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Message in a bottle: long-range retrograde signaling in the nervous system

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In many regions of the nervous system, signals produced by target cells and surrounding glia or in response to injury are received at axon terminals and then retrogradely propagated to cell bodies where they regulate gene transcription and other cellular processes required for development and adult function. The cellular and molecular mechanisms of axonal retrograde signaling in neurons have traditionally been studied in the context of survival signals provided by target-derived neurotrophic factors, in which signaling endosomes containing endocytosed ligand–receptor complexes and downstream effectors are retrogradely transported by dynein motors. In recent years, this notion has been refined and additional mechanisms for long-range retrograde signaling in axons have been described. This article discusses some outstanding issues in the signaling endosome hypothesis as well as recent findings suggesting the existence of a variety of mechanisms for the retrograde propagation of signals in the nervous system.

Introduction

Signal transduction pathways have traditionally been studied in qualitative terms and in isolation from each other, essentially as idealized linear cascades emanating from receptors activated by ligand binding at the cell membrane and devoid of spatial or temporal dimensions. Recently, it has become apparent that growth factor signaling from the plasma membrane to the nucleus involves the intracellular movement and regulated translocation of many different components, including the ligand–receptor complex itself. Intracellular transport of receptor complexes is highly regulated and can follow different routes, depending on the membrane subcompartment in which the receptor is located, the type of signaling activated and the particular cellular context. The realization that highly dynamic intracellular processes occurring throughout space and time can affect the outcome of receptor activity has helped to integrate signal transduction research into a cell biological framework [1–3]. Because of the physical separation between axon terminals and the cell body, ligand–receptor complexes internalized in neurons need to travel relatively long distances to reach downstream effectors localized in the cell soma (Figure 1). Axonal retrograde transport is likely to affect the configuration and activity of receptor complexes, although direct evidence for this is lacking.

After a brief historical account, this review summarizes recent findings supporting the presence of a variety of mechanisms for the retrograde propagation of signals in the nervous system. The emerging links between defects in long-range retrograde signaling and neurodegenerative diseases will not be discussed here, and the reader is directed to a recent review covering this topic [4]. Long-range retrograde signaling is herein meant as the retrograde propagation of signals in axons, from the nerve terminal to the cell body. Retrograde signaling is sometimes also used to denote the short-range propagation of signals from post- to pre-synaptic sites, a topic that will not be covered here but that has been reviewed elsewhere [5,6].

Target-derived neuron survival and retrograde transport

The retrograde propagation of survival signals from targets of innervation to neuronal cell bodies was already implicit in the neurotrophic hypothesis, which was originally formulated to explain the naturally occurring loss of neurons during development as the result of a competition for limiting amounts of target-derived survival factors [7]. Although supported and corroborated by a great amount of data, this concept has since been supplemented with other mechanisms acting in concert with those regulated by survival factors, including cell-death-inducing pathways [8]. Although several different ways can be imagined by which a neuronal cell body could be made aware of the arrival of a survival signal at its axon terminal, early studies showing distal uptake and retrograde transport of radiolabeled nerve growth factor (NGF) suggested that the transport of neurotrophic molecules to neuronal bodies could be significant for survival responses and other trophic effects [9]. After these seminal observations, the main focus was on investigating the basis of the retrograde transport of neurotrophic factors and its underlying mechanisms and pharmacology, mostly using NGF, but also with other neurotrophins and neurotrophic molecules. *In vivo* studies using peripheral nerve ligation initially helped to establish that neurotrophic molecules and their receptors accumulate on the distal side [10,11], but these types of study soon proved inappropriate for investigating receptor-based mechanisms and the dynamics of retrograde transport in quantitative terms. Compartmentalized *in*

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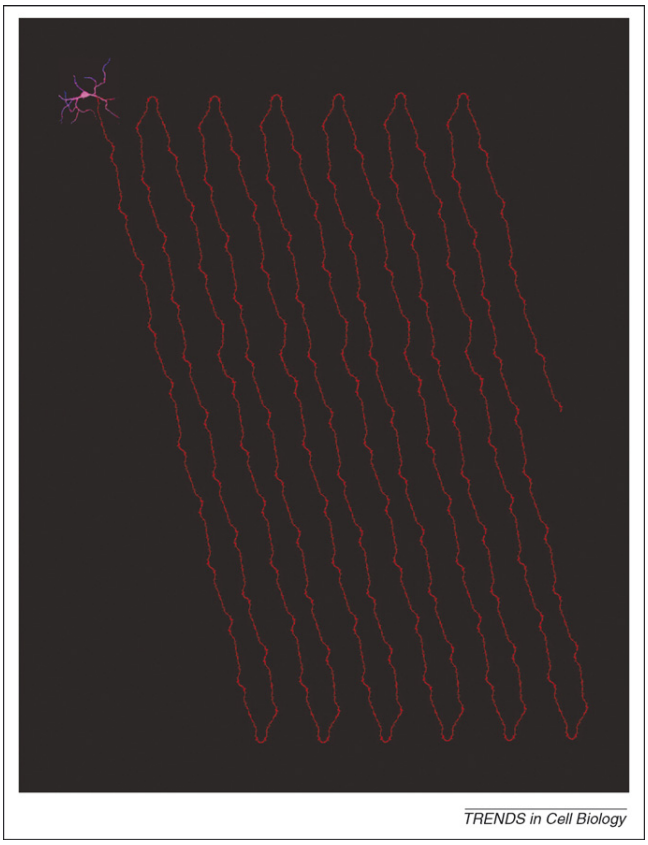

