Jekyll–Hyde neurotrophins: the story of proNGF

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Neurotrophins promote neuronal survival and differentiation by binding to two classes of cell surface receptors: members of the Trk-family receptor tyrosine kinases and p75^{NTR}, a member of the tumor necrosis factor receptor superfamily. The discoveries that the unprocessed proneurotrophin peptide is both a preferential high-affinity ligand for p75^{NTR} (with minimal affinity for trkA) and a potent inducer of p75^{NTR}-mediated neuronal cell death are likely to change our understanding of neurotrophin physiology and our ability to manipulate their signaling pathways.

Some 12 years ago, during my post-doctoral position with the late Håkan Persson, Håkan one day showed a prominent foreign visitor around the laboratory. As they approached my bench, Håkan explained to his guest that my project was to produce and characterize NGF mutant proteins for structure–function studies. Our visitor appeared surprised. 'I didn't know people was still working on NGF', he replied, 'I thought we knew everything about it!' They quickly moved on, and I assume that Håkan improvised an apologetic explanation about the importance of my work. Fortunately for me, and before I had time to feel too depressed about such dispiriting encounter, my timer went off, so I dashed out to the hot room to take care of my precious samples.

Admittedly, by 1990, NGF (the first growth factor to be discovered) had been known about for almost 40 years, and >4000 papers had been published on its biochemical and biological activities. Could anything interesting still come from studying this molecule? Sure enough, we did not have to wait long to verify that our visitor had been rather short sighted, as during the following year the discovery of the Trk family of receptors changed everything we thought we knew about NGF. Since then, an uninterrupted series of breakthroughs has confirmed how little we actually knew about NGF and the other neurotrophins ten years ago. Exciting advances in this field during the past decade include solving the crystal structures of NGF [1] and the NGF–trkA complex [2]; the renaissance of p75 as a signaling receptor [3] with paradoxical cell killing activities [4,5]; and the unexpected discovery that NGF and other neurotrophins have roles in synaptic plasticity [6,7], learning and memory [8,9]. And now, just when some thought the dust had finally settled, the roller-coaster continues: we learn from recent work by Lee *et al.* [10] that NGF has, for all these years, been living a secret double-life.

Neurotrophins and the control of neuronal survival

NGF and the other neurotrophins – brain-derived neurotrophic factor (BDNF), neurotrophin-3 and neurotrophin-4 – regulate neurite outgrowth and neuronal survival during development. They do so by interacting with two types of cell surface molecules: the receptor tyrosine kinases trkA, trkB and trkC, and the p75 neurotrophin receptor (p75^{NTR}), a member of the tumor necrosis factor (TNF) receptor superfamily [11,12]. p75^{NTR} lacks intrinsic catalytic activity, and signals through a series of protein–protein interactions mediated by its intracellular juxtamembrane and death domains [11,12]. In cells expressing Trk receptors, neurotrophins promote cell survival by stimulating sustained activation of the PI-3 kinase–AKT and Ras–ERK pathways, which, in turn, intercept nuclear and mitochondrial cell-death programs [13]. When coexpressed with appropriate Trk receptors, p75^{NTR} increases neurotrophin-binding affinity and assists in ligand discrimination by different Trk family members [14–16]. p75^{NTR} can also contribute to cell survival (by activating the NF-κB pathway) [17], to neurite outgrowth (by regulating Rho activity) [18], and to cell migration [19]. When Trk activation is reduced or absent, high levels of p75 expression can make cells susceptible to apoptotic cell death through increased ceramide production [20], and activation of c-Jun N-terminal kinase (JNK1) and p53 [21–23]. Although the neurotrophins are potent survival factors, typically with subnanomolar EC_{50} values, they are comparatively poor inducers of cell death: concentrations must be at least an order of magnitude greater for activation of p75^{NTR} signaling and cell death than for promotion of cell survival [21]. Even at very high NGF concentrations, activation of NF-κB and JNK1 remains suboptimal [3]. Compared with the robust effects obtained with other well-known activators of these pathways, such as TNF, the cellular responses of p75^{NTR} to purified neurotrophins are modest. This has lead to speculation that the neurotrophins might in fact represent only partial, low potency agonists of p75^{NTR}, and that *bonafide* p75^{NTR} ligands are yet to be discovered. Indeed, during recent years, a number of groups have begun to use cloning and protein-purification approaches in the hope of identifying novel ligands that bind and activate p75^{NTR} with greater efficacy than the neurotrophins. Lee *et al.* now report to have found these elusive molecules close to home, disguised as unprocessed proneurotrophins.

