BRAIN DERIVED NEUROTROPHIC FACTOR TRANSPORT AND PHYSIOLOGICAL SIGNIFICANCE

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THESIS SUMMARY

Neurotrophins are important signaling molecules in neuronal survival and differentiation. The precursor forms of neurotrophins (proneurotrophins) are the dominant form of gene products in animals, which are cleaved to generate prodomain and mature neurotrophins, and are sorted to constitutive or regulated secretory pathway and released. Brain-derived neurotrophic factor (BDNF) plays a pivotal role in the brain development and in the pathogenesis of neurological diseases. In Huntington's disease, the defective transport of BDNF in cortical and striatal neurons and the highly expressed polyQ mutant huntingtin (Htt) result in the degeneration of striatal neurons. The underlying mechanism of BDNF transport and release is remains to be investigated.

Current studies were conducted to identify the mechanisms of how BDNF is transported in axons post Golgi trafficking. By using affinity purification and 2D-DIGE assay, we show Huntingtin-associated protein 1 (HAP1) interacts with the prodomain and mature BDNF. The GST pull-down assays have addressed that HAP1 directly binds to the prodomain but not to mature BDNF and this binding is decreased by PolyQ Htt. HAP1 immunoprecipitation shows that less proBDNF is associated with HAP1 in the brain homogenate of Huntington's disease compared to the control. Co-transfections of HAP1 and BDNF plasmids in PC12 cells show HAP1 is colocalized with proBDNF and the prodomain, but not mature BDNF. ProBDNF was accumulated in the

proximal and distal segments of crushed sciatic nerve in wild type mice but not in HAP1-/- mice. The activity-dependent release of the prodomain of BDNF is abolished in HAP1-/- mice. We conclude that HAP1 is the cargo-carrying molecule for proBDNF-containing vesicles and plays an essential role in the transport and release of BDNF in neuronal cells.

20-30% of people have a valine to methionine mutation at codon 66 (Val66Met) in the prodomain BDNF, which results in the retardation of transport and release of BDNF, but the mechanism is not known. Here, GST-pull down assays demonstrate that HAP1 binds Val66Met prodomain with less efficiency than the wild type and PolyQ Htt further reduced the binding, but the PC12 cells colocalization rate is almost the same between wt prodomain/HAP1 and Val66Met prodomain/HAP1, suggesting that the mutation in the prodomain may reduce the release by impairing the cargo-carrying efficiency of HAP1, but the mutation does not disrupt the sorting process.

Recent studies have shown that proneurotrophins bind p75NTR and sortilin with high affinity, and trigger apoptosis of neurons in vitro. Here, we show that proBDNF plays a role in the death of axotomized sensory neurons. ProBDNF, p75NTR and sortilin are highly expressed in DRG neurons. The recombinant proBDNF induces the dose-dependent death of PC12 cells and the death activity is completely abolished in the presence of antibodies against the prodomain of BDNF. The exogenous proBDNF enhances the death of

axotomized sensory neurons and the antibodies to the prodomain or exogenous sortilin-extracellular domain-Fc fusion molecule reduces the death of axotomized sensory neurons. We conclude that proBDNF induces the death of sensory neurons in neonatal rats and the suppression of endogenous proBDNF rescued the death of axotomized sensory neurons.

DECLARATION

I hereby certify that the work embodied in this thesis is the result of original research obtained while I was enrolled as a Ph.D student at the Department of Human Physiology in Flinders University. This thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university, and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Linyan Wu

I believe that this thesis is properly presented, conforms to the specifications of thesis presentation in the University and is *prima facie* worthy of examination.

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PUBLICATIONS DURING Ph.D STUDY

Journals:

1. Wang H, **Wu LL**, Song XY, Luo XG, Zhong JH, Rush RA, Zhou XF. Axonal transport of BDNF precursor in primary sensory neurons. <u>Eur J Neurosci.</u> 2006 Nov; 24(9):2444-2452 **(H.W. and L.L-y.W contributed equally to this work).**

2. Wang TT, Yuan WL, Ke Q, Song XB, Zhou X, Kang Y, Zhang HT, Lin Y, Hu YL, Feng ZT, **Wu LL**, Zhou XF. Effects of electro-acupuncture on the expression of c-jun and c-fos in spared dorsal root ganglion and associated spinal laminae following removal of adjacent dorsal root ganglia in cats. <u>Neuroscience.</u> 2006 Jul 21; 140(4):1169-76. Epub 2006 May 30.

3. Cai C, Zhou K, Wu Y, **Wu L**. Enhanced liver targeting of 5-fluorouracil using galactosylated human serum albumin as a carrier molecule. <u>J Drug Target.</u> 2006 Feb;14(2):55-61

4. <u>Zhou XF</u>, <u>Li WP</u>, <u>Zhou FH</u>, <u>Zhong JH</u>, <u>Mi JX</u>, **<u>Wu LL</u>**, <u>Xian CJ</u>. Differential effects of endogenous brain-derived neurotrophic factor on the survival of axotomized sensory neurons in dorsal root ganglia: a possible role for the p75 neurotrophin receptor, <u>Neuroscience.</u> 2005;132(3):591-603.

5.   Song XY, Zhou FH, Zhong JH, **Wu LL**, Zhou XF. Knockout of p75(NTR) impairs re-myelination of injured sciatic nerve in mice. <u>J Neurochem.</u> 2006 Feb;96(3):833-42. Epub 2005 Nov 29

6.   Wang TT, Yuan Y, Kang Y, Yuan WL, Zhang HT, **Wu LY**, Feng ZT. Effects of acupuncture on the expression of glial cell line-derived neurotrophic factor (GDNF) and basic fibroblast growth factor (FGF-2/bFGF) in the left sixth lumbar dorsal root ganglion following removal of adjacent dorsal root ganglia. <u>Neurosci Lett.</u> 2005 Jul 15;382(3):236-41. Epub 2005 Apr 8.

Conference Abstracts:

<u>**Wu LL**,Wang H, Song XY, Luo XG, Zhong JH, Rush RA, Zhou XF. Anterotrade and retrograde transport of endogenous and exogenous BDNF precursor in primary sensory neurons. The Society for Neuroscience Annual meeting. 2005, Washington, DC , USA</u>

<u>**Wu LL,** Fang YJ, Li XJ, Zhou XF. Transport mechanism of brain derived neurotrophic factor and its significance. Australian Neuroscience Society Inc 27th Annual meeting and International Brain Research Organisation (IBRO) 7th World Conference of Neuroscience meeting. 2007, Melbourne, Australia.</u>

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ABREVIATIONS

BDNF   Brain-Derived Neurotrophic Factor

CAG   cytosine-adenine-guanine

CAP-Gly   cytoskeleton-associated protein, glycine-rich

CBP   CREB-binding protein

CNS   central nervous system

CPE   carboxypeptidase E

DAPI   Diamidino-2-phenylindole

DRG   dorsal root ganglion

EGF   epidermal growth factor

ER   endoplasmic reticulum

$GABA_AR$ γ-aminobutyric acid type A receptor

GDNF   Glial cell-derived neurotrophic factor

GDP   Guanosine diphosphate

GEF   Guanine exchange factors

GTP   Guanosine-5'-triphosphate

HAP1   Huntingtin-Associated Protein 1

HB   Huntingtin-binding domain

HC   heavy chain

HD   Huntington's disease

Hrs   hepatocyte growth factor-regulated tyrosine kinase

substrate

Htt   huntingtin

ICs	intermediate chains
InsP$_3$R1	type 1 inositol (1,4,5)-trisphosphate receptor
IPTG	isopropyl-b-D-thiogalactopyranoside
IRS1	insulin receptor substrate-1
IV	Intravenously
JIP1	Jnk-interacting protein 1
KAP	kinesin-associated protein
KHC	kinesin heavy chain
KIFs	kinesin superfamily proteins
KIF5	Kinesin superfamily protein 5
KLC	kinesin light chains
LB	Luria-bertani
LCs	light chains
LDCV	Large dense core vesicle
LICs	light intermediate chains
M6PR	Mannose-6-Phosphate Receptor
MAGE	melanoma-associated antigen acting factor
MSN	medium spiny striatal neurons
MVBs	multivesicular bodies
NGF	Nerve Growth Factor
NHS	Normal horse serum
NMDA	N-methyl-D-aspartate
NMDARs	N-methyl-D-aspartate glutamate receptors

NRAGE   neurotrophin receptor p75NTR interacting MAGE

homologue

NRIF   neurotrophin receptor-interacting factor

NRSE   neuron-restrictive silencer element

NRSF   neuronal restrictive silencing factor

NT-3   Neurotrophin-3

NT-4/5   Neurotrophin-4/5

OD   optical density

p75NTR   p75 neurotrophin receptor

PAM   peptidylglycine α-amidating monooxygenase

PCs   proprotein convertases

PNS   peripheral nervous system

polyQ   polyglutamine

proNGF   precursor for NGF

proBDNF   precursor for BDNF

RE1   repressor element 1

REST   RE1-silencing transcription factor

RT   Room temperature

SC   Subcutaneously

TBP   TATA-binding protein

TNF   tumor necrosis factor

Trk   tropomyocin receptor kinase

Val66Mal   valine to methionine at codon 66

CHAPTER 1

INTRODUCTION

1. Neurotrophins

1.1 The Discovery of Neurotrophins

In 1949, Viktor Hamburger and Rita Levi-Montalcini found that factors secreted from tumor cells promote neuronal cell survival and neurite growth. They called those factors "nerve growth promoting factors", later termed Nerve Growth Factor (NGF) (Levi-Montalcini 1949, Cohen & Levi-Montalcini 1957). Later on, Cohen and Levi-Motalcini purified the factor from mice salivary glands. This finding led to the discovery of the neurotrophin family, which was a milestone in the research field of the nervous system.

The neurotrophins are a family of proteins which have similar structure and functions. They have a number of shared characteristics, including similar molecular weights (13.2-15.9 kDa), isoelectric points (in the range of 9-10), and ~50% identity in primary structure. They exist in solution as noncovalently bound dimers. Six cysteine residues conserved in the same relative positions give rise to three intra-chain disulfide bonds (Maisonpierre *et al.* 1990a, Maisonpierre *et al.* 1990b). In addition to NGF, 4 other major neurotrophins have been identified. The second neurotrophin found was brain-derived neurotrophic factor (BDNF) which was purified from pig brain. BDNF is a

factor affecting several neuronal populations not responsive to NGF. Now the family consists of NGF, BDNF, neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5). More recently, members of numerous other families of proteins, such as glial cell-derived neurotrophic factor (GDNF) family, were also discovered and have the capability to regulate neuronal survival, development and other aspects of neuronal function (Ernfors *et al.* 1990, Hohn *et al.* 1990, Maisonpierre et al. 1990a, Berkemeier *et al.* 1991, Dechant & Neumann 2002). Each factor has a distinct trophic effect on the proliferation and survival, axonal and dendritic growth and remodeling, assembly of cytoskeleton, and synapse formation and function in various subpopulations of neurons in the peripheral and central nervous system.