Figure 1. The daunting task of retrograde axonal signaling. Photomontage of a rat hippocampal neuron with dendritic tree (purple) and an axon 1 cm in length (red) shown at scale. Human lumbar motoneurons can have axons exceeding 1 m in length.

vitro culture systems, in which cell bodies and distal axons are separated by an impermeable divider [12], helped to bridge this gap and established for the first time the dynamics of NGF axonal transport in sympathetic neurons [13]. The initial notion that retrogradely transported ligands could by themselves activate signaling mechanisms upon release in the cell body was dismissed by experiments that showed the lack of effects of intracytoplasmic delivery of NGF or NGF-blocking antibodies [14]. Thus, although NGF transport marked retrograde signaling, NGF was not itself the signal, and much work followed to determine whether the transport of NGF was only a side effect or had a more fundamental role in retrograde signal propagation.

Signaling endosomes in long-range retrograde signaling

Upon NGF binding, the receptor tyrosine kinase TrkA is activated, internalized by endocytosis and retrogradely transported in axons of peripheral neurons [10,15–17]. Exposure of axon terminals to ligand results in the accumulation of activated Trk receptors derived from distal axons within cell bodies but not on their surface [18,19]. Disruption of TrkA kinase activity at intermediate axonal segments, but not at distal axons or cell bodies, has no effect on retrograde signal propagation or neuronal survival [20], suggesting that the activity of retrogradely transported TrkA can be regenerated after moving across a region of kinase inhibition. Ligand-mediated internalization of TrkA receptors is required for retrograde signaling and occurs via clathrin-coated vesicles containing activated components of the Ras-mitogen-activated protein (MAP) kinase, Phospholipase C (PLC)-γ and PI3 kinase pathways [20–22]. Vesicle-mediated axonal transport of internalized TrkA receptors has been observed in sensory neurons in the form of small, uncoated vesicles containing TrkA, activated downstream signaling components and immunoreactivity for Rab5, a small GTPase that marks early endosomes [23,24].

The findings summarized above constitute some of the major tenets of what is currently known as the 'signaling endosome hypothesis' of retrograde signaling by neurotrophic factors and is reviewed in greater detail elsewhere [25,26]. This notion has received strong support from a series of studies during the last decade in different neuronal systems and by different laboratories, and thus has grown to become the dominating paradigm for long-range retrograde signaling in neurons. There are, however, several issues and discrepancies that remain incompletely explained concerning the nature, composition and movement of the endosomal carriers as well as the general relevance of signaling endosomes for other signaling systems.

FAQs about signaling endosomes

What stage of the endocytic pathway do signaling endosomes correspond to?

Given our still incomplete understanding of the endocytic pathway (see Box 1 and Figure 2), it is perhaps not surprising that discrepancies have arisen about which stage or stages of the pathway signaling endosomes correspond to. Although there is evidence indicating that signaling endosomes are small, Rab5-positive,

Box 1. A primer on clathrin-mediated receptor endocytosis

Endocytic vesicles that are formed by invagination of clathrin-coated pits become uncoated in the cytoplasm and fuse with specialized endosomal organelles from which they are sorted to various intracellular destinations [27]. Different stages along this pathway are distinguished by the expression of specific sets of endocytic markers: Rab5 and EEA1 mark early endosomes, Rab4 and Rab11 mark recycling endosomes, and Rab7 marks late endosomes (Figure 2). Recent work has indicated that these compartments – particularly early endosomes – are much more dynamic than previously thought, and different subpopulations of Rab5-positive early endosomes of different motilities have been observed [28]. Invagination of early endosomal compartments followed by budding leads to the formation of structures containing multiple vesicles encircled by an external membrane known as multivesicular bodies (MVBs) [29]. MVBs have been widely viewed as intermediate between early and late endosomal compartments, although it has also been pointed out that all endosomes in this pathway might contain multivesicular elements, including regions of early and late endosomes [29]. In addition, direct replacement of Rab5 for Rab7 has also been proposed to underlie the conversion of early into late endosomal vesicles [30], and debate rages in the field on whether endosomal compartments undergo fusion to form the next transport intermediate or whether the earlier compartment converts and matures into a later one. All endosomal compartments are characterized by an acidic pH, although acidity increases towards lysosomes, the final stage for degradation of internalized ligand–receptor complexes.

