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Neurotrophins: A Ticket to Ride for BDNF

The secretion of BDNF from neurons is under activity-dependent control: this is crucial for the formation of appropriate synaptic connections during development and for learning and memory in adults. New evidence shows that interaction between a motif in the tertiary structure of BDNF and the sorting receptor carboxypeptidase E directs this neurotrophin to the regulated secretory pathway.

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Activity-dependent modulation of synapses is critical for brain development and for many cognitive functions in the mature brain. Amongst the neurotrophins, BDNF has for a unique role in long-term potentiation, a form of synaptic plasticity associated with memory formation, and the consolidation of long-term memory [1,2]. Over the past decade it has been established that neuronal activity regulates *bdnf* transcription, transport of BDNF mRNA and protein into neuronal processes and activity-dependent secretion of BDNF to modulate synaptic transmission and synaptogenesis [3–7]. Little, however, is known about the mechanisms that mediate these processes. A recent study by Lou *et al.* [8] highlights the importance of the interaction of a highly conformation-dependent sorting signal in the tertiary structure of BDNF with the sorting receptor carboxypeptidase E [9] for segregating BDNF into the regulated secretory pathway for activity-dependent release.

Secreted proteins transit the Golgi apparatus to the *trans*-Golgi network where two kinds of secretory vesicle are generated: those of the constitutive pathway are transported to and fuse with the plasma membrane to continuously release their contents into the extracellular space, whereas those of the regulated pathway migrate to and accumulate at the plasma membrane until fusion is triggered by signals for regulated secretion. Neurotrophins are synthesized from large precursor proteins that are proteolytically cleaved either intracellularly or extracellularly to yield the mature proteins. Whereas the neurotrophin precursors proNGF, proNT3 and proNT4 are primarily packaged into constitutive vesicles, proBDNF is preferentially packaged into vesicles of the regulated secretory pathway [10–12]. The sorting of BDNF into vesicles of this pathway and their subsequent trafficking to appropriate plasma membrane sites are critical steps for activity-dependent secretion of BDNF. The regulated release of BDNF is crucial for the requirements that BDNF must act locally and specifically at active synapses to modulate synaptic activity and neural connectivity.

Using high resolution X-ray crystallography, Lou *et al.* [8] identified a putative sorting motif in the tertiary structure of BDNF which has similar three-dimensional characteristics to the sorting motifs of two other proteins that undergo regulated release, insulin and pro-opiomelanocortin [13,14]. This motif consists of residues Ile_{16}, Glu_{18}, Ile_{105} and Asp_{106} of the mature BDNF protein sequence with their side chains exposed on the surface of the protein. Molecular modelling predicted that this sorting motif could interact with the signal-binding domain of the sorting receptor carboxypeptidase E, which targets secreted proteins to the regulated secretory pathway. In particular, the acidic side chains Glu_{18} and Asp_{106} of BDNF interacting with the basic residues Asp_{255} and Lys_{260} of carboxypeptidase E.

The importance of this sorting motif for segregating BDNF into secretory vesicles for activity-regulated release was investigated by measuring BDNF secretion from the AtT-20 pituitary cell line transfected with plasmids expressing either wild-type proBDNF or proBDNF in which the two acidic residues of the sorting motif were mutated to uncharged alanine residues [8]. These Glu/Asp→Ala substitutions were found to increase the level of basal, constitutive BDNF secretion and abolished activity-dependent secretion following depolarization with 50 mM K^+. Accordingly, wild-type BDNF co-localized with pro-opiomelanocortin in a punctate pattern along cell processes, whereas mutated BDNF immunostaining was mostly perinuclear and colocalized with the Golgi marker p115.

Significantly, creating a typical sorting motif in NGF by making an acidic Val_{20}→Glu_{20} substitution in the partial sorting motif Val_{18}-Val_{20}-Asp_{105}-Ile_{104} present in this protein significantly reduced constitutive secretion and conferred activity-dependent secretion. Accordingly, like wild-type BDNF, NGF_{Glu20} colocalized with pro-opiomelanocortin, in contrast to the predominant perinuclear localization of wild-type NGF expressed in these cells.