1.2 The structure of neurotrophins

The NGF gene (in mouse) has multiple small 5'exons (exon VIII). Four distinct precursors which have different sequences at the N termini are generated by RNA processing (Selby et al. 1987). The mouse NGF protein exists as a complex (7S) composed of 3 types of polypeptides, termed α, β and γ (Murphy *et al.* 1977). Spectroscopic measurement and X-Ray diffraction analysis revealed the three-dimensional structure of βNGF, and further contributes to the hypothesis of how NGF binds to p75NTR and TrkA

(Williams *et al.* 1982, McDonald *et al.* 1991, Bradshaw *et al.* 1994). The BDNF gene shares about 50% amino acid similarity with NGF, NT-3 and NT-4/5 (Dechant & Barde 2002). The human BDNF gene consists of seven upstream noncoding exons differentially spliced to the downstream coding exon, producing multiple transcripts. Upstream of the noncoding exons are promoters governing differential mechanisms of activation and tissue-specific transcript expression in the CNS (Garzon & Fahnestock 2007, Liu *et al.* 2006). Neurotrophins are synthesized in precursor forms (proneurotrophins) and proteolytically processed to generate the mature neurotrophins. The mature neurotrophins are produced by prohormone convertases, such as furin, through cleaving the proneurotrophins' cutting sites (Lee *et al.* 2001b, Chao & Bothwell 2002, Nykjaer *et al.* 2004). Both mature neurotrophins and uncleaved proneurotrophins are secreted from cells. The bioactivities of proneurotrophins could differ from those of mature, cleaved neurotrophins (Hempstead 2006).

A recent study showed that the precursor for NGF (proNGF) can bind to p75NTR with a high affinity and induces apoptosis of neurons *in vitro* (Nykjaer et al. 2004, Teng *et al.* 2005). Endogenous proNGF is secreted and then binds and activates p75NTR *in vivo*. proNGF expression is induced by CNS injury, and proNGF derived from injured spinal-cord lysates induced apoptosis of cultured oligodendrocytes in a p75NTR-dependent manner (Harrington *et al.*

2004, Beattie *et al.* 2002). proNGF binds to p75NTR *in vivo*, and inhibition of proNGF binding to p75NTR *in vivo* can rescue CNS neurons (Nykjaer et al. 2004, Lee et al. 2001b). However, proNGF was detected abundantly in central nervous system tissues whereas mature NGF was undetectable, suggesting that proNGF may have a function distinct from its role as a precursor (Fahnestock et al. 2001). Studies showed that proNGF also exhibits neurotrophic activity similar to mature 2.5S NGF on murine superior cervical ganglion neurons and PC12 cells, but are approximately fivefold less active. ProNGF binds to the high-affinity receptor, TrkA, as determined by cross-linking to PC12 cells, and is also slightly less active than mature NGF in promoting phosphorylation of TrkA and its downstream signaling effectors, Erk1/2, in PC12 and NIH3T3-TrkA cells (Fahnestock et al. 2004).

BDNF, as well as the other members of the neurotrophin family, is synthesized as a larger precursor (34 kDa), proBDNF, which undergoes posttranslational modifications and proteolytic processing by furin or related proteases. The metabolic labeling of recombinant human proBDNF in AtT-20 cells with $[^{35}SO_4]Na_2$, showed that proBDNF is N-glycosylated and glycosulfated (Lou et al. 2005). Some proBDNF is released extracellularly and biologically acts as mediator in TrkB phosphorylation, activation of ERK1/2, and neurite outgrowth. Pro-BDNF binds to and activates TrkB and could be involved in

TrkB-mediated neurotrophic activity *in vivo* (Fayard et al. 2005). The precursor undergoes N-terminal cleavage within the trans-Golgi network and/or immature secretory vesicles to generate mature BDNF (14 kDa). Small amounts of a 28-kDa protein that is immunoprecipitated with BDNF antibodies are also evident. This protein is generated in the endoplasmic reticulum through N-terminal cleavage of pro-BDNF at the Arg-Gly-Leu-Thr57--Ser-Leu site. Cleavage is abolished when Arg54 is changed to Ala (R54A) by *in vitro* mutagenesis (Mowla et al. 2001). Like mature BDNF, proBDNF is localized at nerve terminals in the superficial layers of the dorsal horn, trigeminal nuclei, nuclei tractus solitarius, amygdaloid complex, hippocampus, hypothalamus and some peripheral tissues (Zhou et al. 2004). These results suggest that proBDNF, like mature BDNF, is anterogradely transported to nerve terminals and may have important functions in synaptic transmission in the spinal cord and brain (Zhou et al. 2004). Some research suggests BDNF exists as a mixture of proBDNF and mature BDNF in all regions tested of human brain. Reduced proBDNF may have functional consequences for the selective neuronal degeneration in Alzheimer's disease brain (Michalski & Fahnestock 2003). Mutation in Val66Met of BDNF results in reduction in hippocampal function of learning and the dysfunction of sorting and secretion of intracellular BDNF (Egan *et al.* 2003, Chen *et al.* 2004).

2.   Receptors

Neurotrophins mainly activate two kinds of cell surface receptors, the high affinity of tropomyocin receptor kinase (Trk) family which includes three members, TrkA, TrkB and TrkC and the low affinity p75 neurotrophin receptor (p75NTR) which is a member of the tumor necrosis factor (TNF) receptor superfamily. The p75NTR binds to mature forms of NGF, BDNF, NT3 and NT4/5 with the same affinity (dissociation constant $K_d \approx 10^{-9}$M), and Trk receptors bind their cognate ligands with different affinities ($K_d = 10^{-9}$-10^{-10}M). The synergistic contribution of TrkA and p75NTR could increase the binding affinity ($K_d = 10^{-11}$M) of mature neurotrophins (Bibel *et al.* 1999, Kalb 2005).

2.1   The structure of neurotrophin receptors

Almost all of the survival-promoting activities stimulated by neurotrophins are activated by Trk receptors, whereas p75NTR can trigger classical apoptotic effects (Kim *et al.* 1999, Dechant *et al.* 1997). Differential splicing of TrkA, TrkB and TrkC mRNAs are translated to the receptor protein with differences in their ligand binding extracellular domains. The differences will affect the selective binding activity of neurotrophins to their receptors. NGF has high binding affinity to TrkA, brain derived neurotrophic factor (BDNF) and NT-

4/5 activate TrkB, and NT-3 activates TrkC but also activates TrkA and TrkB

in certain cellular contexts (Dechant 2001).

The extracellular domains of Trk receptors consist of three tandem leucine-rich

motifs flanked by two cysteine clusters in the amino termini and two IgG-like

domains in the membrane-proximal region. The intracellular domains are

highly conserved sequences with kinase activities (Fig. 1A) (Wiesmann et al.

1999). The p75NTR is one member of the TNF-R/fas family receptor. It has

four cysteine-rich domains in the extracellular part, which binds all the

neurotrophins. Its intracellular domain is similar to the death domains of the

TNF-R/fas receptor family (Fig. 1B). The ligand-receptor binding interface

contains two patches: one binds to all neurotrophins in the same conserved

manner, the other is specific for the NGF binding to TrkA. The research shows

that co-expression of TrkA and p75NTR increases the NGF binding affinity. In

contrast, the binding affinity of NT-3 to TrkA and TrkB and NT-4/5 to TrkB is

reduced when p75NTR exists (Dechant 2001).
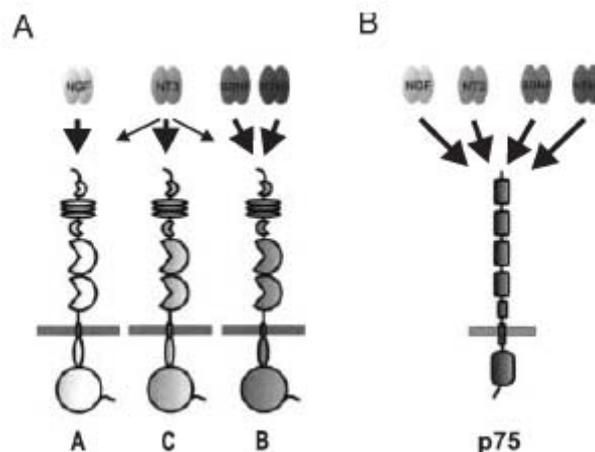
Fig. 1 Neurotrophins bind to two types of transmembrane receptors (Trk and p75NTR). (A) The structure of Trk receptors (A: TrkA, B: TrkB, C: TrkC).   The extracellular domains consist of three tandem repeat leucine-rich motifs which are flanked by two cysteine clusters and two Ig-like C2 type domains which are the main contact area for binding different neurotrophins. The intracellular domains of Trk receptors are highly conserved.   (B) The structure of p75NTR. The four cysteine-rich domains are the binding domain for neurotrophin ligands and its intracellular domain resembles the death domains found in several members of the TNF-R/fas family (Dechant 2001).

2.2   Molecular interactions between neurotrophin receptors

At the cellular level, the responses to neurotrophins are highly diverse. In different contexts neurotrophins can cause cells to proliferate or stop the cell cycle, to grow neurites or collapse them and to protect the cell from apoptosis or accelerate cell death. The view that neuronal survival signals occur via Trk receptor and apoptosis signals via p75NTR is too dogmatic and is not correct in most cases. For Trk receptors, the developing brains of Trk-knockout mice turn out surprisingly intact. For p75NTR knockout, the highly expressed p75NTR

embryonic mice brains seem unaffected. Hence, it seems that the physiological effects of neurotrophins are highly diverse based on the molecular interactions of their receptors in specific cellular environments (Bothwell 1995, Carter *et al.* 1996, Bibel et al. 1999).
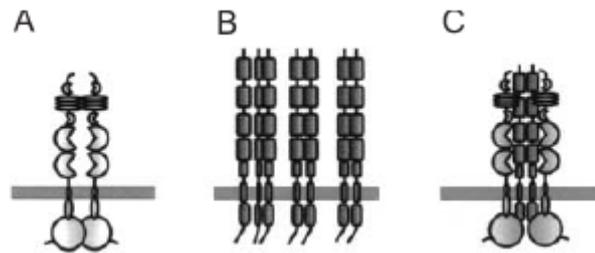
Fig. 2 Neurotrophin receptor complexes. The neurotrophin receptors form complexes within the plasma membrane. Biochemical evidence has been provided for homodimeric Trk receptors (A), for complexes between p75 monomers (B), and for mixed complexes containing both Trk and p75 receptors (C). Formation of these complexes often correlates with higher ligand affinity and specificity (Dechant 2001).

Biochemical experiments show that neurotrophin signals are triggered mainly by three types of complexes: homodimers of Trk receptors (Fig. 2A), trimers of p75NTR (Fig. 2B) and mixed p75NTR-Trk receptor (Fig. 2C). The complexes coexist and are linked through biochemical equilibria, such as receptor phosphorylation and ligand binding. A different receptor complex may cause

the cross talk between different neurotrophin signals, which makes the signaling complicated (Bamji *et al.* 1998, Dechant 2001).

