly, the lesson from the *Drosophila* genome seems to be that one can still evolve a nervous system three orders of magnitude larger than that of *C. elegans* (at least in neuronal numbers), without a need for neurotrophins. Intriguing questions for the future are: to what level of complexity can a CNS evolve without requiring neurotrophic selection of neuron numbers? Do the million or so neurons of a honeybee represent a 'glass ceiling' beyond which insect brains cannot evolve without neurotrophic mechanisms? Fascinating though they might be, the time scales required for experimental analysis of these questions are far beyond the fortitude levels of most funding agencies. We will therefore have to look for insights from comparative genomics and evolutionary studies. A Medline search for invertebrate models from other phyla focuses attention on the organisms presented in Fig. 4, for

which a viable neuroscience research community appears to exist. Comparison of the life cycle and brain size of these organisms highlights the fact that *Drosophila* and *C. elegans* have a short life cycle, compared to research models from most other phyla. Some of these other organisms pose interesting opportunities for future studies of gene families underlying complexity or plasticity in the nervous system. For example, the coelenterate *Hydra* represents the earliest phylum with a nervous system. The *Hydra* nerve net is in a constant flux of differentiation and renewal⁵², and as such provides a contrasting perspective to the hard-wired *C. elegans* system, although both are composed of relatively few neurons. At the other end of the spectrum, the most complex nervous systems in the invertebrate kingdom are undoubtedly those of the cephalopod molluscs (Fig. 4), which rival vertebrates in the intricacy of their brain anatomy², and in the complexity of their behavior^{53,54}. Preliminary evidence for the existence of a trk-like receptor in squid has recently been published²⁵. It is tempting to speculate that one of the 'enabling conditions' underlying the evolution of complex brains in cephalopods has been an expansion of neurotrophin-like signaling systems, in parallel with the situation in vertebrates¹¹. Thus, trophic mechanisms might be one of the qualitative differences between a 'plastic' nervous system with a potential for evolving complexity, versus a more constrained nervous system that cannot evolve beyond certain limitations. An expansion of the genome projects to include representatives of the phyla shown in Fig. 4 should be informative for identifying other gene families that are instrumental for the evolution of complex brains.

Acknowledgements

Our work on evolution and functions of neurotrophic factors in invertebrates has been generously supported by the Human Frontiers Science Program Organization (HFSP), the Israel Science Foundation (ISF), and the USA-Israel Binational Science Foundation (BSF). M.F. is an Allon Foundation Fellow, and the incumbent of the Daniel E. Koshland Sr Career Development Chair at the Weizmann Institute. We apologize to all those whose papers were not cited due to space limitations.

