Published in final edited form as:

Prog Neurobiol. 2011 March ; 93(3): 313–340. doi:10.1016/j.pneurobio.2011.01.003.

Multivesicular Bodies in Neurons: Distribution, Protein Content, and Trafficking Functions

CHRISTOPHER S. VON BARTHELD and AMY L. ALTICK

Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, NV 89557, USA

Summary

Multivesicular bodies (MVBs) are intracellular endosomal organelles characterized by multiple internal vesicles that are enclosed within a single outer membrane. MVBs were initially regarded as purely prelysosomal structures along the degradative endosomal pathway of internalized proteins. MVBs are now known to be involved in numerous endocytic and trafficking functions, including protein sorting, recycling, transport, storage, and release. This review of neuronal MVBs summarizes their research history, morphology, distribution, accumulation of cargo and constitutive proteins, transport, and theories of functions of MVBs in neurons and glia. Due to their complex morphologies, neurons have expanded trafficking and signaling needs, beyond those of “geometrically simpler” cells, but it is not known whether neuronal MVBs perform additional transport and signaling functions. This review examines the concept of compartment-specific MVB functions in endosomal protein trafficking and signaling within synapses, axons, dendrites and cell bodies. We critically evaluate reports of the accumulation of neuronal MVBs based on evidence of stress-induced MVB formation. Furthermore, we discuss potential functions of neuronal and glial MVBs in development, in dystrophic neuritic syndromes, injury, disease, and aging. MVBs may play a role in Alzheimer’s, Huntington’s, and Niemann-Pick diseases, some types of frontotemporal dementia, prion and virus trafficking, as well as in adaptive responses of neurons to trauma and toxin or drug exposure. Functions of MVBs in neurons have been much neglected, and major gaps in knowledge currently exist. Developing truly MVB-specific markers would help to elucidate the roles of neuronal MVBs in intra- and intercellular signaling of normal and diseased neurons.

Keywords

Endosome; Trafficking; Axonal Transport; Sorting; Release; Endocytosis; Degradation

1. Definition of MVBs and scope of this review

Based on their original discovery and historical tradition, MVBs are defined – as their name implies – by morphological criteria at the ultrastructural level. MVBs appear in ultrathin sections as spherical organelles characterized by a single outer (“limiting”) membrane that

© 2011 Elsevier Ltd. All rights reserved.

*Correspondence: Department of Physiology and Cell Biology, Mailstop 352, University of Nevada School of Medicine, Reno, NV 89557 (USA) Phone: (775) 784-6022 FAX: (775) 784-6903 cvonbartheld@medicine.nevada.edu .

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

encloses a variable number of small spherical or ellipsoidal vesicles within a matrix. The entire MVB typically has a diameter of 250–1,000 nm. Most MVBs have a round or slightly oval shape (Fig. 1A-E), although tubular forms or extensions also exist. MVBs contain at least two and up to dozens of smaller vesicles within the membrane-enclosed lumen (an average of 24 internal vesicles/MVB, Cooney *et al.*, 2002). The small vesicles as well as the matrix, the luminal space between the internal vesicles, are typically clear, but they can be of varying electron densities (Roizin *et al.*, 1967). The internal vesicles can have heterogeneous sizes with a diameter of 50–100 nm, although they frequently are uniform in size, about 60–70 nm in diameter. The outer (limiting) membrane is of a similar thickness as the membrane of the internal vesicles and the plasma membrane, with a thickness of about 5.2 nm (Yamamoto, 1963; but see Roizin *et al.*, 1967). MVBs can be surrounded by and be connected to tubular compartments (Cooney *et al.*, 2002). Associations with Golgi stacks, endoplasmic reticulum and secretory vesicles have also been described (Palay, 1960; Peters *et al.*, 1991; Roizin *et al.*, 1967). MVBs move within cellular compartments along microtubules (Gruenberg and Stenmark, 2004; Saito *et al.*, 1997).

MVBs have been described in neurons of numerous animal species and classes, including vertebrates and invertebrates (Roizin *et al.*, 1967). In neurons, MVBs are most commonly located in cell bodies and dendrites. MVBs are occasionally observed in normal axons or axon terminals in vivo (Castel *et al.*, 1992; Delcroix *et al.*, 2003; Kapeller and Mayor, 1969). When they occur in PNS axons, they are more frequent in the axoplasm at nodes of Ranvier than at internodal segments (Altick *et al.*, 2009; Berthold and Rydmark, 1995). With appropriate ultrastructural preservation, the distinct features of MVBs are relatively easy to recognize. Occasionally, MVBs can be mistaken for dense endosomes (when their matrix and vesicles are relatively dense), for phagocytotic vacuoles (Roizin *et al.*, 1967), residual bodies (Roizin *et al.*, 1967; Schmied and Holtzman, 1987), dense lamellar bodies (Smith, 1980), multilamellar, cup-shaped or other endosomes and cisternae (Birks *et al.*, 1972; Holtzman *et al.*, 1973; Weldon, 1975), elongated, membrane-limited profiles (Claude *et al.*, 1982b), or for large vesicles (if there are less than two vesicles within the lumen). Adequate ultrastructural morphology is necessary to distinguish these different types of organelles, and serial thin sections may be required for definitive conclusions (LaVail *et al.*, 1980). Several distinct morphological types of MVBs have been quantified in axons (Altick *et al.*, 2009; LaVail *et al.*, 1980), but not in other neuronal compartments.

Cellular, perikaryal MVBs can stain positive for acid phosphatases and hydrolases (Gatzinsky *et al.*, 1988; Holtzman and Novikoff, 1965; Holtzman *et al.*, 1973; Paula-Barbosa *et al.*, 1978; Roizin *et al.*, 1967; Schmied and Holtzman, 1987). MVBs are thought to function primarily as late or prelysosomal endosomes with sorting functions for internalized transmembrane receptors and their ligands (Katzmann *et al.*, 2002; Mukherjee *et al.*, 1997; Myromslie *et al.*, 2006; Reggiori and Pelham, 2001). Some investigators define MVBs to comprise only one particular subset of late endosomes (Mukherjee *et al.*, 1997) or consider them to be a shuttle/carrier between early and late endosomes in the degradation pathway towards lysosomes (Hurley, 2008; Piper and Katzmann, 2007; Piper and Luzio, 2001; Russell *et al.*, 2006). Other investigators use a broader definition of MVBs and believe that all endosomes along the degradation pathway contain multivesicular elements (Gruenberg and Stenmark, 2004), or recognize two distinct types of MVBs, one for the degradative pathway and one for the recycling/exocytic pathway (Mathivanan *et al.*, 2010). At a functional level, some studies have distinguished the endosomal carrier vesicle, defined as the transport intermediate between early and late endosomes, from MVBs or multivesicular endosomes (Aniento and Gruenberg, 1995). Obviously, investigators apply the term “multivesicular body” to a more or less restricted set of functionally or structurally defined endosomes. On one extreme, use of non-specific MVB markers may cast the net too wide and lead to an unwarranted broad inclusion of endosomes, while a very narrow

functional interpretation may deny MVB status where structural evidence and historical definition would suggest otherwise, thus leading to ambiguity in the field. In this review, we consider primarily work that includes ultrastructural evidence to support conclusions derived in conjunction with biochemistry, physiology, and imaging at the light microscopical level.

Excellent recent reviews are available about the molecular composition of MVBs in non-neuronal cells (Gruenberg, 2001; Gruenberg and Stenmark, 2004; Katzmann *et al.*, 2002; Murk *et al.*, 2002; Piper and Katzmann, 2007; Piper and Luzio, 2001; Raiborg *et al.*, 2003; Sorkin and von Zastrow, 2002). The goal of our review is to compile and organize information available about the morphology, distribution, and potential functions of MVBs specifically in neurons and glia. Neuronal MVBs appear to be highly heterogeneous with distinct functional subtypes both in the somatodendritic compartment (Saito *et al.*, 1997) as well as the axon (Altick *et al.*, 2009). Some molecules such as early endosome antigen 1 (EEA1) and lysobisphosphatidic acid (LBPA) have been suggested to have specific associations with MVBs, but these markers turned out to be stage-dependent for temporally-defined classes of endosomes, i.e. early and late endosomes, respectively (White *et al.*, 2006), rather than for MVBs. Accordingly, these markers are considered ambiguous for MVB identification, because they label subsets of endosomes at different stages of maturation (Maxfield and McGraw, 2004). Compiling information about neuronal MVBs requires considerable effort, because information about these MVBs is currently scattered among various specialty journals. The lack of a comprehensive review of this topic has led to numerous omissions and misconceptions. For example, the association of MVBs with postsynaptic sites has been discovered and re-discovered independently on multiple occasions in the past half century (Cooney *et al.*, 2002; Pappas and Purpura, 1961; Paula-Barbosa *et al.*, 1978; Rind *et al.*, 2005; Schnapp, 1997; von Bartheld *et al.*, 1996). Some conflicting views about the distribution and functions of neuronal MVBs may be based on a lack of awareness of relevant studies. This review is designed to provide a synopsis of previous work, to facilitate access to pertinent original reports, to reveal gaps in current knowledge, and to discuss controversies and recent progress.

2. History of major concepts

The MVB organelle was discovered, first pictured, and briefly described in neurons by Palay and Palade (1955) in their paper entitled *The Fine Structure of Neurons*: “There appears a particular vesicular formation limited by a single membrane and filled with small, circular profiles which may represent membrane invaginations.” This discovery was erroneously attributed in recent reviews to a different article that was authored by Palade in 1955 (see Katzmann *et al.*, 2002; Piper and Katzmann, 2007; Vajjhala *et al.*, 2007; Winter and Hauser, 2006). Soon after Palay and Palade’s 1955 paper, Roberto Sotelo and his colleagues introduced the name “multivesicular body” for these organelles (Estable *et al.*, 1957; Sotelo and Porter, 1959). Although MVBs were first identified in neurons, the majority of subsequent work on MVBs has focused on non-neuronal cells, while neuronal MVBs have been largely neglected. A chronology of major insights regarding MVB functions, biogenesis, protein transport, and their molecular characterization, with emphasis on neurons, is listed in Table 1.

In the earliest studies on neuronal MVBs, these organelles appeared to be associated primarily with Golgi stacks and were thought to be involved in neurosecretion rather than endocytosis (Palay, 1960). This view changed with the observation that tracers such as ferritin, horseradish peroxidase (HRP), and thorium dioxide accumulated in MVBs (Birks *et al.*, 1972; Holtzman and Peterson, 1969; Rosenbluth and Wissig, 1964; Weldon, 1975), and a primary role in the endocytic-degradative pathway was recognized (Holtzman, 1971; Kristensson and Olsson, 1971). Consistent with this notion was the finding of acid

phosphatase and hydrolase activity in at least some MVBs (Gatzinsky and Berthold, 1990; Gatzinsky *et al.*, 1988; Holtzman, 1971; Holtzman and Novikoff, 1965; Overly *et al.*, 1995; Paula-Barbosa *et al.*, 1978; Roizin *et al.*, 1967; Saito, 1983).

In the 1970s and 1980s, it was realized that MVBs accumulate many internalized macromolecules, apparently en route to lysosomal degradation. In neurons, internalized molecules that accumulate in MVBs include endogenous proteins such as nerve growth factor (NGF) (Bernd and Greene, 1983; Claude *et al.*, 1982a; Schwab and Thoenen, 1976), but also exogenous proteins, among them tetanus toxin (Parton *et al.*, 1987; Schwab and Thoenen, 1978) and various neuronal tracers, notably HRP (Berthold and Mellstrom, 1982; Buchner *et al.*, 1987; Bunge, 1977; Bunt and Haschke, 1978; Chan *et al.*, 1980; Chu-Wang and Oppenheim, 1980; Feher, 1984; Kadota *et al.*, 1994; LaVail and LaVail, 1974), and lectins (Broadwell and Balin, 1985; Buchner *et al.*, 1987; Steindler and Cooper, 1986). The HRP studies in particular suggested a role for MVBs in the retrograde axonal transport of macromolecules (Feher, 1984; Grafstein and Forman, 1980), a topic that will be discussed in more detail below (sections 3.5 and 7.2).

MVBs have attracted particular interest because internalized signaling molecules and their receptors accumulate in this organelle. For most of the internalized trophic factors examined, MVBs consistently scored with the highest labeling densities among the subcellular organelles, comparable only with dense endosomes and lysosomes (Butowt and von Bartheld, 2001; Claude *et al.*, 1982a; Claude *et al.*, 1982b; Rind *et al.*, 2005; Schwab and Thoenen, 1977; Schwab *et al.*, 1979; Schwab and Thoenen, 1976; Stieber *et al.*, 1984; von Bartheld *et al.*, 1996).

Several investigators noted an association of MVBs with postsynaptic sites (Fig. 1C-D) (Chicurel and Harris, 1992; Cooney *et al.*, 2002; Pappas and Purpura, 1961; Paula-Barbosa *et al.*, 1978; Rind *et al.*, 2005; Schnapp, 1997; von Bartheld *et al.*, 1996). This association is of interest because it raises the intriguing possibility of a function for MVBs in retrieving, storing and supplying transmembrane receptors for various ligands and transmitters into postsynaptic membranes (see section 4.2). Storage of receptors and their recruitment for membrane insertion has major importance for mechanisms of synaptic plasticity (Cooney *et al.*, 2002; Grampp *et al.*, 2008; Kennedy and Ehlers, 2006), including trafficking of G-protein coupled receptors (Bloch *et al.*, 2003; Eden *et al.*, 2009; Grimes and Miettinen, 2003; Sorkin and von Zastrow, 2002; Woodman, 2009).

Knowledge about the molecular composition and functions of MVBs has advanced primarily in non-neuronal cells, particularly in yeast mutants. However, an important difference between yeast and mammalian cells is that in yeast, proteins targeted to the membranes of the internal vesicles of MVBs are destined for degradation only and are not sorted to recycle back to the limiting MVB membrane or to the cellular membrane (Gruenberg and Stenmark, 2004). Despite a high degree of conservation in the sorting machinery between yeast and mammalian cells (Russell *et al.*, 2006), in order to unravel the full spectrum of sorting mechanisms and signals relevant for multi-cellular organisms and human disease, MVB research cannot solely rely on yeast models. Studies on the trafficking of transferrin, low density lipoprotein (LDL) and epidermal growth factor (EGF) receptors in vertebrate cells proved to be particularly insightful and helped to generate the classic model of the fate of internalized ligand/receptor complexes: The ligand binds to its receptor on the surface membrane; the ligand/receptor complex is internalized; the ligand dissociates from the receptor in the acidic environment of early endosomes; in the endosome the receptor is sorted to either be recycled (considered to be the default and more efficient pathway) or retained to be targeted to the internal vesicles of the late endosome. The soluble ligand remains in the lumen of the endosome and proceeds in the late endosome towards

degradation in the lysosome (Goldstein *et al.*, 1985; Haigler *et al.*, 1979; Maxfield and McGraw, 2004; McKanna *et al.*, 1979; Mukherjee *et al.*, 1997). This simple model has been refined to account for differences between different types of ligands and receptors among signaling molecules (Maxfield and McGraw, 2004; Mayor and Pagano, 2007; Mukherjee *et al.*, 1997; Russell *et al.*, 2006; Sorkin and von Zastrow, 2002).

Recycling may be a particularly important function in neuronal compartments that can be as far away as one meter or more from the cell body. Among the major advances in MVB research was the realization that MVBs are sites for sorting of proteins between recycling and degradation pathways – and that passage through the MVB is not necessarily a one-way street. There is now considerable evidence for additional sorting functions in MVBs (Eden *et al.*, 2010; Felder *et al.*, 1990; Katzmann *et al.*, 2001; Uchil and Mothes, 2005; Vajjhala *et al.*, 2007; Valdez, *et al.*, 2007; Vanlandingham and Ceresa, 2009). Unifying insights into the function of this organelle are emerging as the molecular composition of MVBs is being defined (Babst, 2005; Gruenberg, 2001; Gruenberg and Stenmark, 2004; Hurley, 2008; Katzmann *et al.*, 2002; Maxfield and McGraw, 2004; Piper and Luzio, 2001; Wollert and Hurley, 2010). A molecularly based account of neuronal MVB function is also important for understanding membrane trafficking in normal as well as diseased or dystrophic conditions, with relevance for neurodegenerative diseases (sections 11.2–11.3)

One of the recent controversies in the field of neuronal MVBs has focused on the question whether MVBs are the main retrograde carrier of trophic signals from axon terminals to the cell body. The endosomal signaling hypothesis postulates that endosomes provide platforms for signaling, after and possibly during axonal transport (Campenot and MacInnis, 2004; Grimes and Miettinen, 2003; Howe and Mobley, 2004; Oppenheim and von Bartheld, 2008; Wu *et al.*, 2009; Zweifel *et al.*, 2005). The identity of these endosomes has been a matter of disagreement: are MVBs involved in retrograde axonal transport of trophic signals and are they possibly the main axonal carrier for target-derived trophic factors (Bronfman *et al.*, 2007; Sandow *et al.*, 2000; Weible and Hendry, 2004)? While there is no doubt that neurotrophic factors accumulate in MVBs in cell bodies, until recently it was uncertain whether the neurotrophic factors reside in MVBs already during axonal transport or whether MVBs are generated from trophic factor-carrying axonal endosomes only after reaching the cell body. According to two recent studies, Cui *et al.* (2007) and Altick *et al.* (2009), MVBs in axons are not the retrograde carrier of several major neurotrophic factors, thus indicating that the accumulation of these trophic factors in MVBs within the cell body and dendrites constitutes an event that occurs subsequent to the retrograde axonal transport of the neurotrophic factor in a non-MVB organelle.

Another recent uncertainty concerns potential release mechanisms of MVBs: Do neuronal MVBs release their content via vesicular intermediates with “back-fusion” events (Kleijmeer *et al.*, 2001; Murk *et al.*, 2002; Rind *et al.*, 2005; Uchil and Mothes, 2005), or does the limiting membrane fuse with the plasma membrane and release internal vesicles as exosomes (Faure *et al.*, 2006; Lachenal *et al.*, 2011; Putz *et al.*, 2008; Smalheiser, 2007; Weldon, 1975), or can neurons employ both mechanisms? Modes of release have potentially important disease-relevant implications, for example the release of amyloid-beta 42 and prions from neuronal MVBs in the pathophysiology of Alzheimer’s disease and Prion disease, respectively. These aspects will be discussed with more details in later sections of this review (sections 8.1–8.2; 11.3.2, 11.5).

3. Distribution of MVBs in neuronal compartments

This section describes where MVBs are typically located in neurons and concludes that MVBs are differentially distributed between neuronal compartments

Neuronal MVBs reside predominantly in cell bodies (Holtzman *et al.*, 1973; Peters *et al.*, 1991; Waxman *et al.*, 1995) (Fig. 1A, B) and dendrites (Chicurel and Harris, 1992; Cooney *et al.*, 2002; Pappas and Purpura, 1961; Rind *et al.*, 2005; Roizin *et al.*, 1967) (Fig. 1C-D). MVBs can be present in PNS and CNS axons and terminals, but, as will be discussed in detail below, they are about 50 times more abundant in the soma or dendrites than in axons. Furthermore, the formation of MVBs in axons may occur largely in response to a wide range of non-physiological (“dystrophic”) stimuli (Altick *et al.*, 2009). This section will first review in detail current knowledge about MVBs in cell bodies, dendrites, and PNS and CNS axons.

3.1. Neuronal cell bodies—The fractional area of MVBs (= the relative volume occupied by MVBs, compared to the total space of the compartment analyzed) in vertebrate neuronal somata is similar *in vivo* and *in vitro* and ranges from 0.2% to 1.8% (for references, see Table 2). MVB volume is dynamically regulated and can change with external influences such as pharmacological agents, electrical stimulation and neuronal activity. After treatment with the lysosomal inhibitor, methylamine (10 mM) (Claude *et al.*, 1982b), the frequency of MVBs was normal, but the MVBs were larger and appeared more often “empty.” The volume of MVBs increases in neurosecretory cells when neurohypophysial secretion is stimulated by water deprivation (Theodosios, 1982), when neurons lack prosaposin, a glycoprotein with neurotrophic activities (Chu *et al.*, 2005), or when the membrane trafficking chaperone pincher or the neurotrophin receptor TrkB are overexpressed (Valdez *et al.*, 2005). On the other hand, some other pharmacological agents, notably the lysosomal inhibitor chloroquine, result in a decrease of the fractional area of MVBs (reduced numbers and/or smaller-sized MVBs) (Claude *et al.*, 1982b). The usual location of MVBs has been described as the “cellular cytoplasm within or close to the vicinity of Golgi complex” (Holtzman *et al.*, 1973; Peters *et al.*, 1991; Roizin *et al.*, 1967). MVBs can also be associated with smooth endoplasmic reticulum (Peters *et al.*, 1991; Roizin *et al.*, 1967), vesicles, and tubules (Cooney *et al.*, 2002; Parton *et al.*, 1992). MVBs may also connect with electron-dense membranes or clathrin coats – visible as “plaques” at the ultrastructural level (Peters *et al.*, 1991).

An association or transition of MVBs to secondary lysosomes has been postulated (Gruenberg and Stenmark, 2004; Kaasinen *et al.*, 2008; Morales *et al.*, 1999; Mukherjee *et al.*, 1997; Rink *et al.*, 2005; Roizin *et al.*, 1967; Saxena *et al.*, 2005; Stoorvogel *et al.*, 1991), although this dynamic flux is difficult to verify by morphological techniques (Peters *et al.*, 1991; Roizin *et al.*, 1967) and even more so in the absence of MVB-specific markers. Nevertheless, MVBs in neuronal cell bodies most likely interface between early and late or prelysosomal endosomes, as demonstrated in non-neuronal cells (Hurley, 2008; Katzmann *et al.*, 2002; Mukherjee *et al.*, 1997). This may not be the only function of MVBs, as different subtypes of neuronal MVBs are known to exist (Altick *et al.*, 2009; McCaffrey *et al.*, 2009; Saito *et al.*, 1997).

3.2. Dendrites—MVBs reside in proximal and distal dendrites (Pappas and Purpura, 1961; Rind *et al.*, 2005; Roizin *et al.*, 1967). MVBs have been reported to associate preferentially or specifically with postsynaptic densities on dendrites, including spines and spinules (Chicurel and Harris, 1992; Cooney *et al.*, 2002; Kadota and Kadota, 2002; Pappas and Purpura, 1961; Paula-Barbosa *et al.*, 1978; Rind *et al.*, 2005; Schnapp, 1997; von Bartheld *et al.*, 1996). Four studies have quantified MVBs in dendrites, but only one of them determined

the fractional area (FA) of MVBs (Rind *et al.*, 2005). This study found the dendritic FA of MVBs to be in a similar range as in the neuronal soma (0.2–1.8%, Table 2). The other three studies quantified MVBs in shafts below spines or in spineheads of hippocampal (Chicurel and Harris, 1992; Cooney *et al.*, 2002) and amygdalar dendrites (Ostroff *et al.*, 2010). In the later two studies MVB frequency per dendrite length was 0.1–0.4 MVB per 10 μm or 5–15 MVBs per 10 μm , respectively. Interestingly, MVB frequency increased in dendritic shafts below spines after tetani or mild focal seizures (Kadota and Kadota, 2002; Kraev *et al.*, 2009) and after fear conditioning (Ostroff *et al.*, 2010). Dendritic MVBs can be associated with tubules (Cooney *et al.*, 2002; Parton *et al.*, 1992) and smooth endoplasmic reticulum (Chicurel and Harris, 1992), and MVB-tubule complexes at spine origins have been suggested to be storage, sorting, and recycling centers for membrane-associated proteins, while isolated MVBs (MVBs not associated with tubules) may preferentially carry sorted material to the soma (Cooney *et al.*, 2002). In addition, association of MVBs with electron dense structures (densities or clathrin coats) has been reported, occasionally in neurite growth cones (Birks *et al.*, 1972; Peters *et al.*, 1991; Povolishock, 1976).

MVB formation and/or their preferred association with synapses appear to be influenced by trophic factors obtained from target areas (Fig. 2A–D) (Rind *et al.*, 2005; White *et al.*, 2006). Numerous signaling events depend on endosomal trafficking, as recently reviewed (Sorkin and von Zastrow, 2002, 2009; Wu *et al.*, 2009). It has been shown that dendritic MVBs are transported by a novel kinesin that is restricted to the dendritic compartment (Saito *et al.*, 1997), which is suggestive for a dendrite-specific function of these MVBs. MVBs in dendrites may be organelles en route to the cell body, or they may move from cell body to dendrites, or they may shuttle in both directions (see sections 7.1–7.2, below). It is likely that MVBs carrying cargo into dendrites have different functions than those carrying cargo confined within the soma, since the dendrite-specific kinesin did not co-label with many of the late endosomal or prelysosomal markers (Saito *et al.*, 1997) that can be found in non-neuronal MVBs and are often used for identification of MVBs.

3.3. PNS axons—Seemingly controversial sets of data have been reported describing the distribution of MVBs in PNS axons and terminals. MVBs are rare in most parts of normal PNS axons in vivo (Berthold *et al.*, 1993; Berthold and Rydmark, 1995; Gatzinsky *et al.*, 1988; Hollenbeck, 1993). A recent quantification showed that MVBs are about 50-fold less frequent in PNS axons than in their soma or dendrites (Altick *et al.*, 2009). MVBs have been shown in the presynaptic terminals of axons in sympathetic ganglia after prolonged (1–5 min) electrical stimulation (Kadota and Kadota, 1982). MVBs are consistently found in the distal region of the internodal segment at the node of Ranvier, often in specialized membrane sacs that belong to the “axon-Schwann-cell-network,” a structure that is thought to be a hot spot of membrane and protein exchange and other interactions between axons and their myelinating glia (Berthold *et al.*, 1993; Berthold and Rydmark, 1995; Gatzinsky, 1996; Gatzinsky *et al.*, 1988). The MVBs found at these internodal sites are not localized within the mainstream axoplasm, but rather appear segregated within secluded compartments. Interestingly, this distribution of MVBs is restricted to the PNS domain of the axon; within the CNS pathway of the same myelinated axons, the (rare) MVBs are distributed within the axoplasm without nodal concentration (Gatzinsky *et al.*, 1988). Thus, the accumulation of MVBs at the node of Ranvier in the PNS does not seem to be the consequence of a simple rheologic phenomenon, since similar constrictions and reductions of the effective axonal diameter occur in the CNS portion of the same axons, yet no such accumulation of MVBs occurs there. It has been suggested that these MVBs function to sequester and remove toxic macromolecules that have direct access to PNS terminals and are transported retrogradely, but are degraded before they reach the CNS soma that is protected by the blood-brain-barrier (Gatzinsky, 1996).

3.4. PNS terminals and sensory receptor cells—There are several reports of MVBs in PNS terminals or sensory receptors in vivo, including neuromuscular junctions, mechanoreceptors and free nerve endings, hair cells, photoreceptor cells, and autonomic ganglia. We will comment on each of these categories.

MVBs are rarely seen in normal neuromuscular junctions (Csillik and Knyihar-Csillik, 1980; Lloyd *et al.*, 2002), but the numbers of MVBs are increased in genetically altered, dystrophic, and aging neuromuscular junctions (Boaro *et al.*, 1998; Diaz-Cintra *et al.*, 2004; Fergestad *et al.*, 1999; Jia *et al.*, 1993).

Mechanoreceptors and free nerve endings are, by anatomical criteria, the dendritic (receiving) ends of the bipolar sensory ganglion cells, and thus not directly comparable with “true” efferent axon terminals. However, MVBs in mechanoreceptors are relatively frequent and characteristically located at the base of finger-like protrusions where the sensory cytoplasm of mechanoreceptors and free nerve endings contact the surrounding connective tissue (Andres, 1966; Andres and von Düring, 1973; Byers, 1984, 1985; Byers and Yeh, 1984). The function of MVBs at this location is unknown, but the fact that the fingerlike protrusions in lamellated receptors are exposed to mechanical deformations and are thought to convert such deformations into axon potentials is consistent with the notion (advanced below) that stress can give rise to MVBs.