2.3   Trk receptor-mediated signaling pathways and functions

When neurotrophins bind to the Trk receptors, the receptors become dimerized and autophosphorylated on tyrosines of the intracellular domain, which has tyrosine kinase activity. The cytoplasmic domains of Trk receptors contain several tyrosines that can be phosphorylated by each receptor's tyrosine kinase upon binding of neurotrophins. After phosphorylation, these cytoplasmic tyrosine domains contribute to Trk-mediated signaling. The major signal pathways activated by Trk receptors are Ras, PI3-kinase, PLC-γ1 and their downstream effectors (Huang & Reichardt 2003). Among those cytoplasmic tyrosine kinase domains, two (Y490 and Y785) are the major sites for phosphorylation. The phosphorylated Y490 offers a recruitment site for both Shc and Frs2, which provide links to Ras, MAPK, PI3-kinase and other pathways. The phosphorylated Y785 recruits the enzyme PLC- γ 1 which results in Ca^{2+} and protein kinase C mobilization.

Ras is a small GTP (Guanosine-5'-triphosphate) binding protein. When this molecule is bound to GDP (Guanosine diphosphate), the molecule remains

inactive. Several different adaptors appear to become engaged in promoting Ras activation. Shc is the adaptor protein which is recruited through the PTB domain by phosphorylated Y490, and then phosphorylated Shc induces the phosphorylation of adaptor Grb2 which can bind to the Ras exchange factor son of sevenless (SOS). Additional scaffold proteins, like Src homology-2 domains (APS) and insulin receptor substrate-1 (IRS1), also link to Ras in some cell types. The adaptor proteins activate the intracellular signaling events, including Ras-Raf-Erk, PI3 Kinase-Akt, PLC-4-Ca^{2+}, NFκB and a typical protein kinase C pathway (Qian & Ginty 2001, Kaplan & Miller 2000, Foehr *et al.* 2000, Wooten *et al.* 1999). For example, activated Ras stimulates signaling through c-Raf-Erk, p38MAP kinase and class I PI3 kinase pathway (Xing *et al.* 1998, Vanhaesebroeck *et al.* 2001). Ras activation is required for normal neuronal differentiation and the survival of many neuronal subpopulations. Phosphorylated Y490 also recruits an adaptor called Frs2, which is required for the activation of MAP kinases. This signal pathway is essential for the genes' transcription which is important in the neuron differentiation and survival. PI3 kinase provides phosphoinositides (PI3) phosphorylated, which have various effects on neuronal survival and development. Firstly, those lipid products generated by PI3K recruit some phosphoinositide-dependent kinases, and they activate the protein kinase Akt, which in turn controls several proteins important in promoting cell survival through the reaction of phosphorylation.

In addition, PI3 kinase also affects some targets to promote axon growth and pathfinding, and neuronal differentiation (Yuan & Yankner 2000, Brunet *et al.* 2001, Reichardt 2006).

The phosphorylation of Y785, cytoplasmic tyrosine kinase domains of Trk receptors, leads to the recruitment of the enzyme PLC-r1 which activates IP3 and DAG. IP3 can provide Ca^{2+} release from cytoplasmic stores. DAG can stimulate DAG-regulated isoforms of protein kinase C. Together, signaling through this pathway controls expression and/or activity of many proteins, including ion channels and transcription factors. In addition, neurotrophins also contribute to organization of the cytoskeleton, cell motility, growth cone behaviors and thermal sensitization through some poorly defined pathways (Corbit *et al.* 1999, Minichiello *et al.* 2002, Yuan *et al.* 2003, Klein *et al.* 2005).
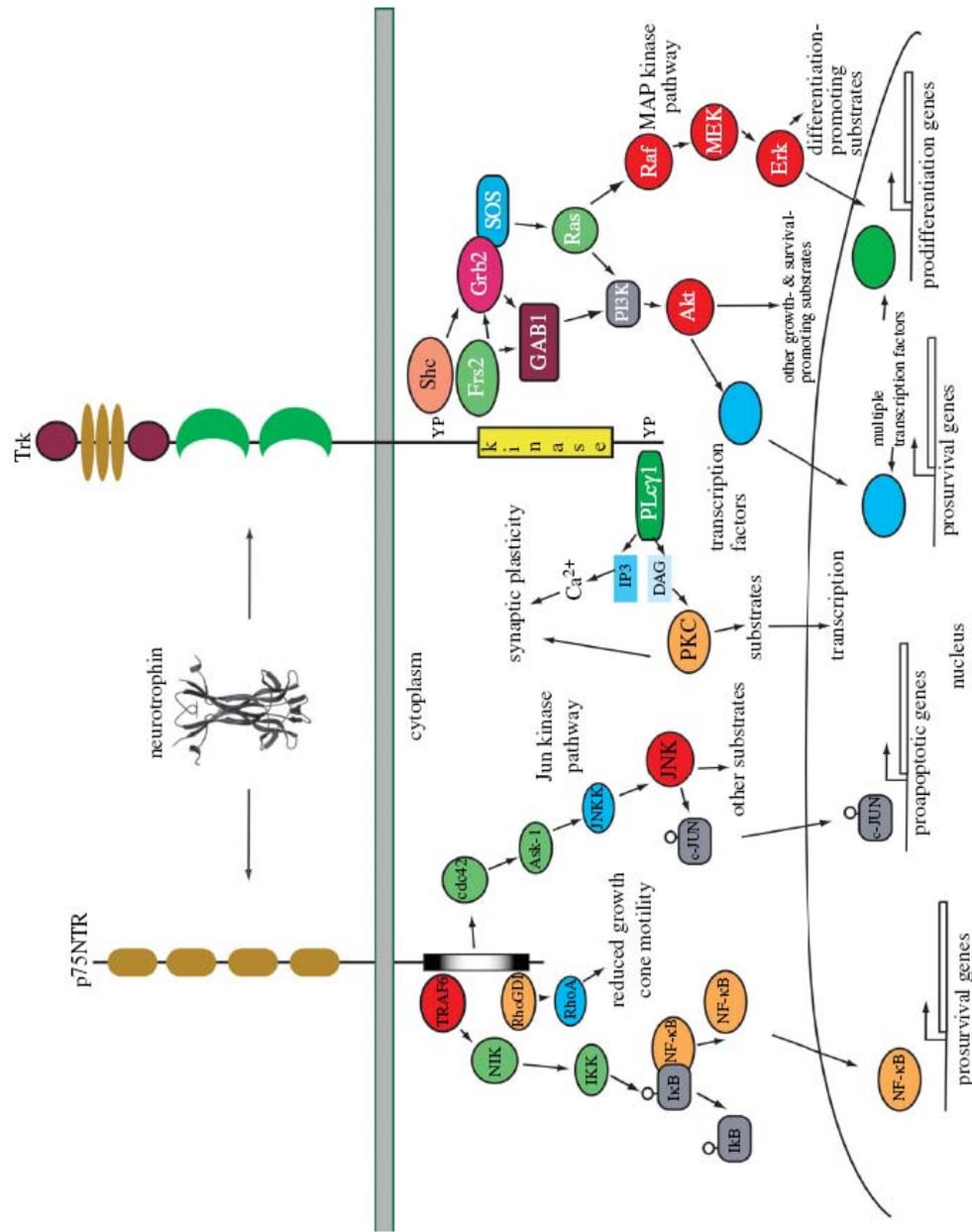
Fig. 3 Neurotrophin signalling. This depicts the interactions of each neurotrophin with Trk and p75NTR receptors and major intracellular signalling pathways activated through each receptor. The p75NTR receptor regulates three major signalling pathways. NF-kB activation results in transcription of multiple genes, including several that promote neuronal survival. Activation of the Jun kinase pathway similarly controls activation of several genes, some of which promote neuronal apoptosis. Ligand engagement of p75NTR also regulates the activity of Rho, which controls growth cone motility. Pro-apoptosis actions of p75NTR appear to require the presence of sortilin, which functions as a co-receptor for the neurotrophins. Sortilin is not depicted in this figure, but is described in the text. Many additional adaptors for p75NTR and Trk receptors have been identified which are not depicted in this figure for simplicity, but are described in more detail in the text (Reichardt 2006).

2.4 The p75NTR-mediated signaling pathways and functions

The p75NTR has lower binding affinity to the mature forms of neurotrophins, but surprisingly has higher binding affinity to the proneurotrophins together with sortilin, a Vps 10-domain containing protein (Nykjaer et al. 2004, Chen *et al.* 2005). The p75NTR inhibits axon growth by the complex with Nogo

receptor and Lingo-1 (Wang *et al.* 2002, Wong *et al.* 2002b, Yamashita *et al.* 2005).

Like Trk receptor multi adaptor proteins, the intracellular domain of p75NTR can activate several signaling pathways by binding to factors such as Traf6, neurotrophin receptor-interacting factor (NRIF), melanoma-associated antigen acting factor (MAGE), neurotrophin receptor p75NTR interacting MAGE homologue (NRAGE) and other proteins (Yamashita et al. 2005). One major signaling pathway activated by p75NTR is the Jun kinase-signaling cascade, which is implicated in neuronal cell death. p53 is activated in this pathway and this results in apoptosis. Furthermore, the activities of the Jun kinase pathway induce the expression of Fas ligand, which also results in apoptosis (Yeiser et al. 2004). The p75NTR can activate RhoA directly to inhibit neurite outgrowth. However, neurotrophins binding to p75NTR can stimulate neurite outgrowth by eliminating p75NTR-dependent activation of RhoA (Yamashita *et al.* 1999, Yamashita & Tohyama 2003). The p75NTR also can promote NF-κB-dependent neuronal survival by activation of NF-κB. In this process, both p62 and the activity of interleukin-1 receptor-associated kinase (IRAK) are necessary (Hamanoue *et al.* 1999, Middleton *et al.* 2000).

2.5   The crosstalk between the p75NTR and Trk receptors

In general, the two different receptors usually have opposing effects: the Trk receptors promote neuronal survival and differentiation, while p75NTR frequently, but not always, generates apoptosis. But this theory is overly simplistic. The life and death destinations are not segregated between one receptor or the other. Many factors contribute to the complexity of cellular responses to neurotrophins.

The Ras signaling pathway activated by the Trk receptor suppresses the major pro-apoptotic-signalling pathway stimulated by p75NTR (Yoon et al. 1998). The intermediates generated from Trk signaling pathway, such as PI3-kinase, Jnk-interacting protein 1 (JIP1) and Akt1, are involved in the various mechanisms through which Trk receptor activation suppresses the apoptotic effects of p75NTR signaling (Patapoutian & Reichardt 2001, Aloyz *et al.* 1998). On the other hand, the p75NTR pathway intermediate NF-κB increases the survival possibility of neurons (Thomas *et al.* 2005, Maggirwar *et al.* 2000) and proteins such as ARMS and caveolin adjust both p75NTR and Trk receptor signaling pathway (Bilderback *et al.* 1999, Du *et al.* 2006, Zampieri & Chao 2006, Kong *et al.* 2001, Chang *et al.* 2004). Together, all these studies about the synergistic effect of both receptors show that the pro-apoptotic signals of p75NTR are largely suppressed by p75NTR-Trk receptor interactions. Much evidence indicates that Trk receptors could also induce cell death (Muragaki *et*

al. 1997, Zampieri & Chao 2006), however the mechanism is still unknown. MAP kinase and PI3 kinase are involved in the death-promoting signaling, but the downstream effectors are unknown. The activated Trk receptors also alter the function of ligand-gated and voltage-gated ion channels (such as the NMDA receptor and $Na_v1.9$) which leads to Na^+ and Ca^{2+} current changes. The cell-type specificity, state of maturation and receptor complex may also contribute to Trk induced apoptosis (Fryer *et al.* 2000, Choi *et al.* 2004b, Kalb 2005).