ProNGF as a novel, high-affinity ligand for p75^{NTR}

The neurotrophins, like many other growth factors, are synthesized as immature precursors that are proteolytically cleaved intracellularly, by furin and other proconvertases, to release mature ligands. Probably owing to their supposedly transient existence, neurotrophin prodomains have been proposed to function only in promotion of protein folding and regulation of neurotrophin secretion [24,25]. The high degree of sequence conservation between neurotrophin prodomains of different species suggested to Lee *et al.* additional biological functions, related to protein–protein recognition. They first demonstrated significant levels of proNGF and proBDNF in various tissue extracts and cell-line supernatants, and showed that the heterogeneous mixture of unprocessed forms can be cleaved by extracellular proteases (including plasmin and several matrix metalloproteinases) to yield mature neurotrophins. In order to obtain enough proNGF for biochemical analysis, Lee *et al.* produced a mutant NGF molecule, which lacked the dibasic furin site that separates the pro- and

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mature regions. Although the resulting protein was still somewhat unstable and sensitive to plasmin and metalloproteinases, it allowed Lee *et al.* to perform receptor-binding studies. The protein displayed at least fivefold greater affinity to $p75^{NTR}$ than did mature NGF, and negligible binding to trkA. Furin-resistant NGF was unable to stimulate trkA tyrosine phosphorylation or differentiation in PC12 cells, but was a potent cell-death inducer in both smooth muscle cells expressing $p75^{NTR}$ and sympathetic neurons. Based on these results, Lee *et al.* proposed that the cleavage-resistant pro-form of NGF is a high-affinity, functional ligand for the pro-apoptotic $p75^{NTR}$ receptor, whereas the proteolytically cleaved mature NGF is the preferred ligand for trkA. The implication of this proposal is that the balance between cell survival and cell death could depend upon the proportions of mature and proNGF available to cells expressing trkA and $p75^{NTR}$ receptors.

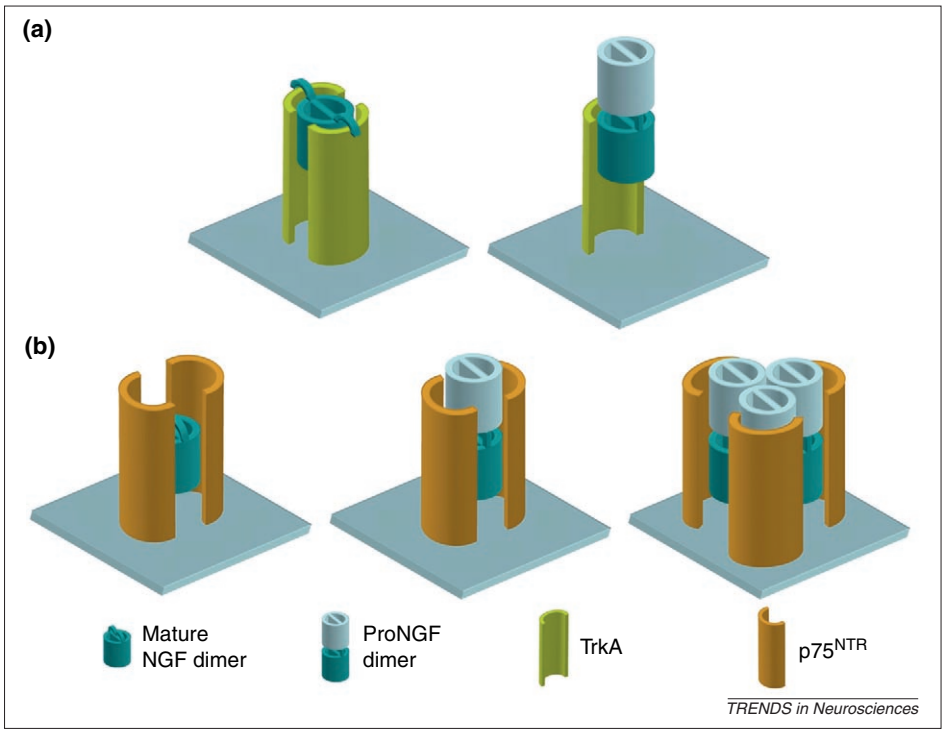

Fig. 1. A model for receptor discrimination by mature NGF and proNGF. Both molecules are proposed to exist as dimers (each half-cylinder represents a monomer); proNGF consists of mature NGF domains (dark blue) attached to prodomains (light blue). (a) Upon binding to trkA, the N-terminal domains in the mature NGF dimer adopt an extended helical conformation, forming essential contacts with the trkA ligand-binding domain. In proNGF, the N-terminal domains of mature NGF are conformationally restricted and cannot contribute to trkA binding (right) – trkA dimerization and activation does not occur. (b) The N-terminal domain of mature NGF does not appear to contribute to $p75^{NTR}$ binding (left). The increased affinity of the proNGF form could be explained by the presence of an extended binding surface so that residues in the NGF prodomain also contributing to binding (centre), or by oligomerization of proNGF molecules (e.g. into trimers of dimers), leading to $p75^{NTR}$ trimerization and enhanced signaling (right).