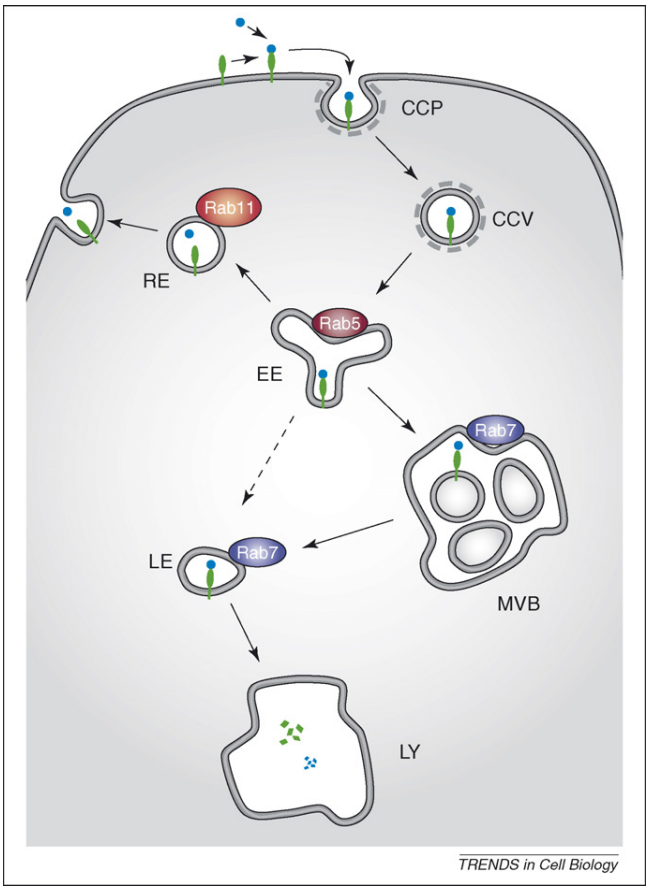

Figure 2. Schematic diagram of clathrin-mediated receptor endocytosis. CCP, clathrin-coated pit; CCV, clathrin-coated vesicle; EE, early endosome; RE, recycling endosome; MVB, multivesicular body; LE, late endosome; LY, lysosome. The dashed line indicates the possible conversion of early into late endosomes by replacement of Rab5 for Rab7 [30].

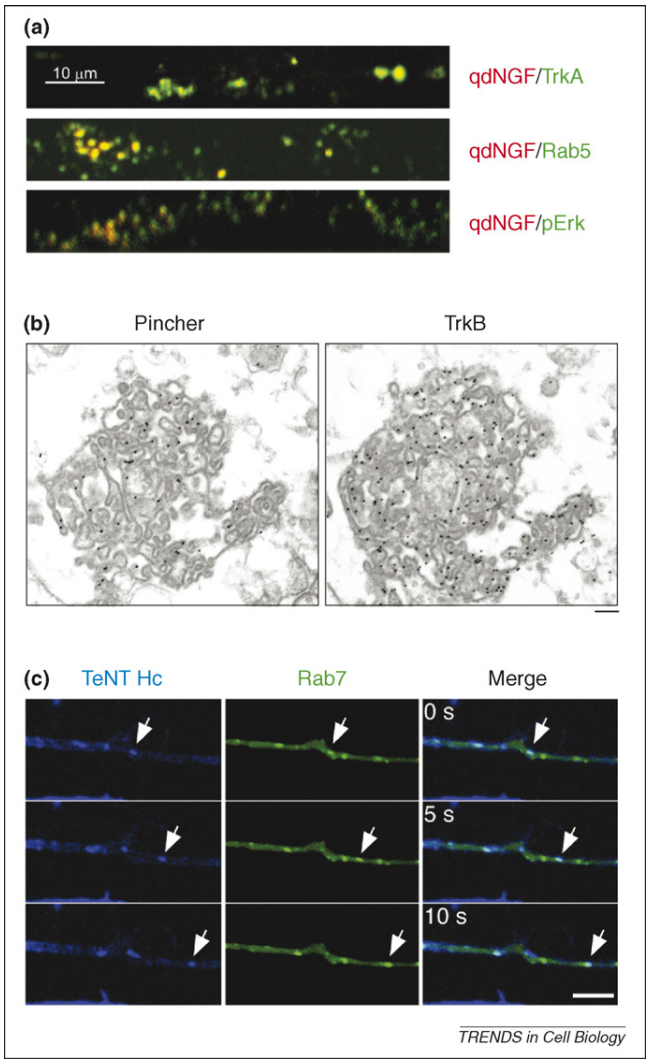

Figure 3. Different types of endosomal organelle can engage in axonal retrograde transport in neurons. **(a)** Signaling endosomes in axons of neurons in the dorsal root ganglion (DRG) grown in compartmentalized cultures retrogradely transporting quantum-dot-NGF (in red) and carrying TrkA, Rab5 or phosphorylated Erk1/2 (all in green). Scale bar represents 10 μm. From Ref. [23]. **(b)** Complex membrane ruffles with pinocytic vesicles – also termed macroendosomes – from cultured hippocampal neurons carrying Pincher and internalized TrkB as visualized by immuno-electron microscopy. Scale bar represents 0.2 μm. From Ref. [32]. **(c)** Time series of signaling endosomes retrogradely transported in axons of DRG neurons labeled with TeNT Hc (blue) and Rab7 (green). Scale bar represents 5 μm. From Ref. [36].

single-membraned vesicles with the characteristics of early endosomes [23,24] (Figure 3a), other studies have indicated that they are larger multivesicular structures resembling multivesicular bodies (MVBs) [4,31–33]. In addition, a large endosomal structure characterized by the presence of the protein Pincher has been implicated in Trk internalization by a clathrin-independent, Rac-mediated, pinocytosis-like mechanism [34,35], leading to the formation of MVBs that engage in retrograde transport ([32] and S. Halegoua, personal commununication) (Figure 3b). Intriguingly, unlike other MVBs previously characterized, Pincher MVBs appear to be resistant to lysosomal degradation and positive for Rab5 but negative for Rab7 [32,35]. It has been argued that, by encapsulating vesicles carrying entire signaling complexes within a second membrane, MVBs might serve to convey to cell bodies a snapshot of the signaling events originally elicited at nerve terminals [33]. However, because vesicles within MVBs are enclosed within a second membrane, the mechanisms by which signaling complexes retrogradely transported within MVBs become exposed in the cell body cytoplasm remain unclear. Finally, yet another set of studies has indicated a role for Rab7-positive endosomes in endosomal trafficking and axonal retrograde transport of neurotrophic signals [36,37]. In particular, a recent study in which axonal trafficking of endosomes was visualized by live-imaging found that Rab5-positive puncta were mostly stationary, whereas Rab7 was present in moving organelles of neutral pH (Figure 3c), suggesting that conversion from Rab5- to Rab7-positive structures could control the generation of axonal retrograde carriers [36]. Some of these discrepancies could presumably be attributed to technical differences – for example intact nerves versus single axons, global versus local ligand stimulation or overexpressed versus native components. Other discrepancies might ultimately be resolved by a better understanding of the dynamics of endocytic networks. In the meantime, it would appear safest to conclude provisionally that more than one type of endosomal organelle might engage in axonal retrograde transport under different conditions.