3. Sensory neurons as a model for neurotrophin studies

3.1 Expression of neurotrophin receptors

The sensory neurons of ganglia are those which are the easiest to access and which also have a precise anatomical location, and the number of neurons in each sensory ganglion is comparatively constant and easy to determine. Moreover, the sensory neurons in dorsal root ganglia (DRG) supply different types of sensory receptors in multiple body locations. Thus, sensory ganglia represent good models for the analysis for neurotrophins effects.

The biological effects of neurotrophins are mediated through Trk receptors. Trk receptor expression was investigated by using *in situ* hibridizaton in DRG

(Tessarollo *et al.* 1993, Mu *et al.* 1993, Farinas *et al.* 1998). The results indicate that the time frame of Trk expression is consistent with neurotophins synthesis at early developmental stages. All Trk receptors begin expression from early embryonic stages, just a few hours after neurogenesis begins in these ganglia. Between E10 and E11, TrkB and TrkC expression is observed and TrkC is expressed in a majority of neurons but this expression stops at E13. The major quantity of TrkA is generated between E11 and E13 in DRGs. The Trk expression pattern of thoracic DRGs is similar to lumber DRGs which have more neurons expressing Trk receptors. At embryonic age, 40% of thoracic neurons express TrkA which are mainly in small neurons, 6% of neurons express TrkB which are mainly in intermediate and large neurons, and 8% of neurons express TrkC which are mainly in large neurons. In embryonic and in adult stages, TrkB and TrkC were observed coexpressed in some neurons.

The p75NTR plays a role in cell death and is constitutively expressed within a subpopulation of DRG sensory neurons. In the C7 and C8 DRG of the newborn rat, p75NTR is expressed in approximately 70% of DRG neurons. The expression of p75NTR is observed in neurons throughout the entire neuronal size range. This is the reason which is speculated to be responsible for why those neurones which constitutively express the highest levels of p75NTR are the most vulnerable following axotomy (Murray & Cheema 2003).

NGF controls survival, differentiation, and target innervation of both peptidergic and nonpeptidergic DRG sensory neurons. The common receptor for GDNF family ligands, Ret, is highly expressed in nonpeptidergic neurons. NGF controls expression of several other genes characteristic of nonpeptidergic neurons, such as TrpC3, TrpM8, MrgD, and the transcription factor Runx1, via a Ret-independent signaling pathway. These findings support a model in which NGF controls maturation of nonpeptidergic DRG neurons through a combination of GFR/Ret-dependent and -independent signaling pathways (Luo *et al.* 2007).

3.2 Neurotrophins are required for the survival of sensory neurons

It has been reported that all members of the neurotrophin family of neuronal growth factors promote survival and neurite outgrowth of dorsal root ganglion (DRG) neurons *in vitro* (Mu et al. 1993). The essential effects of neurotrophins on sensory neuron survival have been investigated *in vivo* by using the neurotrophin or Trk gene knock out animal or by injection of specific neurotrophin antibodies against endogenous neurotrophins (Farinas 1999, Crowley *et al.* 1994, Smeyne *et al.* 1994). Sensory neuron loss was observed in each of the individual neurotrophin and Trk-deficient mice. In the DRGs of

NGF or TrkA-deficient mice, approximately 70% of the normal complement of neurons are missing, while NT-3 absence causes 60-70% neuronal loss in DRG (Farinas *et al.* 1996, Crowley et al. 1994, Smeyne et al. 1994). Both NGF and NT-3 are expressed during early embryogenesis. NGF is expressed in those neurons that have already projected into the limb. The NGF expression is restricted to the limb epidermis. NT-3 is the most abundantly expressed neurotrophin during embryogenesis, but its expression domain changes as axons grow distally. BDNF and NT-4/5 are not clearly detectable at times of gangliogenensis (Coppola et al. 2001). The early death of sensory neurons and the reduction in neuron number suggest that these neurotrophins are critical in the sensory neuron development.

At embryonic stages, NGF and NT-3 contribute greatly in determining the final neuron number. After birth, BDNF plays a role in regulating neuron survival. A lack of TrkB or BDNF causes 30-35% neuron loss at 2 weeks of age (Klein *et al.* 1993, Ernfors *et al.* 1994). This suggests that BDNF is acting to maintain a normal complement of DRG neurons and a deficiency in BDNF production causes the loss of certain type of neurons (LeMaster et al. 1999).

3.3 Response to nerve injury and functions of neurotrophins on injured neurons

Unlike other regions of the body, after a nerve is injured in the periphery, no neuronal mitosis or proliferation occurs (Burnett & Zager 2004). A few hours after injury, axon and myelin start to breakdown progressively. Ultrastructurally, the microtubules, neurofilaments and axonal contour become disarrayed. In this degeneration process, Schwann cells and endoneurial mast cells play pivotal roles. Schwann cells are activated within 24 hours after injury and their mitotic rate is increased. These cells divide rapidly to form de-differentiated daughter cells which can help to remove the degenerated axonal and myelin fractions and pass them to macrophages. Through this process, the site of injury can be cleared. The endoneurial mast cells proliferate quickly within the first 2 weeks after injury. The released histamine and serotonin help macrophage activity. The inflammatory response is also one of the results of nerve injury, which is caused by the local vascular trauma hemorrhage and edema. The proliferated fibroblasts lead to the fusiform swelling of the injured segment and the enlargement of the entire nerve trunk (Richardson *et al.* 1980, Sunderland 1990, Sunderland & Bradley 1950).

Soon after nerve injury, macrophages stimulate the rapidly increasing concentration of NGF mRNA, NGF receptor (TrkA) mRNA in the vast majority of sympathetic and sensory neuron, which leads to the retrograde

transport of NGF to the cell body to act in its survival role (Farinas et al. 1998).

NGF induces motoneuron apoptosis through its receptor p75NTR (Sedel et al.

1999). NGF also acts on Schwann cells to regrow axon sprouts (Yin *et al.*

1998, Burnett & Zager 2004).

The receptor of BDNF (TrkB) is widely expressed in motoneurones. BDNF

increases neurite outgrowth and innervation of motoneurones. All *in vivo* and

in vitro studies strongly suggest the effect of BDNF on axonal and neurite

regeneration, especially on motor nerves (Ye & Houle 1997). After sciatic

nerve transection, BDNF delivered on the injured side improves the rate and

degree of the recovery of sciatic function (Utley et al. 1996).

NT-3 induces survival and differentiation responses in sensory and

parasympathetic neurons and NT-3 increases the regenerative sprouting of

transected corticospinal tract (Henderson *et al.* 1993, Schnell *et al.* 1994). The

combination of NT-3 and BDNF enhances propriospinal axonal regeneration

and more significantly, promotes axonal regeneration of specific distant

populations of brain stem neurons into grafts at the mid-thoracic level in adult

rat spinal cord (Xu et al. 1995).

4. Transport and release of neurotrophins

Generally, neurotrophins come from two sources, autocrine and paracrine. Firstly, during development, neurotrophins are synthesized and secreted by the regions being invaded by axons. This secretion offers trophic support to neurons that are targeting their final destination (Ringstedt *et al.* 1999, Wong et al. 2002b). Secondly, after peripheral nerve injury, the inflammatory response and release of cytokines induces the accurate expression of NGF and other neurotrophins in Schwann cells and fibroblasts within the injured nerve. NGF is also synthesized in mast cells and released following mast cell activation. These phenomena are believed to be essential for the survival and regeneration of injured neurons (Korsching 1993, Levi-Montalcini *et al.* 1996). Thirdly, many neurons themselves synthesize neurotrophins. For example, BDNF acts in an autocrine or paracrine fashion to support DRG sensory neurons, and it is also transported anterogradely, trans-synaptically and retrogradely to effect other neurons in the brain (Altar *et al.* 1997, Brady *et al.* 1999, Huang & Reichardt 2001).

In neuronal cells, following receptor-mediated internalization, neurotrophins can be sorted into several subcellular pathways including being recycled back to the plasma membrane either as a ligand-receptor complex or as the receptor only, they can be rapidly degraded or they can be transported via retrograde

transcytosis (von Bartheld 2004, Butowt & von Bartheld 2001). The ligand-receptor complex internalizes through clathrin-mediated endocytosis. After internalization, some of the vesicles are transported retrogradely to the cell body using a dynein-dependent and microtubule-dependent transport mechanism. The vesicle-associated Trk receptors remain autophosphorylated and capable of promoting a unique set of signals upon arrival at the cell bodies. These include PI3K and Erk5. The alternative model for retrograde signaling is that after neurotrophin binding to Trk receptors on distal axons, a wave of ligand-independent Trk phosphorylation that is retrogradely propagated, or local signals emanating from Trk receptors in distal axons are themselves propagated to cell bodies where they promote activation of cell body-associated Trk and consequently cell surival (Ginty & Segal 2002, Campenot & MacInnis 2004). Once the protein has been transported to either axon terminals or to somatodendritic domains, it can be released from the neuron (von Bartheld 2004, Butowt & von Bartheld 2001).

4.1 Transport of NGF

Axonal retrograde transport of neurotrophins is essential for neuronal growth and survival (Hendry *et al.* 1974a, Hendry *et al.* 1974b). Retrograde transport of NGF is the classic model of neurotrophin transport as a target-derived

survival factor. In adult sympathetic neurons, the endocytosis of NGF is conducted through p75NTR and TrkA (Gatzinsky et al. 2001). Rapid internalization and retrograde trafficking of the NGF-TrkA complex has been demonstrated in a number of systems and this is thought to transmit trophic signals from neuronal terminals to cell bodies. The NGF-p75NTR complex is another internalization pathway. It can be internalized into early endosomes and eventually accumulates in recycling endosomes in the cell (Bronfman *et al.* 2003, Lalli & Schiavo 2002).

4.2 Transport of NT-3

Neurotrophin-3 (NT-3) retrograde transport was tested at adult peripheral nervous system (PNS) and central nervous system (CNS) neuron cell bodies by intracranial and intranerve injections of iodinated NT-3 (Altar & DiStefano 1998). Unlike NGF, NT-3 is produced in a variety of adult neural systems. The investigation of NT-3 localisation by use of highly specific and sensitive antibodies, identified NT-3 proteins in nerve terminals where no NT-3 mRNA was found (Radka et al. 1996). In the chick retinotectal pathway and zebra fish motor-cortical pathway, the anterograde transport of exogenous NT-3 depends on a complex of TrkC and p75NTR and promotes the survival of postsynaptic neurons after axotomy (Johnson *et al.* 1997, von Bartheld *et al.* 1996b, von

Bartheld *et al.* 1996a). Most newly synthesized NT-3 is released through the constitutive secretory pathway as a result of furin-mediated endoproteolytic cleavage of proNT3 in the trans-Golgi network. Sorting of the NT3 precursor can occur in both the constitutive and regulated secretory pathways, which is consistent with NT3 having both survival-promoting and synapse-altering functions (Farhadi et al. 2000).