MVBs have also been noted among the afferent and efferent innervation of outer hair cells within the mammalian inner ear (Omata and Schatzle, 1980) and in cardiac ganglion terminals (Pauziene and Pauza, 2003). Presently, the function of MVBs at these locations is unknown and largely unexamined. MVBs in terminals may contribute to slow turnover of membrane components of the synaptic terminal (Wittich *et al.*, 1994). Ultrastructural studies consistently report MVBs in shedding photoreceptor cells (Calderon *et al.*, 2002; Calman and Chamberlain, 1982; Williams and Blest, 1980; Schmied and Holtzman, 1987). MVB numbers can fluctuate in parallel with circadian shedding and synthesis of the photoreceptor membrane (Williams and Blest, 1980; Calderon *et al.*, 2002; Chamberlain and Barlow, 1984; LaVail, 1976). MVBs are present in the inner segment of photoreceptor cells and seem to have a role in the endocytic pathway, based on work showing MVBs harboring the tracers HRP and gold-labeled interphotoreceptor-retinoid-binding protein, apparently en route to degradation (Hollyfield and Rayborn, 1987; Hollyfield *et al.*, 1985). Moreover, MVBs have been noted in photoreceptor cells of diseased retinas, although their significance has not been explored (Matthes and LaVail, 1989; Montiani-Ferreira *et al.*, 2005). Similar to retinal photoreceptors, light-sensitive pinealocytes contain MVBs, which vary in number with age (Lewczuk *et al.*, 2004; Vigh and Vigh-Teichmann, 1993). The role of MVBs in these cell types remains to be determined.

Besides a physiological function of MVBs in PNS terminals, it needs to be considered that MVBs may be generated artificially, by dystrophic conditions. In some studies (Chu-Wang and Oppenheim, 1980; Omata and Schatzle, 1980; Pauziene and Pauza, 2003), the tissues were not immediately fixed. Delays of up to 8 hours (and lack of oxygen during this delay) could have rendered the terminals ischemic or dystrophic. As noted above, axons in dystrophic conditions have an increased number of MVBs, presumably in response to such insults (Altick *et al.*, 2009; Schroer *et al.*, 1992), in addition to an increase in the number of other organelles, such as autophagosomes and mitochondria (Dixon, 1967; Einheber *et al.*, 2006; Korthals *et al.*, 1978; Marty and Peschanski, 1994; Matthews and Raisman, 1972; Wang *et al.*, 2006). In support of this notion, MVBs can rapidly form de novo (Chu-Wang and Oppenheim, 1980), apparently within minutes (Birks *et al.*, 1972; Piper and Luzio, 2001; Weldon, 1975), and the number of MVBs can increase significantly within hours of a mechanical insult or drug treatment (Altick *et al.*, 2009; Bresnahan, 1978; Claude *et al.*,

1982b). The occurrence of MVBs in PNS terminals is in contrast to the rarity, if not absence, of MVBs along the axon shaft. As a possible explanation for the apparent paucity of MVBs in axon terminals, it has been suggested that MVBs may be rapidly removed from the terminals by retrograde axonal transport to the soma (Holtzman *et al.*, 1973). If this was true, however, then a larger number of MVBs should be visible en route or in transit in the axons shaft – which is not the case (Altick *et al.*, 2009).

3.5. Accumulation of MVBs at axonal ligatures—Accumulation of MVBs at the distal side of axonal ligatures has been reported in multiple studies, and upon superficial consideration, this may be taken as reasonable and convincing evidence that MVBs are the carriers of many, if not all retrogradely transported macromolecules along axons (Bronfman *et al.*, 2007; Kaasinen *et al.*, 2008; LaVail and LaVail, 1974; Parton *et al.*, 1992; Sandow *et al.*, 2000; Smith, 1980; Tsukita and Ishikawa, 1980; Valdez *et al.*, 2005; Weible and Hendry, 2004). This notion, however, needs to be reconciled with the apparently contradictory finding that MVBs are very rare along 99% of normal PNS axons (Altick *et al.*, 2009; Berthold *et al.*, 1993) and along CNS axons (Janetzko *et al.*, 1989). A possible explanation is that MVBs may be rapidly generated from smaller vesicles in response to dystrophic conditions. This was the prevailing notion in the 1960s (Blumcke and Niedorf, 1966; Holtzman and Novikoff, 1965; Kapeller and Mayor, 1969; Smith, 1980; Zelena *et al.*, 1968). Such conditions may include the slowing of axonal transport at sites of mechanical constriction or cooling (Hirokawa *et al.*, 1990; Tsukita and Ishikawa, 1980). Thus, the accumulation of MVBs at ligatures or freeze-blocks in axons could be the result of local slowing of small transport vesicles (Grimes *et al.*, 1997) or other membranous material. Such vesicles en route may not form MVBs under physiological conditions within the axon, but may do so rapidly in response to a wide range of dystrophic conditions. As we will argue below (sections 3.6–7, 11.2), this explanation is entirely consistent with older and more recent experimental evidence.

3.6. CNS axons—MVBs have only rarely been documented in normal CNS axons under physiological conditions (Janetzko *et al.*, 1989; Roizin *et al.*, 1967; Vitalis *et al.*, 2008). Some investigators concluded that MVBs may not exist there at all (Castel *et al.*, 1992; Hirano and Llena, 1995; Pappas and Purpura, 1961). Based on the apparent lack of MVBs in CNS axons, Pappas and Purpura (1961) suggested that MVBs may aid in the ultrastructural distinction between dendritic and axonal processes. When “dystrophic” conditions are generated, by injection of horseradish peroxidase (HRP) or lectins or by other lesions, MVBs have been reported within CNS axons and CNS axon terminals (Bunt and Haschke, 1978; LaVail and LaVail, 1974; LaVail *et al.*, 1980). At the concentrations applied, both HRP and lectins are considered to be toxic for neurons (Bunt and Haschke, 1978), and their internalization, along with the formation of new MVBs, may constitute part of the neuron’s response to a “dystrophic” condition, a response that seeks to remove and thereby eliminate the internalized toxic material by retrograde axonal transport and eventual degradation in lysosomes (Gatzinsky, 1996; Grafstein and Forman, 1980).

3.7. Neurites and growth cones in vitro—Neurites grown in vitro from embryonic or neonatal sympathetic ganglia contain relatively sparse MVBs, some of which are spherical with diameters averaging 750 nm (Bunge, 1977), while others are elongated tubular with diameters of 60–130 nm and up to 2 μ m in length (Birks *et al.*, 1972; Weldon, 1975). In these studies, two types of neurite varicosities were described with one of them containing relatively large numbers of MVBs (Birks *et al.*, 1972). Neurite growth cones contain both spherical and tubular MVBs with no characteristic location within the growth cone (Birks *et al.*, 1972; Povlishock, 1976). Neurites and neurite growth cones also contain cup-shaped bodies (‘C’ bodies) or ‘sacs’ (vesicle-filled structures that often have a curving avascular

portion or “tail”); they are believed to be early (precursor) forms of MVBs (Birks *et al.*, 1972; Bunge, 1977; Weldon, 1975; Wessells *et al.*, 1974 – see section 5). Cup-shaped bodies contain a few vesicles and an occasional myelin figure.

MVBs seem to be more frequently encountered in PNS axon terminals and neurites in vitro than in vivo. The in-vitro conditions may be considered a form of dystrophic state for neurons, and in many in-vitro studies, neurons were furthermore exposed to additional toxic markers or potential inducers of MVBs, such as HRP and thorium dioxide (Birks *et al.*, 1972; Holtzman *et al.*, 1973; Parton *et al.*, 1992; Teichberg and Holtzman, 1973). Thus, results from in-vitro studies may not reflect the normal physiological status of MVBs in axons or axon terminals in vivo.

4. Accumulation of molecules in neuronal MVBs

This section distinguishes and lists two types of molecules: those constitutive for MVBs (housekeeping), and the cargo molecules that are processed

The molecules within MVBs can be classified into two broad categories: constitutive molecules and cargo molecules. Constitutive molecules are not necessarily unique to MVBs, but are essential to make up either the organelle structure or serve house-keeping functions of MVBs, for example vesicle budding, providing an appropriate internal milieu (pH), recognition of signal sequences, ubiquitination and sorting functions, and transport along microtubules. Different families of molecules, for example ESCRT proteins, pincher, kinesins, phosphatases and kinases, accomplish these types of functions (Falguieres *et al.*, 2008; Piper and Katzmann, 2007). Cargo molecules, on the other hand, are the membrane-bound receptors, ligands, internalized proteins and macromolecules that are sorted, processed and transported by MVBs. Different subtypes of MVBs may be composed of different constitutive molecules, and may also accumulate and transport different types or combinations of cargo molecules (McCaffrey *et al.*, 2009; Saito *et al.*, 1997; White *et al.*, 2006). The cargo molecules can be further divided into those that are endogenous to the neurons (e.g., receptors for neurotransmitters or trophic factors, Fig. 2A-D) and those that are exogenous (e.g., HRP tracers or toxins). A large variety of molecules has been shown to accumulate in neuronal MVBs. Accumulation of identified molecules is typically evidenced by radiolabel, immunolabel, or enzyme histochemistry. Major categories and examples of such molecules in neuronal MVBs are listed in Table 3.

4.1. Constitutive molecules—Constitutive proteins associated with MVBs have been identified almost exclusively in non-neuronal cells. Numerous in-vitro studies have characterized molecules necessary for MVB morphogenesis, maintenance, protein sorting, and MVB transport along microtubules. The described features of MVBs most likely also apply to neuronal MVBs. MVBs and the molecules associated with MVB formation and function have been the topic of several excellent recent reviews (Gruenberg and Stenmark, 2004; Hurley, 2008; Katzmann *et al.*, 2002; Maxfield and McGraw, 2004; Piper and Katzmann, 2007; Russell *et al.*, 2006; Williams and Urbe, 2007; Woodman and Futter, 2008). MVBs originate from endosomes that have undergone invagination of the limiting membrane. Four protein complexes, ESCRT 0-III, composed of several subunits each, function to “escort” cargo to endosomes, recruit necessary molecules, such as phosphatidylinositols and respective kinases, sequester lipids and cargo for sorting, and promote invagination and cleavage to form internal vesicles.

Ubiquitination is a major signal for sorting of membrane proteins into MVBs (Babst, 2005; Davies *et al.*, 2009; Hicke and Dunn, 2003; Katzmann *et al.*, 2001; Raiborg *et al.*, 2003; Russell *et al.*, 2006; Woodman, 2009). Ubiquitination of cargo proteins occurs at the plasma membrane or the membrane of early endosomes, which promotes delivery of cargo into the

endosomal-lysosomal system (Davies *et al.*, 2009; Haglund *et al.*, 2003; Longva *et al.*, 2002; Ren *et al.*, 2008; Sigismund *et al.*, 2005; Thelen *et al.*, 2008). De-ubiquitination is necessary for appropriate sorting of cargo by the MVB (Agromayor and Martin-Serrano, 2006; Babst, 2005; Kyuuma *et al.*, 2007; Nikko and Andre, 2007; Richter *et al.*, 2007; Row *et al.*, 2006). The subunits of the ESCRT 0, I and II complexes have ubiquitin binding motifs and are believed to sort cargo via ubiquitin binding (Hurley, 2008; Luzio *et al.*, 2009; Russell *et al.*, 2006; Wollert and Hurley, 2010). While ubiquitination is not the only route into the endosomal-lysosomal system and thus into MVBs (Calistri *et al.*, 2009; Piper and Katzmann, 2007; Reggiori and Pelham, 2001), this route is clearly significant, because mutations of the proteins involved in the ubiquitination/de-ubiquitination activity can lead to neurodegenerative diseases (Lee *et al.*, 2007; Matsuda and Tanaka, 2010; Rubinsztein, 2006; Rusten and Simonsen, 2008; Tamai *et al.*, 2008).

Rab GTPases are another class of proteins that are associated with the endocytic pathway. It has been established that Rabs 4, 5, 7, 9, and 11 function to delineate membrane domains of endosomes which will either invaginate to form internal vesicles, bud off as small vesicles to recycle to the plasma membrane, or become part of lysosomes for degradation (Vonderheist and Helenius, 2005; Zerial and McBride, 2001). Thus Rabs are present at each stage within the endosomal-lysosomal system, including MVBs.

In addition to ESCRT complexes and Rab GTPases, other individual molecules have been identified as being associated with MVBs and/or endosomes. These include the LAMP family, often used to mark the lysosomal pathway (Futter *et al.*, 1996), mannose 6-phosphate receptor (van Deurs *et al.*, 1993), STAM and AMSH (Agromayor and Martin-Serrano, 2006; Kyuuma *et al.*, 2007), LBPA (Kobayashi *et al.*, 1999; Matsuo *et al.*, 2004), and UBPY (Row *et al.*, 2006), and several phosphatidylinositol kinases (Heilmeyer *et al.*, 2003; Odorizzi *et al.*, 1998). These proteins have roles in ubiquitination and de-ubiquitination, sorting, maturation, transport, lysosomal fusion, and exosome release. In spite of their seemingly specific functions, these proteins are found in numerous endosomal and cellular compartments, besides MVBs, thus confounding the molecular definition of MVBs (Maxfield and McGraw, 2004; Sorkin and von Zastrow, 2002, 2009; van Meel and Klumperman, 2008). Interestingly, of all the signaling proteins associated with endosomal compartments as listed by Sorkin and von Zastrow (2002), only one was localized to the endosomal/MVB pathway by electron microscopy, all others were localized by indirect methods, such as immunofluorescence, immunoprecipitation, and subcellular fractionalization. It is a difficult task to assign specific markers to all of the stages of organelle morphology from endosome to lysosome (van Meel and Klumperman, 2008). Thus, MVBs, and possibly MVB subtypes (McCaffrey *et al.*, 2009; White *et al.*, 2006) still await full molecular characterization.

For the most part, neuronal MVBs presumably utilize the same molecules and processes for biogenesis and function as other cell types, but some aspects may be neuron-specific. Studies of neurons have identified several proteins that associate with MVBs, such as neuron-specific kinesin, which moves MVBs anterogradely (– to + direction) along microtubules (Saito *et al.*, 1997); neuron-specific dynein isoform, which moves signaling endosomes retrogradely (+ to – direction) along microtubules (Ha *et al.*, 2008); pincher, which is involved in macroendocytosis of Trks and NogoΔ20 (Joset *et al.*, 2010; Valdez *et al.*, 2005); and Ndfip 1, which is required for exosome secretion (Putz *et al.*, 2008). On the basis of these recent findings, it seems probable that although MVBs are composed of and utilize the same proteins present in other cell types, neuron-specific molecules exist that associate with MVBs in neurons.

4.2. Endogenous cargo molecules—Endogenous cargo molecules within neuronal MVBs may be integral membrane proteins such as channels, transporters and receptors, or they may be soluble molecules. The soluble molecules typically are internalized by receptor-mediated mechanisms. Different categories of endogenous molecules have been identified and localized in neuronal MVBs, including growth factors/trophic factors (Fig. 2A-D) (Butowt and von Bartheld, ²⁰⁰¹, 2009; Claude *et al.*, 1982a; Claude *et al.*, 1982b; Rind *et al.*, 2005; Schwab and Thoenen, 1977; Schwab and Thoenen, 1976; von Bartheld *et al.*, 1996) and their receptors (Pioro *et al.*, 1991; Valdez *et al.*, 2005). Another important category is comprised by receptors for neurotransmitters, ion channels, and G-protein-coupled receptors (Bloch *et al.*, 2003; Grimes and Miettinen, 2003; Sorkin and von Zastrow, 2002, 2009; Vitalis *et al.*, 2008), and their ligands, such as the neuropeptides neurotensin, calcitonin gene-related peptide (CGRP), substance P and small cations, for example calcium (Table 3). Calcium has been localized to the internal vesicles of neuronal MVBs (Buchs *et al.*, 1994; Csillak and Knyihar-Csillak, 1980; Fifkova *et al.*, 1983; Zareba-Kowalska and Gajkowska, 1990). This indicates that MVBs sequester and store calcium. Calcium may participate in MVB housekeeping functions, such as formation of internal vesicles and sorting (Luzio *et al.*, 2007). Neurosecretory material has been localized to MVBs in some experimental paradigms. The number of MVBs in neurosecretory cells increases in parallel with an increase in neurosecretory molecule synthesis, suggesting a role in either processing the neurosecretory molecules or degrading excess material from the previous synthesis and release cycle (Bord and Bell, 1977; Morris and Dyball, 1974; Morris and Steel, 1975, 1977). Another example for endogenous cargo is the LR11 (lipoprotein) receptor which binds apolipoprotein A that is involved in cholesterol metabolism. Cholesterol appears to be required for the diversion of growth factor receptors from MVBs to the Golgi network instead of targeting to lysosomes (Miwako *et al.*, 2001; Offe *et al.*, 2006). This may point to integration of signaling mediated by cholesterol and growth factor receptors at the level of the MVB (Eden *et al.*, 2010; Miwako *et al.*, 2001), a concept that may be relevant for the pathophysiology of Niemann-Pick disease (Kobayashi *et al.*, 1999) as discussed in more detail in section 11.3.4.

Additional membrane and binding proteins that have been localized to neuronal MVBs include interphotoreceptor-retinoid-binding protein (IRBP), amyloid precursor protein (APP), and prion proteins. While IRBPs apparently are destined for degradation (Hollyfield *et al.*, 1985), prion proteins (PrP^C) and APP are targeted to MVBs, from where they may be released as exosomes (Rajendran *et al.*, 2006; Takahashi *et al.*, 2002; Vella *et al.*, 2008). Whether the pathogenic scrapie prion form (PrP^{SC}) follows a similar pathway is unclear (see section 11.5). Cannabinoid receptors accumulate in MVBs in neuronal cell bodies as well as in axonal MVBs (Morozov and Freund, 2003; Vitalis *et al.*, 2008). Transporters for dopamine and monoamine have also been localized to MVBs (Table 3), and the monoamine transporter-2 has been localized to the limiting membrane of MVBs (Nirenberg *et al.*, 1996).

Information about the precise localization of cargo proteins within MVB compartments is available for only a few types of endogenous cargo and one constitutive-type MVB molecule. Such information is important, because growth factors and other signaling molecules may retain signaling capacity when they are targeted to the limiting membrane of MVBs, but not when they are sequestered within MVB's internal vesicles (Mukherjee *et al.*, 1997; Sorkin and von Zastrow, 2002, 2009). In cortical neurons, amyloid-beta 42 accumulates in the limiting membrane of MVBs (Takahashi *et al.*, 2002). In hypoglossal motoneurons, the internalized neurotrophic factors BDNF and GDNF are localized primarily to the internal vesicles of MVBs when those MVBs are located in the neuronal cell body (possibly destined for degradation), while they are preferentially localized at the limiting membranes when the MVBs are located in dendrites (possibly destined for signaling and/or recycling, see Fig. 3A-C) (Rind *et al.*, 2005). Consistent with the notion that tyrosine kinase

activity regulates trafficking into a degradation pathway (Felder *et al.*, 1990), in cultured sympathetic neuronal cell bodies overexpressing the neurotrophin receptor TrkB, most TrkB was immuno-localized to the internal vesicles of MVBs (88%), with only 12% in the limiting membrane (Valdez *et al.*, 2005). In similar experiments, the constitutive MVB molecule and membrane trafficking chaperone, pinchin, on the other hand, was localized primarily to the limiting membrane (77%) and only 23% to internal vesicles (Valdez *et al.*, 2005). As with all over-expression studies, the caveat is that normal pathways may have been overloaded with the additional protein, thus prompting an abnormal load into a non-physiological 'overflow' (Birks *et al.*, 1972). Nevertheless, these studies suggest that sorting of cargo within MVBs is not only stage- but also location-dependent (dendrite vs. soma).

4.3. Exogenous cargo molecules—Exogenous molecules are typically internalized by non-receptor mediated mechanisms: pinocytotic (bulk) uptake/transport. This category includes tracers such as ferritin, thorium dioxide, HRP and lectins (Berthold *et al.*, 1993; Birks *et al.*, 1972; Broadwell and Balin, 1985; Bunge, 1977; Chu-Wang and Oppenheim, 1980; Feher, 1984; Grafstein and Forman, 1980; Haigler *et al.*, 1979; Holtzman and Peterson, 1969; LaVail and LaVail, 1974; Richardson *et al.*, 1997; Rosenbluth and Wissig, 1964; and Weldon, 1975), toxins (Parton *et al.*, 1987; Rind *et al.*, 2005; Schwab and Thoenen, 1977; Schwab and Thoenen, 1976), aminoglycoside antibiotics (de Groot *et al.*, 1990), and viruses (Ashok and Hegde, 2006; Morita and Sundquist, 2004; Uchil and Mothes, 2005). One major purpose for internalization of exogenous cargo into axonal MVBs may be to "detoxify" and protect the CNS (Gatzinsky, 1996). Although exogenous cargo such as tracers (gold, lectin, HRP) is typically found in axonal MVBs, several studies have suggested that a substantial fraction of the MVBs in axons may have formed in response to the endocytosed toxin rather than being recruited to transport the external cargo from the periphery to the CNS (Altick *et al.*, 2009; Birks *et al.*, 1972).

5. Genesis of MVBs

This section provides a brief summary of the steps involved in MVB biogenesis, especially ESCRT functions, as known from studies in non-neuronal cells

The biogenesis of neuronal MVBs was studied in considerable morphological detail in the 1960s and 1970s. These early studies applied electron dense markers to cultured neurons and examined how they were taken up and processed by growth cones and neurites. Markers included ferritin, horseradish peroxidase, and thorium dioxide (Birks *et al.*, 1972; Bunge, 1977; Holtzman and Petersen, 1969; Richardson, *et al.*, 1997; Rosenbluth and Wissig, 1964; Weldon, 1975; Wessells *et al.*, 1974). These studies suggested the following sequence of MVB dynamics: MVBs are formed from pinocytotic vesicles and vacuoles through intermediate structures that are called cup-shaped bodies or sacs ('C'-bodies) (Theodosius, 1982; Wessells *et al.*, 1974), sometimes also called pre-multivesicular bodies (Birks *et al.*, 1972; Weldon, 1975). Coated vesicles and vacuoles of 50–500 nm in diameter are derived from macropinocytosis (Hirsch *et al.*, 1968). Apparently through fluid loss, the vacuoles collapse rapidly (within 3–15 minutes, Weldon, 1975) into cup-shaped bodies. The cup-shaped bodies form internal vesicles to become MVBs. Plasmalemma appears to provide the source for the MVB's limiting membrane (Birks *et al.*, 1972; Bunge, 1977). Based on quantification of organelle populations after tracking uptake and processing of exogenous ferritin, the MVB population turns over within 2 hours (Birks *et al.*, 1972). It needs to be noted that the neurite culture paradigm examines regenerating neurites rather than neurites that advance their very first growth cone, as normally in vivo (Bunge, 1977). This model of MVB biogenesis in cultured neurites was not further explored at the molecular level; therefore, it is not known how pre-MVBs (cup-shaped bodies) relate to current models of

MVB biogenesis (primarily studied in yeast and other non-neuronal model systems) (Gruenberg and Stenmark, 2004; Maxfield and McGaw, 2004; Piper and Katzmann, 2007).

The defining event in MVB biogenesis is the formation of internal vesicles within an endosome (Fig. 4). Internal vesicle formation requires several steps: targeting of endocytosed proteins to specific locations in the membrane of the endosome; sequestering, via tubular extensions, of cargo destined for recycling from other cargo that is targeted for degradation; recruitment of specific Rabs (Vonderheit and Helenius, 2005; Zerial and McBride, 2001); and activity of AAA-type ATPases and phosphatidylinositol-3-phosphate. Both molecules are necessary for invagination of endosomal limiting membrane to form internal vesicles (Babst *et al.*, 1998; Fernandez-Borja *et al.*, 1999). The formation of internal vesicles in an endosome, essentially the genesis of an MVB, is accomplished within 4–30 minutes after endocytosis of markers at the plasma membrane (Mayor *et al.*, 1993; Piper and Luzio, 2001).

Recent reviews provide a detailed account of the machinery that orchestrates MVB formation, including the role and composition of ESCRT complexes, phosphatidylinositols, and Rabs (Babst, 2005; Falguieres *et al.*, 2008; Hurley, 2008; Vonderheit and Helenius, 2005; Williams and Urbe, 2007). Our knowledge about the mechanisms of biogenesis of MVBs is based on evidence from non-neuronal cell types including reticulocytes, endothelial cells, immortalized cell lines, and cells of the immune system (Gruenberg and Stenmark, 2004; Piper and Katzmann, 2007; Russell *et al.*, 2006; Woodman and Futter, 2008). These mechanisms, described below, have not been confirmed in neurons. However, all the molecular and morphological components identified and described in other cells are present in neurons, thus supporting the notion that MVB biogenesis is conserved in different types of cells.

Several lines of work have identified factors that are pertinent for internal vesicle formation: asymmetry in the lipid bilayer; pH gradient between the lumen of the endosome and the cytosol; molecular interactions via ESCRT-III proteins; and specialized lipid composition, such as LBPA, the molecular structure of which can bend membranes (Lawrence *et al.*, 2010; Matsuo *et al.*, 2004; Piper and Luzio, 2001; Theos *et al.*, 2006). Wollert and Hurley (2010) showed distinct roles of each of the ESCRT complexes during MVB formation in a cell-free system. According to their model, ESCRT-0 clusters the cargo, i.e. is involved in sorting, ESCRTs I and II form the membrane neck; then ESCRT-III is recruited to the neck. Three ESCRT-III subunits are necessary for complete scission of the internal vesicle from the MVB limiting membrane (Wollert and Hurley, 2010). In intact cells, it is thought that ESCRT complexes become sequentially associated with endosomes, and cargo is tread-milled along the endosomal membrane until final incorporation into an internal vesicle (Babst, 2005; Vajjhala *et al.*, 2007). A different scenario proposes that ESCRT complexes are arranged concentrically around cargo on the endosomal membrane, thereby effectively sequestering cargo from the cytosol during vesicle formation (Nickerson *et al.*, 2007). The recent work by Wollert and Hurley suggests a third scenario in which cargo is confined by ESCRT-0, then ESCRT-I and II bend the membrane and form the buds at the site of the ESCRT-0-cargo complex. Thereafter, ESCRT-III cleaves the buds to form the internal vesicle containing the cargo, excluding all ESCRT subunits. Several molecules, such as ALIX, Tsg101, and annexins that participate in MVB biogenesis and function are known to interact with calcium-binding proteins. Annexins in particular participate in several different steps of MVB formation and function (Futter and White, 2007).

Regardless of the precise assembly of ESCRTs on an endosome, when ESCRT proteins are lacking or dysfunctional, MVB formation and function are impaired (Babst, 2005; Hurley, 2008; Piper and Katzmann, 2007; Rusten and Simonsen, 2008; Tamai *et al.*, 2008). One

study, using yeast, HEp2, and HeLa cells, found that recognizable MVBs can still form in the absence of individual key subunits of all four ESCRTs (Stuffers *et al.*, 2009). However, trafficking of the EGF receptor is impaired and the morphology of the MVBs is severely altered, possibly compromising proper function of the endosomal-lysosomal system. Cell-free systems designed to recapitulate MVB formation indicate that formation of internal vesicles, the determining feature of MVBs, is dependent upon Hrs and STAM, components of ESCRT-0; and Vps4, an ATP-binding protein (Sun *et al.*, 2010). The plasticity of MVB formation reflects the complexity of the process, and while there seems to be variability in MVB morphogenesis, the relationship of this variability in form to proficient function in physiologically relevant systems is not yet fully understood (Traub, 2010).