Are signaling endosomes distinct organelles specifically dedicated to intracellular signaling?

There are currently several indications that there are specialized endosomal organelles dedicated to the intracellular propagation of signals received at the cell surface. A subpopulation of Rab5-positive endosomes has been characterized on the basis of the presence of two novel multidomain proteins, APPL1 and 2, which might link plasma membrane signaling with nuclear events, such as chromatin remodeling [38]. APPL1 has also been found associated with ligand-activated TrkA in an endosomal subpopulation isolated by two-dimensional centrifugation [39]. The presence and function of APPL proteins in signaling endosomes undergoing axonal retrograde transport remain to be determined. In addition, several of the endosomal compartments mentioned above – such as Pincher MVBs and Rab7 axonal carriers – might also represent unique structures dedicated to sustained intracellular signaling and retrograde transport. Moreover, two-dimensional centrifugation has been used to resolve distinct subpopulations of signaling endosomes containing different receptors, such as TrkA, $p75^{NTR}$, the epidermal growth factor receptor (EGFR) or PACAP type-1 receptor (PAC1) (M. Grimes, personal communication). This finding would be in agreement with observations indicating bidirectional regulation between cargo and the endocytic machinery [3] and indicate that there is a high degree of heterogeneity and specialization among signaling endosomes. The question of whether signaling endosomes represent some specialized type of endosome – using sorting mechanisms that are different from canonical endocytic pathways – remains unresolved. However, the word 'canonical' would seem crucial here, and it might be that, as our knowledge of the endocytic pathway becomes more sophisticated, this distinction might ultimately be rendered dialectical.

Does the ligand have to come along?

As indicated earlier, retrograde signal propagation in axons has been closely linked to ligand retrograde transport from the early days of the NGF studies. However, there is still incomplete agreement about whether the former can occur without the latter, that is whether a neurotrophic signal can reach neuronal cell bodies unaccompanied by the ligand that initiated it. Exposure of distal axons to beads covalently linked to neurotrophins has been shown to elicit a retrograde signal in neuronal cell bodies without any evidence of retrograde transport of the ligand [40,41]. Although these observations suggest that activated receptors can be retrogradely transported in the absence of ligand, they do not resolve the issue of whether this takes place *in vivo*. It has been suggested that beads coupled to neurotrophins could generate endosomes with a high concentration of Trks that – in a similar way to overexpressed receptors – could remain active in the absence of ligand [40], but it is unclear whether the high ligand concentration present on such beads ever occurs in a physiological setting. Moreover, it has also been proposed that the activated state of receptors could be propagated retrogradely in the absence of physical transport [41,42], a possibility to which we shall return later. However, by neutralizing NGF within cell bodies using a membrane-permeable protein delivery system capable of penetrating across intracellular membranes, other studies have shown that ligand and receptor need to be transported together to allow retrograde survival signaling from distal axons [20], and this result also serves to explain how TrkA activation might be reconstituted after traversing a region of kinase inhibition. In addition, a recent study using quantum-dot-labeled NGF has indicated that a single NGF dimer molecule is sufficient to maintain signaling during retrograde transport [23]. Further work will be required to evaluate the properties of quantum-dot-NGF, the size and geometry of which are quite distinct from those of NGF itself. Overall, there is good evidence supporting the requirement of NGF transport for the maintenance of TrkA activity in signaling endosomes, whereas the mechanistic bases of alternative pathways – if they exist – have yet to be resolved.

Can signaling endosome transport explain fast retrograde signal propagation?

Dynein is a large multisubunit protein complex that is responsible for minus-end-directed microtubule-dependent movement [43]. A role of cytoplasmic dynein motors in retrograde axonal transport was first postulated 20 years ago [44]. TrkA receptors associate with dynein [45] and so do signaling endosomes isolated from sensory neurons [24]. Dynein is required for rapid retrograde transport of activated TrkB receptors and for neuronal survival after neurotrophin stimulation of axon terminals, but not of cell bodies [40]. The speed of NGF retrograde transport in compartmentalized cultures of sympathetic neurons has been estimated to be 2.5–5.0 μm/s [13]. The speed of the fastest component of neurotrophin-dependent transport of Trk receptors in sensory neurons has been estimated to be 4 μm/s, using fluorescence recovery after photobleaching (FRAP) [40]. In another study, retrogradely transported endosomes containing quantum-dot NGF were seen by live-imaging to alternate between periods of active movement and pausing – which accounted for ~30% of the time – with speeds ranging from 0.5 to 5 μm/s [23]. These measurements are in good agreement with speeds reported for axonal retrograde transport of NGF to sensory neurons *in vivo* (3.6 μm/s [46]) and within the range of dynein-mediated transport measured in insect and mammalian cells (1.0–1.7 μm/s [47–49]). However, one study reported rapid retrograde tyrosine phosphorylation of TrkA in compartmentalized cultures of sympathetic neurons with speeds of at least 1 mm/min (i.e. >16 μm/s) [50]. It has been argued that this speed, which would appear to exceed those registered for dynein-mediated transport, would preclude the mass transport of any molecular species and that the observed retrograde activation of TrkA receptors in cell bodies, therefore, represented phosphorylation of receptors already present in that compartment at the time of distal NGF application [50]. Although this notion has generated much debate, it is essentially based on one gel published 10 years ago (see Figure 4b in Ref. [50]): no additional evidence has been presented to substantiate this initial observation or its possible underlying mechanisms. Moreover, results from several subsequent studies –mentioned above and