Regulation of neurotrophin function by proteolytic processing

The discovery of Lee *et al.* brings proteolytic processing to centre stage in the regulation of neurotrophin function. The selectivity of postsecretory proteolytic processing of proBDNF observed by Lee *et al.* suggests that regulation of the synthesis and localization of specific proteases could play a major role in the control of neurotrophin activity. This could be true not only during development, but also in nerve injury and synaptic plasticity responses in the adult. Both proNGF and proBDNF are present in large amounts in the brain [26,27], and $p75^{NTR}$ expression and function has been associated with a number of disease-states, including stress and inflammation – conditions that are also known to regulate the activity of several proteases. Regulation of the expression or activation of specific proteases by neurotrophin signaling could also represent a potent feedback mechanism, to limit or amplify distinct neurotrophin activities.

A model for receptor discrimination by unprocessed and mature neurotrophins

At the heart of Lee *et al.*'s discovery is the differential ability of proNGF and mature NGF to selectively interact with the $p75^{NTR}$ and trkA receptors. A better understanding of these interactions is likely to give new insights into the mechanisms of activation of the two NGF receptors. Because mature NGF is released as a dimer, the basic quaternary structure of proNGF is also likely to be dimeric. This is compatible with the close proximity of the two N-terminal domains in the crystal structure of mature NGF [1]. Site-directed mutagenesis [28] and X-ray crystallography [2] studies have shown this N-terminal domain to be vital for trkA binding. Upon receptor interaction, this otherwise disordered domain adopts a helical conformation, providing an extended surface of interaction with the first immunoglobulin-like domain of trkA [2] (Fig. 1a, left). In the proNGF molecule, however, the presence of the prodomain is likely to impose severe restrictions on the conformation of these residues, preventing them from interacting with trkA (Fig. 1a, right), and thereby hindering activation of trkA by proNGF.

How can the increased affinity of proNGF for $p75^{NTR}$ be explained? As the N-terminal domain of mature NGF does not appear to be vital for $p75^{NTR}$ binding (Fig. 1b, left), this receptor could, in principle, interact directly with a proNGF dimer, the increased affinity reflecting additional contacts with residues in the NGF prodomain (Fig. 1b, centre). This model, however, might not readily explain the difference in the activation of intracellular signaling pathways by mature and proNGF. An alternative, not mutually exclusive, possibility could be the oligomerization of proNGF dimers via prodomain interactions. Intriguingly, all other members of the TNFR superfamily, including $p55^{TNFR}$ and Fas, are trimeric receptors that bind trimeric ligands, and trimerization of intracellular death domains is a crucial event in the mechanism of activation of TNF-receptor superfamily proteins [29,30]. Although proNGF molecules are likely to be dimeric, a higher order trimeric complex (i.e. trimers of dimers) is possible, and could in fact be mediated by sequences in the neurotrophin prodomain. High-order

ligand–receptor complexes have consistently been detected in cross-linking experiments using different preparations of radiolabeled neurotrophins [16,31]. Trimeric oligomers of proNGF dimers could present a highly cooperative binding interface to $p75^{NTR}$, which could account for the increased affinity of proNGF for this receptor (Fig. 1b, right). Trimerization of $p75^{NTR}$ death domains could, in turn, result in more potent and efficient activation of intracellular pathways, consistent with the similarity of this receptor to other members of the TNFR superfamily. Preliminary studies using Fas–$p75^{NTR}$ chimeric receptors indicate that a specific orientation of the $p75^{NTR}$ death domains, and not simply trimerization, might be required for optimal intracellular signaling [32].

The road ahead

As with Robert Louis Stevenson's *Dr. Jekyll and Mr. Hyde*, the case of the double identity of the NGF molecule revealed by Lee *et al.* is both shocking and intriguing. It provides a tantalizing explanation for several of the mysteries surrounding the signaling mechanisms and biological activities of $p75^{NTR}$. Many new lines of investigation, discussed here and elsewhere [33], are now open: these include the regulation of neurotrophin proteolytic processing, the molecular basis for receptor-binding specificity of the pro- and mature forms, the signaling pathways activated by proneurotrophins, and the effects *in vivo*. Almost half a century after its discovery, NGF hides still many surprises. Twelve years ago, a visitor in my old lab thought nothing new could come from studying NGF. The latest discovery by Lee *et al.* reminds us, once again, that there are no uninteresting topics in science – just a few uninteresting scientists.

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