During and subsequent to internal vesicle formation and maturation, MVBs vary in size, shape, number of vesicles, electron density of lumen and internal vesicles, subcellular location and protein content (Altick *et al.*, 2009; Clague and Urbe, 2008; Kobayashi *et al.*, 2002; van Meel and Klumperman, 2008; White *et al.*, 2006). It has been observed that with time, MVBs enlarge, increase the number of internal vesicles, become more electron-dense, and localize increasingly within the peri-nuclear region of cells. This series of changes has been called “maturation” (Ohashi *et al.*, 2000; Stoorvogel *et al.*, 1991), which sometimes has been taken to imply that the MVB no longer contains recycled proteins and is not competent to receive additional endocytosed cargo (Woodman and Futter, 2008). Maturation is completed some time after cargo endocytosis, estimated at 8–10 minutes *in vitro* after endocytosis (Mayor *et al.*, 1993). Rab conversion, the replacement of Rab5 with Rab7, is a sign of progression from an “endosomal MVB” to a mature MVB (Deinhardt *et al.*, 2006; Ohashi *et al.*, 2000; Rink *et al.*, 2005; Stoorvogel *et al.*, 1991). This progression is also accompanied by a change in pH. The recycling endosomes have been reported to have a pH of 6.4–6.5, sorting endosomes have been measured at pH 5.9–6.0, and the pH of mature MVBs has been reported to be 5.0–6.0 (Maxfield and McGraw, 2004; Woodman and Futter, 2008). The acidic pH facilitates the dissociation of receptor/ligand pairs as well as initiates protein degradation, which is completed in the lysosome. It is possible that as the luminal matrix of a maturing endosome becomes progressively more acidic, the internal vesicles may form to maintain a protected physiological environment. Until recently, it was thought that any protein sequestered into the lumen of an internal vesicle was destined for degradation (Fader and Colombo, 2009). Some recent findings in neurons and glia suggest that internal vesicles of MVBs may also be released as exosomes (Bulloj *et al.*, 2010; Fevrier and Raposo, 2004; Lachenal *et al.*, 2011; Putz *et al.*, 2008; van Niel *et al.*, 2006) (see section 6.2). In conclusion, genesis of MVBs and particularly the characteristic formation of the MVB’s internal vesicles are orchestrated through complex actions of constitutive molecules, not unlike those that form synaptic vesicles and regulate their fusion for transmitter release (Smith *et al.*, 2008). In fact, it may be speculated that the similar size of synaptic vesicles and MVBs’ internal vesicles point to an optimal ratio of membrane circumference and luminal volume that is common between the processes of exo- and endocytosis.

6. Recycling and release of internal vesicles

This section examines how three pathways may contribute to the processing of cargo within neuronal MVBs

The proteins contained within internal vesicles of MVBs can have one of three fates: extracellular release of the proteins in intact internal vesicles by fusion of the limiting membrane of MVBs to the plasma membrane (exosome release), back-fusion to recycle proteins to the plasma membrane, or degradation in lysosomes (Fig. 4). Early in MVB research it was hypothesized that the internal vesicles may be either released into the cytoplasm, or may be destined for neurosecretion (Palay, 1960). Subsequently, the role of

MVBs in the degradation pathway was discovered, but in the 1990s and early 2000s, recycling to the plasma membrane via the so-called “back-fusion” pathway to the plasma surface membrane moved to the forefront (Murk *et al.*, 2002), only to be followed in most recent years by an emphasis on release as exosomes (for expulsion and/or intercellular communication) (Faure *et al.*, 2006; Lachenal *et al.*, 2011; Mathiavanan *et al.*, 2010; Putz *et al.*, 2008; Smalheiser, 2007; Vingtdeux *et al.*, 2007). In this section, we will discuss both the back-fusion pathway and the exosome release pathway, and examine currently existing evidence for these two pathways of MVBs in neurons.

6.1 Back-fusion/recycling pathway—This pathway is in many ways a reversal of the biogenesis of MVBs (Denzler *et al.*, 2000; Fevrier and Raposo, 2004; Murk *et al.*, 2002; Uchil and Mothes, 2005). It requires a dynamic remodeling of MVBs (Fig. 5). The typical spherical MVBs that contain multiple internal vesicles morph into elongated tubules that are largely depleted of internal vesicles (Murk *et al.*, 2002). According to current models, the limiting membrane expands by the insertion of membrane from the internal vesicles. Such back-fusion allows internally stored proteins to become incorporated into the limiting membrane, from where they can be transported to the surface plasma membrane (Kleijmeer *et al.*, 2001). The final movement from the tubules to the plasma membrane is believed to occur through small transport vesicles. A membrane shuttle mechanism may explain the insertion of proteins from temporary storage in MVBs into the plasma membrane upon stimulation. This mechanism is well established for antigen – e.g., major histocompatibility complex (MHC) class II – presenting cells of the immune system (Murk *et al.*, 2002). Whether other cells such as neurons can use MVBs as temporary protein and membrane storage compartments is not known but is an attractive hypothesis. A storage function of neuronal MVBs is particularly intriguing, given the frequent localization of MVBs at postsynaptic sites (Chicurel *et al.*, 1992; Cooney *et al.*, 2002; Ostroff *et al.*, 2010; Pappas and Purpura, 1961; Paula-Barbosa *et al.*, 1978; Rind *et al.*, 2005; Schnapp, 1997; von Bartheld *et al.*, 1996), the loading of postsynaptic MVBs with neuromodulatory agents (e.g. neurotrophic factors, Rind *et al.*, 2005; von Bartheld *et al.*, 1996), and the evidence that MVBs with tubular extensions exist in neurons and serve distinct regions of synaptic sites within hippocampal (Chicurel *et al.*, 1992; Cooney *et al.*, 2002) and amygdalar dendrites (Ostroff *et al.*, 2010). Three-dimensional reconstruction of ultrathin serial sections provided evidence for tubular extensions of neuronal MVBs and was suggestive for a vesicular shuttle to the plasma membrane (Cooney *et al.*, 2002). Furthermore, the trophic factors BDNF and GDNF reside primarily in the limiting membrane of MVBs in dendrites (Rind *et al.*, 2005, see also Fig. 3), consistent with a back-fusion recycling mechanism. This mechanism could be used to insert receptors into specific sites that are often distant from the cell body, such as axodendritic synapses (Bloch *et al.*, 2003; Cooney *et al.*, 2002). Thus, several lines of circumstantial evidence support back-fusion as a mechanism for protein recycling in neuronal MVBs.

6.2 Exosome release—The concept of exosome release as an alternative or addition to back-fusion has gained considerable attention in the past decade. This mechanism involves fusion of the MVBs outer limiting membrane with the plasma membrane and release of internal vesicles as exosomes (Denzler *et al.*, 2000; Fevrier and Raposo, 2004; Harding *et al.*, 1983; Murk *et al.*, 2002). In this case, proteins associated with internal vesicles are not incorporated (at least not directly) into the plasma membrane, but rather become expelled into the extracellular space. After release, exosomal vesicles may come in contact with the same releasing cell or with other cells. These exosomal vesicles may continue to bind ligands on the outside, with the extracellular domain of receptors facing the outside and the cytoplasmic domain on the inside of the internal vesicle (the vesicular content represents cytoplasmic compartment, see Fig. 4). The continued binding of the ligand to the

transmembrane receptor depends on binding affinities and the milieu of the extracellular environment. Exosome release is an established mode of vesicle release and an important form of intercellular communication for hematopoietic cells of the immune system (Denzer *et al.*, 2000; Fevrier and Raposo, 2004; Murk *et al.*, 2002). It is currently not known whether this mode of release occurs for MVBs in neurons *in vivo*, but a number of recent papers have speculated on the potential significance of exosome release as a mechanism for removal of proteins from neurons as well as for communication between neurons and between neurons and other cells (Bulloj *et al.*, 2010; Faure *et al.*, 2006; Fevrier *et al.*, 2004; Lachenal *et al.*, 2011; Putz *et al.*, 2008; Smalheiser, 2007).

Current evidence for exosome release relies on the isolation of organelles and detection of proteins (known to reside in exosomes) in media of cultured neurons. After isolation, exosomal fractions are often used for immunolabeling followed by electron microscopic analysis (Bulloj *et al.*, 2010; Faure *et al.*, 2006; Fevrier *et al.*, 2004; Lachenal *et al.*, 2011; Putz *et al.*, 2008; Vingetdeux *et al.*, 2007). A potential problem is that some of these exosomal markers are not localized exclusively to exosomes. For example, the heatshock protein Hsp70, is also known to accumulate in synaptosomes (Joshi *et al.*, 2006), and flotillin-1 distributes equally to the pre- and postsynaptic side of synapses (Swanwick *et al.*, 2010). Therefore, it is difficult to unequivocally demonstrate that such organelles are indeed exosomes derived from neuronal MVBs. The use of tetanus toxin C fragment (Lachenal *et al.*, 2011) as an “MVB marker” may also be potentially confounding due to its preferred presynaptic localization (about 10-fold compared to MVBs, Rind *et al.*, 2005). Nevertheless, the notion of exosome release is supported by the size of the vesicles, and the findings that they are immunopositive for proteins known to traffic through MVBs (Mathivanan *et al.*, 2010). Snapshot images of putative exosomes during release (“caught in the act”) are rare (Weldon, 1975; Lachenal *et al.*, 2011), and it is difficult to conclusively identify a partially fused MVB. The recent studies cited above provide evidence consistent with the notion that exosomes are released by neurons *in vitro*, however whether such release would also happen *in vivo* awaits further scrutiny. Direct evidence for release by neurons *in vivo* is preferable to *in vitro* protein correlates (Thery *et al.*, 2002); a truly specific MVB marker would help to resolve such uncertainties. In conclusion, more and preferably direct evidence of neuronal exosome release is desirable – a challenge due to current technological limitations.

7. Neuron-specific transport functions of MVBs

This section reviews possible intraneuronal transport pathways of MVBs and the empirical evidence for which ones are actually utilized

From a morphological standpoint, neurons are specialized cell types with unique geometrical cell shapes unlike any other cell type: they have extremely long processes (axons and dendrites) relative to the size of the cell body, and therefore have extended transport needs, as compared with cells that are geometrically more “simple” (Nixon, 2005). Transport of organelles, membrane proteins and signaling molecules in neurons therefore may require novel strategies or employ modified and expanded transport functions of mechanisms utilized by simple cells. In this context, it is intriguing that neurons, but no other cell type, express an MVB-specific kinesin (KIFC2) that exclusively distributes within the soma and dendrite, but not the axon, and that moves a distinct type of MVBs apparently unrelated to the endosome-to-lysosome pathway (Saito *et al.*, 1997). Furthermore, MVBs in dendrites have been shown to contain molecules that were originally obtained by retrograde axonal transport (Rind *et al.*, 2005). Taken together, this suggests that a novel type of neuron-specific MVBs contributes to transport needs of neurons at a wider and largely unexplored scale when compared to those in simple cells.

How are MVBs involved in trafficking needs of neurons? Theoretically, MVBs may participate in several distinct trafficking pathways as illustrated in Fig. 6. These pathways may be distinguished as (1) intrasomal (somato-somatic); (2) axo-somatic and axo-dendritic; (3) soma-to-dendrite and interdendritic; and (4) soma-to-axon. We will consider the empirical evidence, if available, for each of these categories of MVB trafficking.

(1) Intrasomal and dendro-somal—MVBs may form at the plasma membrane in cell bodies or dendrites and move membranes and internalized proteins from peripheral to central sites within the soma. This is the “classical” endosome-to-lysosome pathway as seen in non-neuronal cells (Katzmann *et al.*, 2002; Maxfield and McGraw, 2004; Mukherjee *et al.*, 1997; Sorkin and von Zastrow, 2002). This pathway is also compatible with the initial delivery of internalized material to the soma from more distant sources (axon or dendrite) via vesicles or endosomes (not necessarily via MVBs), and subsequent transfer to MVBs in the soma (Altick *et al.*, 2009; Rind *et al.*, 2005). There is abundant evidence for apparently “normal” (conventional) MVB composition (endocytotic markers and cargo) in neuronal cell bodies, as summarized in Table 3.

(2) Axo-somatic—This category encompasses all MVB-mediated retrograde axonal transport that originates from either nerve terminals or from the axon shaft, and delivers the axonal MVB to either the soma (axo-somatic), or beyond the soma to proximal or distal dendrites (axo-dendritic). To be considered for this category, the MVB itself must be transported within the axon, not any endosomal (non-MVB) precursors. Evidence for such a MVB transport pathway requires physical evidence that the MVB is transported in the axon, not just components that later accumulate in somal or dendritic MVBs. Such evidence for retrograde axonal transport of tracer-labeled MVBs in vitro was provided by time-lapse video followed by ultrastructural analysis of the transported organelle (Parton *et al.*, 1992). Early studies had indicated that endogenous, receptor-binding proteins such as growth factors tend to reside during axonal transport in small vesicles or tubules rather than MVBs (reviewed in Schwab, 1977; Schwab *et al.*, 1979; Schwab and Thoenen, 1978), while exogenous, bulk-transported proteins and compounds conjugated to tracer molecules reside in MVBs during axonal transport (Breuer *et al.*, 1975; Hirokawa *et al.*, 1990; LaVail and LaVail, 1974; Parton *et al.*, 1992; Sandow *et al.*, 2000; Tsukita and Ishikawa, 1980; Turner and Harris, 1974; Weible and Hendry, 2004). Recent work (Altick *et al.*, 2009) confirms this earlier notion of a more restricted role of MVBs for retrograde axonal transport and points to a small vesicular transport endosome, consistent with studies characterizing NGF- and TrkA-containing endosomes (Cui *et al.*, 2007; Delcroix *et al.*, 2003; Grimes *et al.*, 1997). Retrograde transport of cannabinoids in MVBs may be an exception to this general rule (Vitalis *et al.*, 2008). Thus, although MVBs can move retrogradely along axons, only a select type of cargo appears to utilize them as retrograde carriers.

(3) Soma-to-dendrite or interdendritic—Endocytosed material (delivered from the soma plasma membrane and/or from other dendrites or axons) accumulates in MVBs, which transport the cargo to dendrites. This category also includes the special case of MVB transport between adjacent dendrites or between adjacent spines within the same dendrite. The most convincing (albeit indirect) evidence for such a transport function of MVBs is provided by the work of Cooney *et al.*, 2002 who showed that collections of adjacent spines are supplied by MVBs – likely involved in recycling of signaling molecules and synaptic plasticity. Taken together with evidence of a neuron-specific type of MVBs in the soma/dendrite (Saito *et al.*, 1997) and recent studies showing that postsynaptic MVBs can contain trophic factors derived from the axonal target (Rind *et al.*, 2005), these data are highly suggestive for a distinct MVB type specialized for somatic and dendritic transport and storage. This is consistent with the surprising lack of endocytotic markers in this MVB type

(Saito *et al.*, 1997), apparently differing from other MVB types in the soma. The “dendritic” MVB type appears to lack proteins that are typically found in MVBs in the soma of non-neuronal cells, including Rab5, dynamin, Lamp2, and Trk as well as proteins typically found in organelles of the endosome-to-lysosome pathway, such as transferrin receptor and Lamp1 (Saito *et al.*, 1997). Thus, neurons appear to utilize a novel type of MVB for dendritic functions that differ considerably from the conventional somal MVB type.

(4) Soma-to-axon (anterograde axonal)—There is currently no evidence that MVBs are transported anterogradely within axons, based on HRP transport experiments or ligation and cooling experiments of peripheral nerves, since there was virtually no HRP labeling of MVBs indicative of anterograde MVB movement (LaVail and LaVail, 1974), and no accumulation of MVBs proximal to axonal ligatures or cooling sites (Hirokawa *et al.*, 1990; Smith, 1980; Tsukita and Ishikawa, 1980).

8. Multiple functions of neuronal MVBs

This section argues that MVBs in neurons may have expanded, neuron-specific functions beyond those seen in non-neuronal cells

It is obvious from the above accounts that neuronal MVBs are involved in numerous fundamental cell biological processes, and therefore are likely to have multiple functions, based on cellular and subcellular context. The primary role of MVBs is traditionally placed in the endocytic pathway, where MVBs are heavily involved in sorting and adequate routing of endocytosed cargo. Since endosomal and membrane trafficking are closely intertwined with cellular signaling (Scita and Di Fiore, 2010; Sorkin and von Zastrow, 2002, 2009; Zwang and Yarden, 2009), it is not surprising that MVBs, and in particular neuronal MVBs, appear to have important functions in intracellular – and, possibly, in intercellular – signaling. Such functions appear to involve sorting/recycling; regulation of receptor surface expression, therefore implications for synaptic plasticity; intra- and intercellular communication, via intercompartmental transport and exosome release; neurosecretion, via degradation and/or processing of neurosecretory materials; signal termination; and clearance of toxins and unnecessary proteins, via degradation or export to the extracellular space or neighboring cells. In this section, we will summarize these multiple functions of neuronal MVBs and examine the empirical evidence.

Sorting/recycling—MVBs are part of the endocytic pathway. In the endo-lysosomal pathway receptors and ligands internalized from the plasma membrane are sorted and then targeted into particular pathways. After sorting in the MVB, cargo can be routed back to the plasma membrane, moved to internal vesicles for exosome release, or retained within the membrane of internal vesicles for eventual lysosomal degradation (Grant and Donaldson, 2009; Gruenberg and Stenmark, 2004; Piper and Katzmann, 2007; Raiborg *et al.*, 2003; Russell *et al.*, 2006). Thus, MVBs sort between “garbage” and proteins that can be recycled – thus saving energy for replacement and transport. Clutter is removed and housekeeping functions prevent accumulation of dementia-inducing protein aggregates, as summarized in a recent commentary in the *Journal of Cell Biology*, suitably entitled “clean-up or go crazy” (Williams, 2007). MVBs keep the cellular machinery functional, reduce inventory, and help with “just-in-time” delivery of cargo where it is needed. As shown in Table 3, numerous proteins have been localized in neuronal MVBs, but only few studies have examined sorting/recycling in neurons (Butowt and von Bartheld, 2009; Cooney *et al.*, 2002; Grimes and Miettinen, 2003; Rind *et al.*, 2005; Valdez *et al.*, 2005). Nevertheless, this fundamental MVB function is believed to be applicable to neurons as it is to other cell types, as mentioned above.

Regulation of receptor surface expression—After signal termination, receptors and ligands are recycled to the plasma membrane, degraded, or possibly released via exosomes. Degradation conclusively terminates signaling and alters a cell's molecular make-up as compared to recycling, which returns the receptors to the plasma membrane, re-setting the signaling competence of the cell. In cultured hippocampal cells, endocytosed receptors, e.g., TrkA vs. EGF receptor, follow different routes within MVBs, distinguished by the co-localization with specific Rab proteins (Valdez *et al.*, 2007). Dendritic MVBs accumulate and recycle internalized markers from neighboring spines, thus serving as a local store for endocytosed material (Cooney *et al.*, 2002). In neurons, protein degradation and recycling play important roles in synaptic potentiation, depression, and circuit maintenance.

Intra- and intercellular communication—Neuron morphology places extreme demands on an efficient transportation system between neuronal compartments. Communication between axon terminals and the cell body, between dendrites and the cell body, and between axons and dendrites is necessary for cell homeostasis as well as cellular plasticity related to circuitry development, maintenance and synaptic plasticity. MVBs are a major retrograde transport organelle, although many cargo types still remain to be defined. It is known that MVBs in dendrites contain proteins that were originally endocytosed in axon terminals (Rind *et al.*, 2005; von Bartheld *et al.*, 1996), suggesting that MVBs not only sort material, but also participate in transport of endocytosed material between neuronal compartments. Exosome release, an alternative to degradation and recycling, has potential significance for intercellular communication as well as a possible function for exporting unwanted proteins and toxins from the cell (Thery *et al.*, 2002) and has been documented in cultured neurons (Bullock *et al.*, 2010; Faure *et al.*, 2006; Fevrier *et al.*, 2004; Lachenal *et al.*, 2011; Putz *et al.*, 2008; Vingtdeux *et al.*, 2007), see section 6.2 above for full discussion.

Neurosecretion—In neurosecretory cells, the number of MVBs increases with neurosecretory activity and then decreases during the recovery period of secretion (Borg and Bell, 1977; LaVail, 1976; Morris and Dyball, 1974; Morris and Steel, 1975, 1977). Some MVBs appear to contain neurosecretory granules and/or aggregates (Morris and Dyball, 1974; Morris and Steel 1975, 1977), suggesting that MVBs process or sort secretory molecules originating in the Golgi and cytoplasm.

Signal termination—After endocytosis at the plasma membrane and cellular signaling, incorporation of signaling molecules into internal vesicles of an MVB is believed to terminate signaling (Mukherjee *et al.*, 1997; Sorkin and von Zastrow, 2002, 2009). Signal termination could be accomplished in two different ways: 1) release of ligand from its receptor after the receptor has been endocytosed at the plasma membrane and has been incorporated into the outer membrane of the MVB. This process reverses the orientation of the receptor/ligand pair (Fig. 4), with the ligand now facing the luminal side of the MVB's outer membrane and thus being exposed to a lower pH (5.9–6.0), which inhibits ligand-receptor interactions. 2) sequestration of the entire receptor/ligand complex into internal vesicles of MVBs. In this case, the ligand faces the luminal matrix of the MVB, and the cytoplasmic domain of the receptor extends into the lumen of the internal vesicle (Fig. 4), where signaling is blocked from the cytoplasm. Formation of MVBs in non-neuronal cells has been observed within minutes of ligand/receptor endocytosis (Maxfield and McGraw, 2004). The rapid formation is possible because endocytosis and MVB biogenesis are independent of gene transcription (Zwang and Yarden, 2009). Therefore this mechanism of signal termination is fast and efficient.

Clearance of toxins and unnecessary proteins—Some neurons of the peripheral nervous system extend processes out of the central nervous system, thus beyond the blood-

brain barrier. It has been suggested that MVBs in axons, specifically MVBs at the node of Ranvier, function as a back-up barrier system. Based on evidence from HRP transport and acid phosphatase studies, it appears that MVBs in peripheral axons accumulate toxins and other exogenous substances and route them out of the neuron and into neighboring Schwann cells (Berthold and Mellstrom, 1982; Gatzinsky, 1996; Gatzinsky *et al.*, 1997).

MVBs in neurons have been shown to contain protein-aggregates that accumulate in Huntington's, Parkinson's and Alzheimer's disease as well as frontotemporal dementia (Alegre-Abarrategui *et al.*, 2009; Filimonenko *et al.*, 2007; Nixon, 2005; Takahashi *et al.*, 2002). In neurons, the dysfunction of either MVBs or autophagosomes can lead to neurodegeneration. Recent work has connected the autophagosome pathway with the endosomal-lysosomal pathway; specifically ESCRT subunits are necessary for both pathways. Mutations in ESCRT subunits result in accumulation of non-membrane bound protein aggregates (Filimonenko *et al.*, 2007). Similarly, loss of ESCRT-III subunit Msnf7-2 causes autophagosome accumulation and neuronal cell loss (Lee *et al.*, 2007). These observations, along with others from non-neuronal cells, have led to the hypothesis that the endosomal-lysosomal pathway and the autophagy pathway are interactive and interdependent most likely via their mutual use of ESCRT proteins (Fader and Colombo, 2009; Rusten and Simonsen, 2008). Thus, MVBs in neurons are integral to proper protein clearance and aggregation prevention, which is essential for neuron viability.

9. MVBs in glial cells

In this section, we review information about the role of MVBs in glial cells and their function in glia-neuron homeostasis

MVBs have been described in all major types of glial cells, including Schwann cells (Pannese *et al.*, 1988a; Pannese *et al.*, 1988b), oligodendrocytes (Ogawa *et al.*, 2004; Trapp *et al.*, 1989), astrocytes (Ng *et al.*, 2009), microglia (Poticchio *et al.*, 2005), Müller glia (LaVail and Margolis, 1987), and Bergmann glia (Baude *et al.*, 1994; Herndon, 1964; Lively and Brown, 2010). Tanycytes, an ependymal/glial cell type, also contain MVBs that are often connected to tubular structures – possibly reflecting the extensive endocytotic and transcytotic trafficking in this cell type (Rodriguez *et al.*, 2005). MVBs in glial cells contain proteolipid protein, a myelin membrane component (Trajkovic *et al.*, 2006); myelin-associated glycoprotein (Trapp *et al.*, 1989); cathepsin D, a lysosomal enzyme (Trapp *et al.*, 1989); and SCI/Hevin, an extracellular matrix protein (Lively and Brown, 2010). Some of these proteins are not destined for degradation, but rather are being used for neuropil construction and maintenance. There is accumulating evidence that oligodendrocytes, astrocytes, and microglia release exosomes, and that these exosomes are internal luminal vesicles derived from glial MVBs (Faure *et al.*, 2006; Hsu *et al.*, 2010; Poticchio *et al.*, 2005; Tomboli *et al.*, 2010; Trajkovic *et al.*, 2008; Tytell, 2005).

Glial MVBs have been implicated in specific cellular functions related to neurons. It has been proposed that glial MVBs are involved in the transport of material from axons to Schwann cells (Fabricius *et al.*, 1993; Gatzinsky, 1996; Gatzinsky *et al.*, 1997; Pannese *et al.*, 1988a). This idea is supported by the findings that the tracer HRP and fluorescent nanospheres injected into hind limb muscle localize to MVBs at the nodes of Ranvier and in the axon-Schwann cell network (Berthold and Mellstrom, 1982; Gatzinsky *et al.*, 1997). Consistent with this hypothesis, we have observed that quantum dots, endocytosed at the axon terminal, can reach neighboring glial cells (A.L. Altick, L.M. Wiggins, C.S. von Bartheld, unpublished). Likewise, it has been shown that axonal transport along myelinated nerve fibers in the CNS, including transport of MVBs, is dependent upon oligodendrocytes (Edgar *et al.*, 2004). Signals originating in the neuron result in myelin membrane exocytosis from glial cells (Trajkovic *et al.*, 2006). In related work, it has been shown that LDLs use a

novel transport pathway to cross the blood-brain-barrier. The pathway involves MVBs in the epithelial cells of the blood vessels in the brain. Activated astrocytes regulate the uptake and transcytosis of LDL from the blood. In this case, the cargo, LDL, accumulates within the luminal matrix, but outside the internal vesicles of glial MVBs (Candela *et al.*, 2008). Myelin-associated glycoprotein that accumulates in glial MVBs has been suggested as a candidate for transducing axonal influences onto oligodendrocytes (Trapp *et al.*, 1989), but also, in the reverse direction, in potentially transferring trophic signals from oligodendrocytes via exosomes to axons (Kramer-Albers *et al.*, 2007). Therefore, glial MVBs appear to assist in functional neuron-glial relationships and possibly inter-cellular trafficking.

Much information with regard to MVBs in glial cells has been gained from studies on brain diseases. MVBs are frequently mentioned in studies documenting changes in oligodendrocytes in animals with myelinating pathology (Lassmann *et al.*, 1997; Nakadate *et al.*, 2006), from models of Niemann-Pick disease (Suzuki *et al.*, 2003), and in astrocytes and oligodendrocytes of the lateral geniculate nucleus in aged rats (de la Roza *et al.*, 1985). MVBs are increased in number, enlarged, and have aberrant morphology in glia of prion-infected animals (Ersdal *et al.*, 2009). MVBs in oligodendrocytes are associated with cholesterol trafficking, and disruptions in this trafficking can lead to neurodegeneration (Liu *et al.*, 2010). Finally, following a kindling protocol simulating pre-epileptic activity, MVBs are enlarged in dendrites contacting activated astrocytes, and translocate to the neck of the dendritic spine (Kraev *et al.*, 2009). MVB's potential association with diverse pathologies, from demyelinating diseases to neurodegeneration, such as Alzheimer's and Niemann-Pick diseases (see section 11.3.2 – 11.3.3 below), suggests that MVBs are critical for both glial and neuronal cell homeostasis.

10. MVBs: repertoire of techniques

This section compiles information about the range of techniques used to examine neuronal MVBs and comments on advantages and disadvantages

As mentioned previously, MVBs can currently be identified with certainty only by electron-microscopic analysis. A specific marker for MVBs would be tremendously useful for light-microscopic studies, but is yet lacking. Here, we summarize the types of techniques that have been and are currently used to examine MVBs, and particularly neuronal MVBs.

Morphological techniques—MVBs can be identified in thin sections, with standard processing for electron microscopic analysis. This approach can be expanded to serially reconstruct individual MVBs (Cooney *et al.*, 2002; LaVail *et al.*, 1980). MVBs in thin sections can also be analyzed by quantitative techniques, e.g., to determine fractional areas or volumes within defined compartments such as cell bodies, dendrites and axons (for references, see Table 2), to compare changes in MVBs in different groups, such as experimental treatment or age (Boaro *et al.*, 1998; de la Roza *et al.*, 1985; Johnson *et al.*, 1975; Nakadate *et al.*, 2006; Piori *et al.*, 1991). Researchers have examined protein content of neuronal MVBs by using a variety of techniques (Table 3). Enzyme histochemistry (e.g. for acid phosphatase), immunocytochemistry with antibodies labeled by biotin-streptavidin-HRP, gold-conjugated antibodies, or quantum-dot conjugated proteins or antibodies are the most commonly used techniques to localize specific proteins to MVBs (Altick *et al.*, 2009; Chu-Wang and Oppenheim, 1980; Holtzman, 1971; Neutra *et al.*, 1985; Vu *et al.*, 2005). Such techniques have the advantage of providing high-resolution spatial information, e.g., whether internal vesicles within MVBs contain proteins of interest, or the limiting membranes of MVBs, or both (Mobius *et al.*, 2003). One can also ask whether MVBs accumulate certain (neuronal) tracers that can be visualized by various methods, e.g. HRP, lectins, utilizing natural electron-dense properties (e.g., quantum dots), or fluorescence that

can be photo-converted to electron dense properties (Fomina *et al.*, 2003; Maranto, 1982; von Bartheld *et al.*, 1990). The content of MVBs can be further explored by introducing radioactive isotopes such as ^{125}I into proteins and localizing them by autoradiography at the electron-microscopic level after coating thin sections with a monolayer of photographic emulsion to develop silver grains (von Bartheld, 2001). This technique allows for quantification of labeling densities between organelles that lend themselves for easy statistical analysis, and group analysis even allows one to enhance practical resolution to distinguish labeling of internal vesicles of MVBs versus label that is localized to the limiting membranes (Rind *et al.*, 2005). Techniques that visualize proteins within MVBs differ significantly in their resolution, sensitivity, and the degree of preservation of structural integrity.