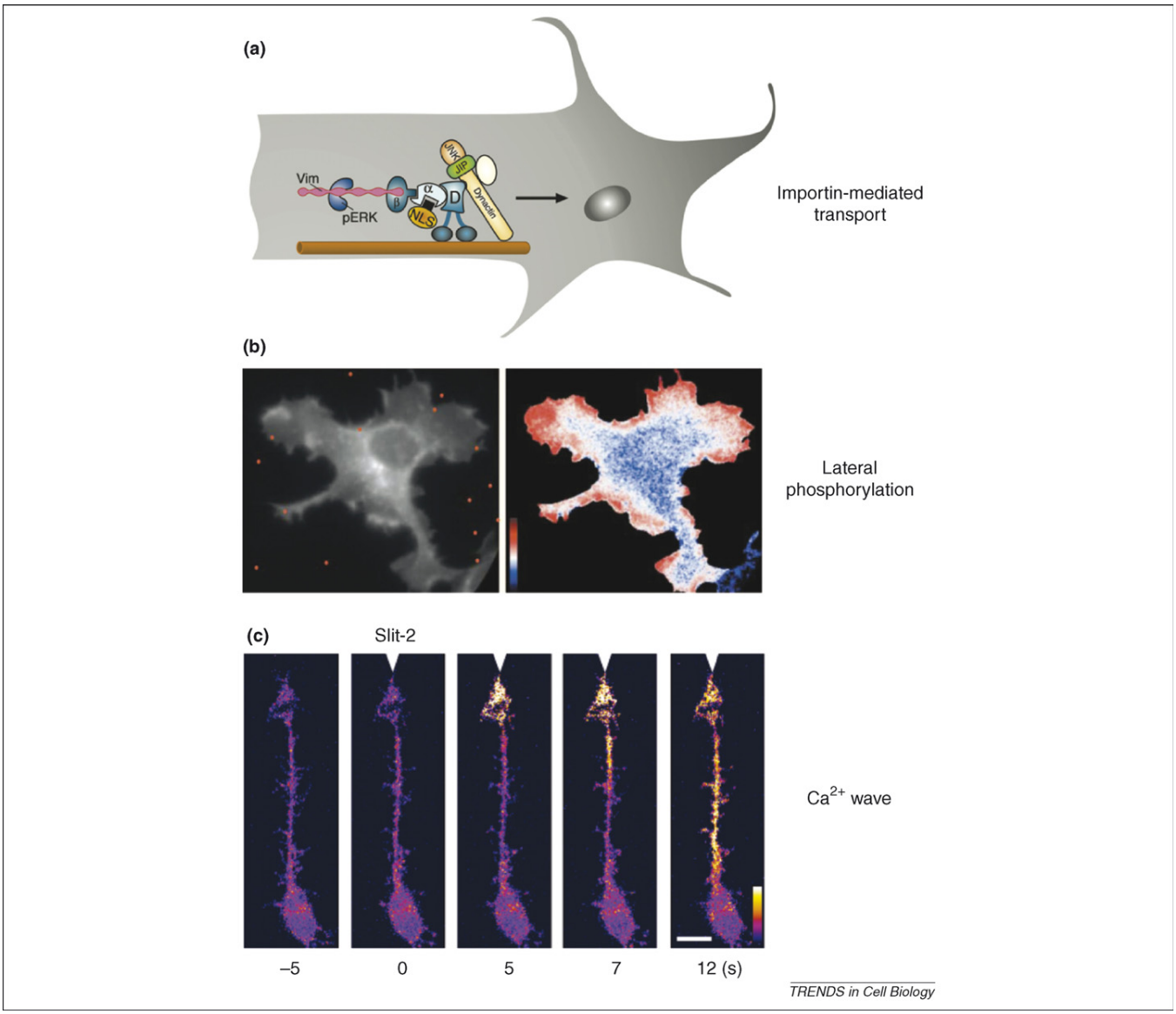

Figure 4. Non-endosomal mechanisms of retrograde signaling. **(a)** Schematic of importin-mediated transport of NLS-containing cargo and vimentin–pErk complex bound to dynein motor moving retrogradely on microtubules. Binding of the JNK–JIP complex to dynactin is also shown. From Ref. [75]. **(b)** Spreading of EGFR signaling by lateral phosphorylation. Cells locally stimulated with EGF-coated beads (red dots in left panel) provoke EGFR phosphorylation as visualized by FRET throughout the whole cell (red signal in right panel). From Ref. [83]. **(c)** Ca^{2+} wave stimulated by distal application of Slit-2 moving retrogradely to the cell body of a migrating cerebellar granule neuron. Scale bar, 10 μm. From Ref. [84].

reviewed elsewhere [25,26] – would appear to refute some of its main predictions. Nevertheless, the general concept of intracellular signal propagation over extended distances in the absence of physical transport of molecular species remains tantalizing, and several ideas have recently emerged from work on other systems (see below). Finally, it should also be noted that fast retrograde signal propagation might still be within a reasonable biological range for dynein motors because speeds up to 19 μm/s have been reported for axonemal dyneins isolated from cilia and flagella [51,52].

