Regulation of signal transduction by endocytosis

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Endocytosis of ligand-activated receptors has generally been considered a mechanism to attenuate signaling. There is now a growing body of evidence suggesting that this process is much more sophisticated and that endocytic membrane trafficking regulates both the intensity of signaling and the co-localization of activated receptors with downstream signaling molecules.

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Abbreviations

β_2AR	β_2 adrenergic receptor
EBP50	ERM-binding phosphoprotein-50
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ERK 1/2	extracellular regulated kinases 1 and 2
ERM	ezrin–radixin–moesin
G protein	guanine-nucleotide-binding protein
GPCR	G-protein-coupled receptor
IRS-1	insulin receptor substrate-1
NDF	Neu differentiation factor
PI3-K	phosphatidyl inositol 3-kinase
PLD	phospholipase D
RTK	receptor tyrosine kinase
TGF-α	transforming growth factor-α

Introduction

Cell surface receptors are the molecules through which changes in the extracellular environment are communicated within the cell. Among the diverse cellular responses to ligand-mediated signaling events are the uptake of nutrients and ions, the regulation of protein and DNA synthesis, and decisions about the proliferation or death of the cell. These responses are triggered when intracellular signaling molecules are activated or generated through signaling pathways initiated by ligand-bound cell surface receptors. However, it remains poorly understood how these resultant signaling pathways are regulated to form a co-ordinated, receptor-specific response. A cell can express a variety of cell surface receptors, which utilize a limited number of directed and regulated signaling pathways; yet each receptor produces a distinct response in cell physiology. The interactions of activated cell surface receptors with downstream effectors needed to amplify and transduce biochemical signals are governed by such diverse cellular processes as membrane trafficking, compartmentalization and regulated protein expression. This review focuses on the interplay between membrane trafficking and signaling by cell surface receptors. We discuss how membrane trafficking regulates signal transduction and how signaling events, in turn, regulate distinct steps in membrane trafficking.

Caveolae as coordinators of signaling molecules

One well-studied example of compartmentalized signaling occurs from caveolae, which are morphologically defined as 'omega'-shaped invaginations of the plasma membrane. Biochemically, these membrane domains are defined by their association with a family of cholesterol-binding proteins called caveolins, which function to establish and/or maintain these structures. Caveolae constitute microdomains of the plasma membrane that are enriched in cholesterol, glycosphingolipids and lipid-anchored membrane proteins. With over 30 membrane receptors, signaling molecules and membrane transporters localized to caveolae, these lipid- and protein-dense cell surface microdomains are natural candidates for centers of signaling activity (reviewed in [1]).

Recent compelling evidence that caveolae are signaling centers comes from analysis of the direct consequences of modulating endogenous levels of caveolin or interfering with caveolin function in signaling. For instance, using an antisense strategy to inhibit the expression of caveolin-1 in NIH-3T3 cells causes their transformation by facilitating anchorage-independent growth and hyperactivation of extracellular regulated kinases 1 and 2 (ERK 1/2) [2]. Signaling through these pathways was restored when caveolin-1 returned to endogenous levels. Correspondingly, overexpression of caveolin-1 suppresses ERK 1/2 signaling [3]. Caveolin overexpression does not have an inhibitory effect on all signaling pathways. The expression of recombinant caveolin-1 in NIH-3T3 cells causes an increase in phospholipase D1 (PLD1) activity [4].

A more striking example of the specificity of caveolae-dependent signaling events is the finding that the disruption of caveolin function or caveolae structure, caused either by overexpression of dominant-negative caveolin-3 mutants or by depletion of cellular cholesterol, interferes with the activation of the protein kinase raf by activated H-ras without affecting its activation by the almost identical isoform K-ras [5•]. The difference between these two ras isoforms is their localization at the plasma membrane: H-ras, but not K-ras, is palmitylated, a modification that targets it to the cholesterol-rich lipid microdomains associated with caveolin. A functional relationship between caveolin expression and cholesterol was also observed in *Caenorhabditis elegans*, in which either reduction of caveolin expression by RNA interference or depletion of cholesterol perturbs ras signaling through the MAP kinase pathway [6•].