Physiological and biochemical techniques—Movement of neuronal MVBs can be examined in vitro by fluorescent label at the light-microscopic level in time-lapse video microscopy, followed by morphological analysis of such identified organelles, including MVBs, at the ultrastructural level (Lively and Brown, 2010; Tsukita *et al.*, 1980). Endosomes, including MVBs, have been examined by biochemical means, i.e. purification followed by Western blots or mass spectrometry (McCaffrey *et al.*, 2009). Finally, structural components of MVBs can be examined by biochemical or molecular techniques to identify MVB components such as exosomes, although concerns about reliability and validation persist (Thery *et al.*, 2002; Traub, 2010).

Dynamic imaging techniques—Organelles can be labeled with fluid-phase markers (ovalbumin-gold and/or HRP) and tracked in vitro by time-lapse video, followed by ultrastructural analysis to identify the transported organelle (Parton *et al.*, 1992). Similarly, quantum dot-labeled NGF has been tracked in axons and the endosomes were subsequently identified by electron microscopy, taking advantage of the fact that quantum dots are both fluorescent and electron dense (Cui *et al.*, 2007). Caveats are that the markers used to track the organelle movement may affect the trafficking or sorting (Neutra *et al.*, 1985), and that with current techniques the organelle has to be identified retrospectively as an MVB by electron microscopy. Therefore, an authentic MVB marker would be exceptionally valuable for dynamic imaging studies of neuronal MVBs.

Genetic/molecular techniques—Constitutive MVB proteins can be mutated, deleted or altered by transfection or genetic manipulations, and the effects of such manipulations on MVB structure and function can be assessed. Most of these approaches have utilized simple model systems and in-vitro settings (e.g., yeast, tissue culture; Almeida *et al.*, 2006; Cataldo *et al.*, 2008; Liao *et al.*, 2007), but naturally occurring mutations that are of relevance for MVB function can also be studied in human populations (e.g., humans with neurodegenerative disease, Skibinski *et al.*, 2005; Urwin *et al.*, 2010a, b).

In summary, although a variety of morphological techniques are available for analysis of neuronal MVBs, one major handicap is the lack of a sensitive and specific molecular marker that would allow researchers to unambiguously identify MVBs, and possibly distinct types of MVBs in fluorescence and confocal light microscopy. Such a tool would without doubt accelerate the pace of MVB research and allow further insights into this multifaceted organelle.

11. Neuronal MVBs in development, diseases and aging

This section examines how MVBs have been implicated in aging and disease-related processes such as amyloid beta processing in Alzheimer's, huntingtin in Huntington's, and prion protein trafficking

MVBs are essential organelles for normal cell function, but they may also be significant for cells during vulnerable stages (development or aging) and for cells' reaction to adverse or challenging conditions. Therefore it is not surprising that MVBs are not only present, but may participate in adaptive responses aimed at maintaining homeostasis in developing as well as mature, diseased, and aging neurons.

11. 1. Development—MVBs have been described in growth cones of neurites in the PNS (Birks *et al.*, 1972; Bunge, 1977; Estable *et al.*, 1957; Weldon, 1975) and also the CNS (Povlishock, 1976), and in dendrites and cell bodies of developing (embryonic) neurons (Butowt and von Bartheld, 2001; Chu-Wang and Oppenheim, 1980; Rind *et al.*, 2005; Roizin *et al.*, 1967; Vitalis *et al.*, 2008). Mutations and/or knock-out of various components of ESCRT complexes, the machinery necessary for MVB biogenesis and function, have been studied during development in a range of multi-cellular animal models including *Drosophila*, *C. elegans*, mouse, and *Arabidopsis* (Michelet *et al.*, 2010). There are numerous and disparate developmental effects of mutations in ESCRTs, and thus MVBs, in each of these models, possibly reflecting multiple functions of MVBs in different cell types and animal models. In developing neurons of cortex and hippocampus, a specialized vesicle containing markers for recycling endosomes, but not early or late endosomes, has been implicated in growth cone formation and membrane trafficking necessary for neurite outgrowth (Alberts and Galli, 2003; Alberts *et al.*, 2006; Hernandez-Deviez *et al.*, 2007). An additional study identified a novel protein that interacts with Rab11 and is necessary for neuritogenesis and membrane trafficking in neurons isolated from embryonic mouse cerebral cortex (Shirane and Nakayama, 2006). Rab11 is located on recycling endosomes (Rink *et al.*, 2005; Vonderheit and Helenius, 2005) after cargo has been sorted by the MVB, thus this work suggests a role for MVBs in neuron morphogenesis and development. As a caveat, none of these Rab studies analyzed the neurons at the ultrastructural level, and therefore the identity of these vesicles as MVBs, recycling endosomes, or as another type of endosomes is unclear.

In hippocampal neurons, the number of MVBs per spine (MVB density) was higher in younger than in older rats (Cooney *et al.*, 2002). In the developing spinal cord, MVBs were found in myelin layers during ensheathment by oligodendrocytes, but MVBs were not present in myelin layers in the adult spinal cord (Trapp *et al.*, 1989), suggesting a function of glial MVBs during myelin formation. Developmental functions of MVBs have been examined with most scrutiny in *Drosophila* for notch signaling (Kramer, 2002; Wilkin *et al.*, 2008), and shub signaling for dendritic arborization (Sweeney *et al.*, 2006). Because few studies have specifically compared the number or distribution of MVBs between different developmental stages, it is not known whether MVB distribution and/or numbers differ significantly in neurons of the developing CNS/PNS as compared with the mature CNS/PNS, and whether MVBs have any specific or unique developmental roles. It is intriguing to speculate that MVBs may provide specialized signaling platforms for crucial morphogenetic events (Traub, 2010).

11.2. Dystrophic neurites—It has been known for over half a century that MVBs are particularly prominent in injured and regenerating axons (Estable *et al.*, 1957; Holtzman and Novikoff, 1965; Inoue, 1983; Kapeller and Mayor, 1969; Smith, 1980; Yagashita, 1979). The phenomenon of MVB accumulation in PNS axons after interruption of axonal transport

by mechanical ligature or cooling has already been discussed above (see section 3.5 and Edgar *et al.*, 2004; Heerssen *et al.*, 2004; Hirokawa *et al.*, 1990). Dystrophic axons in the PNS of diabetic patients show large numbers of MVBs (Schroer *et al.*, 1992). When CNS axons are deprived of their target, MVBs appear in large numbers in the target-deprived axon terminals as early as four days later and remain at elevated levels for at least 20 days after target ablation (Marty and Peschanski, 1994). In the CNS of genetically epilepsy-prone rat, neuronal somata, dendrites and axons display an abundance of MVBs (Roberts and Ribak, 1988) consistent with an *in vivo* study simulating epileptic activity (Kraev *et al.*, 2009). This is consistent with earlier studies showing increased MVBs after prolonged (1-5 min) electrical stimulation (Kadota and Kadota, 1982; Kadota *et al.*, 1994) or in *Drosophila* potassium channel mutants with dramatically increased motoneuron firing and transmitter release (Jia *et al.*, 1993). Likewise, MVBs increase in number when a *stoned* mutation is introduced in *Drosophila*, apparently due to defective synaptic vesicle recycling and increased demands on membrane trafficking (Fergestad *et al.*, 1999). Increase of MVB size and/or number thus appears to be a general adaptive response of neurons to injury or insult of axons (see, e.g., Birks *et al.*, 1972; Cavalli *et al.*, 2005; Yagashita, 1979). Our own work, which quantified MVBs in axons, supports this hypothesis by the finding that in normal, immediately fixed and processed axons, MVBs were rare, while in axons that were subjected to a period of hypoxia before fixing, MVBs were significantly increased (Altick *et al.*, 2009).

11.3. Neurodegeneration—Neuronal cell death is the hallmark of numerous diseases, including Parkinson's, Huntington's, Alzheimer's, Niemann-Pick disease, amyotrophic lateral sclerosis, and frontotemporal dementia. MVBs have been found to play a role in all of these pathologies. It is becoming increasingly clear that appropriate and efficient sorting and degradation of cellular proteins is vital to the health of neurons (Nixon *et al.*, 2008). Mutations or disruptions that affect the endosomal-lysosomal pathway, which relies on MVB function, result in aberrant endosomal sorting and possible neuron death (Bronfman *et al.*, 2007; Filimonenko *et al.*, 2007; Lee and Gao, 2008; Liu *et al.*, 2010; Parkinson *et al.*, 2006; Rusten and Simonsen, 2008; Schindowski *et al.*, 2008; Skibinski *et al.*, 2005). However, it has also been postulated that the aggregation of proteins in MVBs and/or late endosomes and lysosomes is actually neuroprotective, by sequestering the aberrant protein (Truant *et al.*, 2008), and cell death may occur via another mechanism that happens to be concurrent with aggregation. Neurons stressed by hypoxia or ischemia (Altick *et al.*, 2009), epileptic discharges (Kraev, *et al.*, 2009; Roberts and Ribak, 1988; Schulze-Bonhage *et al.*, 1995), or simply age (de la Roza *et al.*, 1985; Johnson *et al.*, 1975; Nakadate *et al.*, 2006; Schroer *et al.*, 1992) show an increase in size or number of MVBs. Thus, MVBs seem to be a general indicator of neuronal stress, as they are dynamic in both size and frequency. Due to their pivotal role in protein sorting, recycling and degradation, it is not surprising that many brain pathologies are associated with changes in MVBs.

11.3.1. Huntington's disease: Huntington's disease (HD) is a progressive neurodegenerative genetic disorder, with degeneration of neurons primarily in the corpus striatum. A role for MVBs in Huntington's disease was first discovered in 1997, when it was revealed that huntingtin, the protein that is mutated in Huntington's disease, accumulates in MVBs (Aronin *et al.*, 1999; Sapp *et al.*, 1997). Huntingtin has also been located to other membrane structures, including the plasma membrane, lipid rafts, endoplasmic reticulum, and late endosomes (Atwal and Truant, 2008; Kegel *et al.*, 2000; Valencia *et al.*, 2010). Over-expression of huntingtin in clonal striatal cells of rats stimulates the endosomal-lysosomal pathway as seen by increased tubulation of endosomal membranes and increases in the number, size and morphological variability of autophagic vacuoles and lysosome-like bodies (Kegel *et al.*, 2000). Huntingtin-associated protein, HAP1, interacts with ESCRT-0,

and when over-expressed, causes enlarged endosomes and impaired trafficking of the EGF receptor through MVBs and lysosomes (Li *et al.*, 2002). Similarly, dysfunction of Rab11, which is necessary for trafficking through the recycling endosome, is a feature of HD transgenic mouse models. Without Rab11 activity, the glutamate/cysteine transporter EAAC1 fails to be recycled to the plasma membrane, resulting in oxidative stress and neurodegeneration (Li *et al.*, 2010). It seems that the pathology of HD is closely tied to the MVB function of recycling and sorting cellular proteins and maintaining efficient endosomal-lysosomal trafficking (Kegel *et al.*, 2000; Li *et al.*, 2010; Rusten *et al.*, 2008).

11.3.2. Alzheimer's disease: The hallmark of Alzheimer's disease is the degeneration of neurons in the cholinergic basal forebrain as well as in cortex. Although MVBs were seen in neurites of senile plaques in early studies (Kidd, 1964), an involvement of MVBs in Alzheimer's disease was first suggested in the 1970s with the finding that MVBs were much enlarged and their number increased in cortical neurons from the forebrain of Alzheimer's patients (Paula-Barbosa *et al.*, 1978). Subsequently, MVBs have been found to accumulate amyloid beta 42 (A β 42, Takahashi *et al.*, 2002), the peptide fragment that is the main constituent of the plaques characteristic of Alzheimer's disease. Intracellular A β 42 accumulates in the outer membrane of MVBs prior to synaptic dystrophy (Takahashi *et al.*, 2002; Takahashi *et al.*, 2004), suggesting that intracellular A β 42 accumulation may be an early and causative step prior to neuronal degeneration (Li *et al.*, 2007). The export (Bullock *et al.*, 2010; Rajendran *et al.*, 2006) or leakage of A β 42 from MVBs is now hypothesized to be one or maybe "the" neurotoxic event involved in the pathogenesis of Alzheimer's disease (Friedrich *et al.*, 2010; LaFerla *et al.*, 2007; Nixon, 2005; Nixon *et al.*, 2005; Offe *et al.*, 2006; Pastorino *et al.*, 2006; Perez *et al.*, 2005; Takahashi *et al.*, 2002; Vingtdoux *et al.*, 2007). Ultrastructural analysis of normal, aging, and transgenic mice shows the accumulation of amyloid-beta in MVBs of neurons (Langui *et al.*, 2004), specifically in pre- and post-synaptic compartments (Takahashi *et al.*, 2002), suggesting that MVBs are involved in the normal routing of amyloid-beta. In a cell culture model for Alzheimer's disease, amyloid-beta plaques first accumulate within MVBs, then penetrate the MVB membrane to eventually invade the cytoplasm, resulting in cell death and deposition of the aggregate extracellularly (Friedrich *et al.*, 2010). Accumulation of amyloid-beta in MVBs is known to impair protein sorting (Almeida *et al.*, 2006), and deregulated endocytosis initiated by a Rab5 mutation causes downstream perturbations in protein sorting and degradation (Cataldo *et al.*, 2008). The potential significance of MVBs in the development of Alzheimer's disease may be attributed to their role in appropriate sorting and degradation of amyloid-beta. However, it is not known whether the MVB first fails to process A β 42, or whether the rapid accumulation of amyloid-beta causes the failure of efficient sorting and degradation, and thus impairs the clearance of amyloid-beta aggregates, or if some other cellular dysfunction occurs simultaneously with amyloid-beta aggregation.

11.3.3. Frontotemporal Dementia: Frontotemporal dementia (FTD) describes a partially heritable, diverse, neurodegenerative disease that targets both the frontal and temporal lobes. Neuropathology of this disease shows an overall cortical and central atrophy, and a majority of cases have either tau or ubiquitin pathology (Urwin *et al.*, 2010b). The correlation of a disruption in ESCRT functions with neurodegeneration has recently linked numerous neuropathologies (Lee *et al.*, 2007; Lee and Gao, 2008; Rusten and Simonsen, 2008). ESCRTs are necessary for MVB biogenesis and function (see sections 5 and 8), and ESCRTs are necessary for proper autophagy (Filimonenko *et al.*, 2007; Lee *et al.*, 2007). MVBs are part of both the endosomal-lysosomal and autophagosomal systems (Fader and Colombo, 2009; Yi and Tang, 1999), thus functional MVBs are critical for neuronal viability. MVB function has been linked to FTD via a mutation in CHMP2B, a subunit of ESCRT-III (Skibinski *et al.*, 2005). Recent work shows that CHMP2B mutations cause abnormal MVBs and prevent fusion of MVBs with lysosomes in fibroblasts from FTD

patients (Urwin *et al.*, 2010a), thus suggesting a direct role of MVBs in FTD pathology. Additionally, several variations of CHMP2B mutations have been found in other familial pedigrees of FTD (van der Zee *et al.*, 2008). Studies on a human neuroblastoma cell line using mutant CHMP2B proteins (van der Zee *et al.*, 2008) showed similar aberrant endosomal structures as seen previously in fibroblasts from FTD patients (Urwin *et al.*, 2010a). Thus, this line of work suggests that FTD is likely the result of loss of proper MVB function.

11.3.4. Niemann-Pick Disease: Niemann-Pick disease is a fatal inherited metabolic disorder that affects the CNS as well as other organs. Symptoms reflect abnormal lipid storage in certain types of neurons, e.g. ataxia and dementia are seen when cerebellum and cortex are affected, respectively. Unlike Parkinson's, Huntington's, and Alzheimer's disease, proteins do not tend to aggregate, instead lipids accumulate in late endosomes or MVBs and lysosomes (Chiulli *et al.*, 2007; Liao *et al.*, 2007; Lusa *et al.*, 2001). Three genes are known to cause Niemann-Pick disease type C: NPC1 and NPC2 (Ko *et al.*, 2001; Lusa *et al.*, 2001) and sphingomyelin phosphodiesterase (SMPD1, Sikora *et al.*, 2003; Simonaro *et al.*, 2002). These three genes encode proteins that function in the processing of membrane components, namely cholesterol (Liao *et al.*, 2007; Liu *et al.*, 2010; Lusa *et al.*, 2001) and sphingomyelin (Chiulli *et al.*, 2007; Jones *et al.*, 2008). For NPC1 and NPC2 the build-up of cholesterol in late endosomes and lysosomes is thought to be due to a failure to recycle LDL-cholesterol to the plasma membrane (Lusa *et al.*, 2001; Pentchev *et al.*, 1984). For SMPD1, which resides in membranes of late endosomes (Jones *et al.*, 2008), the mechanism that leads to neurodegeneration has been suggested to involve a build-up of a sphingomyelin metabolite, which then activates astrocytes, creating a chain of events resulting in neurotoxicity (Chiulli *et al.*, 2007). In Niemann-Pick pathology, the sorting system seems to be functional, but the specific carriers and enzymes responsible for the processing of cargo and membrane components malfunction, and thus MVB function is compromised due to aberrant cargo delivery, membrane recycling, and protein processing (Devlin *et al.*, 2010).

11.4. Toxic or drug insults—Drugs can be neurotoxic, either when they are introduced intentionally as for chemotherapy, or inadvertently as through pesticides and occupational exposure. Ultrastructural studies on neuronal responses to drug insults commonly report a set of abnormalities that are “typical features of neurodegeneration”, which includes an increased number and/or size of MVBs (Lampert, 1967; Lieberman, 1971). Neurons contained an increased number of MVBs when they were exposed to toxic levels of aluminum (Deloncle *et al.*, 2001), acrylamide with axonal ligation (Cavanagh and Gysbers, 1980, 1981; Schaumburg *et al.*, 1974), to the pesticide dichlorodiphenyltrichloroethane (DDT) (Williams and Chung, 1987), or to hydrocarbon solvents (reviewed in Spencer *et al.*, 2002). In a study of antibiotic-treated cultured astrocytes, abnormal mitochondria and MVBs were noted. In support of the hypothesis that the antibiotics added to the culture medium were responsible for the structural defects, it was found that abnormalities increased with increasing doses of antibiotics, gentamicin more so than streptomycin (Robert and Hevor, 2007). Inhibitors specific for cathepsin B and L were found to selectively kill (via apoptosis) neuroblastoma cells, and apoptosis was preceded by an accumulation of MVBs in the cytoplasm (Colella *et al.*, 2010). When neurons are exposed to alcohol, MVBs increase in size or frequency, as documented for adult rat hippocampal cells after chronic alcohol consumption (Paula-Barbosa *et al.*, 1986), cultured mouse sensory neurons exposed to either ethanol or acetaldehyde (Smith and Wubetu, 1991), and Purkinje cells from fetal rats whose mothers were given alcohol (Koksal *et al.*, 2005). Ventral tegmental neurons of rats exposed to morphine, either acutely or chronically, showed reduced recycling and degradation of glutamate receptors, possibly due to decreased numbers of MVBs in postsynaptic dendrites (Lane *et al.*, 2008). Neuronal toxicity via drug or alcohol results in cellular adaptations,

which are typical of neuronal responses to stress, injury, or disease. This includes changes in the size or number of MVBs, perhaps reflecting a compensatory mechanism for altered demand on sorting, recycling, or degradation. It remains to be determined if MVBs are a direct target of the toxin, or if the alterations of the MVB pathway reflect adaptive responses of neurons.

11.5. Virus and prion transmission—Viruses use the endosomal-lysosomal system to both enter and exit a cell (Calistri *et al.*, 2009; Le Blanc *et al.*, 2005; McDonald and Martin-Serrano, 2009; Morita and Sundquist, 2004). Viruses can also use cellular organelles as sites for replication (Chu *et al.*, 2009; Dilley *et al.*, 2010; Joshi *et al.*, 2009; Novoa *et al.*, 2005). The cytopathic vacuoles (CPVs), “virus factories,” harbor some of the same molecular markers that are used to identify late endosomes and lysosomes, leading to the idea that CPVs are formed from endosomal or lysosomal organelles (Kujala *et al.*, 2001). In neurons, virus- and cell type-specific evidence indicates that viruses recruit the endocytosis machinery to gain entry into cells and then exploit ESCRTs, internal vesicle formation, and exosome release by MVBs. The alpha herpesvirus pseudorabies virus that is known to infect neurons has been located to “neurovesicles” in primary neuron cultures (Maresch *et al.*, 2010). CVS-11, an experimental rabies virus model, causes neurodegenerative changes with incorporation of MVBs into autophagosomes in dorsal root ganglion neurons (Rossiter *et al.*, 2009). A study on HIV-1 neuropathology has shown that the HIV-1 TAT protein binds to low-density lipoprotein receptor, the same receptor used by amyloid precursor protein, and thus uses the endosomal-lysosomal system to enter neurons via receptor-mediated endocytosis (Liu *et al.*, 2000). HIV-1 nef protein has since been shown to actively interact with CD4 on the surface of HeLa cells and target CD4 to MVBs for degradation, thus down-regulating CD4 (daSilva *et al.*, 2009). In rat primary hippocampal neurons, HIV-1 TAT protein promotes the secretion of A β 42 from the cell and the release of A β 42 aggregates from the cell surface (Aksenov *et al.*, 2010). HIV-1 exacerbates Alzheimer’s disease (AD) pathology in a mouse model for AD (Giunta *et al.*, 2009), and, as discussed above in section 11.3.2, AD pathology involves MVBs. Ultrastructural evidence links HIV-1 proteins with MVBs in a range of physiologically relevant cell types (but not neurons), and shows that MVBs can support virus assembly (Joshi *et al.*, 2009). Additionally, HIV-1 neuropathology may be indirectly linked to MVBs via amyloid-beta interactions, low-density lipoprotein receptor, or by reducing the CD4 surface expression by shunting CD4 to MVBs for eventual lysosomal degradation.

Prion diseases, such as scrapie and Creutzfeldt-Jakob disease, are incurable neurodegenerative diseases. The cellular prion protein (PrP^C) takes on a toxic “scrapie” conformation (PrP^{SC}), which eventually results in neuron death, causing large spongiform vacuoles in brain tissue. In brains from normal and infected animals, electron microscopy has shown PrP^C and PrP^{SC} located in MVBs as well as other cellular membranes and structures (Ersdal *et al.*, 2009; Laine *et al.*, 2001; Laszlo *et al.*, 1992). In the infected animals, the MVBs have been reported to be abnormal and increased in number (Ersdal *et al.*, 2009; Laszlo *et al.*, 1992). In studies that specifically track PrP^C in neurons, a variety of methods have been used to locate PrP^C and/or PrP^{SC}. Ultrastructural analysis of radiolabeled PrP^C showed labeled MVBs in the neuronal soma (Butowt *et al.*, 2006), by Western blotting of neuronal cell lysates and culture media, PrP^C was located to exosomes assumed to be released by MVBs (Faure *et al.*, 2006; Fevrier *et al.*, 2004), and fluorescent microscopy showed colocalization of specific markers of endosomal compartments, namely PrP^{SC} in compartments positive for Rab7, a marker for late endosomal MVB, (but see discussion above) (Magalhaes *et al.*, 2005). MVBs in mouse hippocampal neurons (Mironov *et al.*, 2003), and mouse hippocampal cellular layers and neuropil (Godsave *et al.*, 2008) have been shown to contain gold-labeled PrP^C and/or PrP^{SC}, however MVB abnormalities were not noted in these studies. In a similar study, a PrP^C protease-resistant peptide was not

associated with MVBs when fluorescent microscopy was used to colocalize the marker Lamp-1 with the peptide in a neuroblastoma cell line (Wilson *et al.*, 2007). Again, using endosomal compartment markers, in this case Lamp-2, to mark lysosomes, PrP^{SC} fibrils were identified in lysosomes in sensory neurons (Jen *et al.*, 2010), but not within MVBs. The discrepancies in findings could be due to the form of PrP^{SC} being used, i.e. peptide or fibrils, or due to the ambiguity of markers used for endosomal/ lysosomal compartments, as well as the difficulty in exactly determining co-localization using fluorescence. However, altogether, the data regarding PrP^C and the toxic form, PrP^{SC}, support roles for MVBs in prion trafficking, processing, and function. The ultrastructural studies confirm that MVBs contain prion protein, both in its native conformation (Butowt *et al.*, 2006; Laine *et al.*, 2001; Mironov *et al.*, 2003) as well as in its pathogenic conformation (Godsave *et al.*, 2008; Marijanovic *et al.*, 2009). The findings that MVBs are both abnormal and increased in the prion disease state (Ersdal *et al.*, 2009; Laszlo *et al.*, 1992) strongly suggest a role for MVBs in the progression of prion pathology.

11.6. Aging—Aging of normal neurons is associated with degenerative changes. An increase in MVBs is a common finding in degenerating neurons. Studies that focused on neuronal changes at the ultrastructural level have noted an increase in MVBs along with other signs of neuron degeneration. An increase in MVBs was found in aging mouse neuromuscular junctions (Boaro *et al.*, 1998), dorsal column nuclei (Johnson *et al.*, 1975), and axon terminals of sympathetic ganglia (Schmidt *et al.*, 2008). Similar work in rat neurons (Jung *et al.*, 1999; Nakadate *et al.*, 2006) and oligodendrocytes (de la Roza *et al.*, 1985) also found an increase or accumulation of MVBs along with other degenerative changes in cell bodies or processes. An increase in MVBs as a response to aging, specifically degeneration, was also noted in monkeys (Townes-Anderson and Raviola, 1978) and in humans (Schroer *et al.*, 1992). However, in monkeys, the differences noted in autonomic neuromuscular junctions were not correlated to the age of the monkey, but rather to the state of degeneration or regeneration of the axon. These findings are in line with two studies that found no changes in MVBs, as measured by relative or absolute volume when comparing Purkinje cells (Monteiro, 1991) or neurons of the auditory cortex (Vaughan and Vincent, 1979) in young and old rats. Ultrastructural changes in aged brains, specifically an increase in MVBs in neurons from aged animals, are most likely related to neurodegenerative changes that occur with aging. It is becoming increasingly clear that neurodegeneration and the concurrent structural changes in the endosomal-lysosomal system inherent in the aging process are the basis for many neurodegenerative diseases in the elderly (Nixon *et al.*, 2008).

12. Outlook

This review has summarized compelling evidence for multiple ways in which neuronal MVBs contribute to important cell biological functions related to protein transport, sorting and signaling. However, our review has also revealed numerous gaps in knowledge that require further studies. We expect to see progress in the coming years in neuronal MVB research in particular to address the following topics: In what way and to what extent are neuronal MVBs used to transport proteins – possibly in a neuron-specific fashion – between different neuronal compartments? And, in a related question, do MVB axonal and dendritic subtypes exist that have distinct morphological and functional specializations? How are the internal vesicles and their protein content released – to what extent does backfusion and exosome release contribute? Is there, and if so, to what extent, a physiological role of retrograde axonal transport for MVBs? How do neuronal MVBs contribute to prion transport and dissemination within the nervous system? What is the relevance of MVBs in Schwann cells for myelination? What is the precise role of neuronal MVBs in responses of neurons to stress and dystrophic conditions? What is their role in the sequence of

pathophysiological events, and could this be a target for therapeutic improvement? Most importantly, how do neuronal MVBs process amyloid-beta and why do they seem to fail to process this molecule in neurodegenerative diseases such as Alzheimer's disease? How could better knowledge and appreciation of MVB's role in this process be used to design novel therapies for this type of disease? For each of the above questions, it would be tremendously helpful to find a sensitive and truly specific marker for each functional stage of MVBs, so that these organelles can be definitively identified at the light-microscopic, and not only the ultrastructural level. We hope that our review will contribute to a better understanding of the roles that MVBs play in cellular housekeeping functions to promote neuronal integrity and function, so that neuronal MVBs do not remain the neglected stepchild among MVBs as in current research model systems.