Do signaling endosomes play a general role in retrograde signaling by different receptor systems?

Most studies on endocytosis, intracellular trafficking and retrograde transport of ligand–receptor complexes have focused on receptors with intrinsic tyrosine kinase activity, such as the EGFR – for endocytosis – and members of the Trk family – for retrograde signaling in neurons. However, axons express many different classes of receptors that affect functions and transcriptional programs in the cell body. Unfortunately, there is relatively little information on intracellular trafficking and transport for many of those receptor systems, particularly in neurons.

The p75 neurotrophin receptor ($p75^{NTR}$) is a member of the death-receptor superfamily – which includes a couple of dozen receptors related to Fas and the tumor necrosis factor receptor (TNFR) – and can bind all the neurotrophins as well as other ligands [53,54]. A possible involvement of $p75^{NTR}$ in the retrograde transport of neurotrophins in sensory neurons has been indicated [55], but the significance and mechanisms underlying $p75^{NTR}$ trafficking are unclear.

More recent work has demonstrated that binding of NGF to $p75^{NTR}$ results in internalization of ligand–receptor complexes with a kinetics that is at least three times slower than that of the TrkA complex [56], although this could be due to a slow association of the ligand with the receptor, rather than to slow internalization *per se* [57]. In PC12 cells, $p75^{NTR}$ complexes internalize independently of TrkA via clathrin-coated pits into early endosomes together with $p75^{NTR}$-interacting molecules such as necdin and NRAGE [56], suggesting that signaling endosomes containing $p75^{NTR}$ are temporally and spatially distinct from those containing Trk receptors. Internalization of $p75^{NTR}$ has also been visualized in hippocampal [4] and sympathetic [57] neurons, although in the latter both clathrin coats and cholesterol appear to play a role. In motoneurons, $p75^{NTR}$ has been seen to undergo constitutive, clathrin-independent internalization and plasma membrane recycling. In the presence of NGF, a fraction of $p75^{NTR}$ molecules was internalized instead by clathrin-mediated endocytosis and diverted to a fast axonal transport pathway [58]. The extent to which $p75^{NTR}$ undergoes ligand-independent recycling in other cellular systems is unclear, although this could possibly account for some of the clathrin-independent internalization observed in sympathetic neurons [57]. Visualization of retrograde transport in living motoneurons – which lack TrkA – has shown that NGF and $p75^{NTR}$ travel retrogradely in endocytic carriers that are shared with the C-terminal binding fragment of tetanus neurotoxin (TeNT HC), and characterized by a neutral pH and expression of Rab7 [36,59]. On the whole, there would now appear to be good evidence for the existence of $p75^{NTR}$ signaling endosomes and their possible role in retrograde signal propagation in axons. It has not yet been established whether other $p75^{NTR}$ ligands, such as unprocessed pro-neurotrophins and myelin components, also induce the formation of $p75^{NTR}$ endosomes and retrograde signaling in neurons. However, an indication that this might be the case has recently been provided by the demonstration that pro-NT3 – the unprocessed precursor of neurotrophin-3 – can induce retrograde cell death in a $p75^{NTR}$-dependent manner when applied to distal axons of sympathetic neurons growing in compartmentalized cultures (K. Teng, personal communication).

In the transforming growth factor-β (TGF-β) ligand superfamily, internalization of receptor complexes occurs via both clathrin-dependent and –independent mechanisms [60,61]. Although receptor internalization is apparently not required for downstream phosphorylation of Smad effector proteins [62], clathrin-dependent endocytosis into EEA1-positive early endosomes has been shown to promote TGF-β signaling [60]. This pathway would then appear to generate a signaling endosome in which heteromeric Ser-Thr kinase receptors, Smad proteins and additional scaffolding components – such as SARA – are enriched. However, the mechanistic significance of this step, whether it facilitates Smad nuclear translocation or some other event, remains unclear. Likewise, the contribution of clathrin-independent internalization to downstream signaling is also uncertain, with evidence presented favoring a role in TGF-β receptor degradation [60] or Smad-independent BMP signaling [61]. Although the relevance of these findings for retrograde signaling in neurons has not yet been defined, work in *Drosophila* has indicated that the activity of the BMP-like ligand Gbb on the growth and strength of neuromuscular synapses requires retrograde axonal transport, because both of these effects as well as nuclear accumulation of phospho-Smad were disrupted by a dominant negative inhibitor of dynein-mediated transport [63]. Similar results were obtained by studies of Gbb regulation of neuropeptide gene expression in the *Drosophila* CNS [64,65]. More recently, retrograde signaling by BMP4 could be demonstrated directly in trigeminal neurons growing in compartmentalized cultures by the appearance of phosphorylated Smads in neuronal cell bodies after stimulation of distal axons [66]. It remains an open question whether signaling endosomes undergo axonal retrograde transport in these systems, and whether phospho-Smads travel with them or are first produced in the cell body. Given that phospho-Smads undergo importin-mediated nuclear translocation [67,68], an interesting alternative suggested by recent work on retrograde injury signaling via axonal importins [69] (Box 2) is the direct retrograde transport of a phospho-Smad–importin complex by dynein motors.