4.3 Transport of BDNF

Nerve growth factor (NGF) is released through the constitutive secretory pathway from cells in peripheral tissues and nerves where it can act as a target-derived survival factor. In contrast, brain-derived neurotrophic factor (BDNF) appears to be processed in the regulated secretory pathway of brain neurons and secreted in an activity-dependent manner to play a role in synaptic plasticity (Mowla et al. 1999). After being synthesized, unlike most proNGF efficiently cleaved by the endoprotease furin which is also the preferred proprotein convertases (PCs) of proBDNF in the trans-Golgi network (TGN), BDNF is mainly secreted as proBDNF (Hempstead 2006, Lee et al. 2001b, Lee *et al.* 2001a). The secreted proBDNF can be converted to mature BDNF by the action of extracellular tPA/plasmin (Lee et al. 2001b, Pang *et al.* 2004). There also has a controversial study showed that proBDNF was effectively processed

by furin, PACE4 and PC516-B in coexpressed either the kidney epithelial cell line BSC40 or the furin activity-deficient colon carcinoma cell line LoVo (Seidah et al. 1996). As it was discussed in the paper, those experiments performed under conditions where both the substrate and the convertase were expressed at high levels. In vivo, the enzyme to substrate ratios may differ widely from one type of cell to the other, which may influence the nature of the convertase responsible for the processing of each neurotrophin precursor.

Although little is known about the bidirectional transport mechanisms of BDNF, many groups are investigating this issue. In the PNS, convincing studies using a double ligation of sciatic nerve have shown that endogenous BDNF accumulated on both proximal and distal sides of the ligature (Zhou & Rush 1996, Tonra *et al.* 1998). It also has been shown that the injury of sensory neurons can upregulate the expression and anterograde transport of BDNF (Tonra et al. 1998). In the CNS, anterograde transport is a common feature of BDNF trafficking. Many regions, such as medial habenula, central nucleus of the amygdala, bed nucleus of stria terminalis, lateral septum and spinal cord, were detected by immunohistochemistry containing extremely heavy BDNF-immunoreactive fiber/terminal labeling that lacked BDNF mRNA. This observation suggested that in these regions BDNF was derived from anterograde axonal transport by afferent systems (Conner *et al.* 1997, Altar et

al. 1997). In all, BDNF is bidirectionally transported in axons, but the mechanisms of how BDNF is transported is not known.

4.3.1 Anterograde transport

Anterograde transport is the process of transport of molecules from the cell body to axons and dendrites. Many kinesin superfamily proteins (KIFs) participate in this sort of transport as molecular motors. In axons and dendrites, microtubules act as rails along which a variety of cargoes including protein complexes, vesicular and cytoskeletal components can be transported (Schliwa & Woehlke 2003). Microtubules in axons and distal dendrites are unipolar, and their plus ends orient distal to the cell body. However, microtubules in proximal dendrites are of mixed polarity (Baas et al. 1989). During transport, the neurotrophins may be packed in the vesicles or fused into organelles (Mukherjee *et al.* 1997, Gunawardena & Goldstein 2004a, von Bartheld 2004).

The mechanism of anterograde transport is still incompletely understood and the identity of transport cargoes is not entirely clear. Large dense core vesicles (LDCV) may be one of these cargoes (Shakiryanova *et al.* 2005, Li *et al.* 1999a). In axon terminals, LDCVs have been associated with anterogradely transported neurotrophins. But in some cell types such as hippocampal

neurons, LDCVs rarely exist, suggesting that newly synthesized trophic factors may not be packaged and transported in LDCVs in hippocampal neurons (Smith et al. 1997).

4.3.2 Retrograde transport

Retorgrade transport is the process of transport of molecules from the axonal or dendritic terminals to the cell body. Many dynein superfamily proteins participate in this sort of transport as molecular motors (Gunawardena & Goldstein 2004a). Recent studies indicate endosomes are the main retrograde transport vehicles (Wahle *et al.* 2005, Seaman *et al.* 1998), while other studies identify multivesicular bodies (MVBs) as the major trafficking organelles for retrograde transport (Gabriely et al. 2007). Neurotrophins bind and activate their receptors at axon terminals and are internalized into cells through receptor-dependent mechanisms. Endocytosed neurotrophins and receptors enter the retrograde transport pathway along the axon. Sciatic nerve ligation experiments showed that p75NTR was transported both anterogradely and retrogradely (Johnson et al. 1987). Accumulation of Trk receptors was detected at the distal site after the sciatic nerve ligature, indicating its retrograde transport (Yano & Chao 2004). Although the retrograde transport of Trk receptors and p75NTR has been observed, the molecular mechanism of

transport has not been fully understood. Tctex-1, the light chain of the dynein motor complex, was found to interact with the juxtamembrane domain of the TrkB receptor by yeast two-hybrid screening, co-immunoprecipitation and sciatic nerve ligature experiments (Yano *et al.* 2001, Bhattacharyya *et al.* 2002). So far, whether other motor molecules are involved in the retrograde transport remains unknown.

4.3.3 Release

In general, the newly synthesized neurotrophins are packaged into vesicles and are then transported to either pre-synaptic axon terminals or post-synaptic dendrites for local secretion. The two principal cellular secretion pathways for neurotrophins are constitutive and regulated release, depending on whether the release occurs spontaneously or in response to stimuli. In acute hippocampal slices and cultured neurons overexpressing NGF, there is a transient increase above baseline of extracellular NGF following stimulation with high concentrations of potassium, glutamate or carbachol (Blochl & Thoenen 1995). Similarly, regulated BDNF secretion is also observed by high frequency burst stimulation in hippocampal slices and cultured neurons overexpressing BDNF (Griesbeck *et al.* 1999, Balkowiec & Katz 2002). After potassium-mediated

depolarization and an increase in intracellular calcium levels, the regulated secretion of NT-3 was observed in neurons (Boukhaddaoui et al. 2001).

With the development of techniques, synaptic secretion of neurotrophin was visualized by using overexpression of GFP-tagged BDNF in cultured neurons (Horch & Katz 2002, Kojima *et al.* 2001). In these studies, BDNF-GFP accumulated at glutamatergic synaptic junctions and underwent activity-dependent release. BDNF-GFP was also reported to be released from a pre-synaptic to post-synaptic neuron (Kohara et al. 2001). There exists no data for NGF, NT-3 or NT-4/5 secretion at synapses.

The different releasing pathway of BDNF from dendrites seems to have different neuronal effects than BDNF released from synapses. The dendrite-to-dendrite release of BDNF helps nearby dendritic branching without any effect on axons or spines (Horch & Katz 2002), whereas neurotrophin secretion from synapses may affect synaptic plasticity and formation of new spines (Schinder et al. 2000).

4.3.4 Proteins involved in intracellular BDNF trafficking

Two proteins have been identified to interact with BDNF within neurons. Amino acids 44-103 of the prodomain of BDNF are the BDNF-sortilin interaction domain, and this domain is required for efficient regulated BDNF secretion. Furthermore, the highly expressed sortilin in HEK 293 cells increases the amount of BDNF secreted. Alltogether, sortilin interacts with the prodomain of BDNF and is involved in sorting to the regulated secretory pathway and release of BDNF (Chen et al. 2005).

The other interaction protein is carboxypeptidase E (CPE). CPE is the sorting receptor in secretory granule membranes and interacts with mature BDNF. It contains a three-dimensional sorting signal motif, $I_{16}E_{18}I_{105}D_{106}$, which is required for the sorting and release of BDNF to the regulated pathway for activity-dependent secretion. The mutation of the two acidic residues (E_{18} and D_{106}) results in missorting of proBDNF to the constitutive pathway (Lou et al. 2005).

5. Htt and HAP1 take part in the transport of BDNF

BDNF transportation is distinctly decreased in Huntington's disease (HD) (Charrin *et al.* 2005, Bossy-Wetzel *et al.* 2004). Htt is an important regulator in BDNF transport along microtubules in the cell. Htt could enhance its efficiency

of microtubule based transport, whereas polyQ-htt could inhibit this process (Gauthier et al. 2004). This htt-mediated vesicular transport requires HAP1 and P150[Glued]. The complex of Htt/HAP1/P150[Glued] enables the BDNF-containing vesicles to move along microtubules. PolyQ-htt inhibits HAP1/P150[Glued] dynactin binding to microtubules, and this could lead to the BDNF transport deficit and result in a loss of neurotrophic support and toxicity (Gauthier et al. 2004).

5.1 Huntington's Disease

 HD is an inherited neurodegenerative disease caused by mutant htt with cytosine-adenine-guanine (CAG) trinucleotide repeats in exon 1 which codes for polyglutamine (polyQ) expansion. The normal htt protein with 6 to 34 polyQs tract does not cause the disease whereas disease symptoms can be observed when polyQ extension is greater than 40 in the N-terminal fragment of htt (Karlin & Burge 1996, Bertaux *et al.* 1998, Davies & Ramsden 2001). The polyQ region contributes to the modification of the three-dimensional structure of the whole protein and differences in the physiological function of the protein (Chen 2003, Weibezahn *et al.* 2004). This is why the number of ployQ repeats plays a key role in the disease pathogenesis.

HD is characterized by cognitive decline, chorea, dementia, and other psychiatric symptoms. In HD, the mutant huntingtin is ubiquitously expressed in the brain and peripheral organs. Although the disease affects a number of brain regions such as the cortex, thalamus and subthalamic nuclei, the neuropathological hallmark of HD is the severe atrophy of the striatum. In the striatum, medium spiny GABAergic projection neurons in the basal ganglia are preferentially lost, whereas spiny interneurons are relatively spared. In the cerebral cortex, large neurons in layer VI are most affected. The disease typically has a mean onset at an age of 35 years, and ultimately leads to death after 15-20 years of progressive neurodegeneration (Davies & Ramsden 2001, Cattaneo *et al.* 2005).

The pathological mechanisms of HD are not fully understood but many theories are proposed. Excitotoxicity plays a critical role in the pathogenesis of the disease. Nuclear inclusions form after different types of proteolytic cleavage which causes abnormal protein-protein interaction in HD. Oxidative stress, energy metabolism, apoptosis also change during the progress of HD (Davies & Ramsden 2001, Sadri-Vakili *et al.* 2006).

5.2 Huntingtin regulates neuronal gene transcription

Wild type and mutant huntingtin (Htt) are localized in several organelles as well as the cytoplasm and nucleus of neurons. Mutant huntingtin causes transcriptional dysregulation in HD by interacting with numerous nuclear transcription factors, such as CREB-binding protein (CBP) and TATA-binding protein (TBP). The interaction of mutant huntingtin with transcription factors has been demonstrated to cause the downregulation of specific gene transcription in the brains of R6/2 and N171-82Q HD mouse models (Steffan *et al.* 2000, Nucifora *et al.* 2001, Sadri-Vakili et al. 2006).