Acknowledgments

The authors are grateful for advice, insightful discussions, comments or other support by the following colleagues: Mark Bothwell, Rafal Butowt, Margaret Byers, Elizabeth Fisher, Mark Grimes, Adrian Isaacs, Alex Krüttgen, Howard Rind, Larisa Wiggins, Chengbiao Wu, the late Sanford Palay, and the late Lesnick Westrum. Particular thanks go to Margaret Byers and Lesnick Westrum (both at the University of Washington) for introducing the senior author to MVBs. Our own work was supported by NIH grants HD 043347 and EY 12841 to C.S.v.B. and, in part, by an IDEA Network of Biomedical Research Excellence Grant (P20RR016464 from the NCRR), and an award from the Sanford Center for Aging, University of Nevada, Reno.

Supported by NIH grants HD 043347 and EY 12841 to C.S.v.B. and, in part, by an IDEA Network of Biomedical Research Excellence Grant (P20RR016464 from the NCRR), and an award from the Sanford Center for Aging, University of Nevada, Reno.

Abbreviation List

Aβ42	amyloid-beta 42 peptide
AD	Alzheimer's disease
CNS	central nervous system
ESCRT	endosomal sorting complex required for transport
FA	fractional area
HD	Huntington's Disease
HRP	horseradish peroxidase
LBPA	lysobisphosphatidic acid
MVB	multivesicular body
NGF	nerve growth factor
PNS	peripheral nervous system

References

- Agromayor M, Martin-Serrano J. Interaction of AMSH with ESCRT-III and deubiquitination of endosomal cargo. *J Biol Chem* 2006;281:23083–23091. [PubMed: 16760479]
- Aguado F, Sanchez-Franco F, Cacicedo L, Fernandez T, Rodrigo J, Martinez-Murillo R. Subcellular localization of insulin-like growth factor I (IGF-I) in Purkinje cells of the adult rat: an immunocytochemical study. *Neurosci Lett* 1992;135:171–174. [PubMed: 1625789]
- Agulhon C, Rostaing P, Ravassard P, Sagne C, Triller A, Giros B. Lysosomal amino acid transporter LYAAT-1 in the rat central nervous system: an in situ hybridization and immunohistochemical study. *J Comp Neurol* 2003;462:71–89. [PubMed: 12761825]

- Aksenov MY, Aksenova MV, Mactutus CF, Booze RM. HIV-1 protein-mediated amyloidogenesis in rat hippocampal cell cultures. *Neurosci Lett*. 2010
- Alberts P, Galli T. The cell outgrowth secretory endosome (COSE): a specialized compartment involved in neuronal morphogenesis. *Biol Cell* 2003;95:419–424. [PubMed: 14597259]
- Alberts P, Rudge R, Irinopoulou T, Danglot L, Gauthier-Rouviere C, Galli T. Cdc42 and actin control polarized expression of TI-VAMP vesicles to neuronal growth cones and their fusion with the plasma membrane. *Mol Biol Cell* 2006;17:1194–1203. [PubMed: 16381811]
- Alegre-Abarrategui J, Christian H, Lufino MM, Mutihac R, Venda LL, Ansorge O, Wade-Martins R. LRRK2 regulates autophagic activity and localizes to specific membrane microdomains in a novel human genomic reporter cellular model. *Hum Mol Genet* 2009;18:4022–4034. [PubMed: 19640926]
- Almeida CG, Takahashi RH, Gouras GK. Beta-amyloid accumulation impairs multivesicular body sorting by inhibiting the ubiquitin-proteasome system. *J Neurosci* 2006;26:4277–4288. [PubMed: 16624948]
- Altick AL, Baryshnikova LM, Vu TQ, von Bartheld CS. Quantitative analysis of multivesicular bodies (MVBs) in the hypoglossal nerve: evidence that neurotrophic factors do not use MVBs for retrograde axonal transport. *J Comp Neurol* 2009;514:641–657. [PubMed: 19363811]
- Andres KH. On the microstructure of receptors on sinus hair. *Z Zellforsch Mikrosk Anat* 1966;75:338–365. [PubMed: 5966248]
- Andres, KH.; von Doring, M. Morphology of Cutaneous Receptors. In: Iggo, A., editor. *Somatosensory System*. Springer-Verlag; Berlin: 1973. p. 1-28.
- Aniento F, Gruenberg J. Membrane transport from early to late endosomes. *Cold Spring Harb Symp Quant Biol* 1995;60:205–209. [PubMed: 8824392]
- Aronin N, Kim M, Laforet G, DiFiglia M. Are there multiple pathways in the pathogenesis of Huntington's disease? *Philos Trans R Soc Lond B Biol Sci* 1999;354:995–1003. [PubMed: 10434298]
- Ashok A, Hegde RS. Prions and retroviruses: an endosomal rendezvous? *EMBO Rep* 2006;7:685–687. [PubMed: 16819462]
- Atwal RS, Truant R. A stress sensitive ER membrane-association domain in Huntingtin protein defines a potential role for Huntingtin in the regulation of autophagy. *Autophagy* 2008;4:91–93. [PubMed: 17986868]
- Babst M. A protein's final ESCRT. *Traffic* 2005;6:2–9. [PubMed: 15569240]
- Babst M, Wendland B, Estepa EJ, Emr SD. The Vps4p AAA ATPase regulates membrane association of a Vps protein complex required for normal endosome function. *EMBO J* 1998;17:2982–2993. [PubMed: 9606181]
- Baude A, Molnar E, Latawiec D, McIlhinney RA, Somogyi P. Synaptic and nonsynaptic localization of the GluR1 subunit of the AMPA-type excitatory amino acid receptor in the rat cerebellum. *J Neurosci* 1994;14:2830–2843. [PubMed: 8182442]
- Bernard V, Laribi O, Levey AI, Bloch B. Subcellular redistribution of m2 muscarinic acetylcholine receptors in striatal interneurons in vivo after acute cholinergic stimulation. *J Neurosci* 1998;18:10207–10218. [PubMed: 9822774]
- Bernd P, Greene LA. Electron microscopic radioautographic localization of iodinated nerve growth factor bound to and internalized by PC12 cells. *J Neurosci* 1983;3:631–643. [PubMed: 6827313]
- Berthold CH, Fabricius C, Rydmark M, Andersen B. Axoplasmic organelles at nodes of Ranvier. I. Occurrence and distribution in large myelinated spinal root axons of the adult cat. *J Neurocytol* 1993;22:925–940. [PubMed: 7507975]
- Berthold CH, Mellstrom. Distribution of peroxidase activity at nodes of Ranvier after intramuscular administration of horseradish peroxidase in the cat. *Neuroscience* 1982;7:45–54. [PubMed: 6176902]
- Berthold, CH.; Rydmark, M. Morphology of normal peripheral axons. In: Waxman, SG.; Kocsis, JD.; Stys, PK., editors. *The Axon. Structure, Function and Pathophysiology*. Oxford University Press; New York: 1995. p. 13-48.
- Birks RI, Mackey MC, Weldon PR. Organelle formation from pinocytotic elements in neurites of cultured sympathetic ganglia. *J Neurocytol* 1972;1:311–340. [PubMed: 8530968]

- Bloch B, Bernard V, Dumartin B. "In vivo" intraneuronal trafficking of G protein coupled receptors in the striatum: regulation by dopaminergic and cholinergic environment. *Biol Cell* 2003;95:477–488. [PubMed: 14597266]
- Blumcke S, Niedorf HR. Electron microscope studies of Schwann cells during the Wallerian degeneration with special reference to the cytoplasmic filaments. *Acta Neuropathol* 1966;6:46–60. [PubMed: 5966679]
- Boaro SN, Soares JC, Konig B Jr. Comparative structural analysis of neuromuscular junctions in mice at different ages. *Ann Anat* 1998;180:173–179. [PubMed: 9587641]
- Borg TK, Bell RA. Ultrastructure of the neurosecretory cells in the brain of diapausing pupae of the tobacco hornworm, *Manduca sexta* (L). *Tissue Cell* 1977;9:567–574. [PubMed: 929583]
- Bresnahan JC. An electron-microscopic analysis of axonal alterations following blunt contusion of the spinal cord of the rhesus monkey (*Macaca mulatta*). *J Neurol Sci* 1978;37:59–82. [PubMed: 99494]
- Breuer AC, Christian CN, Henkart M, Nelson PG. Computer analysis of organelle translocation in primary neuronal cultures and continuous cell lines. *J Cell Biol* 1975;65:562–576. [PubMed: 48515]
- Broadwell RD, Balin BJ. Endocytic and exocytic pathways of the neuronal secretory process and trans-synaptic transfer of wheat germ agglutinin-horseradish peroxidase in vivo. *J Comp Neurol* 1985;242:632–650. [PubMed: 2418083]
- Bronfman FC, Escudero CA, Weis J, Kruttgen A. Endosomal transport of neurotrophins: roles in signaling and neurodegenerative diseases. *Dev Neurobiol* 2007;67:1183–1203. [PubMed: 17514710]
- Buchner K, Seitz-Tutter D, Schonitzer K, Weiss DG. A quantitative study of anterograde and retrograde axonal transport of exogenous proteins in olfactory nerve C-fibers. *Neuroscience* 1987;22:697–707. [PubMed: 3670606]
- Buchs PA, Stoppini L, Parducz A, Siklos L, Muller D. A new cytochemical method for the ultrastructural localization of calcium in the central nervous system. *J Neurosci Methods* 1994;54:83–93. [PubMed: 7815822]
- Bulloj A, Leal MC, Xu H, Castano EM, Morelli L. Insulin-degrading enzyme sorting in exosomes: a secretory pathway for a key brain amyloid-beta degrading protease. *J Alzheimers Dis* 2010;19:79–95. [PubMed: 20061628]
- Bunge MB. Initial endocytosis of peroxidase or ferritin by growth cones of cultured nerve cells. *J Neurocytol* 1977;6:407–439. [PubMed: 894333]
- Bunt AH, Haschke RH. Features of foreign proteins affecting their retrograde transport in axons of the visual system. *J Neurocytol* 1978;7:665–678. [PubMed: 731278]
- Butowt R, Abdelraheim S, Brown DR, von Bartheld CS. Anterograde axonal transport of the exogenous cellular isoform of prion protein in the chick visual system. *Mol Cell Neurosci* 2006;31:97–108. [PubMed: 16203158]
- Butowt R, von Bartheld CS. Sorting of internalized neurotrophins into an endocytic transcytosis pathway via the Golgi system: Ultrastructural analysis in retinal ganglion cells. *J Neurosci* 2001;21:8915–8930. [PubMed: 11698603]
- Butowt R, von Bartheld CS. Fates of neurotrophins after retrograde axonal transport: phosphorylation of p75NTR is a sorting signal for delayed degradation. *J Neurosci* 2009;29:10715–10729. [PubMed: 19710323]
- Byers MR. Dental sensory receptors. *Int Rev Neurobiol* 1984;25:39–94. [PubMed: 6206015]
- Byers MR. Sensory innervation of periodontal ligament of rat molars consists of unencapsulated Ruffini-like mechanoreceptors and free nerve endings. *J Comp Neurol* 1985;231:500–518. [PubMed: 3968252]
- Byers MR, Yeh Y. Fine structure of subepithelial "free" and corpuscular trigeminal nerve endings in anterior hard palate of the rat. *Somatosens Res* 1984;1:265–279. [PubMed: 6494667]
- Caldero J, Casanovas A, Sorribas A, Esquerda JE. Calcitonin gene-related peptide in rat spinal cord motoneurons: subcellular distribution and changes induced by axotomy. *Neuroscience* 1992;48:449–461. [PubMed: 1603329]

- Calderon C, Mohamed F, Munoz E, Fogal T, Pelzer L, Penissi A, Piezzi R. Daily morphological variations in the viscacha (*Lagostomus maximus maximus*) retina. Probable local modulatory action of melatonin. *Anat Rec* 2002;266:198–206. [PubMed: 11920382]
- Calistri A, Salata C, Parolin C, Palu G. Role of multivesicular bodies and their components in the egress of enveloped RNA viruses. *Rev Med Virol* 2009;19:31–45. [PubMed: 18618839]
- Calman BG, Chamberlain SC. Distinct lobes of *Limulus* ventral photoreceptors. II. Structure and ultrastructure. *J Gen Physiol* 1982;80:839–862. [PubMed: 7175491]
- Campanot RB, MacInnis BL. Retrograde transport of neurotrophins: fact and function. *J Neurobiol* 2004;58:217–229. [PubMed: 14704954]
- Candela P, Gosselet F, Miller F, Buee-Scherrer V, Torpier G, Cecchelli R, Fenart L. Physiological pathway for low-density lipoproteins across the blood-brain barrier: transcytosis through brain capillary endothelial cells in vitro. *Endothelium* 2008;15:254–264. [PubMed: 19065317]
- Castel MN, Woulfe J, Wang X, Laduron PM, Beaudet A. Light and electron microscopic localization of retrogradely transported neurotensin in rat nigrostriatal dopaminergic neurons. *Neuroscience* 1992;50:269–282. [PubMed: 1279459]
- Cataldo AM, Mathews PM, Boiteau AB, Hassinger LC, Peterhoff CM, Jiang Y, Mullaney K, Neve RL, Gruenberg J, Nixon RA. Down syndrome fibroblast model of Alzheimer-related endosome pathology: accelerated endocytosis promotes late endocytic defects. *Am J Pathol* 2008;173:370–384. [PubMed: 18535180]
- Cavalli V, Kujala P, Klumperman J, Goldstein LS. Sunday Driver links axonal transport to damage signaling. *J Cell Biol* 2005;168:775–787. [PubMed: 15738268]
- Cavanagh JB, Gysbers MF. “Dying back” above a nerve ligature produced by acrylamide. *Acta Neuropathol* 1980;51:169–177. [PubMed: 7445971]
- Cavanagh JB, Gysbers MF. Ultrastructural changes in axons caused by acrylamide above a nerve ligature. *Neuropathol Appl Neurobiol* 1981;7:315–326. [PubMed: 7279142]
- Chamberlain SC, Barlow RB Jr. Transient membrane shedding in *Limulus* photoreceptors: control mechanisms under natural lighting. *J Neurosci* 1984;4:2792–2810. [PubMed: 6502204]
- Chan KY, Bunt AH, Haschke RH. Endocytosis and compartmentation of peroxidases and cationized ferritin in neuroblastoma cells. *J Neurocytol* 1980;9:381–403. [PubMed: 7441298]
- Chavrier P, Parton RG, Hauri HP, Simons K, Zerial M. Localization of low molecular weight GTP binding proteins to exocytic and endocytic compartments. *Cell* 1990;62:317–329. [PubMed: 2115402]
- Chicurel ME, Harris KM. Three-dimensional analysis of the structure and composition of CA3 branched dendritic spines and their synaptic relationships with mossy fiber boutons in the rat hippocampus. *J Comp Neurol* 1992;325:169–182. [PubMed: 1460112]
- Chiulli N, Codazzi F, Di Cesare A, Gravaghi C, Zacchetti D, Grohovaz F. Sphingosylphosphocholine effects on cultured astrocytes reveal mechanisms potentially involved in neurotoxicity in Niemann-Pick type A disease. *Eur J Neurosci* 2007;26:875–881. [PubMed: 17666077]
- Chu H, Wang JJ, Spearman P. Human immunodeficiency virus type-1 gag and host vesicular trafficking pathways. *Curr Top Microbiol Immunol* 2009;339:67–84. [PubMed: 20012524]
- Chu Z, Sun Y, Kuan CY, Grabowski GA, Qi X, Saposin C. Neuronal effect and CNS delivery by liposomes. *Ann N Y Acad Sci* 2005;1053:237–246. [PubMed: 16179529]
- Chu-Wang IW, Oppenheim RW. Uptake, intra-axonal transport and fate of horseradish peroxidase in embryonic spinal neurons of the chick. *J Comp Neurol* 1980;193:753–776. [PubMed: 6160167]
- Clague MJ, Urbe S. Multivesicular bodies. *Curr Biol* 2008;18:R402–404. [PubMed: 18492464]
- Claude P, Hawrot E, Dunis DA, Campanot RB. Binding, internalization, and retrograde transport of 125I-nerve growth factor in cultured rat sympathetic neurons. *J Neurosci* 1982a;2:431–442. [PubMed: 7069465]
- Claude P, Hawrot E, Parada I. Ultrastructural studies on the intracellular fate of 125I-nerve growth factor in cultured rat sympathetic neurons. *J Cell Biochem* 1982b;20:1–13. [PubMed: 6761346]
- Colella R, Lu G, Glazewski L, Korant B, Matlapudi A, England MR, Craft C, Frantz CN, Mason RW. Induction of cell death in neuroblastoma by inhibition of cathepsins B and L. *Cancer Lett*. 2010

- Cooney JR, Hurlburt JL, Selig DK, Harris KM, Fiala JC. Endosomal compartments serve multiple hippocampal dendritic spines from a widespread rather than a local store of recycling membrane. *J Neurosci* 2002;22:2215–2224. [PubMed: 11896161]
- Csillik B, Knyihar-Csillik E. Fine structural localization of calcium binding sites in the neuromuscular junction. *Acta Biol Acad Sci Hung* 1980;31:49–56. [PubMed: 7223245]
- Cui B, Wu C, Chen L, Ramirez A, Bearer EL, Li WP, Mobley WC, Chu S. One at a time, live tracking of NGF axonal transport using quantum dots. *Proc Natl Acad Sci U S A* 2007;104:13666–13671. [PubMed: 17698956]
- daSilva LLP, Sougrat R, Burgos PV, Janvier K, Mattera R, Bonifacino JS. Human immunodeficiency virus type 1 Nef protein targets CD4 to the multivesicular body pathway. *J Virol* 2009;83:6578–6590. [PubMed: 19403684]
- Davies BA, Lee JR, Oestreich AJ, Katzmann DJ. Membrane protein targeting to the MVB/lysosome. *Chem Rev* 2009;109:1575–1586. [PubMed: 19243135]
- de Groot JC, Meeuwse F, Ruizendaal WE, Veldman JE. Ultrastructural localization of gentamicin in the cochlea. *Hear Res* 1990;50:35–42. [PubMed: 2076981]
- de la Roza C, Cano J, Reinoso-Suarez F. An electron microscopic study of astroglia and oligodendroglia in the lateral geniculate nucleus of aged rats. *Mech Ageing Dev* 1985;29:267–281. [PubMed: 3990383]
- Dehnes Y, Chaudhry FA, Ullensvang K, Lehre KP, Storm-Mathisen J, Danbolt NC. The glutamate transporter EAAT4 in rat cerebellar Purkinje cells: a glutamate-gated chloride channel concentrated near the synapse in parts of the dendritic membrane facing astroglia. *J Neurosci* 1998;18:3606–3619. [PubMed: 9570792]
- Deinhardt K, Salinas S, Verastegui C, Watson R, Worth D, Hanrahan S, Bucci C, Schiavo G. Rab5 and Rab7 control endocytic sorting along the axonal retrograde transport pathway. *Neuron* 2006;52:293–305. [PubMed: 17046692]
- Delcroix JD, Valletta JS, Wu C, Hunt SJ, Kowal AS, Mobley WC. NGF signaling in sensory neurons: evidence that early endosomes carry NGF retrograde signals. *Neuron* 2003;39:69–84. [PubMed: 12848933]
- Donne, K. T. Delle; Sesack, SR.; Pickel, VM. Ultrastructural immunocytochemical localization of neurotensin and the dopamine D2 receptor in the rat nucleus accumbens. *J Comp Neurol* 1996;371:552–566. [PubMed: 8841909]
- Deloncle R, Huguet F, Fernandez B, Quillard N, Babin P, Guillard O. Ultrastructural study of rat hippocampus after chronic administration of aluminum L-glutamate: an acceleration of the aging process. *Exp Gerontol* 2001;36:231–244. [PubMed: 11226739]
- Denzer K, Kleijmeer MJ, Heijnen HF, Stoorvogel W, Geuze HJ. Exosome: from internal vesicle of the multivesicular body to intercellular signaling device. *J Cell Sci* 2000;113(Pt 19):3365–3374. [PubMed: 10984428]
- Devlin C, Pipalia NH, Liao X, Schuchman EH, Maxfield FR, Tabas I. Improvement in Lipid and Protein Trafficking in NPC1 Cells by Correction of a Secondary Enzyme Defect. *Traffic*. 2010
- Diaz-Cintra S, Yong A, Aguilar A, Bi X, Lynch G, Ribak CE. Ultrastructural analysis of hippocampal pyramidal neurons from apolipoprotein E-deficient mice treated with a cathepsin inhibitor. *J Neurocytol* 2004;33:37–48. [PubMed: 15173631]
- Dilley KA, Gregory D, Johnson MC, Vogt VM. An LYPSL late domain in the Gag protein contributes to the efficient release and replication of Rous sarcoma virus. *J Virol*. 2010
- Dixon JS. “Phagocytic” lysosomes in chromatolytic neurones. *Nature* 1967;215:657–658. [PubMed: 6050233]
- Eden ER, White IJ, Futter CE. Down-regulation of epidermal growth factor receptor signalling within multivesicular bodies. *Biochem Soc Trans* 2009;37:173–177. [PubMed: 19143625]
- Eden ER, White IJ, Tsapara A, Futter CE. Membrane contacts between endosomes and ER provide sites for PTP1B-epidermal growth factor receptor interaction. *Nat Cell Biol* 2010;12:267–272. [PubMed: 20118922]
- Edgar JM, McLaughlin M, Yool D, Zhang SC, Fowler JH, Montague P, Barrie JA, McCulloch MC, Duncan ID, Garbern J, Nave KA, Griffiths IR. Oligodendroglial modulation of fast axonal

transport in a mouse model of hereditary spastic paraplegia. *J Cell Biol* 2004;166:121–131. [PubMed: 15226307]

- Einheber S, Bhat MA, Salzer JL. Disrupted axo-glial junctions result in accumulation of abnormal mitochondria at nodes of Ranvier. *Neuron Glia Biol* 2006;2:165–174. [PubMed: 17460780]
- Ersdal C, Goodsir CM, Simmons MM, McGovern G, Jeffrey M. Abnormal prion protein is associated with changes of plasma membranes and endocytosis in bovine spongiform encephalopathy (BSE)-affected cattle brains. *Neuropathol Appl Neurobiol* 2009;35:259–271. [PubMed: 19473293]
- Estable C, Acosta-Ferreira W, Sotelo JR. An electron microscope study of the regenerating nerve fibers. *Z Zellforsch Mikrosk Anat* 1957;46:387–399. [PubMed: 13531602]
- Fabricius C, Berthold CH, Rydmark M. Axoplasmic organelles at nodes of Ranvier. II. Occurrence and distribution in large myelinated spinal cord axons of the adult cat. *J Neurocytol* 1993;22:941–954. [PubMed: 7507976]
- Fader CM, Colombo MI. Autophagy and multivesicular bodies: two closely related partners. *Cell Death Differ* 2009;16:70–78. [PubMed: 19008921]
- Falguieres T, Luyet PP, Gruenberg J. Molecular assemblies and membrane domains in multivesicular endosome dynamics. *Exp Cell Res*. 2008
- Faure J, Lachenal G, Court M, Hirrlinger J, Chatellard-Causse C, Blot B, Grange J, Schoehn G, Goldberg Y, Boyer V, Kirchhoff F, Raposo G, Garin J, Sadoul R. Exosomes are released by cultured cortical neurones. *Mol Cell Neurosci* 2006;31:642–648. [PubMed: 16446100]
- Feher E. Electron microscopic study of retrograde axonal transport of horseradish peroxidase. *Int Rev Cytol* 1984;90:1–29. [PubMed: 6389411]
- Felder S, Miller K, Moehren G, Ullrich A, Schlessinger J, Hopkins CR. Kinase activity controls the sorting of the epidermal growth factor receptor within the multivesicular body. *Cell* 1990;61:623–634. [PubMed: 2344614]
- Fergestad T, Davis WS, Broadie K. The stoned proteins regulate synaptic vesicle recycling in the presynaptic terminal. *J Neurosci* 1999;19:5847–5860. [PubMed: 10407025]
- Fernandez-Borja M, Wubbolts R, Calafat J, Janssen H, Divecha N, Dusseljee S, Neefjes J. Multivesicular body morphogenesis requires phosphatidylinositol 3-kinase activity. *Curr Biol* 1999;9:55–58. [PubMed: 9889123]
- Fevrier B, Raposo G. Exosomes: endosomal-derived vesicles shipping extracellular messages. *Curr Opin Cell Biol* 2004;16:415–421. [PubMed: 15261674]
- Fevrier B, Vilette D, Archer F, Loew D, Faigle W, Vidal M, Laude H, Raposo G. Cells release prions in association with exosomes. *Proc Natl Acad Sci U S A* 2004;101:9683–9688. [PubMed: 15210972]
- Fifkova E, Markham JA, Delay RJ. Calcium in the spine apparatus of dendritic spines in the dentate molecular layer. *Brain Res* 1983;266:163–168. [PubMed: 6189559]
- Filimonenko M, Stuffers S, Raiborg C, Yamamoto A, Malerod L, Fisher EM, Isaacs A, Brech A, Stenmark H, Simonsen A. Functional multivesicular bodies are required for autophagic clearance of protein aggregates associated with neurodegenerative disease. *J Cell Biol* 2007;179:485–500. [PubMed: 17984323]
- Fomina AF, Deerinck TJ, Ellisman MH, Cahalan MD. Regulation of membrane trafficking and subcellular organization of endocytic compartments revealed with FM1-43 in resting and activated human T cells. *Exp Cell Res* 2003;291:150–166. [PubMed: 14597416]
- Friedrich RP, Tepper K, Ronicke R, Soom M, Westermann M, Reymann K, Kaether C, Fandrich M. Mechanism of amyloid plaque formation suggests an intracellular basis of Abeta pathogenicity. *Proc Natl Acad Sci U S A* 2010;107:1942–1947. [PubMed: 20133839]
- Futter CE, Pearse A, Hewlett LJ, Hopkins CR. Multivesicular endosomes containing internalized EGF-EGF receptor complexes mature and then fuse directly with lysosomes. *J Cell Biol* 1996;132:1011–1023. [PubMed: 8601581]
- Futter CE, White IJ. Annexins and endocytosis. *Traffic* 2007;8:951–958. [PubMed: 17547702]
- Gatzinsky KP. Node-paranode regions as local degradative centres in alpha-motor axons. *Microsc Res Tech* 1996;34:492–506. [PubMed: 8842019]