The importance of BDNF's interaction with carboxypeptidase E for activity-dependent release was demonstrated in binding experiments and studies of BDNF secretion and localization in cell cultures prepared from wild-type and carboxypeptidase E-deficient mice [8]. BDNF was shown to bind to bovine pituitary secretory granule membranes, a rich source of carboxypeptidase E, and bound to membranes from sf9 cells expressing recombinant carboxypeptidase E much more effectively than did NGF. Whereas membrane-bound BDNF was efficiently displaced by a peptide that contains the pro-opiomelanocortin sorting motif, this peptide did not significantly affect NGF binding. Pituitary and cortical cells from carboxypeptidase E-deficient mice exhibited enhanced

constitutive release of endogenously synthesized BDNF and no activity-dependent secretion, indicating missorting of endogenous BDNF to the constitutive pathway in the absence of carboxypeptidase E. Hippocampal neurons from carboxypeptidase E-deficient mice exhibited cell body fluorescence following infection with a viral vector encoding wtBDNF–EGFP fusion protein, whereas virus-infected wild-type neurons additionally exhibited punctate fluorescence in neurites colocalised with staining for secretogranin II, a marker of vesicles of the regulated secretory pathway.

In addition to the sorting motif in mature BDNF, the proBDNF sequence also influences sorting. A valine-to-methionine substitution in the prodomain impairs BDNF sorting from the Golgi complex into secretogranin II-positive vesicles, markedly reducing activity-dependent secretion from neurons [15] . This substitution is a recognized polymorphism of the human *bdnf* gene which is associated with memory impairment and increased susceptibility to neuropsychiatric disorders [16]. It is unclear, however, whether the missorting phenotype of this polymorphism implies the existence of an additional and separate sorting motif in the prodomain or whether the polymorphism causes misfolding of the nascent protein, impairing formation of the sorting motif in the mature domain or preventing its interaction with carboxypeptidase E in the unprocessed protein.

In contrast to previous studies of BDNF processing and secretion, the study by Lou *et al*. [8] has employed a very sensitive pulse-chase paradigm to track and quantify the synthesis, processing and secretion of endogenous BDNF in primary neuron cultures. This powerful approach avoids the potentially misleading consequences of overexpressing tagged proteins that could be processed, sorted, trafficked and secreted differently from their endogenous

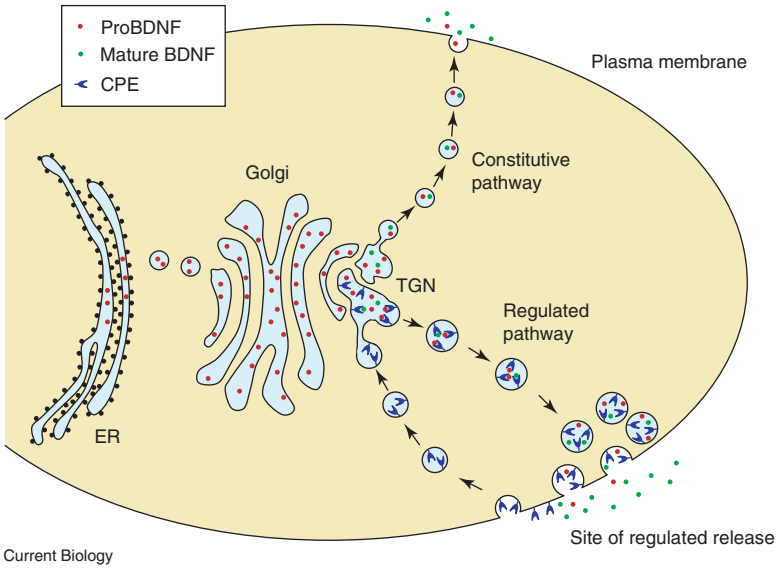

Figure 1. BDNF synthesis, processing, sorting, transport and secretion in neurons.