References

- Barde, Y.A. (1994) Neurotrophic factors: an evolutionary perspective. *J. Neurobiol.* 25, 1329–1333
- Allman, J.M. (1999) *Evolving Brains*, W.H. Freeman
- Koch, C. and Laurent, G. (1999) Complexity and the nervous system. *Science* 284, 96–8
- Adami, C. *et al.* (2000) Evolution of biological complexity. *Proc. Natl. Acad. Sci. U. S. A.* 97, 4463–4468
- Ibanez, C.F. (1998) Emerging themes in structural biology of neurotrophic factors. *Trends Neurosci.* 21, 438–444
- Levi-Montalcini, R. (1987) The nerve growth factor 35 years later. *Science* 237, 1154–1162
- McKay, S.E. *et al.* (1999) Regulation of synaptic function by neurotrophic factors in vertebrates and invertebrates: implications for development and learning. *Learn. Mem.* 6, 193–215
- Levi-Montalcini, R. *et al.* (1996) Nerve growth factor: from neurotrophin to neurokinine. *Trends Neurosci.* 19, 514–520
- Barde, Y.A. (1988) What, if anything, is a neurotrophic factor? *Trends Neurosci.* 11, 343–346
- Korsching, S. (1993) The neurotrophic factor concept: a reexamination. *J. Neurosci.* 13, 2739–2748
- Hallbook, F. (1999) Evolution of the vertebrate neurotrophin and Trk receptor gene families. *Curr. Opin. Neurobiol.* 9, 616–621
- Chao, M.V. (2000) Trophic factors: an evolutionary cul-de-sac or door into higher neuronal function? *J. Neurosci. Res.* 59, 353–355
- The *C. elegans* Sequencing Consortium (1998) Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* 282, 2012–2018
- Adams, M.D. *et al.* (2000) The genome sequence of *Drosophila melanogaster*. *Science* 287, 2185–2195
- Hayashi, I. *et al.* (1992) Neurotrophic factor-like activity in *Drosophila*. *Biochem. Biophys. Res. Commun.* 184, 73–79
- Ridgeway, R.L. *et al.* (1991) Nerve growth factor (NGF) induces sprouting of specific neurons of the snail, *Lymnaea stagnalis*. *J. Neurobiol.* 22, 377–390
- Hahn, U.K. *et al.* (1996) An invertebrate (Molluscan) plasma protein that binds to vertebrate immunoglobulins and its potential for yielding false-positives in antibody-based detection systems. *Dev. Comp. Immunol.* 20, 39–50
- Ruvkun, G. and Hobert, O. (1998) The taxonomy of developmental control in *Caenorhabditis elegans*. *Science* 282, 2033–2041
- Myers, E.W. *et al.* (2000) A whole-genome assembly of *Drosophila*. *Science* 287, 2196–2204
- Pulido, D. *et al.* (1992) Dtrk, a *Drosophila* gene related to the trk family of neurotrophin receptors, encodes a novel class of neural cell adhesion molecule. *EMBO J.* 11, 391–404
- Chisholm, A. and Tessier-Lavigne, M. (1999) Conservation and divergence of axon guidance mechanisms. *Curr. Opin. Neurobiol.* 9, 603–615
- Oishi, I. *et al.* (1997) A novel *Drosophila* receptor tyrosine kinase expressed specifically in the nervous system – unique structural features and implication in developmental signaling. *J. Biol. Chem.* 272, 11916–11923
- Wilson, C. *et al.* (1993) Dror, a potential neurotrophic receptor gene, encodes a *Drosophila* homolog of the vertebrate Ror family of Trk-related receptor tyrosine kinases. *Proc. Natl. Acad. Sci. U. S. A.* 90, 7109–7113
- Lucini, C. *et al.* (1999) Neuronal and non-neuronal Trk neurotrophin receptor-like proteins in *Eisenia foetida* (Annelida Oligochaeta). *Neurosci. Lett.* 261, 163–166
- Moreno, H. *et al.* (1998) Nerve growth factor acutely reduces chemical transmission by means of postsynaptic TrkA-like receptors in squid giant synapse. *Proc. Natl. Acad. Sci. U. S. A.* 95, 14997–15002
- van Kesteren, R. *et al.* (1998) Early evolutionary origin of the neurotrophin receptor family. *EMBO J.* 17, 2534–2542
- Adoutte, A. *et al.* (2000) The new animal phylogeny: reliability and implications. *Proc. Natl. Acad. Sci. U. S. A.* 97, 4453–4456