- Gatzinsky KP, Berthold CH. Lysosomal activity at nodes of Ranvier during retrograde axonal transport of horseradish peroxidase in alpha-motor neurons of the cat. *J Neurocytol* 1990;19:989–1002. [PubMed: 1705579]
- Gatzinsky KP, Berthold CH, Corneliussen O. Acid phosphatase activity at nodes of Ranvier in alpha-motor and dorsal root ganglion neurons of the cat. *J Neurocytol* 1988;17:531–544. [PubMed: 3193130]
- Gatzinsky KP, Persson GH, Berthold CH. Removal of retrogradely transported material from rat lumbosacral alpha-motor axons by paranodal axon-Schwann cell networks. *Glia* 1997;20:115–126. [PubMed: 9179596]
- Giunta B, Hou H, Zhu Y, Rrapo E, Tian J, Takashi M, Commins D, Singer E, He J, Fernandez F, Tan J. HIV-1 Tat contributes to Alzheimer's disease-like pathology in PSAPP mice. *Int J Clin Exp Pathol* 2009;2:433–443. [PubMed: 19294002]
- Godsave SF, Wille H, Kujala P, Latawiec D, DeArmond SJ, Serban A, Prusiner SB, Peters PJ. Cryo-immunogold electron microscopy for prions: toward identification of a conversion site. *J Neurosci* 2008;28:12489–12499. [PubMed: 19020041]
- Goldstein JL, Brown MS, Anderson RG, Russell DW, Schneider WJ. Receptor-mediated endocytosis: concepts emerging from the LDL receptor system. *Annu Rev Cell Biol* 1985;1:1–39. [PubMed: 2881559]
- Grafstein B, Forman DS. Intracellular transport in neurons. *Physiol Rev* 1980;60:1167–1283. [PubMed: 6159657]
- Gramp T, Notz V, Broll I, Fischer N, Benke D. Constitutive, agonist-accelerated, recycling and lysosomal degradation of GABA(B) receptors in cortical neurons. *Mol Cell Neurosci*. 2008
- Grant BD, Donaldson JG. Pathways and mechanisms of endocytic recycling. *Nat Rev Mol Cell Biol* 2009;10:597–608. [PubMed: 19696797]
- Grimes ML, Beattie E, Mobley WC. A signaling organelle containing the nerve growth factor-activated receptor tyrosine kinase, TrkA. *Proc Natl Acad Sci U S A* 1997;94:9909–9914. [PubMed: 9275225]
- Grimes ML, Miettinen HM. Receptor tyrosine kinase and G-protein coupled receptor signaling and sorting within endosomes. *J Neurochem* 2003;84:905–918. [PubMed: 12603816]
- Gruenberg J. The endocytic pathway: a mosaic of domains. *Nat Rev Mol Cell Biol* 2001;2:721–730. [PubMed: 11584299]
- Gruenberg J, Griffiths G, Howell KE. Characterization of the early endosome and putative endocytic carrier vesicles in vivo and with an assay of vesicle fusion in vitro. *J Cell Biol* 1989;108:1301–1316. [PubMed: 2538480]
- Gruenberg J, Stenmark H. The biogenesis of multivesicular endosomes. *Nat Rev Mol Cell Biol* 2004;5:317–323. [PubMed: 15071556]
- Ha J, Lo KW, Myers KR, Carr TM, Humsi MK, Rasoul BA, Segal RA, Pfister KK. A neuron-specific cytoplasmic dynein isoform preferentially transports TrkB signaling endosomes. *J Cell Biol* 2008;181:1027–1039. [PubMed: 18559670]
- Haglund K, Sigismund S, Polo S, Szymkiewicz I, Di Fiore PP, Dikic I. Multiple monoubiquitination of RTKs is sufficient for their endocytosis and degradation. *Nat Cell Biol* 2003;5:461–466. [PubMed: 12717448]
- Haigler HT, McKanna JA, Cohen S. Direct visualization of the binding and internalization of a ferritin conjugate of epidermal growth factor in human carcinoma cells A-431. *J Cell Biol* 1979;81:382–395. [PubMed: 313931]
- Harding C, Heuser J, Stahl P. Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes. *J Cell Biol* 1983;97:329–339. [PubMed: 6309857]
- Heerssen HM, Pazyra MF, Segal RA. Dynein motors transport activated Trks to promote survival of target-dependent neurons. *Nat Neurosci* 2004;7:596–604. [PubMed: 15122257]
- Heilmeyer LM Jr, Vereb G Jr, Vereb G, Kakuk A, Szivak I. Mammalian phosphatidylinositol 4-kinases. *IUBMB Life* 2003;55:59–65. [PubMed: 12749687]
- Hernandez-Deviez D, Mackay-Sim A, Wilson JM. A Role for ARF6 and ARNO in the regulation of endosomal dynamics in neurons. *Traffic* 2007;8:1750–1764. [PubMed: 17897316]

- Herndon RM. The Fine Structure of the Rat Cerebellum. II. The Stellate Neurons, Granule Cells, and Glia. *J Cell Biol* 1964;23:277–293. [PubMed: 14222815]
- Hersch SM, Yi H, Heilman CJ, Edwards RH, Levey AI. Subcellular localization and molecular topology of the dopamine transporter in the striatum and substantia nigra. *J Comp Neurol* 1997;388:211–227. [PubMed: 9368838]
- Hicke L, Dunn R. Regulation of membrane protein transport by ubiquitin and ubiquitin-binding proteins. *Annu Rev Cell Dev Biol* 2003;19:141–172. [PubMed: 14570567]
- Hirano, A.; Llena, JF. Morphology of central nervous system. In: Waxman, SG.; Kocsis, JD.; Stys, PK., editors. *The Axon. Structure, Function and Pathophysiology*. Oxford University Press; New York: 1995. p. 46–67.
- Hirokawa N, Sato-Yoshitake R, Yoshida T, Kawashima T. Brain dynein (MAP1C) localizes on both anterogradely and retrogradely transported membranous organelles in vivo. *J Cell Biol* 1990;111:1027–1037. [PubMed: 2143999]
- Hirsch JG, Fedorko ME, Cohn ZA. Vesicle fusion and formation at the surface of pinocytotic vacuoles in macrophages. *J Cell Biol* 1968;38:629–632. [PubMed: 5664229]
- Hiura A, Ishizuka H. Changes in features of degenerating primary sensory neurons with time after capsaicin treatment. *Acta Neuropathol* 1989;78:35–46. [PubMed: 2735188]
- Hollenbeck PJ. Products of endocytosis and autophagy are retrieved from axons by regulated retrograde organelle transport. *J Cell Biol* 1993;121:305–315. [PubMed: 7682217]
- Hollyfield JG, Rayborn ME. Endocytosis in the inner segment of rod photoreceptors: analysis of *Xenopus laevis* retinas using horseradish peroxidase. *Exp Eye Res* 1987;45:703–719. [PubMed: 3428395]
- Hollyfield JG, Varner HH, Rayborn ME, Bridges CD. Participation of photoreceptor cells in retrieval and degradation of components in the interphotoreceptor matrix. *Prog Clin Biol Res* 1985a; 190:171–175. [PubMed: 2864698]
- Hollyfield JG, Varner HH, Rayborn ME, Liou GI, Bridges CD. Endocytosis and degradation of interstitial retinol-binding protein: differential capabilities of cells that border the interphotoreceptor matrix. *J Cell Biol* 1985b;100:1676–1681. [PubMed: 4039328]
- Holtzman E. Cytochemical studies of protein transport in the nervous system. *Philos Trans R Soc Lond B Biol Sci* 1971;261:407–421. [PubMed: 4401620]
- Holtzman E, Novikoff AB. Lysosomes in the rat sciatic nerve following crush. *J Cell Biol* 1965;27:651–669. [PubMed: 5885432]
- Holtzman E, Peterson ER. Uptake of protein by mammalian neurons. *J Cell Biol* 1969;40:863–869. [PubMed: 5765774]
- Holtzman E, Teichberg S, Abrahams SJ, Citkowitz E, Crain SM, Kawai N, Peterson ER. Notes on synaptic vesicles and related structures, endoplasmic reticulum, lysosomes and peroxisomes in nervous tissue and the adrenal medulla. *J Histochem Cytochem* 1973;21:349–385. [PubMed: 4574997]
- Howe CL, Mobley WC. Signaling endosome hypothesis: A cellular mechanism for long distance communication. *J Neurobiol* 2004;58:207–216. [PubMed: 14704953]
- Hsu C, Morohashi Y, Yoshimura S, Manrique-Hoyos N, Jung S, Lauterbach MA, Bakhti M, Gronborg M, Mobius W, Rhee J, Barr FA, Simons M. Regulation of exosome secretion by Rab35 and its GTPase-activating proteins TBC1D10A-C. *J Cell Biol* 2010;189:223–232. [PubMed: 20404108]
- Hurley JH. ESCRT complexes and the biogenesis of multivesicular bodies. *Curr Opin Cell Biol* 2008;20:4–11. [PubMed: 18222686]
- Inoue K. Fine structure at the cut ends following section of the sensory component of the vagus nerve of mice. *Anat Anz* 1983;154:387–395. [PubMed: 6660552]
- Janetzko A, Zimmermann H, Volknandt W. Intraneuronal distribution of a synaptic vesicle membrane protein: antibody binding sites at axonal membrane compartments and trans-Golgi network and accumulation at nodes of Ranvier. *Neuroscience* 1989;32:65–77. [PubMed: 2586752]
- Jen A, Parkyn CJ, Mootoosamy RC, Ford MJ, Warley A, Liu Q, Bu G, Baskakov IV, Moestrup S, McGuinness L, Emptage N, Morris RJ. Neuronal low-density lipoprotein receptor-related protein 1 binds and endocytoses prion fibrils via receptor cluster 4. *J Cell Sci* 2010;123:246–255. [PubMed: 20048341]

- Jia XX, Gorczyca M, Budnik V. Ultrastructure of neuromuscular junctions in *Drosophila*: comparison of wild type and mutants with increased excitability. *J Neurobiol* 1993;24:1025–1044. [PubMed: 8409967]
- Johnson JE, Mehler WR, Miquel J. A fine structural study of degenerative changes in the dorsal column nuclei of aging mice. Lack of protection by vitamin E. *J Gerontol* 1975;30:395–411. [PubMed: 1141671]
- Jones I, He X, Katouzian F, Darroch PI, Schuchman EH. Characterization of common SMPD1 mutations causing types A and B Niemann-Pick disease and generation of mutation-specific mouse models. *Mol Genet Metab* 2008;95:152–162. [PubMed: 18815062]
- Joset A, Dodd DA, Halegoua S, Schwab ME. Pincher-generated Nogo-A endosomes mediate growth cone collapse and retrograde signaling. *J Cell Biol* 2010;188:271–285. [PubMed: 20083601]
- Joshi A, Ablan SD, Soheilian F, Nagashima K, Freed EO. Evidence that productive human immunodeficiency virus type 1 assembly can occur in an intracellular compartment. *J Virol* 2009;83:5375–5387. [PubMed: 19297499]
- Joshi G, Perluigi M, Sultana R, Agrippino R, Calabrese V, Butterfield DA. In vivo protection of synaptosomes by ferulic acid ethyl ester (FAEE) from oxidative stress mediated by 2,2-azobis(2-amidino-propane)dihydrochloride (AAPH) or Fe(2+)/H(2)O(2): insight into mechanisms of neuroprotection and relevance to oxidative stress-related neurodegenerative disorders. *Neurochem Int* 2006;48:318–327. [PubMed: 16386335]
- Jung H, Lee EY, Lee SI. Age-related changes in ultrastructural features of cathepsin B- and D-containing neurons in rat cerebral cortex. *Brain Res* 1999;844:43–54. [PubMed: 10536260]
- Kaasinen SK, Harvey L, Reynolds AJ, Hendry IA. Autophagy generates retrogradely transported organelles: a hypothesis. *Int J Dev Neurosci* 2008;26:625–634. [PubMed: 18499388]
- Kadota T, Kadota K. Membrane retrieval by macropinocytosis in presynaptic terminals during transmitter release in cat sympathetic ganglia in situ. *J Electron Microsc (Tokyo)* 1982;31:73–80. [PubMed: 6152696]
- Kadota T, Kadota K. Rapid structural remodeling of shaft synapses associated with long-term potentiation in the cat superior cervical ganglion in situ. *Neurosci Res* 2002;43:135–146. [PubMed: 12067749]
- Kadota T, Mizote M, Kadota K. Dynamics of presynaptic endosomes produced during transmitter release. *J Electron Microsc (Tokyo)* 1994;43:62–71. [PubMed: 8077873]
- Kapeller K, Mayor D. An electron microscopic study of the early changes distal to a constriction in sympathetic nerves. *Proc R Soc Lond B Biol Sci* 1969;172:53–63. [PubMed: 4388103]
- Katzmann DJ, Babst M, Emr SD. Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I. *Cell* 2001;106:145–155. [PubMed: 11511343]
- Katzmann DJ, Odorizzi G, Emr SD. Receptor downregulation and multivesicular-body sorting. *Nat Rev Mol Cell Biol* 2002;3:893–905. [PubMed: 12461556]
- Kegel KB, Kim M, Sapp E, McIntyre C, Castano JG, Aronin N, DiFiglia M. Huntingtin expression stimulates endosomal-lysosomal activity, endosome tubulation, and autophagy. *J Neurosci* 2000;20:7268–7278. [PubMed: 11007884]
- Kennedy MJ, Ehlers MD. Organelles and Trafficking Machinery for Postsynaptic Plasticity. *Annu Rev Neurosci*. 2006
- Kidd M. Alzheimer's Disease--an Electron Microscopical Study. *Brain* 1964;87:307–320. [PubMed: 14188276]
- Kleijmeer M, Ramm G, Schuurhuis D, Griffith J, Rescigno M, Ricciardi-Castagnoli P, Rudensky AY, Ossendorp F, Melief CJ, Stoorvogel W, Geuze HJ. Reorganization of multivesicular bodies regulates MHC class II antigen presentation by dendritic cells. *J Cell Biol* 2001;155:53–63. [PubMed: 11581285]
- Ko DC, Gordon MD, Jin JY, Scott MP. Dynamic movements of organelles containing Niemann-Pick C1 protein: NPC1 involvement in late endocytic events. *Mol Biol Cell* 2001;12:601–614. [PubMed: 11251074]

- Kobayashi T, Beuchat MH, Chevallier J, Makino A, Mayran N, Escola JM, Lebrand C, Cosson P, Gruenberg J. Separation and characterization of late endosomal membrane domains. *J Biol Chem* 2002;277:32157–32164. [PubMed: 12065580]
- Kobayashi T, Beuchat MH, Lindsay M, Frias S, Palmiter RD, Sakuraba H, Parton RG, Gruenberg J. Late endosomal membranes rich in lysobisphosphatidic acid regulate cholesterol transport. *Nat Cell Biol* 1999;1:113–118. [PubMed: 10559883]
- Koksal M, Ilgaz C, Erdogan D, Ozogul C, Tong EK, Kalender H. Ultrastructure of rat pup's Purkinje neurons whose mothers were exposed to ethanol during pregnancy and lactation. *Int J Neurosci* 2005;115:1669–1686. [PubMed: 16287633]
- Korthals JK, Korthals MA, Wisniewski HM. Peripheral nerve ischemia: Part 2. Accumulation of organelles. *Ann Neurol* 1978;4:487–498. [PubMed: 742848]
- Kraev IV, Godukhin OV, Patrushev IV, Davies HA, Popov VI, Stewart MG. Partial kindling induces neurogenesis, activates astrocytes and alters synaptic morphology in the dentate gyrus of freely moving adult rats. *Neuroscience* 2009;162:254–267. [PubMed: 19447163]
- Kramer H. Sorting out signals in fly endosomes. *Traffic* 2002;3:87–91. [PubMed: 11929598]
- Kramer-Albers EM, Bretz N, Tenzer S, Winterstein C, Mobius W, Berger H, Nave KA, Schild H, Trotter J. Oligodendrocytes secrete exosomes containing major myelin and stress-protective proteins: Trophic support for axons? *Proteomics Clinical Applications* 2007;1:1446–1461. [PubMed: 21136642]
- Kristensson K, Olsson Y. Uptake and retrograde axonal transport of peroxidase in hypoglossal neurons. Electron microscopical localization in the neuronal perikaryon. *Acta Neuropathol* 1971;19:1–9. [PubMed: 4107378]
- Kujala P, Ikaheimonen A, Ehsani N, Vihinen H, Auvinen P, Kaariainen L. Biogenesis of the Semliki Forest virus RNA replication complex. *J Virol* 2001;75:3873–3884. [PubMed: 11264376]
- Kyuuma M, Kikuchi K, Kojima K, Sugawara Y, Sato M, Mano N, Goto J, Takeshita T, Yamamoto A, Sugamura K, Tanaka N. AMSH, an ESCRT-III associated enzyme, ubiquitinates cargo on MVB/late endosomes. *Cell Struct Funct* 2007;31:159–172. [PubMed: 17159328]
- Lachenal G, Pernet-Gallay K, Chivet M, Hemming FJ, Belly A, Bodon G, Blot B, Haase G, Goldberg Y, Sadoul R. Release of exosomes from differentiated neurons and its regulation by synaptic glutamatergic activity. *Mol Cell Neurosci*. 2011
- LaFerla FM, Green KN, Oddo S. Intracellular amyloid-beta in Alzheimer's disease. *Nat Rev Neurosci* 2007;8:499–509. [PubMed: 17551515]
- Laine J, Marc ME, Sy MS, Axelrad H. Cellular and subcellular morphological localization of normal prion protein in rodent cerebellum. *Eur J Neurosci* 2001;14:47–56. [PubMed: 11488948]
- Lampert PW. A comparative electron microscopic study of reactive, degenerating, regenerating, and dystrophic axons. *J Neuropathol Exp Neurol* 1967;26:345–368. [PubMed: 5229871]
- Lane DA, Lessard AA, Chan J, Colago EE, Zhou Y, Schlussman SD, Kreek MJ, Pickel VM. Region-specific changes in the subcellular distribution of AMPA receptor GluR1 subunit in the rat ventral tegmental area after acute or chronic morphine administration. *J Neurosci* 2008;28:9670–9681. [PubMed: 18815253]
- Langui D, Girardot N, El Hachimi KH, Allinquant B, Blanchard V, Pradier L, Duyckaerts C. Subcellular topography of neuronal Aβ peptide in APPxPS1 transgenic mice. *Am J Pathol* 2004;165:1465–1477. [PubMed: 15509518]
- Lassmann H, Bartsch U, Montag D, Schachner M. Dying-back oligodendroglialopathy: a late sequel of myelin-associated glycoprotein deficiency. *Glia* 1997;19:104–110. [PubMed: 9034827]
- Laszlo L, Lowe J, Self T, Kenward N, Landon M, McBride T, Farquhar C, McConnell I, Brown J, Hope J, et al. Lysosomes as key organelles in the pathogenesis of prion encephalopathies. *J Pathol* 1992;166:333–341. [PubMed: 1355530]
- LaVail JH, LaVail MM. The retrograde intraaxonal transport of horseradish peroxidase in the chick visual system: a light and electron microscopic study. *J Comp Neurol* 1974;157:303–357. [PubMed: 4138789]
- LaVail JH, Margolis TP. The anterograde axonal transport of wheatgerm agglutinin as a mode for transcellular transport in neurons. *Neurology & Neurobiology* 1987;25:311–326.

- LaVail JH, Rapisardi S, Sugino IK. Evidence against the smooth endoplasmic reticulum as a continuous channel for the retrograde axonal transport of horseradish peroxidase. *Brain Res* 1980;191:3–20. [PubMed: 6155169]
- LaVail JH, Sugino IK, McDonald DM. Localization of axonally transported 125I-wheat germ agglutinin beneath the plasma membrane of chick retinal ganglion cells. *J Cell Biol* 1983;96:373–381. [PubMed: 6187749]
- LaVail MM. Rod outer segment disk shedding in rat retina: relationship to cyclic lighting. *Science* 1976;194:1071–1074. [PubMed: 982063]
- Lawrence SP, Bright NA, Luzio JP, Bowers K. The sodium/proton exchanger NHE8 regulates late endosomal morphology and function. *Mol Biol Cell* 2010;21:3540–3551. [PubMed: 20719963]
- Le Blanc I, Luyet PP, Pons V, Ferguson C, Emans N, Petiot A, Mayran N, Demaurex N, Faure J, Sadoul R, Parton RG, Gruenberg J. Endosome-to-cytosol transport of viral nucleocapsids. *Nat Cell Biol* 2005;7:653–664. [PubMed: 15951806]
- Lee JA, Beigneux A, Ahmad ST, Young SG, Gao FB. ESCRT-III dysfunction causes autophagosome accumulation and neurodegeneration. *Curr Biol* 2007;17:1561–1567. [PubMed: 17683935]
- Lee JA, Gao FB. Roles of ESCRT in autophagy-associated neurodegeneration. *Autophagy* 2008;4:230–232. [PubMed: 18094607]
- Lewczuk B, Przybylska-Gornowicz B, Brzostowski H. Qualitative and quantitative studies on the ultrastructure of ovine pinealocytes during postnatal development. *Neuro Endocrinol Lett* 2004;25:127–134. [PubMed: 15159696]
- Li M, Chen L, Lee DH, Yu LC, Zhang Y. The role of intracellular amyloid beta in Alzheimer's disease. *Prog Neurobiol* 2007;83:131–139. [PubMed: 17889422]
- Li X, Valencia A, Sapp E, Masso N, Alexander J, Reeves P, Kegel KB, Aronin N, Difiglia M. Aberrant Rab11-dependent trafficking of the neuronal glutamate transporter EAAC1 causes oxidative stress and cell death in Huntington's disease. *J Neurosci* 2010;30:4552–4561. [PubMed: 20357106]
- Li Y, Chin LS, Levey AI, Li L. Huntingtin-associated protein 1 interacts with hepatocyte growth factor-regulated tyrosine kinase substrate and functions in endosomal trafficking. *J Biol Chem* 2002;277:28212–28221. [PubMed: 12021262]
- Liao G, Yao Y, Liu J, Yu Z, Cheung S, Xie A, Liang X, Bi X. Cholesterol accumulation is associated with lysosomal dysfunction and autophagic stress in *Npc1* ^{-/-} mouse brain. *Am J Pathol* 2007;171:962–975. [PubMed: 17631520]
- Lieberman AR. The axon reaction: a review of the principal features of perikaryal responses to axon injury. *Int Rev Neurobiol* 1971;14:49–124. [PubMed: 4948651]
- Liste I, Bernard V, Bloch B. Acute and chronic acetylcholinesterase inhibition regulates in vivo the localization and abundance of muscarinic receptors m2 and m4 at the cell surface and in the cytoplasm of striatal neurons. *Mol Cell Neurosci* 2002;20:244–256. [PubMed: 12093157]
- Liu JP, Tang Y, Zhou S, Toh BH, McLean C, Li H. Cholesterol involvement in the pathogenesis of neurodegenerative diseases. *Mol Cell Neurosci*. 2010
- Liu Y, Jones M, Hingtgen CM, Bu G, Laribee N, Tanzi RE, Moir RD, Nath A, He JJ. Uptake of HIV-1 tat protein mediated by low-density lipoprotein receptor-related protein disrupts the neuronal metabolic balance of the receptor ligands. *Nat Med* 2000;6:1380–1387. [PubMed: 11100124]
- Lively S, Brown IR. The extracellular matrix protein SC1/Hevin localizes to multivesicular bodies in Bergmann glial fibers in the adult rat cerebellum. *Neurochem Res* 2010;35:315–322. [PubMed: 19757034]
- Lloyd TE, Atkinson R, Wu MN, Zhou Y, Pennetta G, Bellen HJ. Hrs regulates endosome membrane invagination and tyrosine kinase receptor signaling in *Drosophila*. *Cell* 2002;108:261–269. [PubMed: 11832215]
- Longva KE, Blystad FD, Stang E, Larsen AM, Johannessen LE, Madshus IH. Ubiquitination and proteasomal activity is required for transport of the EGF receptor to inner membranes of multivesicular bodies. *J Cell Biol* 2002;156:843–854. [PubMed: 11864992]
- Lusa S, Blom TS, Eskelinen EL, Kuismanen E, Mansson JE, Simons K, Ikonen E. Depletion of rafts in late endocytic membranes is controlled by NPC1-dependent recycling of cholesterol to the plasma membrane. *J Cell Sci* 2001;114:1893–1900. [PubMed: 11329376]

- Luzio JP, Bright NA, Pryor PR. The role of calcium and other ions in sorting and delivery in the late endocytic pathway. *Biochem Soc Trans* 2007;35:1088–1091. [PubMed: 17956286]
- Luzio JP, Piper SC, Bowers K, Parkinson MD, Lehner PJ, Bright NA. ESCRT proteins and the regulation of endocytic delivery to lysosomes. *Biochem Soc Trans* 2009;37:178–180. [PubMed: 19143626]
- Magalhaes AC, Baron GS, Lee KS, Steele-Mortimer O, Dorward D, Prado MA, Caughey B. Uptake and neuritic transport of scrapie prion protein coincident with infection of neuronal cells. *J Neurosci* 2005;25:5207–5216. [PubMed: 15917460]
- Maranto AR. Neuronal mapping: a photooxidation reaction makes Lucifer yellow useful for electron microscopy. *Science* 1982;217:953–955. [PubMed: 7112109]
- Maresch C, Granzow H, Negatsch A, Klupp BG, Fuchs W, Teifke JP, Mettenleiter TC. Ultrastructural analysis of virion formation and anterograde intraaxonal transport of the alphaherpesvirus pseudorabies virus in primary neurons. *J Virol*. 2010
- Marijanovic Z, Caputo A, Campana V, Zurzolo C. Identification of an intracellular site of prion conversion. *PLoS Pathog* 2009;5:e1000426. [PubMed: 19424437]
- Martinez-Murillo R, Fernandez T, Alguacil MM, Aguado F, Achaval M, Bovolenta P, Rodrigo J, Nieto-Sampedro M. Subcellular localization of nerve growth factor receptors in identified cells of the rat nucleus basalis magnocellularis: an immunocytochemical study. *Neuroscience* 1991;42:463–472. [PubMed: 1654534]
- Marty S, Peschanski M. Fine structural alteration in target-deprived axonal terminals in the rat thalamus. *Neuroscience* 1994;62:1121–1132. [PubMed: 7845590]
- Mathivanan S, Ji H, Simpson RJ. Exosomes: extracellular organelles important in intercellular communication. *J Proteomics* 2010;73:1907–1920. [PubMed: 20601276]
- Matsuda N, Tanaka K. Does impairment of the ubiquitin-proteasome system or the autophagy-lysosome pathway predispose individuals to neurodegenerative disorders such as Parkinson's disease? *J Alzheimers Dis* 2010;19:1–9. [PubMed: 20061621]
- Matsuo H, Chevallier J, Mayran N, Le Blanc I, Ferguson C, Faure J, Blanc NS, Matile S, Dubochet J, Sadoul R, Parton RG, Vilbois F, Gruenberg J. Role of LBPA and Alix in multivesicular liposome formation and endosome organization. *Science* 2004;303:531–534. [PubMed: 14739459]
- Matthes MT, LaVail MM. Inherited retinal dystrophy in the RCS rat: composition of the outer segment debris zone. *Prog Clin Biol Res* 1989;314:315–330. [PubMed: 2608665]
- Matthews MR, Raisman G. A light and electron microscopic study of the cellular response to axonal injury in the superior cervical ganglion of the rat. *Proc R Soc Lond B Biol Sci* 1972;181:43–79. [PubMed: 4402334]
- Maxfield FR, McGraw TE. Endocytic recycling. *Nat Rev Mol Cell Biol* 2004;5:121–132. [PubMed: 15040445]
- Mayor S, Pagano RE. Pathways of clathrin-independent endocytosis. *Nat Rev Mol Cell Biol* 2007;8:603–612. [PubMed: 17609668]
- Mayor S, Presley JF, Maxfield FR. Sorting of membrane components from endosomes and subsequent recycling to the cell surface occurs by a bulk flow process. *J Cell Biol* 1993;121:1257–1269. [PubMed: 8509447]
- McCaffrey G, Welker J, Scott J, der Salm L, Grimes ML. High-resolution fractionation of signaling endosomes containing different receptors. *Traffic* 2009;10:938–950. [PubMed: 19416476]
- McDonald B, Martin-Serrano J. No strings attached: the ESCRT machinery in viral budding and cytokinesis. *J Cell Sci* 2009;122:2167–2177. [PubMed: 19535732]
- McKanna JA, Haigler HT, Cohen S. Hormone receptor topology and dynamics: morphological analysis using ferritin-labeled epidermal growth factor. *Proc Natl Acad Sci U S A* 1979;76:5689–5693. [PubMed: 230489]
- Michelet X, Djeddi A, Legouis R. Developmental and cellular functions of the ESCRT machinery in pluricellular organisms. *Biol Cell* 2010;102:191–202. [PubMed: 20059450]
- Milner TA, Lee A, Aicher SA, Rosin DL. Hippocampal alpha2a-adrenergic receptors are located predominantly presynaptically but are also found postsynaptically and in selective astrocytes. *J Comp Neurol* 1998;395:310–327. [PubMed: 9596526]