BDNF is synthesized in the endoplasmic reticulum (ER) as a 32 kDa precursor protein (proBDNF) that moves though the Golgi apparatus to the trans Golgi network (TGN), from where it passes into the constitutive and regulated secretory pathways. Binding of BDNF to the lipid-raft-associated sorting receptor carboxypeptidase E (CPE) in the TGN is necessary for sorting into secretory vesicles of the regulated pathway. These vesicles are subsequently transported to appropriate sites for activity-dependent secretion. Most BDNF in the regulated secretory pathway is transported to postsynaptic dendrites and spines, but it also undergoes anterograde axonal transport and activity-dependent transfer from pre- to postsynaptic sites. The contents of these vesicles are eventually released upon triggering signals for regulated secretion. CPE is subsequently internalized and transported through the endocytic recycling compartment back to the TGN. Distinct protein convertases within the TGN and secretory vesicles of the regulated pathway cleave off the amino-terminal pro-domain of proBDNF to yield mature BDNF (13 kDa). Proteolytic conversion of proBDNF to mature BDNF continues following secretion by the action of extracellular proteases such as plasmin. Mature BDNF interacts with both pre- and post-synaptic TrkB receptors to activate intracellular secondary messenger cascades. There is evidence that proBDNF interacts with the pan neurotrophin receptor $p75^{NTR}$ to affect distinct cellular responses.

counterparts. Not only do they demonstrate activity-dependent secretion of endogenously synthesized BDNF in cortical neurons and its dependence on carboxypeptidase E, they quantify the extent of proteolytic conversion of proBDNF to the mature protein and provide information on where this takes place.

The finding that the 2:1 ratio of mature BDNF:proBDNF secreted from cortical neurons was reduced to a ratio of 1:1 from carboxypeptidase E-deficient neurons suggests a substantial level of processing occurs intracellularly, independently of sorting into the regulated pathway and that additional processing takes place in the secretory granules of this pathway. Although it is possible that differences in the extent of intracellular BDNF processing occur from one kind of neuron to another, it was observed in studies of PC12 cells overexpressing dual-tagged proBDNF that the vast majority of BDNF secreted from the regulated pathway was the pro form [15]. Quantification of the extent of intracellular and extracellular processing of endogenously synthesized BDNF in physiologically relevant neuronal systems is important because this has profound implications for synaptic function, as once released, proBDNF and mature BDNF bind preferentially to different receptors (TrkB and $p75^{NTR}$, respectively), activate different intracellular pathways and initiate different cellular processes [17]. Whereas mature BDNF plays a key role in eliciting long-lasting

enhancement of synaptic transmission in the hippocampus, it has been suggested that elevated extracellular levels of proBDNF can lead to the negative regulation of synaptic plasticity [18].

The clear demonstration of a carboxypeptidase E-interacting sorting motif in BDNF [8] is an important step in understanding how BDNF is channelled into the regulated secretory pathway. Although disruption of this motif abolishes activity-dependent release of endogenous BDNF from cortical neurons, it may not be the only structural feature of BDNF that influences its intracellular distribution and trafficking. The finding that a point mutation in the prodomain of BDNF also affects sorting has raised the intriguing possibility of an additional sorting structure in pro-BDNF.

Separate sorting structures in the pro and mature regions could function synergistically or independently of each other in different neuronal settings. Indeed, neuron-type-specific differences in sorting, transport and processing of endogenously synthesized BDNF would have important consequences for neural function and plasticity in the mature brain. Elucidating and quantifying such differences, understanding the molecular mechanisms that underlie them and relating this to the neurophysiology of individual neurons and the circuits of which they form a part are important challenges.

In hippocampal neurons, NGF, which possesses a partial sorting motif, is apparently secreted in part by the regulated pathway [19], but how this occurs is not understood. Additionally, it has yet to be demonstrated whether sorting for regulated secretion via the interaction of BDNF with carboxypeptidase E occurs in the developing nervous system. Critically, the relative importance of regulated and constitutive release of BDNF in the control of neuronal survival and the establishment of appropriate connectivity in the developing nervous system and plasticity in the mature brain remains to be established. Further advances in the cell biology of neurotrophin secretion will undoubtedly have wide ranging implications for understanding synaptogenesis and synaptic function in the developing and mature nervous system.

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