Although several additional axonal receptors are known to be internalized in different systems, including Notch

Box 2. Retrograde injury signaling

In addition to signals controlling normal neuronal development and function, a different set of signals is generated upon lesion of adult axons and this set is also retrogradely propagated to initiate repair and regenerative responses in cell bodies. It is thought that the first indication that a lesion has taken place is provided by the retrograde propagation of electrophysiological responses elicited by rapid ion influx at the site of injury. A later phase provides injury signals in the form of activated or modified proteins generated at the lesion site (reviewed in Ref. [75]). Until recently, the nature of those signals and the ways in which they reach the cell bodies of injured neurons had remained elusive. Members of the importin family have been found in peripheral nerve axoplasm where they can link to dynein motors for retrograde transport of NLS-containing cargos. Although some importins are present constitutively in axons, importin β appears to be generated upon injury by local translation of axonal mRNA [69]. One of the cargos retrogradely transported upon injury by this system has recently been identified as a complex between phosphorylated Erk1/2 and a soluble fragment of the intermediate filament protein vimentin, which binds directly to importins, thereby providing a link to the retrograde transport machinery [76]. The Vimentin–Erk interaction protects phospho-Erk from dephosphorylation, which enables it to activate the Elk-1 transcription factor and thereby induce transcriptional responses in the cell body.

Other injury signals generated at sites of lesion in peripheral axons involve activation of the c-Jun kinase JNK. Recent work has found that the scaffolding protein Sunday Driver (Syd) mediates axonal retrograde transport of locally activated JNK3 by interacting with the dynactin complex, one of the components of the dynein retrograde motor [77]. Unlike the case of the importins, however, here JNK3 and Syd appear to be associated with vesicles that resemble MVBs at the EM level [77]. It is unclear what other components might be transported in those vesicles or whether they are distinct from signaling endosomes characterized by other methods. Part of the JNK injury response includes activation of the ATF2 and ATF3 transcription factors, for which there is also evidence of retrograde transport from axons to cell bodies after peripheral nerve lesion [78]. It remains to be established whether these factors travel along with Syd vesicles or are transported by some other mechanism, such as NLS binding to axonal importins.

[70], Ephs [71,72], and receptors for Sonic Hedgehog [73] and Wnts [74], it is unclear whether they form signaling endosomes or how their signaling is retrogradely propagated in axons.

Beyond endosomes: a message without bottle

At least two different means of retrograde signal propagation that do not entail endosomes can be envisioned. The first means involves axonal transport of activated signaling molecules or complexes, such as those generated downstream of receptors, independently of vesicular transport (Figure 4a). The second means relies upon self-propagating waves of increased phosphorylation or calcium in the absence of physical transport of molecular species (Figure 4b,c). Recent studies have provided evidence for the existence of both types of mechanisms in neurons.

Details of a mechanism for retrograde transport of distally activated signaling molecules carrying nuclear localization signals (NLS) – such as transcription factors – have begun to emerge from work on retrograde injury signaling in lesioned peripheral nerves [69] (Figure 4a). Local expression and *de novo* synthesis of NLS-binding importins – the main mediators of NLS-dependent nuclear import – have been detected in lesioned axons in complex with the retrograde motor dynein. In addition, retrograde transport of synthetic NLS peptides has been observed in lesioned nerves, suggesting a mechanism by which locally activated NLS-containing cargos could be retrogradely transported to the neuronal cell body. It has been proposed that this mechanism plays an important role in signaling the regeneration response in sensory neurons [75] (Box 2), but would also seem to be well suited to enable the retrograde transport of NLS-containing signaling molecules locally activated in axons, such as phosphorylated Smads or the Notch intracellular domain, both of which contain functional NLS [68,79].

Lateral propagation of receptor tyrosine kinase signaling has been detected in various cell lines upon local stimulation with ligand [80,81]. A wave of EGFR phosphorylation – the only receptor system studied so far – has been observed to extend from a local site of ligand application to the whole cell (Figure 4b). A reaction–diffusion mechanism has been proposed in which a local threshold stimulus triggers the coupled propagation along the plasma membrane of receptor kinase activation – by receptor transphosphorylation – and phosphatase inhibition – by hydrogen peroxide [82]. Although lateral phosphorylation has been proposed as a possible mechanism for fast retrograde signal propagation in axons [83], it is currently unclear whether it might be functional in cells expressing physiological levels of receptors on their surface, as it has been argued that only cancer cells with abnormally high levels of receptors might be capable of sustaining lateral propagation of receptor phosphorylation [80]. Moreover, it is not clear how such a mechanism could explain the directionality of the propagation in axons of neurotrophic signals, which have never been observed to move anterogradely from cell bodies to axons. Experimental evidence against a major role for lateral phosphorylation in retrograde signaling by NGF/TrkA in axons has been provided by the demonstration that disruption of TrkA kinase activity at intermediate axonal segments – unlike inhibition in distal axons or cell bodies – has no effect on retrograde signal propagation or neuronal survival [20]. Notwithstanding these considerations, it is possible that lateral phosphorylation could still play an important role in axonal retrograde signaling by other receptor systems.