Further investigations conducted in vitro and in vivo show that the wild type huntingtin stimulates BDNF expression in cortical neurons through regulating the activity of the BDNF promoter exon II, which consists of a sequence named repressor element 1 (RE1, also called neuron-restrictive silencer element, NRSE). Wild type huntingtin can competitively bind to this sequence with RE1-silencing transcription factor (REST, also called neuronal restrictive silencing factor, NRSF) which acts as a transcriptional silencer, whereas mutant huntingtin can not. As a consequence, REST suppresses the expression of BDNF in neurons which express mutant huntitngtin. Taken together, wild type huntingtin promotes BDNF transcription through inhibition of REST. Nevertheless, mutant huntingtin reduces the transcription by the formation of RE1/REST complex in the nucleus (Zuccato *et al.* 2001, Sugars & Rubinsztein

2003, Zuccato *et al.* 2003, Zucker *et al.* 2005, Sadri-Vakili et al. 2006, Sadri-Vakili & Cha 2006).

5.3 Htt plays a role in BDNF vesicle transport

Htt can act as a scaffold protein which enables the packaging of various proteins for transport along microtubules. As one of the components of cargo-motor molecules, Htt is required for the efficient anterograde and retrograde transport of neurotrophins (Gunawardena & Goldstein 2004a). BDNF is the most important factor supporting the survival of striatal neurons. BDNF located in striatal neurons is synthesized in and transported from the cerebral cortex (Altar et al. 1997). Down-regulation of wild type htt by RNAi can attenuate BDNF transport, the effect is gene dosage-dependent and BDNF transport is completely abolished by knockout of huntingtin (Gauthier et al. 2004). The mutant huntingtin in neuronal cells may impair the trafficking of BDNF-containing vesicles (Gauthier et al. 2004, Trushina *et al.* 2004).

Mutant Htt could interrupt the complex of wild type huntingtin/HAP1/p150Glued and thus interfere with microtubule transport. The pathogenic polyQ expansion blocks the axonal transport pathway, and retards BDNF transport to striatal neurons (Davies & Ramsden 2001, Gauthier et al.

2004, Gunawardena & Goldstein 2004b, Trushina et al. 2004). This lack of BDNF transport is one of the reasons why medium spiny neurons die in the striatum. Thus enhancing the transportation of neurotrophins is a potential therapeutic strategy for HD. There is evidence indicating that increased BDNF, GDNF, NT-3 and NT-4/5 support provides structural and functional neuroprotection in various models of HD (Cattaneo et al. 2005).

On the other hand, as TrkB gene transcription is reduced by mutant Htt in a polyQ-length-dependent manner (Gines et al. 2006), TrkB levels in striatum and cortex were also found to be decreased in both transgenic HD mouse models and in human HD (Gines et al. 2006). This is why applying BDNF into the brains of HD patients is not as effective as expected (Kells et al. 2004). Nevertheless, in neurotoxin-injured striatal neurons and astrocytes, the GDNF receptors, GFRα-1 and Ret, are upregulated (Marco et al. 2002). The viral delivery of GDNF improves behavior and protects striatal neurons in HD transgenic mouse models (Gines et al. 2006, McBride *et al.* 2006).

6. Discovery of Huntingtin-Associated Protein 1 (HAP1)

A number of proteins are known to interact with Htt. One of these is HAP1 which was first identified in a yeast two-hybrid system as an interacting partner

for Htt. It interacts with both Htt and mutant Htt, but the binding ability is increased by the polyQ expansion (Li et al. 1998c). HAP1 consists of two isoforms (HAP1-A, 75kDa and HAP1-B, 85kDa) which have different C-terminal sequences. HAP1-A has a unique sequence of 21 amino acids, whereas HAP1-B has a different sequence of 51 amino acids (Li *et al.* 1995, Nasir *et al.* 1998).

6.1 Localization of HAP1

HAP1 is mainly present in the somatodendritic cytoplasm of neurons (Martin et al. 1999). The mRNA localization is extremely discrete (Page et al. 1998). The most prominent localizations of HAP1 mRNA are in basal forebrain, cerebral cortex, cerebellum, the accessory olfactory bulb and the pedunculopontine nuclei. HAP1 is highly expressed in the olfactory bulb, the hypothalamus, and the supraoptic nucleus (Page et al. 1998, Li *et al.* 1996). In the rat brain, the expression is more intense in the stratum, brainstem, olfactory bulb, thalamus and ventral forebrain than in cerebral cortex, hippocampus, cerebellum and spinal cord (Li *et al.* 1998a, Gutekunst *et al.* 1998). The ratios of HAP1-A to HAP1-B are different in various regions. In the olfactory bulb and spinal cord, the level of HAP1-A is lower than that of HAP1-B (Li et al. 1998a). In the striatum and other regions their levels are almost the same. The

68kDa isoform is predominant in the cerebral cortex, striatum, hippocampus and thalamus (Li et al. 1998b).

HAP1 is highly expressed throughout the cell cycle of striatal cells. In dividing cells, it radiates from spindle poles to microtubules in mitotic spindles. In the earliest stages of mitotic striatal cells, HAP1 is associated with condensed chromatin in the nucleus and large amount of punctate structures in the perinuclear and peripheral cytoplasm. During postmitosis, HAP1 is absent from the nucleus and appears on endoplasmic reticulum (ER)/Golgi-like membranes in the perinuclear region, and has a punctuate distribution in the cytoplasm (Martin et al. 1999).

In adult mouse brain neurons, HAP1 is highly enriched in large dense organelles, large endosomes (multivesicular bodies) and moderately expressed in small vesicles, tubulovesicular structures, plasma membrane, coated and budding vesicles and microtubules (Martin et al. 1999).

6.2 Molecular structure of HAP1

The common region of both HAP1 isoforms contains three predicted coiled-coil domains (H1, H2 and H3) and a huntingtin-binding domain (HB) as shown in Fig. 4.
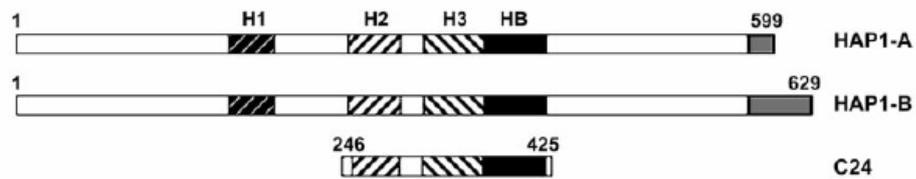
Fig. 4 Domain structure of HAP1. HAP1 isoforms, HAP1-A and HAP1-B, differ only at the C terminus. Three putative coiled-coil domains (H1,H2 and H3) and the HB are indicated (Li et al. 2002b).

6.3   The function of HAP1

6.3.1   Postnatal lethality

HAP1 plays an important role in early postnatal life. The majority of HAP1 knock out mice die at P2, and ~80% die before P3, and none can survive past P9 (Li et al. 1998b, Chan *et al.* 2002). However restored HAP1 expression in neuronal cells before birth rescues the early lethality (Dragatsis et al. 2004). A few hours after birth, HAP1 mutant mice exhibit abnormal feeding behavior (Chan et al. 2002). HAP1 is indispensable for the initiation and/or maintenance of sucking in neonatal mice (Dragatsis et al. 2004). The mechanism of the phenotype still remains to be investigated.

Nipple-searching behavior and attachment to the nipple, in most mammals, is believed to be mediated primarily by olfactory and tactile cues (Larson & Stein 1984). Absence of olfactory bulbs or surgical lesions in the olfactory system in newborns lead to a reduction in nipple attachment efficiency, and consequent early postnatal lethality due to starvation (Hongo et al. 2000). All HAP1 knock out pups exhibited a normal rooting reflex in response to manual stimulation of their mouth region, indicating normal tactile sensation and motor control. By eliminating the competition of milk sucking between wild type and heterozygous pups, HAP1 knock out pups survived into adulthood and did not display any feeding, behavioral or brain abnormalities as adults. Moreover, HAP1 knock out pups' mothers do nest, crouch over their pups in a typical nursing manner, and collect them when they are scattered, further indicating that olfaction is not affected in HAP1 knock out mice (Dragatsis et al. 2004).

Furthermore, neuronal cell number in the trigeminal ganglion, which could affect the sucking behavior, is not reduced. There is no change in the levels of satiety peptides within the hypothalamus of P1 HAP1 pups nor was any change found in the morphology of peptide-containing neurons. However, more neuronal death was found in the hypothalamus of HAP1 knockout mice (Li et al. 2002b). The precise role of HAP1 in the feeding behavior of pups needs to be thoroughly studied. For example, whether the gustatory responses have been

altered is not known. It is also possible that changes in neurotransmitter release play a role in the abnormal feeding behavior in HAP1 knock-out mice. It is known that the HAP1 knock out can alter serotonin function through the hypothalamic-pituitary-adrenal axis (Dragatsis et al. 2004).

6.3.2   Intracellular transport

Duo which is a membrane cytoskeletal protein was identified by using the yeast two-hybrid system as a HAP1-binding proteins (Goodman et al. 1995) and belongs to RhoGEF superfamily (Erickson & Cerione 2004, Colomer *et al.* 1997). Guanine exchange factors (GEF) stimulate Rho and Rac signal transduction molecules by switching them from the inactive (GDP-bound) to the active (GTP-bound) form. These molecules are often involved in organizing the cytoskeleton and act as axon guidance molecules (Schmidt & Hall 2002). Duo contains at least four or five spectrin-like repeats which enable it to bind to actin, one GEF domain (Matsudaira 1991, Goodman et al. 1995), peptidylglycine α-amidating monooxygenase (PAM) binding region, and HAP1 binding region. The cytoplasmic domain of PAM, which binds Duo is believed to be involved in the biogenesis of secretory granules and has a sorting signal for internalization from the cell surface (Milgram et al. 1996). Duo is a rac1-specific binding protein, which regulates cytoskeleton (actin)

organization, endocytosis, exocytosis and free radical production. Thus HAP1 is proposed to play a role in vesicle trafficking and cytoskeletal functions, and takes part in a ras-related signaling pathway (Colomer et al. 1997).

Furthermore, HAP1 binds to P150^{Glued}, a subunit of dynactin, which binds to both microtubules and dynein, and induces the microtubule-dependent retrograde transport of membranous organelles. HAP1 may influence the transport of various proteins that bind to P150^{Glued} (Engelender *et al.* 1997, Li et al. 1998b).

In investigating the function of two HAP1 isoforms, it was found that the common region of HAP1-A and HAP1-B binds to other molecules which constitute the cytoplasmic inclusions, however the C-terminus of HAP1-B takes the role for inhibition of the formation of inclusions whereas the unique C-terminal region of HAP1-A seems to be critical for inclusion formation. Both isoforms can aggregate in different proportions or self-associate *in vivo*, where HAP1-A accelerates the formation of inclusions and HAP1-B suppresses this formation simultaneously. Whether there are inclusions in the cell body depends on the proportion of the two isoforms (Li et al. 1998a). The expression level of HAP1-B is normally higher than that of HAP1-A in most brain regions. This could explain in part why the majority of native HAP1 in

the brain is cytosolic and diffusely distributed in the neurons (Li et al. 1998a).