The dramatic and diverse effects that caveolin function has on regulating signaling molecules are probably the result of using extreme measures to manipulate a system

that is, under normal conditions, more delicately balanced by much smaller changes in caveolin function. In NIH-3T3 and Rat1 cells, mutationally activated c-erbB2 (c-neu) causes the downregulation of caveolin-1, but not caveolin-2 [7]. These decidedly more subtle changes are probably part of a complex feedback mechanism to regulate transformation potential under prolonged growth factor stimulation.

The exact role of caveolin and caveolae remains enigmatic. There is good evidence that they play direct roles in regulating plasma membrane cholesterol levels [8]. Although caveolae are clearly regions enriched with signaling proteins, it remains uncertain whether these structures function to spatially coordinate signaling events or whether there exists a more direct role for cholesterol and membrane subdomain composition itself in controlling the activity of signaling complexes.

Endocytosis as a regulator of signal transduction

Ligand-mediated endocytosis is characteristically an early response in the signaling pathways triggered by a diverse group of cell surface receptors, including heterotrimeric guanine-nucleotide-binding protein (G protein)-coupled receptors (GPCRs), receptor tyrosine kinases (RTKs) and cytokine receptors [9,10]. Upon binding of the ligand (hormone, neuropeptide, growth factor, odorant, etc.) to its cognate cell surface signaling receptor, the activated receptors are targeted to clathrin-coated membrane invaginations, which, through a series of highly regulated, yet still not fully understood, biochemical events, eventually pinch off to form a clathrin-coated vesicle. Subsequent membrane fusion and budding reactions deliver the contents of the vesicle through sequential endosomal compartments. During progression along the endocytic pathway, the endosomes are modified in protein composition and pH, and their contents are sorted for shipment to the appropriate cellular destination. Among these fates are retention in the endosomal compartment, recycling back to the plasma membrane and delivery toward a lysosomal degradation pathway.

Ligand-mediated endocytosis plays at least two functions in receptor signaling. First, it can serve as a biophysical mechanism for attenuating the signaling of an activated cell surface receptor. Discussed in this review is the evolving story of the ErbB RTK family, which illustrates how controlled receptor trafficking regulates the potency of mitogenic signaling. Second, endocytosis plays a role in placing the activated cell surface receptor in the appropriate cellular location to interact with downstream signaling molecules. Signaling to ERK 1/2 by the internalized β_2 adrenergic receptor (β_2AR) and the epidermal growth factor (EGF) receptor (EGFR) not only demonstrates this phenomena but also provides insight into how these interactions might be regulated.

Endocytosis as a means of regulating receptor activity

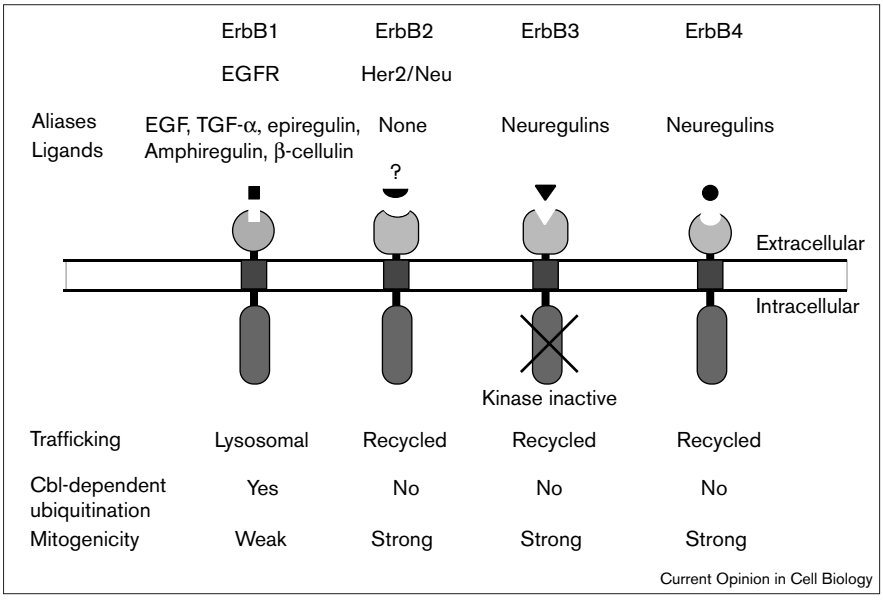
An excellent example of how endocytic membrane trafficking can regulate signaling comes from studies on the ErbB family of RTKs. ErbB family members, including the EGFR (ErbB1), are activated upon dimerization induced by binding their ligands, which are EGF, transforming growth factor-α (TGF-α) and Neu differentiation factor (NDF). The specific ligand determines the composition of the dimeric receptor (Figure 1).