- Mironov A Jr, Latawiec D, Wille H, Bouzamondo-Bernstein E, Legname G, Williamson RA, Burton D, DeArmond SJ, Prusiner SB, Peters PJ. Cytosolic prion protein in neurons. *J Neurosci* 2003;23:7183–7193. [PubMed: 12904479]
- Miwako I, Yamamoto A, Kitamura T, Nagayama K, Ohashi M. Cholesterol requirement for cation-independent mannose 6-phosphate receptor exit from multivesicular late endosomes to the Golgi. *J Cell Sci* 2001;114:1765–1776. [PubMed: 11309206]
- Mobius W, van Donselaar E, Ohno-Iwashita Y, Shimada Y, Heijnen HF, Slot JW, Geuze HJ. Recycling compartments and the internal vesicles of multivesicular bodies harbor most of the cholesterol found in the endocytic pathway. *Traffic* 2003;4:222–231. [PubMed: 12694561]
- Monteiro RA. Age-related quantitative changes in the organelles of rat neocerebellar Purkinje cells. *Histol Histopathol* 1991;6:9–20. [PubMed: 1806060]
- Montiani-Ferreira F, Fischer A, Cernuda-Cernuda R, Kiupel M, DeGrip WJ, Sherry D, Cho SS, Shaw GC, Evans MG, Hocking PM, Petersen-Jones SM. Detailed histopathologic characterization of the retinopathy, globe enlarged (rge) chick phenotype. *Mol Vis* 2005;11:11–27. [PubMed: 15660021]
- Morales CR, Zhao Q, Lefrancois S. Biogenesis of lysosomes by endocytic flow of plasma membrane. *Biocell* 1999;23:149–160. [PubMed: 10904540]
- Morita E, Sundquist WI. Retrovirus budding. *Annu Rev Cell Dev Biol* 2004;20:395–425. [PubMed: 15473846]
- Morozov YM, Freund TF. Post-natal development of type 1 cannabinoid receptor immunoreactivity in the rat hippocampus. *Eur J Neurosci* 2003;18:1213–1222. [PubMed: 12956720]
- Morris GP, Steel CG. Ultrastructure of neurosecretory cells in the pars intercerebralis of *Rhodnius prolixus* (Hemiptera). *Tissue Cell* 1975;7:73–90. [PubMed: 47198]
- Morris GP, Steel CG. Sequence of ultrastructural changes induced by activation in the posterior neurosecretory cells in the brain of *Rhodnius prolixus* with special reference to the role of lysosomes. *Tissue Cell* 1977;9:547–561. [PubMed: 337573]
- Morris JF, Dyball RE. A quantitative study of the ultrastructural changes in the hypothalamo-neurohypophyseal system during and after experimentally induced hypersecretion. *Cell Tissue Res* 1974;149:525–535. [PubMed: 4367857]
- Mukherjee S, Ghosh RN, Maxfield FR. Endocytosis. *Physiol Rev* 1997;77:759–803. [PubMed: 9234965]
- Mullock BM, Bright NA, Fearon CW, Gray SR, Luzio JP. Fusion of lysosomes with late endosomes produces a hybrid organelle of intermediate density and is NSF dependent. *J Cell Biol* 1998;140:591–601. [PubMed: 9456319]
- Murk JL, Stoorvogel W, Kleijmeer MJ, Geuze HJ. The plasticity of multivesicular bodies and the regulation of antigen presentation. *Semin Cell Dev Biol* 2002;13:303–311. [PubMed: 12243730]
- Murphy RF. Maturation models for endosome and lysosome biogenesis. *Trends Cell Biol* 1991;1:77–82. [PubMed: 14731781]
- Myromslien FD, Grovdal LM, Raiborg C, Stenmark H, Madhus IH, Stang E. Both clathrin-positive and -negative coats are involved in endosomal sorting of the EGF receptor. *Exp Cell Res* 2006;312:3036–3048. [PubMed: 16859684]
- Nakadate K, Noda T, Sakakibara S, Kumamoto K, Matsuura T, Joyce JN, Ueda S. Progressive dopaminergic neurodegeneration of substantia nigra in the zitter mutant rat. *Acta Neuropathol* 2006;112:64–73. [PubMed: 16609850]
- Neutra MR, Ciechanover A, Owen LS, Lodish HF. Intracellular transport of transferrin and asialoorosomucoid-colloidal gold conjugates to lysosomes after receptor-mediated endocytosis. *J Histochem Cytochem* 1985;33:1134–1144. [PubMed: 2997327]
- Ng EL, Ng JJ, Liang F, Tang BL. Rab22B is expressed in the CNS astroglia lineage and plays a role in epidermal growth factor receptor trafficking in A431 cells. *J Cell Physiol* 2009;221:716–728. [PubMed: 19725050]
- Nickerson DP, Russell MR, Odorizzi G. A concentric circle model of multivesicular body cargo sorting. *EMBO Rep* 2007;8:644–650. [PubMed: 17603537]
- Nikko E, Andre B. Evidence for a direct role of the Doa4 deubiquitinating enzyme in protein sorting into the MVB pathway. *Traffic* 2007;8:566–581. [PubMed: 17376168]

- Nirenberg MJ, Vaughan RA, Uhl GR, Kuhar MJ, Pickel VM. The dopamine transporter is localized to dendritic and axonal plasma membranes of nigrostriatal dopaminergic neurons. *J Neurosci* 1996;16:436–447. [PubMed: 8551328]
- Nixon RA. Endosome function and dysfunction in Alzheimer's disease and other neurodegenerative diseases. *Neurobiol Aging* 2005;26:373–382. [PubMed: 15639316]
- Nixon RA, Cataldo AM. The endosomal-lysosomal system of neurons: new roles. *Trends Neurosci* 1995;18:489–496. [PubMed: 8592758]
- Nixon RA, Wegiel J, Kumar A, Yu WH, Peterhoff C, Cataldo A, Cuervo AM. Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study. *J Neuropathol Exp Neurol* 2005;64:113–122. [PubMed: 15751225]
- Nixon RA, Yang DS, Lee JH. Neurodegenerative lysosomal disorders: a continuum from development to late age. *Autophagy* 2008;4:590–599. [PubMed: 18497567]
- Novoa RR, Calderita G, Arranz R, Fontana J, Granzow H, Risco C. Virus factories: associations of cell organelles for viral replication and morphogenesis. *Biol Cell* 2005;97:147–172. [PubMed: 15656780]
- Odorizzi G, Babst M, Emr SD. Fab1p PtdIns(3)P 5-kinase function essential for protein sorting in the multivesicular body. *Cell* 1998;95:847–858. [PubMed: 9865702]
- Offe K, Dodson SE, Shoemaker JT, Fritz JJ, Gearing M, Levey AI, Lah JJ. The lipoprotein receptor LR11 regulates amyloid beta production and amyloid precursor protein traffic in endosomal compartments. *J Neurosci* 2006;26:1596–1603. [PubMed: 16452683]
- Ogawa T, Suzuki M, Matoh K, Sasaki K. Three-dimensional electron microscopic studies of the transitional oligodendrocyte associated with the initial stage of myelination in developing rat hippocampal fimbria. *Brain Res Dev Brain Res* 2004;148:207–212.
- Ohashi M, Miwako I, Yamamoto A, Nagayama K. Arrested maturing multivesicular endosomes observed in a Chinese hamster ovary cell mutant, LEX2, isolated by repeated flow-cytometric cell sorting. *J Cell Sci* 2000;113(Pt 12):2187–2205. [PubMed: 10825292]
- Omata T, Schatzle W. Afferent and efferent nerve endings of the outer hair cells in the rabbit. *Arch Otorhinolaryngol* 1980;229:175–182. [PubMed: 7469942]
- Oppenheim, RW.; von Bartheld, CS. Programmed cell death and neurotrophic factors. In: Squire, L., editor. *Fundamental Neuroscience*. Elsevier; San Diego: 2008. p. 437–467.
- Ostroff LE, Cain CK, Bedont J, Monfils MH, Ledoux JE. Fear and safety learning differentially affect synapse size and dendritic translation in the lateral amygdala. *Proc Natl Acad Sci U S A*. 2010
- Overly CC, Lee KD, Berthiaume E, Hollenbeck PJ. Quantitative measurement of intraorganelle pH in the endosomal-lysosomal pathway in neurons by using ratiometric imaging with pyranine. *Proc Natl Acad Sci U S A* 1995;92:3156–3160. [PubMed: 7724533]
- Palay SL. The fine structure of secretory neurons in the preoptic nucleus of the goldfish (*Carassius auratus*). *Anat Rec* 1960;138:417–443. [PubMed: 13732184]
- Palay SL, Palade GE. The fine structure of neurons. *J Biophys Biochem Cytol* 1955;1:69–88. [PubMed: 14381429]
- Pannese E, Ledda M, Arcidiacono G, Frattola D, Rigamonti L, Procacci P. Qualitative and quantitative observations on the structure of the Schwann cells in myelinated fibres. *Acta Anat (Basel)* 1988a; 131:314–326. [PubMed: 3376737]
- Pannese E, Rigamonti L, Procacci P, Ledda M, Arcidiacono G, Frattola D. The structure of Schwann cells in unmyelinated fibres. A qualitative and quantitative electron microscope study. *J Submicrosc Cytol Pathol* 1988b;20:325–333. [PubMed: 3395971]
- Pappas GD, Purpura DP. Fine structure of dendrites in the superficial neocortical neuropil. *Exp Neurol* 1961;4:507–530. [PubMed: 14483755]
- Parkinson N, Ince PG, Smith MO, Highley R, Skibinski G, Andersen PM, Morrison KE, Pall HS, Hardiman O, Collinge J, Shaw PJ, Fisher EM. ALS phenotypes with mutations in CHMP2B (charged multivesicular body protein 2B). *Neurology* 2006;67:1074–1077. [PubMed: 16807408]
- Parton RG, Ockleford CD, Critchley DR. A study of the mechanism of internalisation of tetanus toxin by primary mouse spinal cord cultures. *J Neurochem* 1987;49:1057–1068. [PubMed: 3114428]
- Parton RG, Simons K, Dotti CG. Axonal and dendritic endocytic pathways in cultured neurons. *J Cell Biol* 1992;119:123–137. [PubMed: 1527164]

- Pastorino L, Sun A, Lu PJ, Zhou XZ, Balastik M, Finn G, Wulf G, Lim J, Li SH, Li X, Xia W, Nicholson LK, Lu KP. The prolyl isomerase Pin1 regulates amyloid precursor protein processing and amyloid-beta production. *Nature* 2006;440:528–534. [PubMed: 16554819]
- Paula-Barbosa MM, Borges MM, Cadete-Leite A, Tavares MA. Giant multivesicular bodies in the rat hippocampal pyramidal cells after chronic alcohol consumption. *Neurosci Lett* 1986;64:345–349. [PubMed: 3008045]
- Paula-Barbosa MM, Cardoso R, Mota, Faria R, Cruz C. Multivesicular bodies in cortical dendrites of two patients with Alzheimer's disease. *J Neurol Sci* 1978;36:259–264. [PubMed: 650258]
- Pauziene N, Pauza DH. Electron microscopic study of intrinsic cardiac ganglia in the adult human. *Ann Anat* 2003;185:135–148. [PubMed: 12725437]
- Pentchev PG, Boothe AD, Kruth HS, Weintroub H, Stivers J, Brady RO. A genetic storage disorder in BALB/C mice with a metabolic block in esterification of exogenous cholesterol. *J Biol Chem* 1984;259:5784–5791. [PubMed: 6325448]
- Perez SE, Lazarov O, Koprich JB, Chen EY, Rodriguez-Menendez V, Lipton JW, Sisodia SS, Mufson EJ. Nigrostriatal dysfunction in familial Alzheimer's disease-linked APP^{swe}/PS1^{DeltaE9} transgenic mice. *J Neurosci* 2005;25:10220–10229. [PubMed: 16267229]
- Peters, A.; Palay, SL.; Webster, H. d. *The Fine Structure of the Nervous System*. Oxford University Press; New York: 1991.
- Pickel VM, Joh TH, Chan J. Substance P in the rat nucleus accumbens: ultrastructural localization in axon terminals and their relation to dopaminergic afferents. *Brain Res* 1988;444:247–264. [PubMed: 2451997]
- Pioro EP, Ribeiro-Da-Silva A, Cuello AC. Similarities in the ultrastructural distribution of nerve growth factor receptor-like immunoreactivity in cerebellar Purkinje cells of the neonatal and colchicine-treated adult rat. *J Comp Neurol* 1991;305:189–200. [PubMed: 1851186]
- Piper RC, Katzmman DJ. Biogenesis and function of multivesicular bodies. *Annu Rev Cell Dev Biol*. 2007
- Piper RC, Luzio JP. Late endosomes: sorting and partitioning in multivesicular bodies. *Traffic* 2001;2:612–621. [PubMed: 11555415]
- Potolicchio I, Carven GJ, Xu X, Stipp C, Riese RJ, Stern LJ, Santambrogio L. Proteomic analysis of microglia-derived exosomes: metabolic role of the aminopeptidase CD13 in neuropeptide catabolism. *J Immunol* 2005;175:2237–2243. [PubMed: 16081791]
- Povlishock JT. The fine structure of the axons and growth cones of the human fetal cerebral cortex. *Brain Res* 1976;114:379–379i. [PubMed: 953762]
- Putz U, Howitt J, Lackovic J, Foot N, Kumar S, Silke J, Tan SS. Nedd4 family-interacting protein 1 (Ndfip1) is required for the exosomal secretion of Nedd4 family proteins. *J Biol Chem* 2008;283:32621–32627. [PubMed: 18819914]
- Raiborg C, Rusten TE, Stenmark H. Protein sorting into multivesicular endosomes. *Curr Opin Cell Biol* 2003;15:446–455. [PubMed: 12892785]
- Rajendran L, Honsho M, Zahn TR, Keller P, Geiger KD, Verkade P, Simons K. Alzheimer's disease beta-amyloid peptides are released in association with exosomes. *Proc Natl Acad Sci U S A* 2006;103:11172–11177. [PubMed: 16837572]
- Reggiori F, Pelham HR. Sorting of proteins into multivesicular bodies: ubiquitin-dependent and -independent targeting. *EMBO J* 2001;20:5176–5186. [PubMed: 11566881]
- Ren J, Pashkova N, Winistorfer S, Piper RC. DOA1/UFD3 plays a role in sorting ubiquitinated membrane proteins into multivesicular bodies. *J Biol Chem* 2008;283:21599–21611. [PubMed: 18508771]
- Richardson GP, Forge A, Kros CJ, Fleming J, Brown SD, Steel KP. Myosin VIIA is required for aminoglycoside accumulation in cochlear hair cells. *J Neurosci* 1997;17:9506–9519. [PubMed: 9391006]
- Richter C, West M, Odorizzi G. Dual mechanisms specify Doa4-mediated deubiquitination at multivesicular bodies. *EMBO J* 2007;26:2454–2464. [PubMed: 17446860]
- Rind HB, Butowt R, von Bartheld CS. Synaptic targeting of retrogradely transported trophic factors in motoneurons: comparison of glial cell line-derived neurotrophic factor, brain-derived

- neurotrophic factor, and cardiotrophin-1 with tetanus toxin. *J Neurosci* 2005;25:539–549. [PubMed: 15659589]
- Rink J, Ghigo E, Kalaidzidis Y, Zerial M. Rab conversion as a mechanism of progression from early to late endosomes. *Cell* 2005;122:735–749. [PubMed: 16143105]
- Robert F, Hevor TK. Abnormal organelles in cultured astrocytes are largely enhanced by streptomycin and intensively by gentamicin. *Neuroscience* 2007;144:191–197. [PubMed: 17049172]
- Roberts RC, Ribak CE. The ultrastructure of the central nucleus of the inferior colliculus of the genetically epilepsy-prone rat. *Epilepsy Res* 1988;2:196–214. [PubMed: 2848697]
- Rodriguez EM, Blazquez JL, Pastor FE, Pelaez B, Pena P, Peruzzo B, Amat P. Hypothalamic tanycytes: a key component of brain-endocrine interaction. *Int Rev Cytol* 2005;247:89–164. [PubMed: 16344112]
- Roizin L, Nishikawa K, Koizumi J, Keoseian S. The fine structure of the multivesicular body and their relationship to the ultracellular constituents of the central nervous system. *J Neuropathol Exp Neurol* 1967;26:223–249. [PubMed: 4960545]
- Rosenbluth J, Wissig SL. The distribution of exogenous ferritin in toad spinal ganglia and the mechanism of Itsuptake by neurons. *J Cell Biol* 1964;23:307–325. [PubMed: 14222816]
- Rossiter JP, Hsu L, Jackson AC. Selective vulnerability of dorsal root ganglia neurons in experimental rabies after peripheral inoculation of CVS-11 in adult mice. *Acta Neuropathol* 2009;118:249–259. [PubMed: 19252919]
- Row PE, Prior IA, McCullough J, Clague MJ, Urbe S. The ubiquitin isopeptidase UBPY regulates endosomal ubiquitin dynamics and is essential for receptor down-regulation. *J Biol Chem* 2006;281:12618–12624. [PubMed: 16520378]
- Rubinsztein DC. The roles of intracellular protein-degradation pathways in neurodegeneration. *Nature* 2006;443:780–786. [PubMed: 17051204]
- Russell MR, Nickerson DP, Odorizzi G. Molecular mechanisms of late endosome morphology, identity and sorting. *Curr Opin Cell Biol* 2006;18:422–428. [PubMed: 16781134]
- Rusten TE, Filimonenko M, Rodahl LM, Stenmark H, Simonsen A. ESCRTing autophagic clearance of aggregating proteins. *Autophagy* 2008;4:233–236.
- Rusten TE, Simonsen A. ESCRT functions in autophagy and associated disease. *Cell Cycle* 2008;7:1166–1172. [PubMed: 18418046]
- Saito K. Fine structure of the sensory epithelium of guinea-pig organ of Corti: subsurface cisternae and lamellar bodies in the outer hair cells. *Cell Tissue Res* 1983;229:467–481. [PubMed: 6839349]
- Saito N, Okada Y, Noda Y, Kinoshita Y, Kondo S, Hirokawa N. KIFC2 is a novel neuron-specific C-terminal type kinesin superfamily motor for dendritic transport of multivesicular body-like organelles. *Neuron* 1997;18:425–438. [PubMed: 9115736]
- Sandow SL, Heydon K, Weible MW 2nd, Reynolds AJ, Bartlett SE, Hendry IA. Signalling organelle for retrograde axonal transport of internalized neurotrophins from the nerve terminal. *Immunol Cell Biol* 2000;78:430–435. [PubMed: 10947869]
- Sapp E, Schwarz C, Chase K, Bhide PG, Young AB, Penney J, Vonsattel JP, Aronin N, DiFiglia M. Huntingtin localization in brains of normal and Huntington's disease patients. *Ann Neurol* 1997;42:604–612. [PubMed: 9382472]
- Saxena S, Howe CL, Cosgaya JM, Steiner P, Hirling H, Chan JR, Weis J, Kruttgen A. Differential endocytic sorting of p75NTR and TrkA in response to NGF: a role for late endosomes in TrkA trafficking. *Mol Cell Neurosci* 2005;28:571–587. [PubMed: 15737746]
- Schaumburg HH, Wisniewski HM, Spencer PS. Ultrastructural studies of the dying-back process. I. Peripheral nerve terminal and axon degeneration in systemic acrylamide intoxication. *J Neuropathol Exp Neurol* 1974;33:260–284. [PubMed: 4362700]
- Schindowski K, Belarbi K, Buee L. Neurotrophic factors in Alzheimer's disease: role of axonal transport. *Genes Brain Behav* 2008;7(Suppl 1):43–56. [PubMed: 18184369]
- Schmidt RE, Parvin CA, Green KG. Synaptic ultrastructural alterations anticipate the development of neuroaxonal dystrophy in sympathetic ganglia of aged and diabetic mice. *J Neuropathol Exp Neurol* 2008;67:1166–1186. [PubMed: 19018240]

- Schmied R, Holtzman E. A phosphatase activity and a synaptic vesicle antigen in multivesicular bodies of frog retinal photoreceptor terminals. *J Neurocytol* 1987;16:627–637. [PubMed: 2826708]
- Schnapp BJ. Retroactive motors. *Neuron* 1997;18:523–526. [PubMed: 9136762]
- Schroer JA, Plurad SB, Schmidt RE. Fine structure of presynaptic axonal terminals in sympathetic autonomic ganglia of aging and diabetic human subjects. *Synapse* 1992;12:1–13. [PubMed: 1411960]
- Schulze-Bonhage A, Wiemann M, Altrup U, Wittkowski W, Speckmann EJ. Epileptic discharges induced by pentylenetetrazol: ultrastructural alterations in identified neurons and glial cells (Helix pomatia). *Epilepsy Res* 1995;22:23–34. [PubMed: 8565964]
- Schwab M, Thoenen H. Selective trans-synaptic migration of tetanus toxin after retrograde axonal transport in peripheral sympathetic nerves: a comparison with nerve growth factor. *Brain Res* 1977;122:459–474. [PubMed: 66083]
- Schwab ME. Ultrastructural localization of a nerve growth factor-horseradish peroxidase (NGF-HRP) coupling product after retrograde axonal transport in adrenergic neurons. *Brain Res* 1977;130:190–196. [PubMed: 69476]
- Schwab ME, Suda K, Thoenen H. Selective retrograde transsynaptic transfer of a protein, tetanus toxin, subsequent to its retrograde axonal transport. *J Cell Biol* 1979;82:798–810. [PubMed: 92475]
- Schwab ME, Thoenen H. Electron microscopic evidence for a transsynaptic migration of tetanus toxin in spinal cord motoneurons: an autoradiographic and morphometric study. *Brain Res* 1976;105:213–227. [PubMed: 1260442]
- Schwab ME, Thoenen H. Selective binding, uptake, and retrograde transport of tetanus toxin by nerve terminals in the rat iris. An electron microscope study using colloidal gold as a tracer. *J Cell Biol* 1978;77:1–13. [PubMed: 659508]
- Scita G, Di Fiore PP. The endocytic matrix. *Nature* 2010;463:464–473. [PubMed: 20110990]
- Shirane M, Nakayama KI. Protrudin induces neurite formation by directional membrane trafficking. *Science* 2006;314:818–821. [PubMed: 17082457]
- Sigismund S, Woelk T, Puri C, Maspero E, Tacchetti C, Transidico P, Di Fiore PP, Polo S. Clathrin-independent endocytosis of ubiquitinated cargos. *Proc Natl Acad Sci U S A* 2005;102:2760–2765. [PubMed: 15701692]
- Sikora J, Pavlu-Pereira H, Elleder M, Roelofs H, Wevers RA. Seven novel acid sphingomyelinase gene mutations in Niemann-Pick type A and B patients. *Ann Hum Genet* 2003;67:63–70. [PubMed: 12556236]
- Simonaro CM, Desnick RJ, McGovern MM, Wasserstein MP, Schuchman EH. The demographics and distribution of type B Niemann-Pick disease: novel mutations lead to new genotype/phenotype correlations. *Am J Hum Genet* 2002;71:1413–1419. [PubMed: 12369017]
- Skibinski G, Parkinson NJ, Brown JM, Chakrabarti L, Lloyd SL, Hummerich H, Nielsen JE, Hodges JR, Spillantini MG, Thusgaard T, Brandner S, Brun A, Rossor MN, Gade A, Johannsen P, Sorensen SA, Gydesen S, Fisher EM, Collinge J. Mutations in the endosomal ESCRTIII-complex subunit CHMP2B in frontotemporal dementia. *Nat Genet* 2005;37:806–808. [PubMed: 16041373]
- Smalheiser NR. Exosomal transfer of proteins and RNAs at synapses in the nervous system. *Biol Direct* 2007;2:35. [PubMed: 18053135]
- Smith RA, Wubetu T. Cytoplasmic changes in primary cultured adult mouse sensory neurons induced by ethanol and acetaldehyde treatments. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1991;61:209–215. [PubMed: 1685282]
- Smith RS. The short term accumulation of axonally transported organelles in the region of localized lesions of single myelinated axons. *J Neurocytol* 1980;9:39–65. [PubMed: 6162922]
- Smith SM, Renden R, von Gersdorff H. Synaptic vesicle endocytosis: fast and slow modes of membrane retrieval. *Trends Neurosci* 2008;31:559–568. [PubMed: 18817990]
- Sorkin A, von Zastrow M. Signal transduction and endocytosis: close encounters of many kinds. *Nat Rev Mol Cell Biol* 2002;3:600–614. [PubMed: 12154371]

- Sorkin A, von Zastrow M. Endocytosis and signalling: intertwining molecular networks. *Nat Rev Mol Cell Biol* 2009;10:609–622. [PubMed: 19696798]
- Sotelo JR, Porter KR. An electron microscope study of the rat ovum. *J Biophys Biochem Cytol* 1959;5:327–342. [PubMed: 13654454]
- Spencer PS, Kim MS, Sabri MI. Aromatic as well as aliphatic hydrocarbon solvent axonopathy. *Int J Hyg Environ Health* 2002;205:131–136. [PubMed: 12018006]
- Steindler DA, Cooper NG. Wheat germ agglutinin binding sites in the adult mouse cerebellum: light and electron microscopic studies. *J Comp Neurol* 1986;249:170–185. [PubMed: 3755449]
- Stieber A, Hickey WF, Hogue-Angeletti R, Gonatas NK. Endocytosis of nerve growth factor by 'differentiated' PC12 cells studied by quantitative ultrastructural autoradiography. *Brain Res* 1984;310:223–234. [PubMed: 6488018]
- Stoorvogel W, Strous GJ, Geuze HJ, Oorschot V, Schwartz AL. Late endosomes derive from early endosomes by maturation. *Cell* 1991;65:417–427. [PubMed: 1850321]
- Stuffers S, Sem Wegner C, Stenmark H, Brech A. Multivesicular endosome biogenesis in the absence of ESCRTs. *Traffic* 2009;10:925–937. [PubMed: 19490536]
- Sun W, Vida TA, Sirisaengtaksin N, Merrill SA, Hanson PI, Bean AJ. Cell-Free Reconstitution of Multivesicular Body Formation and Receptor Sorting. *Traffic*. 2010
- Suzuki H, Sakiyama T, Harada N, Abe M, Tadokoro M. Pathologic changes of glial cells in murine model of Niemann-Pick disease type C: immunohistochemical, lectin-histochemical and ultrastructural observations. *Pediatr Int* 2003;45:1–4. [PubMed: 12654060]
- Swanwick CC, Shapiro ME, Vicini S, Wenthold RJ. Flotillin-1 promotes formation of glutamatergic synapses in hippocampal neurons. *Dev Neurobiol* 2010;70:875–883. [PubMed: 20669324]
- Sweeney NT, Brenman JE, Jan YN, Gao FB. The coiled-coil protein shrub controls neuronal morphogenesis in *Drosophila*. *Curr Biol* 2006;16:1006–1011. [PubMed: 16713958]
- Takahashi RH, Almeida CG, Kearney PF, Yu F, Lin MT, Milner TA, Gouras GK. Oligomerization of Alzheimer's beta-amyloid within processes and synapses of cultured neurons and brain. *J Neurosci* 2004;24:3592–3599. [PubMed: 15071107]
- Takahashi RH, Milner TA, Li F, Nam EE, Edgar MA, Yamaguchi H, Beal MF, Xu H, Greengard P, Gouras GK. Intraneuronal Alzheimer abeta42 accumulates in multivesicular bodies and is associated with synaptic pathology. *Am J Pathol* 2002;161:1869–1879. [PubMed: 12414533]
- Tamai K, Toyoshima M, Tanaka N, Yamamoto N, Owada Y, Kiyonari H, Murata K, Ueno Y, Ono M, Shimosegawa T, Yaegashi N, Watanabe M, Sugamura K. Loss of hrs in the central nervous system causes accumulation of ubiquitinated proteins and neurodegeneration. *Am J Pathol* 2008;173:1806–1817. [PubMed: 19008375]
- Tamboli IY, Barth E, Christian L, Siepmann M, Kumar S, Singh S, Tolksdorf K, Heneka MT, Lutjohann D, Wunderlich P, Walter J. Statins promote the degradation of extracellular amyloid {beta}-peptide by microglia via stimulation of exosome-associated insulin-degrading enzyme (IDE) secretion. *J Biol Chem* 2010;285:37405–37414. [PubMed: 20876579]
- Teichberg S, Holtzman E. Axonal agranular reticulum and synaptic vesicles in cultured embryonic chick sympathetic neurons. *J Cell Biol* 1973;57:88–108. [PubMed: 4347980]
- Thelen K, Georg T, Bertuch S, Zelina P, Pollerberg GE. Ubiquitination and endocytosis of cell adhesion molecule DM-GRASP regulate its cell surface presence and affect its role for axon navigation. *J Biol Chem* 2008;283:32792–32801. [PubMed: 18790729]
- Theodosius DT. Secretion-related accumulation of horseradish peroxidase in magnocellular cell bodies of the rat supraoptic nucleus. *Brain Res* 1982;233:3–16. [PubMed: 7059805]
- Theos AC, Truschel ST, Tenza D, Hurbain I, Harper DC, Berson JF, Thomas PC, Raposo G, Marks MS. A lumenal domain-dependent pathway for sorting to intralumenal vesicles of multivesicular endosomes involved in organelle morphogenesis. *Dev Cell* 2006;10:343–354. [PubMed: 16516837]
- Thery C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. *Nat Rev Immunol* 2002;2:569–579. [PubMed: 12154376]
- Townes-Anderson E, Raviola G. Degeneration and regeneration of autonomic nerve endings in the anterior part of rhesus monkey ciliary muscle. *J Neurocytol* 1978;7:583–600. [PubMed: 102735]