It is reported that the common region also binds to Htt and P150^{Glued} (Li et al. 1998b). From the colocalization experiment of HAP1 and P150Glued in transfected cells, the cytoplasmic inclusions were found to be colocalized with HAP1. Thus the cytoplasmic inclusions could be transported along microtubules with HAP1 and P150^{Glued} (Li et al. 1998b).

During synaptic vesicle trafficking in axonal terminals, Htt and HAP1 may act as scaffold proteins which bind to synaptic vesicles. Mutant Htt has a much higher binding affinity to synaptic vesicles than normal Htt and reduces the association of HAP1 with the vesicle (Li *et al.* 2003a, DiFiglia *et al.* 1995). The tightly bonded complexes of mutant Htt and vesicles are separated and released, thus create a physical barrier that interferes with organelle transport and vesicle recycling in axons (Li *et al.* 2001, Li et al. 2003a). Such an effect could alter the release and uptake of various neurotransmitters and could lead to axonal degeneration (Li et al. 2003a).

HAP1 also interacts with hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs), an early endosome-associated phosphoprotein which plays a role in the regulation of vesicular trafficking and signal transduction (Komada & Kitamura 2001, Raiborg *et al.* 2001) , and regulates endocytic trafficking

through early endosomes (Li et al. 2002b). HAP1 is involved in both constitutive or ligand-induced receptor-mediated endocytosis and the endosome to lysosome trafficking of epidermal growth factor (EGF) receptors (Li et al. 2002b). Endosomes containing translocated EGF receptors activate cell proliferation and survival (Li et al. 2003b). The overexpression of HAP1 blocks vesicular trafficking from early to late endosomes, causes the formation of enlarged early endosomes and inhibits the trafficking of endosomes to lysosomes. Thus, HAP-1 can inhibit the degradation of internalized EGF receptors but not affect continuous EGF receptor internalization (Li et al. 2002b). The overexpression of HAP1 could protect against the lack of EGF receptor-induced degeneration. On the other hand, the increased expression of mutant Htt causes abnormal interactions of HAP1 with Hrs, resulting in aberrant endocytic trafficking (Li et al. 2002b, Li et al. 2003b).

HAP1 may interact directly with some membrane receptors. One of these is γ-aminobutyric acid type A receptor (GABA$_A$R) which regulates neuronal excitability by its level of stability on the cell surface (Moss & Smart 2001, Sieghart & Sperk 2002). HAP1 binds the GABA$_A$R β subunit specifically (Kittler et al. 2004). During the cell signalling pathway of GABA$_A$R, HAP1 inhibits receptor lysosomal degradation and at the same time facilitates receptor recycling back to the cell membrane. In this way, HAP1 increases

GABA$_A$R cell surface number, therefore affecting neuronal excitability (Kittler et al. 2004). The type 1 inositol (1,4,5)-trisphosphate receptor (InsP$_3$R1) is another membrane receptor that also binds to HAP1 (Tang et al. 2003). InsP$_3$R1 is an intracellular Ca^{2+} release channel which is very important in the neuronal Ca^{2+} signal pathway (Berridge 1998). Htt may directly interact with the InsP$_3$R1 C-terminus, and this binding of Htt to the InsP$_3$R1 C-terminus is dependent on both the presence of HAP1 and the polyQ expansion. Mutant Htt can bind to the InsP$_3$R1 C-terminus either directly or indirectly through HAP1 (Tang et al. 2004). But the interesting finding is that the functional effects of mutant Htt on InsP$_3$R1-mediated Ca^{2+} release are attenuated in medium spiny striatal neurons (MSN) of HAP1 knock out mice when compared with wild-type mice MSN. Thus, HAP1 potentiates functional effects of mutant Htt on InsP$_3$R1 function in vivo. As is already known, increases in neuronal Ca^{2+} represent early events in the pathogenesis of HD (Tang et al. 2003, Zeron *et al.* 2002). HAP1 facilitates functional effects of mutant Htt on InsP$_3$R1 and suggests a role for HAP1 in altered neuronal Ca^{2+} signalling, which potentially leads to selective neuronal loss in HD (Tang et al. 2004).

7. Molecular motors

Organelle transport in cell processes is mainly controlled by molecular motors including kinesin which are plus-end-directed motors (anterograde transport) and dynein which are minus-end-directed motors (retrograde transport). Structurally, molecular motors consist of two functional parts, a motor domain and a tail domain. The motor domain binds to cytoskeletal filaments and also offers chemical energy for movement. The tail domain is quite divergent in different motors that allow it to recognize different cargoes either directly or through accessory light chains (Gunawardena & Goldstein 2004b).

Axonal and dendritic transport is extremely complicated as some cargoes are transported selectively whereas others are transported nonselectively. Which sequences of selectively transported proteins function as selective targeting signals and what mechanism underlies the selective transport or selective retention of cargoes remains to be fully elucidated. With regard to how motors differentiate axons from dendrites, research shows that microtubules play a role in selective transport. At the transition site from the cell body to the axon there is a deficiency in rough endoplasmic reticulum compared with other parts of the cell body. This special structure has a remarkably high affinity for microtubule-associated proteins EB1 (Nakata & Hirokawa 2003). It is possible that Kinesin superfamily protein 5 (KIF5) also recognizes the same special structure as EB1 which leads to KIF5 carrying its cargo selectively to the axon.

However, both selective and nonselective transport occurs in most cases, many sequences for selective transport have been identified, but such sequence identification has not always clarified the underlying sorting mechanism (Hirokawa & Takemura 2004).

7.1 Kinesin motors

7.1.1 The structure of kinesin

Kinesins are the largest superfamily of microtubule-dependent motors for anterograde transport, with 45 members in mice and human, and they are the most abundant motors in many cell types. The kinesin superfamily can be divided into three groups on the basis of the positions of the motor domains: the amino (N)-terminal motor, middle motor and carboxy (C)-terminal motor types (referred to as N-kinesins, M-kinesins and C-kinesins). There are 39 N-kinesins, 3 M-kinesins and 3 C-kinesins. Kinesin also can be classified based on 14 large families, kinesin1 to 14 (Hirokawa & Takemura 2005). It is thought that different kinesins carry different subpolulatins of cargoes and have different anterograde pathways. Conventional kinesin, kinesin I, was originally discovered in the context of vesicle transport in axons. It is a tetramer consisting of a kinesin heavy chain (KHC) dimer and two kinesin light chains

(KLC) (Fig. 5). In mammals, kinesin I KHC consist of three different subunits (KIF5A, KIF5B, and KIF5C) (Xia et al. 1998) and kinesin I KLC consists of at least three subunits (KLC1, KLC2, and KLC3) (Rahman et al. 1998). All KIFs have a globular motor domain, an α-helical coiled coil stalk domain and a C-terminal tail domain. The globular motor domains have high degrees of homology in all KIFs and contain a microtubule-binding sequence and an ATP-binding sequence. The C-terminal has a unique sequence and interacts with the N-terminal coiled-coil domain of KLC. This diversity of domains is thought to regulate motor activity and binding to different cargoes. The C-terminal of KLC consists of six tetratrico peptide repeat domains, which are involved in protein-protein interactions and are proposed to link KLC to receptor proteins on vesicular cargoes (Schnapp 2003, Hirokawa & Takemura 2005).
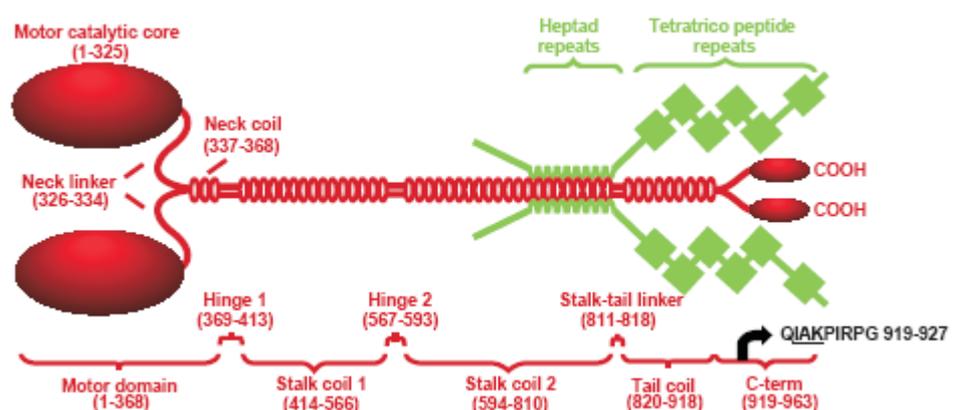

Fig. 5 Domain organization of kinesin I. Heavy and light polypeptide chains are shown in red and green, respectively. Cargo-binding domains (CBD) are located at C-terminus (Schnapp 2003).

7.1.2 Kinesins mediate cargo transport in axons

In the axon, many membranous organelles are transported from the cell body to synaptic terminals. After proteins are synthesized in the Golgi apparatus, they are packaged in different vesicles and anterogradely transported to the synaptic terminal.

KIF1A and KIF1Bβ transport synaptic vesicle precursors along axons and then the precursors are assembled into synaptic vesicles at synaptic terminals. Mutant mice that lack either KIF1A or KIF1Bβ have reduced synaptic vesicle density at nerve terminals, and sensory and motor nerve function is impaired (Yonekawa *et al.* 1998, Zhao *et al.* 2001). Moreover, *in vivo* experiments in heterozygous KIF1Bβ mutant mice show the transport of synaptic vesicle precursors is significantly decreased (Zhao et al. 2001). KIF1Bα, an isoform derived from the same gene as KIF1Bβ by alternative splicing, transports mitochondria anterogradely (Nangaku et al. 1994). KIF5 can also transport mitochondria (Tanaka *et al.* 1998, Kanai *et al.* 2000). The hypothesis is that mitochondria may have many binding sites for motor proteins. KIF5 also transports other cargoes, including vesicles that contain apolipoprotein E receptor 2, GAP43 and VAMP2 and oligomeric tubulin in a large transport

complex at a speed that is compatible with slow axonal transport. The kinesin complex KIF3A-KIF3B-KAP3 transports large vesicles with diameters of 90-160 nm, which are associated with fodrin through an interaction between KAP3 and fodrin. These sorts of transport are essential for neurite extension (Nangaku et al. 1994, Yonekawa et al. 1998, Takeda *et al.* 2000, Nakata & Hirokawa 2003, Hirokawa & Takemura 2005).

7.1.3 Kinesins mediate cargo transport in dendrites

In dendrites, there are many molecules transported from the cell body by different kinesins, including those associated with postsynaptic densities, neurotransmitter receptors, ion channels and specific mRNAs. KIF17 transports NMDA (N-methyl-D-aspartate) glutamate receptors (NMDARs). KIF17 is an N-kinesin and co-exists mainly in dendrites with the NR2B subunit of NMDARs which it anterogradely transports to postsynaptic regions (Guillaud et al. 2003). The overexpression of KIF17 enhances working or episodic-like memory and spatial learning and memory in transgenic mice (Wong et al. 2002a). This might be due to the increased phosphorylation of a transcription factor, cyclic AMP responsive element binding protein (CREB) (Wong et al. 2002a, Guillaud et al. 2003). KIF5s (KIF5A, KIF5B and KIF5C) can transport AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic

acid) glutamate receptors (AMPARs) and a large multisubunit complex of 42

proteins that includes the mRNAs for αCaMKII and ARC (Kanai et al. 2004).