Another reaction–diffusion mechanism based on the propagation of Ca^{2+} waves has recently been proposed [84]. In this case, application of a gradient of the chemorepellent Slit-2 in front of the leading process of cultured cerebellar granule neurons generated a Ca^{2+} wave that propagated retrogradely from the growth cone to the soma, resulting in growth cone collapse and reversal of neuronal migration (Figure 4c). Interestingly, the Ca^{2+} wave was both required and in itself sufficient for the Slit-2 effect on migration reversal, which was mediated by a relocalization of active RhoA away from the leading process. The propagation of Ca^{2+} waves in these cells appeared to be based on a Ca^{2+}-induced Ca^{2+} release (CICR) mechanism and moved at an average speed of 8 μm/s, which is similar to the speed previously observed in other non-neuronal systems. Although Ca^{2+} waves propagated by CICR are likely to move both retrogradely and anterogradely, directionality in this system appears to depend on the preferential location of the Slit-2 receptor Robo2 at the growth cone of the leading neurite in migrating granule neurons. Because several other signaling systems are known to activate Ca^{2+} signaling, this mechanism might also be relevant to their ability to trigger fast retrograde signal propagation in axons. However, as the leading process in migrating neurons is relatively short, it will be important to determine whether Ca^{2+} waves could support signal propagation over longer distances, such as those more typical of mammalian axons, that is in the mm and cm range.

What next?

Despite the important advances made during the last decade, there is still much to be learnt about long-range retrograde signaling in neurons, both concerning the best established paradigms, such as signaling endosomes, and other less well-characterized systems of signal propagation. Studies in several laboratories are underway to characterize the inventory of molecular components present in signaling endosomes isolated from different sources using mass spectrometry techniques, after recent pioneering work on synaptic vesicles [85]. The results of that work are likely to offer answers to several of the questions raised above, particularly concerning the identity and uniqueness of signaling endosomes. Besides the proteomic aspect, more work is needed to determine the circumstances that result in retrograde transport of early endosomes, late endosomes or MVBs – whether it depends on, for example, the type of ligand and receptor, the stimulation regime, the cargo transported or the cell type – and the functional significance of the transport of each type of vesicle. A related question concerns the coupling between endocytosis and retrograde transport, particularly the mechanism(s) by which signaling endosomes escape the recycling and degradative routes and are instead diverted to the axonal transport pathway. It will also be important

Box 3. Compartmentalization and diversification

Long-range retrograde signaling contributes not only to the propagation of signals generated in axon terminals to cell bodies but also to the diversification of signaling events in neurons, allowing different downstream effects to be elicited by the same receptor, depending on whether this is activated in the cell body or in distal axons and then retrogradely transported. For example, whereas neurotrophins stimulate local activation of both Erk1/2 and Erk5 in axons as well as cell bodies, only Erk5 is activated during retrograde signaling in sensory neurons [87]. Likewise, in sympathetic neurons, glial-cell-line-derived neurotrophic factor (GDNF) locally stimulates the activities of both Erk1/2 and AKT in axons and cell bodies, but only activation of AKT is detected in cell bodies after stimulation of distal axons [88]. Whether these effectors travel from distal axons in activated form or are generated in cell bodies upon the arrival of signaling endosomes is unclear at present. The particular inability of Erk1/2 to be activated in cell bodies by retrograde signaling is intriguing, and it is possible that signaling endosomes arriving from distal axons by retrograde transport – as opposed to those locally generated – are unable to activate these kinases in cell bodies. However, other studies have found activated forms of Erk1/2 in retrogradely transported signaling endosomes isolated from peripheral nerves [24]. In these cases, the endosomes analyzed originated from a mix of sensory and motor axons, and could have been generated by diverse target- and nerve-derived signals – not only from neurotrophins. It is also possible that these MAP kinases do not accumulate to sufficient levels to be detected in cell bodies after retrograde transport, or become dephosphorylated upon arrival.

There is also evidence that different signaling events elicited in different compartments might trigger different biological outcomes. Thus, for example, whereas increased brain-derived neurotrophic factor (BDNF) in the target of retinal ganglion cells promotes arborization of both their axons and dendrites, increased BDNF within the retina itself inhibits dendritic arborization and has no anterograde effect on axons, indicating differential actions of BDNF in the two compartments [89]. In sympathetic neurons, both NGF and NT3 can activate the TrkA receptor, but whereas NGF supports TrkA internalization, retrograde signaling and neuronal survival, local activation by NT3 fails to trigger TrkA endocytosis and survival but instead elicits axon growth [90]. This finding also indicates that, depending on the ligand that activates them, some receptors might or might not internalize, thus linking endocytic trafficking to ligand-specific responses. The contribution of retrograde signaling to the diversification of cellular responses is not restricted to neurotrophic factors and has been observed in other systems. For example, in the cerebellar granule cell system discussed above, it has been shown that stimulation of growth cones with Slit-2 triggers growth cone collapse locally, but migration reversal distally, after retrograde propagation of a Ca^{2+} wave to cell bodies [84]. Interestingly, the Ca^{2+} wave was by itself capable of inducing only migration reversal, and not growth cone collapse, indicating that Slit-2 signaling in different compartments produces different effects.

to know more about the significance of local versus distal signaling in different settings (Box 3), how retrograde propagation modifies the signal, and which pathways and biological effects require retrograde signaling or are specifically activated by it. Some of these issues are likely to be better addressed by a combination of experimental and computational approaches, as shown by some recent studies [82,86].

The few neurotrophin signaling endosomes that have been characterized so far are almost certainly just the tip of the iceberg, and unique insights will probably be obtained from studies on long-range retrograde signaling by other systems, such as TGF-β, Eph, Notch, Shh and Wnt. Finally, the mechanistic bases and significance of several of the different systems for non-endosomal signal propagation that have been proposed need to be resolved, such as the nature of NLS- and non-NLS-containing cargos that use importins for retrograde transport, additional means of coupling activated signaling complexes to dynein motors, the existence and possible relevance of lateral phosphorylation in axons, and the prevalence and functional importance of Ca^{2+} waves, among others.

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