- Trajkovic K, Dhaunchak AS, Goncalves JT, Wenzel D, Schneider A, Bunt G, Nave KA, Simons M. Neuron to glia signaling triggers myelin membrane exocytosis from endosomal storage sites. *J Cell Biol* 2006;172:937–948. [PubMed: 16520383]
- Trajkovic K, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F, Schwille P, Brugger B, Simons M. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science* 2008;319:1244–1247. [PubMed: 18309083]
- Trapp BD, Andrews SB, Cootauco C, Quarles R. The myelin-associated glycoprotein is enriched in multivesicular bodies and periaxonal membranes of actively myelinating oligodendrocytes. *J Cell Biol* 1989;109:2417–2426. [PubMed: 2478568]
- Traub LM. The reverse logic of multivesicular endosomes. *EMBO Rep* 2010;11:79–81. [PubMed: 20062001]
- Truant R, Atwal RS, Desmond C, Munsie L, Tran T. Huntington's disease: revisiting the aggregation hypothesis in polyglutamine neurodegenerative diseases. *FEBS J* 2008;275:4252–4262. [PubMed: 18637947]
- Tsukita S, Ishikawa H. The movement of membranous organelles in axons. Electron microscopic identification of anterogradely and retrogradely transported organelles. *J Cell Biol* 1980;84:513–530. [PubMed: 6153657]
- Turner PT, Harris AB. Ultrastructure of exogenous peroxidase in cerebral cortex. *Brain Res* 1974;74:305–326. [PubMed: 4134532]
- Tytell M. Release of heat shock proteins (Hsps) and the effects of extracellular Hsps on neural cells and tissues. *Int J Hyperthermia* 2005;21:445–455. [PubMed: 16048841]
- Uchil P, Mothes W. Viral entry: a detour through multivesicular bodies. *Nat Cell Biol* 2005;7:641–642. [PubMed: 15990891]
- Urwin H, Authier A, Nielsen JE, Metcalf D, Powell C, Froud K, Malcolm DS, Holm I, Johannsen P, Brown J, Fisher EM, van der Zee J, Bruyland M, Van Broeckhoven C, Collinge J, Brandner S, Futter C, Isaacs AM. Disruption of endocytic trafficking in frontotemporal dementia with CHMP2B mutations. *Hum Mol Genet* 2010a;19:2228–2238. [PubMed: 20223751]
- Urwin H, Josephs KA, Rohrer JD, Mackenzie IR, Neumann M, Authier A, Seelaar H, Van Swieten JC, Brown JM, Johannsen P, Nielsen JE, Holm IE, Dickson DW, Rademakers R, Graff-Radford NR, Parisi JE, Petersen RC, Hatanpaa KJ, White CL 3rd, Weiner MF, Geser F, Van Deerlin VM, Trojanowski JQ, Miller BL, Seeley WW, van der Zee J, Kumar-Singh S, Engelborghs S, De Deyn PP, Van Broeckhoven C, Bigio EH, Deng HX, Halliday GM, Kril JJ, Munoz DG, Mann DM, Pickering-Brown SM, Doodeman V, Adamson G, Ghazi-Noori S, Fisher EM, Holton JL, Revesz T, Rossor MN, Collinge J, Mead S, Isaacs AM. FUS pathology defines the majority of tau- and TDP-43-negative frontotemporal lobar degeneration. *Acta Neuropathol* 2010b;120:33–41. [PubMed: 20490813]
- Utvik JK, Haglerod C, Mylonakou MN, Holen T, Kropf M, Hirling H, Skare O, Laake P, Ottersen OP, Haug FM, Davanger S. Neuronal enriched endosomal protein of 21 kDa colocalizes with glutamate receptor subunit GLUR2/3 at the postsynaptic membrane. *Neuroscience* 2009;158:96–104. [PubMed: 19063943]
- Vajjhala PR, Catchpoole E, Nguyen CH, Kistler C, Munn AL. Vps4 regulates a subset of protein interactions at the multivesicular endosome. *FEBS J* 2007;274:1894–1907. [PubMed: 17408385]
- Valdez G, Akmentin W, Philippidou P, Kuruvilla R, Ginty DD, Halegoua S. Pincher-mediated macroendocytosis underlies retrograde signaling by neurotrophin receptors. *J Neurosci* 2005;25:5236–5247. [PubMed: 15917464]
- Valdez G, Philippidou P, Rosenbaum J, Akmentin W, Shao Y, Halegoua S. Trk-signaling endosomes are generated by Rac-dependent macroendocytosis. *Proc Natl Acad Sci U S A* 2007;104:12270–12275. [PubMed: 17640889]
- Valencia A, Reeves PB, Sapp E, Li X, Alexander J, Kegel KB, Chase K, Aronin N, DiFiglia M. Mutant huntingtin and glycogen synthase kinase 3-beta accumulate in neuronal lipid rafts of a presymptomatic knock-in mouse model of Huntington's disease. *J Neurosci Res* 2010;88:179–190. [PubMed: 19642201]
- van der Zee J, Urwin H, Engelborghs S, Bruyland M, Vandenberghe R, Dermaut B, De Pooter T, Peeters K, Santens P, De Deyn PP, Fisher EM, Collinge J, Isaacs AM, Van Broeckhoven C.

- CHMP2B C-truncating mutations in frontotemporal lobar degeneration are associated with an aberrant endosomal phenotype in vitro. *Hum Mol Genet* 2008;17:313–322. [PubMed: 17956895]
- van Deurs B, Holm PK, Kayser L, Sandvig K, Hansen SH. Multivesicular bodies in HEp-2 cells are maturing endosomes. *Eur J Cell Biol* 1993;61:208–224. [PubMed: 8223712]
- van Meel E, Klumperman J. Imaging and imagination: understanding the endo-lysosomal system. *Histochem Cell Biol* 2008;129:253–266. [PubMed: 18274773]
- van Niel G, Porto-Carreiro I, Simoes S, Raposo G. Exosomes: a common pathway for a specialized function. *J Biochem* 2006;140:13–21. [PubMed: 16877764]
- Vanlandingham PA, Ceresa BP. Rab7 regulates late endocytic trafficking downstream of multivesicular body biogenesis and cargo sequestration. *J Biol Chem* 2009;284:12110–12124. [PubMed: 19265192]
- Vaughan DW, Vincent JM. Ultrastructure of neurons in the auditory cortex of ageing rats: a morphometric study. *J Neurocytol* 1979;8:215–228. [PubMed: 469574]
- Vella LJ, Sharples RA, Nisbet RM, Cappai R, Hill AF. The role of exosomes in the processing of proteins associated with neurodegenerative diseases. *Eur Biophys J* 2008;37:323–332. [PubMed: 18064447]
- Vigh B, Vigh-Teichmann I. Development of the photoreceptor outer segment-like cilia of the CSF-contacting pinealocytes of the ferret (*Putorius furo*). *Arch Histol Cytol* 1993;56:485–493. [PubMed: 8129982]
- Vingtdeux V, Hamdane M, Loyens A, Gele P, Drobeck H, Begard S, Galas MC, Delacourte A, Beauvillain JC, Buee L, Sergeant N. Alkalinizing drugs induce accumulation of amyloid precursor protein by-products in luminal vesicles of multivesicular bodies. *J Biol Chem* 2007;282:18197–18205. [PubMed: 17468104]
- Vitalis T, Laine J, Simon A, Roland A, Leterrier C, Lenkei Z. The type 1 cannabinoid receptor is highly expressed in embryonic cortical projection neurons and negatively regulates neurite growth in vitro. *Eur J Neurosci* 2008;28:1705–1718. [PubMed: 18973587]
- von Bartheld CS. Tracing with radiolabeled neurotrophins. *Methods Mol Biol* 2001;169:195–216. [PubMed: 11142011]
- von Bartheld CS, Byers MR, Williams R, Bothwell M. Anterograde transport of neurotrophins and axodendritic transfer in the developing visual system. *Nature* 1996;379:830–833. [PubMed: 8587607]
- von Bartheld CS, Cunningham DE, Rubel EW. Neuronal tracing with DiI: decalcification, cryosectioning, and photoconversion for light and electron microscopic analysis. *J Histochem Cytochem* 1990;38:725–733. [PubMed: 2185313]
- Vonderheit A, Helenius A. Rab7 associates with early endosomes to mediate sorting and transport of Semliki forest virus to late endosomes. *PLoS Biol* 2005;3:e233. [PubMed: 15954801]
- Vu TQ, Maddipati R, Blute TA, Nehilla BJ, Nusblat L, Desai TA. Peptide-conjugated quantum dots activate neuronal receptors and initiate downstream signaling of neurite growth. *Nano Lett* 2005;5:603–607. [PubMed: 15826094]
- Wang QJ, Ding Y, Kohtz DS, Mizushima N, Cristea IM, Rout MP, Chait BT, Zhong Y, Heintz N, Yue Z. Induction of autophagy in axonal dystrophy and degeneration. *J Neurosci* 2006;26:8057–8068. [PubMed: 16885219]
- Waxman, SG.; Kocsis, JD.; Stys, PK., editors. *The Axon. Structure, Function and Pathophysiology*. Oxford University Press; New York: 1995.
- Weible MW 2nd, Hendry IA. What is the importance of multivesicular bodies in retrograde axonal transport in vivo? *J Neurobiol* 2004;58:230–243. [PubMed: 14704955]
- Weldon PR. Pinocytotic uptake and intracellular distribution of colloidal thorium dioxide by cultured sensory neurites. *J Neurocytol* 1975;4:341–356. [PubMed: 1133592]
- Wessells NK, Luduena MA, Letourneau PC, Wrenn JT, Spooner BS. Thorotrast uptake and transit in embryonic glia, heart fibroblasts and neurons in vitro. *Tissue Cell* 1974;6:757–776. [PubMed: 4477415]
- White IJ, Bailey LM, Aghakhani MR, Moss SE, Futter CE. EGF stimulates annexin 1-dependent inward vesiculation in a multivesicular endosome subpopulation. *Embo J* 2006;25:1–12. [PubMed: 16052208]

- Wilkin M, Tongngok P, Gensch N, Clemence S, Motoki M, Yamada K, Hori K, Taniguchi-Kanai M, Franklin E, Matsuno K, Baron M. *Drosophila* HOPS and AP-3 complex genes are required for a Deltex-regulated activation of notch in the endosomal trafficking pathway. *Dev Cell* 2008;15:762–772. [PubMed: 19000840]
- Williams CS, Chung RA. Ultrastructural effects of DDT on cells grown in vitro. *J Environ Pathol Toxicol Oncol* 1987;7:35–58. [PubMed: 3559916]
- Williams DS, Blest AD. Extracellular shedding of photoreceptor membrane in the open rhabdom of a tipulid fly. *Cell Tissue Res* 1980;205:423–438. [PubMed: 7357581]
- Williams RL. Clean up or go crazy. *Journal of Cell Biology* 2007;179:360.
- Williams RL, Urbe S. The emerging shape of the ESCRT machinery. *Nat Rev Mol Cell Biol* 2007;8:355–368. [PubMed: 17450176]
- Wilson R, Bate C, Boshuizen R, Williams A, Brewer J. Squalastatin alters the intracellular trafficking of a neurotoxic prion peptide. *BMC Neurosci* 2007;8:99. [PubMed: 18034899]
- Winter V, Hauser MT. Exploring the ESCRTing machinery in eukaryotes. *Trends Plant Sci* 2006;11:115–123. [PubMed: 16488176]
- Wittich B, Volkandt W, Zimmermann H. SV2 and o-rab3 remain associated with recycling synaptic vesicles. *J Neurochem* 1994;63:927–937. [PubMed: 7914225]
- Wollert T, Hurley JH. Molecular mechanism of multivesicular body biogenesis by ESCRT complexes. *Nature*. 2010
- Woodman P. ESCRT proteins, endosome organization and mitogenic receptor down-regulation. *Biochem Soc Trans* 2009;37:146–150. [PubMed: 19143620]
- Woodman PG, Futter CE. Multivesicular bodies: co-ordinated progression to maturity. *Curr Opin Cell Biol* 2008;20:408–414. [PubMed: 18502633]
- Wu C, Cui B, He L, Chen L, Mobley WC. The coming of age of axonal neurotrophin signaling endosomes. *J Proteomics* 2009;72:46–55. [PubMed: 19028611]
- Yagashita S. Ultrastructural observations on axonal swelling in the human gracile nucleus. *Virchows Arch A Pathol Anat Histol* 1979;382:217–226. [PubMed: 157609]
- Yamamoto T. On the thickness of the unit membrane. *J Cell Biol* 1963;17:413–421. [PubMed: 14002339]
- Yi J, Tang XM. The convergent point of the endocytic and autophagic pathways in leydig cells. *Cell Res* 1999;9:243–253. [PubMed: 10628833]
- Zareba-Kowalska A, Gajkowska B. Cytochemical localization of calcium in the corpus Luysi of the rat after ischemia. *J Hirnforsch* 1990;31:515–524. [PubMed: 2254660]
- Zelena J, Lubinska L, Gutmann E. Accumulation of organelles at the ends of interrupted axons. *Z Zellforsch Mikrosk Anat* 1968;91:200–219. [PubMed: 4894061]
- Zerial M, McBride H. Rab proteins as membrane organizers. *Nat Rev Mol Cell Biol* 2001;2:107–117. [PubMed: 11252952]
- Zwang Y, Yarden Y. Systems biology of growth factor-induced receptor endocytosis. *Traffic* 2009;10:349–363. [PubMed: 19183301]
- Zweifel LS, Kuruvilla R, Ginty DD. Functions and mechanisms of retrograde neurotrophin signalling. *Nat Rev Neurosci* 2005;6:615–625. [PubMed: 16062170]

Fig. 1. A-E Examples of multivesicular bodies (MVBs) in neurons at the ultrastructural level
A. Two profiles of MVBs (arrows) in a neuronal soma. The MVBs have a single limiting outer membrane, unlike the double membrane of mitochondria (M). The MVBs contain internal vesicles of homogeneous size. **B.** This MVB is located near the cell nucleus (N), the endoplasmic reticulum (ER) and the Golgi apparatus (G). **C and D.** Dendritic MVBs in close vicinity of presynaptic terminals (S), a preferred location of MVBs (Rind *et al.*, 2005). Note the varying amount of internal vesicles within MVBs. The internal vesicles in the dendritic MVB have a similar diameter as the synaptic vesicles (SV). **E.** MVB within a myelinated axon; my = myelin sheath. All images are from postnatal rat brain hypoglossal motoneurons. Scale bars A-E = 250 nm.

Fig. 2. A-D. Trophic factors accumulate in neuronal MVBs of the soma and dendrites after axonal transport

A. The radiolabeled trophic factor GDNF, visualized by an autoradiographic silver grain, localizes within an MVB of a hypoglossal motoneuron dendrite after retrograde axonal transport of the GDNF from the tongue muscle and after trans-somal transport, likely within an MVB, to reach the dendrite. **B.** In the soma, radiolabeled trophic factor BDNF localizes in a MVB after retrograde transport from motoneuron terminals. **C.** MVBs located in close vicinity of postsynaptic densities of a synapse (S) contain the radiolabeled trophic factor GDNF that arrived there by retrograde axonal transport, followed by transport across the soma and apparently along microtubules (mt) into the dendrite. **D.** Example of a dendritic MVB that accumulated the radiolabeled trophic factor NT-3, following internalization of this trophic factor by retinal ganglion cells and anterograde axonal transport along the optic nerve to the midbrain tectum. The radiolabeled NT-3 crossed the retinotectal synapse to accumulate in an MVB within the postsynaptic dendrite. Panel D was reproduced with permission (von Bartheld *et al.*, 1996). Abbreviations: M, mitochondrion; mt, microtubule; S, synapse. Scale bars A-D = 200 nm.

Fig. 3. A-C. Neurotrophic factors reside primarily on the outer membrane of MVBs in dendrites, but inside MVBs located in the soma

A. Example of a silver grain (asterisk) representing the trophic factor BDNF residing on the outer membrane of a dendritic MVB. **B.** Example of a silver grain representing the trophic factor GDNF residing on the inside (asterisk) of a somal MVB. **C.** The location of silver grains was quantified in a histogram for a total of 119 silver grains representing either radiolabeled BDNF or GDNF that were injected in the tongue muscle and transported retrogradely to the soma and dendrites of hypoglossal motoneurons (Rind *et al.*, 2005). The method (LaVail *et al.*, 1983) relies on measuring the distance of the center of silver grain from the outer membrane, plotted separately for dendritic and somatic MVBs. The data for BDNF and GDNF was combined, as there was no significant difference between the two data sets. Somal and dendritic MVBs showed statistically significant differences in trophic factor location. Panel C was reproduced with permission (Rind *et al.*, 2005). Scale bars in A, B = 250 nm.

Fig. 4. MVB biogenesis and three possible MVB sorting pathways: exosome release (1), back fusion/recycling (2), and degradation (3)

Left side: Endosome formation with membrane invagination to form internal vesicles. Center: The MVB may progress through one of three maturation or sorting stages: **(1)** The limiting membrane of the MVB may fuse with the plasma membrane and release internal vesicles as exosomes. **(2)** The MVB may extend tubular processes formed by membranes from internal vesicles. The tubular extensions “back-fuse” to insert limiting membrane into the cell surface membrane, thereby recycling ligand/receptor to the plasma membrane. **(3)** MVBs can target internalized ligand/receptors for degradation in the lysosome either by fusion with lysosomes or maturation into a lysosome. None of these three pathways have been directly demonstrated in neurons, but there is suggestive evidence from non-neuronal cell types. For details on exosomes and back fusion, see Faure *et al.*, 2005; Fevrier *et al.*, 2004; Harding, *et al.*, 1983; Murk *et al.*, 2002; and Putz *et al.*, 2008. For maturation, see Murphy, 1991; van Deurs *et al.*, 1993; for fusion/sorting, see Gruenberg *et al.*, 1989; Mullock *et al.*, 1998; Vonderheit & Helenius 2005. Red square: ligand; blue dot: cytoplasmic domain of receptor; black: transmembrane domain of receptor; white circle: internal vesicle.

Fig. 5. A-D. Dynamic MVB morphology suggestive of biogenesis and recycling

Examples of neuronal MVBs with invaginations (A) or tubular extensions (B-D). The limiting membranes and internal vesicles are outlined for clarity in the lower panel, with arrows pointing to the invagination or tubular extension. These MVB forms indicate biogenesis or membrane recycling according to the biogenesis and back-fusion models of MVB functions (Murk *et al.*, 2002). Note that the MVB is nearly devoid of internal vesicles in the area next to the tubular extension, consistent with the notion that the tubular extensions are generated by fusion of internal vesicles with the limiting membrane. For other examples of neuronal MVBs in transition, see Roizin *et al.*, 1967. Scale bar A-D = 250 nm.

Fig. 6. Synopsis of known and potential MVB trafficking pathways in neurons

Different types of MVBs may be involved in different trafficking patterns within neurons, numbered from 1 to 4 as indicated in the legend. Routes are based on presumed or known pathways (1-3, solid lines) as shown in studies tracking internalized markers and their localization within neuronal MVBs. There is no indication that MVBs move along axons in the anterograde direction (4, stippled); all other pathways are either proven or likely (solid lines). Evidence is primarily based on Altick *et al.*, 2009; Cooney *et al.*, 2002, LaVail and LaVail, 1974; Parton *et al.*, 1992; and Rind *et al.*, 2005.

TABLE 1

History of major concepts of MVBs, focus on neurons, with major discoveries in non-neuronal cells included.

Year	Authors	Significance
1955	Palay & Palade	First description of MVBs – coincidentally in neurons
1957	Estable <i>et al.</i>	Name “multivesicular body” (MVB) introduced
1961	Pappas & Purpura	MVBs are associated with postsynaptic sites
1964	Rosenbluth & Wissig	MVBs take up macromolecules
1965	Holtzman & Novikoff	MVBs are prelysosomal and contain acid phosphatases
1974	LaVail & LaVail	MVBs are carriers for retrograde axonal HRP transport
1979	Haigler <i>et al.</i>	MVBs move cargo to internal vesicles
1983	Harding <i>et al.</i>	MVBs release exosomes
1990	Felder <i>et al.</i>	MVBs sort endocytosed EGF receptor
1990	Chavrier <i>et al.</i>	Rabs mark specific endosomal compartments
1991	Stoorvogel <i>et al.</i>	MVBs are formed by endosome maturation
1992	Parton <i>et al.</i>	MVBs transport between nerve terminals and cell body
1995	Nixon & Cataldo	Review: Endosomal-lysosomal system involved in neuropathologies
1996	Futter <i>et al.</i>	MVBs fuse with lysosomes
1997	Saito <i>et al.</i>	KIFC2 is a neuron-specific kinesin associated with dendritic MVBs
2000	Denzer <i>et al.</i>	Review: Exosome – intercellular signaling device
2001	Piper & Luzio	Review: MVB markers, morphogenesis and function
2001	Katzmann <i>et al.</i>	ESCRT-I associates with MVBs in mammalian cells
2002	Murk <i>et al.</i>	Review: Back-fusion pathway of MVBs in non-neuronal cells
2002	Cooney <i>et al.</i>	MVBs serve multiple dendritic spines by recycling cargo
2002	Takahashi <i>et al.</i>	MVBs accumulate amyloid beta – cause of Alzheimer’s?
2005	Skibinski <i>et al.</i>	ESCRT mutation causes MVB malfunction and dementia
2005	Rind <i>et al.</i>	MVBs recycle target-derived trophic factors at synapses
2006	Faure <i>et al.</i>	Cultured neurons release exosomes
2007	Filimonenko <i>et al.</i>	Failure of MVBs to clear protein aggregates causes neurodegenerative disease
2008	Nixon <i>et al.</i>	Review: MVB’s role in neurodegenerative diseases
2008	Hurley	Review: ESCRTs role in MVB biogenesis

TABLE 2

MVB fractional areas in neuronal and glial compartments in vivo and in vitro.

in vivo		
Adult Rat Motoneuron Soma	0.6%	Schwab and Thoenen, 1976
Adult Rat Adrenergic Ganglion Soma	0.2% - 0.4%	Schwab and Thoenen, 1977
Adult Rat Pyramidal Cell Soma	0.2-0.6%	Paula-Barbosa <i>et al.</i> , 1986
With ethanol abuse	0.5-0.9%	Paula-Barbosa <i>et al.</i> , 1986
Adult Lizard Schwann Cell Soma	0.4%	Pannese <i>et al.</i> , 1988a
Adult Lizard Schwann Cell Soma	0.26 %	Pannese <i>et al.</i> , 1988b
Adult Cat PNS Axon		
Internodal	*ROA= 0	Berthold <i>et al.</i> , 1993
Paranode-node-paranode	*ROA=60	Berthold <i>et al.</i> , 1993
P5 Rat Motoneuron Soma	1.5% - 1.7%	Rind <i>et al.</i> , 2005
P5 Rat Motoneuron Dendrite	0.5% - 1.4%	Rind <i>et al.</i> , 2005
P7 Rat Motoneuron Axon	0.013% - 0.039%	Altick <i>et al.</i> , 2009
in vitro		
Neonatal Rat SCG Soma	0.35% - 0.4%	Claude <i>et al.</i> , 1982a
	0.5% - 1.8%	Claude <i>et al.</i> , 1982b
Neonatal Rat SCG Soma		
With 0.05 mM Chloroquin	0.04%	Claude <i>et al.</i> , 1982b
With 10 mM Methylamine	3.6%	Claude <i>et al.</i> , 1982b
PC12 Cell Soma in vitro	0.12% - 0.29%	Stieber <i>et al.</i> , 1984

* ROA, relative organelle abundance within specific axonal region SCG, superior cervical ganglion; PC12, pheochromocytoma 12 cell line

TABLE 3

Accumulation of endogenous and exogenous cargo molecules in neuronal MVBs.

ENDOGENOUS	
Trophic factors/receptors	
Neurotrophin receptors	Pioro <i>et al.</i> , 1991; Martinez-Murillo 1991; Valdez <i>et al.</i> , 2005
Neurotrophic factors	
NGF	Claude <i>et al.</i> , 1982 ^{a, b} ; Sandow <i>et al.</i> , 2000; Butowt and von Bartheld, 2009
BDNF	Butowt and von Bartheld, 2001, 2009
NT3	von Bartheld <i>et al.</i> , 1996; Schnapp, 1997
NT4	Butowt and von Bartheld, 2009
IGF	Aguado <i>et al.</i> , 1992
GDNF	Rind <i>et al.</i> , 2005
Receptors for transmitters	
Acetylcholine receptors	Bernard <i>et al.</i> , 1998; Liste, 2002
Ion channels/Glutamate receptors	Baude <i>et al.</i> , 1994
Adrenergic receptors	Milner <i>et al.</i> , 1998
Cannabinoid receptors	Vitalis <i>et al.</i> , 2008
Transporters (dopamine/monoamine)	Nirenberg <i>et al.</i> , 1996; Hersch <i>et al.</i> , 1997; Dehnes <i>et al.</i> , 1998; Agulhon <i>et al.</i> , 2003; Bloch <i>et al.</i> , 2003; Grimes and Miettinen, 2003;
Neuropeptides	
Neurotensin	Castel <i>et al.</i> , 1992; Delle Donne <i>et al.</i> , 1996
CGRP (Calcitonin-gene-related-protein)	Caldero <i>et al.</i> , 1992
Substance P	Pickel <i>et al.</i> , 1988
Calcium	Csillik and Knyihar-Csillik, 1980; Fifkova <i>et al.</i> , 1983; Zareba-Kowalska <i>et al.</i> , 1990; Buchs <i>et al.</i> , 1994
Miscellaneous Proteins	
Interphotoreceptor retinoid-binding protein	Hollyfield <i>et al.</i> , 1985
NEEP21 (endosomal protein)	Utvik <i>et al.</i> , 2008
LR11 (lipoprotein receptor)	Offe <i>et al.</i> , 2006
Amyloid (A β 40, 42)	Takahashi <i>et al.</i> , 2002, 2004
Prion proteins	Laine <i>et al.</i> , 2001; Mironov <i>et al.</i> , 2003; Vella <i>et al.</i> , 2008
SV2 (synaptic vesicle protein)	Wittich <i>et al.</i> , 1994
Synaptophysin	Kadota <i>et al.</i> , 1994
EXOGENOUS	
Tracers	
HRP (horseradish peroxidase)	Holtzman and Peterson, 1969; LaVail and LaVail, 1974; Bunge, 1977; LaVail <i>et al.</i> , 1980; Chu-Wang and Oppenheim, 1980; Berthold, 1982; Grafstein, 1980
Lectins	Broadwell 1985; Steindler and Cooper, 1986
Ferritin	Rosenbluth and Wissig, 1964; Birks <i>et al.</i> , 1972; Bunge, 1977; Richardson <i>et al.</i> , 1997
Thorium dioxide	Birks <i>et al.</i> , 1972; Weldon, 1975
Toxins	
Tetanus toxin	Schwab and Thoenen, 1976, 1977; Parton <i>et al.</i> , 1987

Ethanol	Paula-Barbosa <i>et al.</i> , 1986; Koksai, 2005
Capsaicin	Hiura, 1989
Mutant huntingtin	Sapp <i>et al.</i> , 1997; Aronin <i>et al.</i> , 1999
Aminoglycoside antibiotics	de Groot <i>et al.</i> , 1990
Viruses [*]	Uchil, 2005; Morita and Sunquist, 2004; Ashok and Hegde, 2006; Chu <i>et al.</i> , 2009

^{*} Viruses that can affect neurons, but examples are from non-neuronal cells.