The proteins in this complex include those associated with RNA transport,

those associated with protein synthesis, RNA helicases, heterogeneous nuclear

ribonucleoproteins, and other RNA-associated proteins. Furthermore, KIF21B

and KIFC2 are also included in dendritic transport (Setou *et al.* 2002,

Hirokawa & Takemura 2005).

7.1.4 Adaptor/scaffolding proteins for cargo recognition by motors

Although transmembrane cargo proteins can bind directly to specific motors,

adaptor/scaffolding proteins also participate in cargo-motor binding. For

example, KIF13A transports vesicles containing the mannose-6-phosphate

receptor (M6PR). The cytoplasmic side of M6PR binds to the AP1 (adaptor

protein 1) adaptor complex which also binds to KIF13A. Therefore, the AP1

adaptor complex serves as an adaptor for both the motor and vesicle. Another

example is that the interaction between KIF17 and its cargo vesicles which

contains NMDARs is mediated by a tripartite protein complex that contains

LIN10, LIN2 and LIN7 (Bonifacino et al. 1996). All those three proteins

contain PDZ domains which do not bind to each other but recruit other proteins

(Garner *et al.* 2000, Hung & Sheng 2002). The C-terminal tail of KIF17 binds

directly with the first PDZ domain of LIN10, which then sequentially binds

with LIN2 and LIN7. LIN7 binds to the C-terminal of NMDAR subunite

NR2B via a PDZ domain. In this way the vesicles containing NMDARs are

transported by KIF17 through the scaffolding protein complex (Jo *et al.* 1999,

Hirokawa & Takemura 2005).

7.2. Dynein motor

7.2.1 The structure of Dynein

Dyneins are the other major family of microtubule motor proteins. There are

about 15 forms of dynein in vertebrates, but only two forms are cytoplasmic,

dynein 1 and dynein 2. All forms of dynein contain the largest subunit heavy

chain (HC) polypeptide of >500 kDa, which is termed the motor domain. The

large motor domain consists of multiple ATPase units and other structures and

this structure is highly conserved in all dynein heavy chain isoforms (Fig. 6)

(Neuwald et al. 1999). The cargo-binding domain is diverse and is responsible

for cargo binding. Dynein also has a variety of accessory subunits named

intermediate, light intermediate and light chains (ICs, LICs and LCs), which

take part in cargo binding through interaction with the cargo binding domain

(Fig. 6) (Samso *et al.* 1998, Neuwald et al. 1999, Burgess *et al.* 2003, Vallee *et al.* 2004).
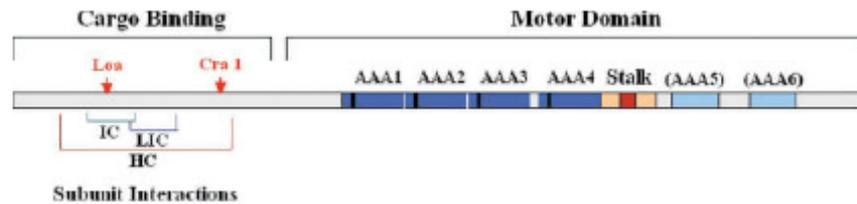

Fig. 6 Structural organization of cytoplasmic dynein. Diagram shows functional domains and binding sites within cytoplasmic dynein heavy chain. All of the accessory subunits of cytoplasmic dynein are associated with the N-terminal portion of the HC, which constitutes the base of the dynein particle. (Sites for binding of IC, LIC, and for HC dimerization are indicated by brackets.) The C-terminal portion of the HC contains six AAA ATPase domains arranged in a ring (Burgess et al. 2003).

7.2.2 Dynactin - the adaptor protein for dynein

Dynactin is one of most important multisubunit protein complex that is a necessary accessory for the dynein motor movement. Dynactin is a multifunctional adapter protein helping the cellular component to connect with microtubules and motors (Schroer 2004).

One typical structure of dynactin consists of the Arp1 rod (Arp1, Arp11, Actin, CapZ, p62, p27 and p25) and the projecting arm (p150^{Glued}, Dynamitin, p24/p22). Arp1 (actin-related protein) can hydrolyze ATP and form remarkably stable, very short and of uniform length filaments and associates with spectrin-family proteins which can be found on Golgi membranes. This could be one mechanism by which dynactin binds a variety of subcellular structures. The primary sequence of p62 contains a zinc-binding motif (RING or LIM domain) near the N terminus, which commonly supports protein-protein interactions. So p62 may participate in the binding to dynactin subunits or other subcellular structures (Schroer 2004).

p150Glued is the largest subunint of dynactin which consists of two globular heads serving as microtubule binding sites and is located in the very tip of the dynactin arm. p150Glued binds microtubules and the intermediate chain of dynein, and is thought to be responsible for the movement of many cargo organelles through dynein. The globular heads contain a CAP-Gly (cytoskeleton-associated protein, glycine-rich) motif (Fig. 7), which contributes to enhancing the reaction of the dynein motor, microtubule minus-end anchoring at interphase centrosomes (Quintyne *et al.* 1999, Quintyne & Schroer 2002) and mitotic spindle poles (Gaglio et al. 1997). The p150^{Glued} CAP-Gly domain also binds microtubule-binding protein, such as EB1 and

CLIP-170. The two coiled coil domains (aa~220-550, aa~925-1050) (Fig. 7) support binding interactions within dynein-dynactin, and also provide a binding platform for interactions with multiple microtubule-based motors (Waterman-Storer *et al.* 1995, McGrail *et al.* 1995). The region of aa 1023-1223 has been reported to interact with HAP1 (Li *et al.* 2002a, Vaughan *et al.* 2002, Engelender et al. 1997).
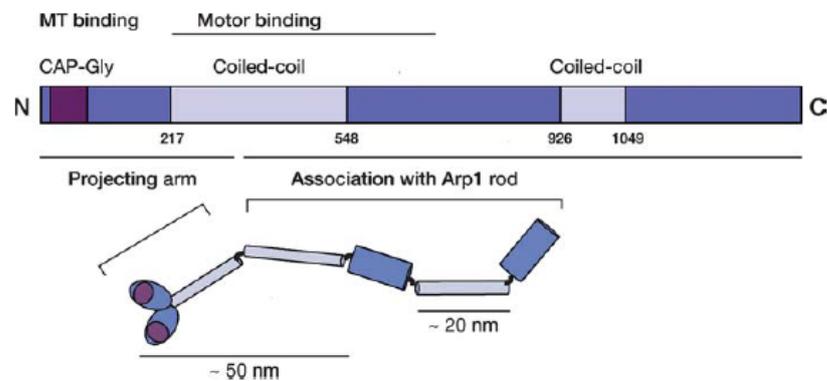

Fig. 7 Schematic illustration of the domain organization and proposed functions of p150Glued. The different functional and structural domains are defined as described in the text (Schroer 2004).

Dynactin's two structural domains are linked by dynamitin. Dynamitin associates with p150^Glude and p24/22 via a series of three coiled-coil motifs. Disruption of dynamitin can cause p150Glued and p24/22 to be released from the rest of the dynactin structure. Conversely, overexpression of dynamitin could also disrupt the binding between p150Glude and p24/22. p24/22 is one of

dynactin's smallest subunits and it binds dynamitin directly (Schroer 2004).

7.2.3 Dynactin polices two-way organelle traffic

The generally accepted theory is that membranous organelles are transported anterogradely by motor proteins of the kinesin superfamily and retrogradely by motor proteins of the dynein family. In most cells, organelles do not travel directly in one direction, but change direction frequently. The mechanism of this phenomenon is not very clear. But one clue is that dynactin complexes also interact with kinesin II, except for dynein, which is important for plus end-directed anterograde transport. p150Glued is the largest and most important subunit of dynactin. The treatment of extruded squid axoplasm with antibody against p150Glued inhibits both anterograde and retrograde organelle transport (Waterman-Storer et al. 1997). Drosophila embryos expressing a mutant p150^{Glued} protein display impaired bidirectional organelle transport (Gross et al. 2002). It is suggested that dynactin might be involved in coordination of bidirectional transport (Dell 2003). More detailed evidence is provided by investigations using Xenopus melanophores and showing that the COOH-terminal domain of kinesin-associated protein (KAP, which is one of the motor subunits comprising kinesin II) competitively binds the same region of p150Glued (aa600-811) as dynein intermediate chain (Deacon et al. 2003). This

interaction is important for kinesin II-mediated anterograde transport of melanosomes (Dell 2003).

8. Research aims and plans

Although we already know that Htt, HAP1 and p150Glued are involved in BDNF transport and polyQ Htt could interrupt the microtubule-based BDNF transport and wild type Htt could act as an anti-apoptotic factor by rescuing axonal transport (Gauthier et al. 2004), the process of how those molecules interact with each other to carry BDNF forward and backward along axons remains to be investigated. It is not known which molecule interacts with BDNF. It is also not known whether the prodomain of BDNF or mature BDNF interacts with any of these molecules. It is believed that BDNF is present within vesicles and packaged as a cargo which is carried by molecular motors. How BDNF vesicles interact with the motor accessary molecules is not clear. The first project (Chapter 2) aims to find out how BDNF interacts with molecular motor accessary proteins and how it is transported from the cell body to nerve terminals within axons. During the course of this study it was found that both the prodomain and mature BDNF interact with HAP1. Thus, the following questions have been addressed with a variety of techniques and approaches: Do BDNF fragments interact with HAP1 directly or indirectly? Do

BDNF fragments co-localize with HAP1 after cotransfection? Does BDNF co-localize with HAP1 in vivo and does the ablation of HAP1 alter the localization pattern of BDNF? Does recombinant mutant htt or mutant htt from human HD brains interfere with the interaction between HAP1 and BDNF? Is HAP1 required for the axonal transport and activity-dependent release of BDNF? Does the mutation of V66M in the BDNF prodomain affect the interaction and colocalization with HAP1?

Recent studies have shown that proneurotrophins bind p75NTR and sortilin with high affinity and trigger apoptosis of neurons *in vitro*. As the proneurotrophins are a dominant form of gene products in developing and adult animals, we wish to find out if proneurotrophins have any physiological roles *in vivo*. The second project (Chapter 3) aims to find out whether proBDNF acts as a death factor *in vivo* in a physiological condition. In this chapter, the *in vitro* role of proBDNF is investigated by using PC12 cells as a model to test whether proBDNF induces cell death and whether the apoptotic effect could be abolished by antibodies against proBDNF. Secondly, p75NTR, sortilin and proBDNF expression in DRG is investigated. Thirdly, we use the axotomized sensory neurons of neonatal rats as a model in which we have examined the potential role of proBDNF in neuronal survival *in vivo*. Finally, we examined

whether soluble sortilin extracellular domain receptor rescues axotomized sensory neurons from death.