Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Cell Biology

Effects of endocytosis on receptor-mediated signaling

Roshanak Irannejad[1,2], Nikoleta G Tsvetanova[1,2],
Braden T Lobingier[1,2] and Mark von Zastrow[1,2]

Cellular mechanisms of membrane traffic and signal transduction are deeply interconnected. The present review discusses how membrane trafficking in the endocytic pathway impacts receptor-mediated signaling. Examples of recent progress are highlighted, focusing on the endocytosis-signaling nexus in mammals.

Addresses
[1] Department of Psychiatry, University of California, San Francisco School of Medicine, 600 16th Street, San Francisco, CA 94158-2140, USA
[2] Department of Cellular & Molecular Pharmacology, University of California, San Francisco School of Medicine, 600 16th Street, San Francisco, CA 94158-2140, USA

Corresponding author: von Zastrow, Mark (mark.vonzastrow@ucsf.edu)

Current Opinion in Cell Biology 2015, **35**:137–143

This review comes from a themed issue on **Cell organelles**

Edited by **Maya Schuldiner** and **Wei Guo**

For a complete overview see the Issue and the Editorial

Available online 6th June 2015

http://dx.doi.org/10.1016/j.ceb.2015.04.005

Introduction

Close relationships between endocytosis and receptor-mediated cellular signaling have been recognized since early investigations of ligand-induced down-regulation of epidermal growth factor receptors (EGFRs, reviewed in [1•]), and the identification of endosomes as discrete membrane compartments containing internalized growth factors and activated growth factor receptors [2•,3,4•,5•]. Subsequent studies have verified and extended this relationship in many systems, as reviewed previously (e.g. [6–9]). The present discussion seeks to minimize duplication by focusing on recent developments and restricting scope to results from mammalian systems.

We will begin with a brief review of mechanisms determining the molecular sorting of signaling receptors in endosomes, and the role of these mechanisms in modulating long-term cellular signaling responsiveness. We will then discuss the hypothesis that endosomes serve, additionally, as sites of active signal initiation. There are other interesting examples of intracellular signaling that do not require receptor endocytosis *per se* (such as nutrient sensing by lysosomes); these are not discussed here but excellent reviews have appeared elsewhere (e.g. [10]).

Endosomes as sorting stations determining long-term cellular signaling responsiveness

Endocytosis of signaling receptors is widely recognized to confer long-term homeostatic control on cellular signaling responsiveness by adjusting the total cellular receptor complement, or surface-accessible complement, in accord with the cell's history of cognate ligand exposure or overall activation state. Ligand-induced activation typically increases receptor endocytic rate, and internalized receptors engage molecular sorting machineries that specify subsequent transport via divergent lysosomal and recycling routes. These events, in turn, determine the degree to which cellular ligand responsiveness is attenuated ('down-regulated') or sustained ('re-sensitized') under conditions of prolonged or repeated ligand exposure.

Many signaling receptors internalize via clathrin-coated pits and a considerable amount is now known about this mechanism (reviewed in [11]). However, it has been recognized for many years that additional endocytic mechanisms exist [12], and one area of recent progress is toward identifying alternate mechanisms relevant to signaling receptors. One that has been described recently requires endophilin but not clathrin, and is called 'fast endophilin-mediated endocytosis' (FEME) to distinguish it from clathrin-mediated endocytosis (CME) [13••]. FEME is outwardly similar to CME in that dynamin and local actin polymerization contribute to endocytic membrane scission, but FEME occurs through the formation of distinct tubulovesicular structures lacking clathrin, with endophilin providing the major force for membrane deformation [14••]. FEME also differs from CME in its mechanism of cargo selection. CME is generally engaged by receptor association with clathrin adaptor proteins [11], whereas FEME appears to be engaged by binding of proline-rich sequences in the receptor to the SH3 domain of endophilin [13••]. Identification of the FEME mechanism is an exciting development and a remarkable number of signaling receptors appear to engage it, but questions remain. For example, the D4 dopaminergic receptor (DRD4) is a putative FEME cargo but its SH3 domain-interacting sequences were found previously to inhibit, rather than promote, endocytosis of receptors. In addition, mutating these motifs to fully destabilize SH3 domain binding results in a ligand-independent endocytic phenotype [15]. These observations, not easily reconciled with the present understanding of cargo engagement with FEME, suggest that still more remains to be learned about diversity and specificity in mechanisms of signaling receptor endocytosis.