- 28 Steele, R.E. *et al.* (1999) Appearance and disappearance of Syk family protein-tyrosine kinase genes during metazoan evolution. *Gene* 239, 91–97
- 29 Bergner, A. *et al.* (1996) Crystal structure of a coagulogen, the clotting protein from horseshoe crab: a structural homologue of nerve growth factor. *EMBO J.* 15, 6789–6797
- 30 Murray-Rust, J. *et al.* (1993) Topological similarities in TGF- β 2, PDGF-BB and NGF define a superfamily of polypeptide growth factors. *Structure* 1, 153–159
- 31 Sato, A. *et al.* (1997) Cystine knot of the gonadotropin alpha subunit is critical for intracellular behavior but not for *in vitro* biological activity. *J. Biol. Chem.* 272, 18098–18103
- 32 DeLotto, Y. and DeLotto, R. (1998) Proteolytic processing of the Drosophila Spatzle protein by easter generates a dimeric NGF-like molecule with ventralising activity. *Mech. Dev.* 72, 141–148
- 33 Mizuguchi, K. *et al.* (1998) Getting knotted: a model for the structure and activation of Spatzle. *Trends Biochem. Sci.* 23, 239–242
- 34 Halfon, M.S. and Keshishian, H. (1998) The Toll pathway is required in the epidermis for muscle development in the Drosophila embryo. *Dev. Biol.* 199, 164–174
- 35 Rose, D. and Chiba, A. (2000) Synaptic target recognition at Drosophila neuromuscular junctions. *Microsc. Res. Tech.* 49, 3–13
- 36 Wiesmann, C. *et al.* (1999) Crystal structure of nerve growth factor in complex with the ligand-binding domain of the TrkA receptor. *Nature* 401, 184–188
- 37 Windisch, J.M. *et al.* (1995) Brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4 bind to a single leucine-rich motif of TrkB. *Biochemistry* 34, 11256–11263
- 38 Sugaya, R. *et al.* (1994) A Drosophila homolog of human proto-oncogene ret transiently expressed in embryonic neuronal precursor cells including neuroblasts and CNS cells. *Mech. Dev.* 45, 139–145
- 39 Schweitzer, R. and Shilo, B.Z. (1997) A thousand and one for the Drosophila EGF receptor. *Trends Genet.* 13, 191–196
- 40 Forrester, W.C. *et al.* (1999) A *C. elegans* Ror receptor tyrosine kinase regulates cell motility and asymmetric cell division. *Nature* 400, 881–885
- 41 Kurada, P. and White, K. (1998) Ras promotes cell survival in Drosophila by downregulating *hid* expression. *Cell* 95, 319–329
- 42 Bergmann, A. *et al.* (1998) The Drosophila gene *hid* is a direct molecular target of ras-dependent survival signaling. *Cell* 95, 331–341
- 43 Hermann, P.M. *et al.* (2000) Neurotrophic actions of a novel molluscan epidermal growth factor. *J. Neurosci.* 20, 6355–6364
- 44 Fainzilber, M. *et al.* (1996) CRNF, a molluscan neurotrophic factor that interacts with the p75 neurotrophin receptor. *Science* 274, 1540–1543
- 45 Takahashi, T. *et al.* (2000) A novel neuropeptide, Hym-355, positively regulates neuron differentiation in Hydra. *Development* 127, 997–1005
- 46 Metzstein, M.M. *et al.* (1998) Genetics of programmed cell death in *C. elegans*: past, present and future. *Trends Genet.* 14, 410–416
- 47 Weeks, J.C. (1999) Steroid hormones, dendritic remodeling and neuronal death: insights from insect metamorphosis. *Brain Behav. Evol.* 54, 51–60
- 48 Draizen, T.A. *et al.* (1999) Genetic and hormonal regulation of the death of peptidergic neurons in the Drosophila central nervous system. *J. Neurobiol.* 38, 455–465
- 49 Robinow, S. *et al.* (1997) Genes that induce apoptosis: transcriptional regulation in identified, doomed neurons of the Drosophila CNS. *Dev. Biol.* 190, 206–213
- 50 Thorn, R.S. and Truman, J.W. (1994) Sexual differentiation in the CNS of the moth, *Manduca sexta*. II. Target dependence for the survival of the imaginal midline neurons. *J. Neurobiol.* 25, 1054–1066
- 51 Campos, A.R. *et al.* (1992) Survival of photoreceptor neurons in the compound eye of Drosophila depends on connections with the optic ganglia. *Development* 114, 355–366
- 52 David, C.N. and Hager, G. (1994) Formation of a primitive nervous system: nerve cell differentiation in the polyp hydra. *Perspect. Dev. Neurobiol.* 2, 135–140
- 53 Fiorito, G. and Scotto, P. (1992) Observational learning in Octopus vulgaris. *Science* 256, 545–547
- 54 Hanlon, R.T. and Messenger, J.B. (1996) *Cephalopod Behaviour*. Cambridge University Press

Prion and prejudice: normal protein and the synapse

David R. Brown

The word prion has become synonymous with unusual diseases, such as bovine spongiform encephalopathy and Creutzfeldt–Jakob disease. However, there is also a normal prion protein that does not cause disease. Until recently this highly conserved and widely expressed glycoprotein has been overshadowed by its rogue isoform. Now it is emerging that not only is this protein important for understanding prion disease but it is also important for a healthy brain. The normal cellular isoform of the prion protein is expressed at high levels at synapses suggesting an important role in neuronal function. There is increasing evidence that the normal prion protein binds copper and the resulting complex possesses anti-oxidant activity, and that this, in turn, might have vital implications for synaptic homeostasis.

The genetic code of the prion protein (PrP^c) was identified only after the isolation of an abnormal isoform (PrP^{Sc}) from brains of mice that were infected with the disease scrapie¹. Scrapie, bovine spongiform

encephalopathy (BSE), and Creutzfeldt–Jakob disease (CJD) are examples of prion diseases or transmissible spongiform encephalopathies (TSEs). These diseases result in fatal neurodegenerative conditions². Discovery of the prion protein gene led to the realization that a misfolded form of a normal brain protein was involved in these diseases^{2,3}. However, the role of the normal protein in the brain has remained a mystery for the past fifteen years. Instead, most research on these diseases has focussed on PrP^{Sc} and the process by which it is derived from PrP^c. PrP^{Sc} is readily detected as aggregates of misfolded protein in the brains of animals or humans that have all forms of prion diseases. The nature and possible aetiology of prion disease have been reviewed in detail elsewhere^{2,3}. This review discusses new evidence suggesting that PrP^c is a copper-binding protein concentrated at synapses that protects them from oxidative damage. In spite of prions being seen as a 'curse' to the survival of ageing mammals, the normal isoform is possibly a 'blessing' to a stressed nervous system.

The search for PrP^c function

PrP^c at the synapse

PrP^c is a glycoprotein expressed on the surface of many cell types^{4–8}. Therefore, a 'neurone only function' for PrP^c is not valid. However, the fact that the protein is expressed in neurones at higher levels