Progress has also been made recently toward more fully understanding how signaling receptors are sorted after endocytosis. Ubiquitin-directed engagement of the endosomal sorting complex required for transport (ESCRT) is an important mechanism driving lysosomal down-regulation and is highly conserved, including in yeast where many components of this machinery were first identified [16]. However, it has been suspected for some time that additional mechanisms operate in higher eukaryotes. This appears particularly likely for the GPCR family, which is ~1000-fold more diverse in mammals than in yeast.

Early evidence suggesting the existence of additional endosomal sorting machinery emerged through the study of GPCR down-regulation leading to identification of a putative 'GPCR-associated sorting protein' (GASP) that binds various GPCR cytoplasmic tails without requiring ubiquitination [17•]. GASP-1 (or GPRASP1) is the founding member of a small protein family that is widely expressed in mammals but not found in yeast [18]. The precise cellular function(s) of GASPs remain poorly understood, but recent studies suggest interesting possibilities. GASP-1 binds Beclin2, a mammalian-restricted paralogue of Beclin1 (ATG6), through a Beclin2-specific N-terminal domain [19••]. Beclin2 is otherwise similar to Beclin1, including in its ability to regulate the endosomal type III PI3-kinase (VPS34) and bind ATG14 that functions as a tethering protein in autophagosome–endolysosome fusion [20]. GASP-1 can also bind dysbindin as well as the stimulatory heterotrimeric G protein, Gs. These interactions appear to promote GPCR degradation by engaging the ESCRT through additional association with HRS, providing a path of alternate receptor connectivity to the ESCRT that does not require ubiquitination and is regulated by heterotrimeric G protein [21,22•].

Studies of GPCR recycling provided further evidence for additional mechanisms of signaling receptor sorting in mammals. A PDZ and PX domain-containing protein called sorting nexin 27 (SNX27) was identified as a key protein that binds beta-adrenergic receptors in endosomes and promotes receptor recycling [23]. SNX27 associates with the WASH–Arp2/3 actin nucleation complex and this interacts, in turn, with the retromer complex. This 'actin module-SNX27-retromer tubule module' (ASRT) machinery assembles at the base of specialized membrane tubules that extend from the endosome limiting membrane and mediate cargo exit from endosomes [24]. SNX27 interacts not only with WASH but also with retromer directly through the arrestin-like protein VPS26, and the integrated ASRT machinery appears to mediate specific endosome-to-plasma membrane transport of various signaling receptors as well as other specialized membrane cargoes such as the Glut1 (SLC2A1) glucose transporter [25]. Physiological roles of this mechanism are only beginning to be explored but are likely considerable. For example, the ASRT machinery was shown recently to mediate a discrete route of localized membrane delivery to the postsynaptic plasma membrane that is required for functional surface expression of excitatory neurotransmitter receptors at synapses [26•]. It is also interesting to note that human genetic studies have linked core components of retromer, as well as SNX27 and WASH components, to neurological and neurodegenerative syndromes (reviewed in [27]).

Endosomes as sites of receptor-mediated signal initiation

As noted above, it was proposed from the earliest investigations that endosomes may themselves function as active signaling sites. This idea, formalized in the 'signaling endosome' hypothesis, has been supported by many subsequent studies. However, two fundamental questions remain incompletely resolved. First, are endosomes bona fide sites of significant signal initiation under normal physiological conditions? Second, does the endosome signal confer functional effects different from the plasma membrane signal?

Perhaps the strongest support, overall, for an affirmative answer to both questions comes from the study of retrograde neurotrophin signaling (reviewed in [7]). Sympathetic neurons require stimulation by ligands released from peripheral targets that they innervate. Absent such signals, neurons undergo apoptotic cell death and are eliminated. One trophic signaling ligand, nerve growth factor (NGF), is a polypeptide that activates the TrkA tyrosine kinase receptor. TrkA activity is required to induce an anti-apoptotic transcriptional program mediated by a downstream MAP kinase cascade. Using a compartmentalized culture system, NGF applied selectively to distal axons was shown sufficient to elicit an anti-apoptotic transcriptional response in the neuronal cell body. Further, NGF was shown to internalize together with TrkA, and endosomes containing both cargoes were shown to move from the axon to cell body. The current model is that TrkA is continuously ligand-activated and phosphorylated in signaling endosomes during retrograde movement, effectively carrying the trophic signal from the cell periphery to nucleus. Considerable evidence supports this model, including recent work elaborating features of cytoskeletal control that are required for both retrograde endosome movement and the functional trophic signaling response [28]. However, this model is not beyond reproach. For example, some results suggest that chemical inhibition of TrkA kinase activity in the cell body is not sufficient to block the trophic signal initiated in distal axons; thus it has been suggested that the