It has been appreciated for some time that different ErbB receptor ligands invoke different signaling potencies, particularly in terms of their mitogenic potential [11]. This has been convincingly demonstrated and mechanistically explored by stably transfecting cells lacking ErbB family members with ErbB1 alone or in combination with either ErbB2 or ErbB3 [12•]. Cells expressing only ErbB1 were less proliferative in response to EGF than in response to TGF-α. When either ErbB2 or ErbB3 was co-expressed with ErbB1, the cells became significantly more responsive to EGF, without altering their response to TGF-α. These increases in mitogenic response correlate with the increased recycling and decreased downregulation of ErbB1 homodimers that occur in the presence of TGF-α or when ErbB2- or ErbB3-containing heterodimers are activated by either EGF or TGF-α.

As implied by these results, endosome sorting is regulated by interaction with both lumenal and cytoplasmic domains of the ErbB family members. Two lines of evidence suggest that endosome acidification and the pH-dependent dissociation of receptor–ligand complexes are central in controlling this sorting decision. First, TGF-α and NDF dissociate from their receptors at a relatively higher pH than EGF, suggesting that this dissociation would occur in early endosomal compartments, which are involved in receptor recycling. Second, treating cells with the ionophore monensin, which increases the pH of endosomal sorting compartments, leads to increased downregulation of receptors activated with TGF-α and NDF [12•,13•]. Studies with chimaeric receptors encoding the ligand-binding extracellular domain of ErbB1 and the intracellular domain of ErbB2 [14] or other ErbB family members [15] indicate that sorting signals in the ErbB1 cytoplasmic domain are required for receptor degradation.

One candidate molecule that may recognize sorting determinants in ErbB1 is c-Cbl or its *C. elegans* ortholog Sli-1. c-Cbl has been shown to be a downstream substrate and negative regulator of a number of cell surface receptors, although its mechanism has been poorly understood [16,17]. Levkowitz *et al.* [18] found that c-Cbl is recruited to endosomes in cells transiently expressing the lysosomally directed ErbB1, but not the recycling ErbB3. c-Cbl is required for ligand-dependent ubiquitination of ErbB1 in endosomes, a modification that may target the protein to the lysosome degradative pathway. Importantly, mutants of either the ErbB1 RTK or c-Cbl

Figure 1



Structural and signaling properties of the ErbB receptor tyrosine kinase family (see text for details).

that disrupt their interactions [18,19,20••] result in decreased ErbB1 degradation and increased mitogenic signaling [19] (Figure 2a). Results using other cell surface signaling receptors suggest a general role for ubiquitination in regulating endocytic membrane trafficking [21].

Pathophysiological consequences of receptor tyrosine kinase trafficking

The physiological significance of differential RTK trafficking has been demonstrated by studies utilizing cultured breast cancer cell lines expressing varying ratios of endogenous ErbB1 and ErbB2 RTKs. Through a series of biochemical and immunocytochemical studies, Wang *et al.* [22•] demonstrate that, despite auto-tyrosine-phosphorylation of both family members upon EGF treatment, endocytosis of dimeric receptor complexes is inversely proportional to ErbB2 expression. The physiological consequence of the differential cellular trafficking is increased mitogenic signaling owing to the prolonged activation state of ligand–receptor complexes involving ErbB2 or ErbB3 subunits [12•,13•,23].

This selective degradation of only ErbB1 receptors may explain the more carcinogenic nature of other ErbB family members. For instance, expression of ErbB2 has long been correlated with many carcinomas and poor prognosis [24,25]. One plausible explanation for ErbB2's carcinogenic effect is its ability to increase the ratio of ErbB1–ErbB2 heterodimers over ErbB1 homodimers. In doing so, alterations in membrane trafficking would result in enhanced mitogenicity.

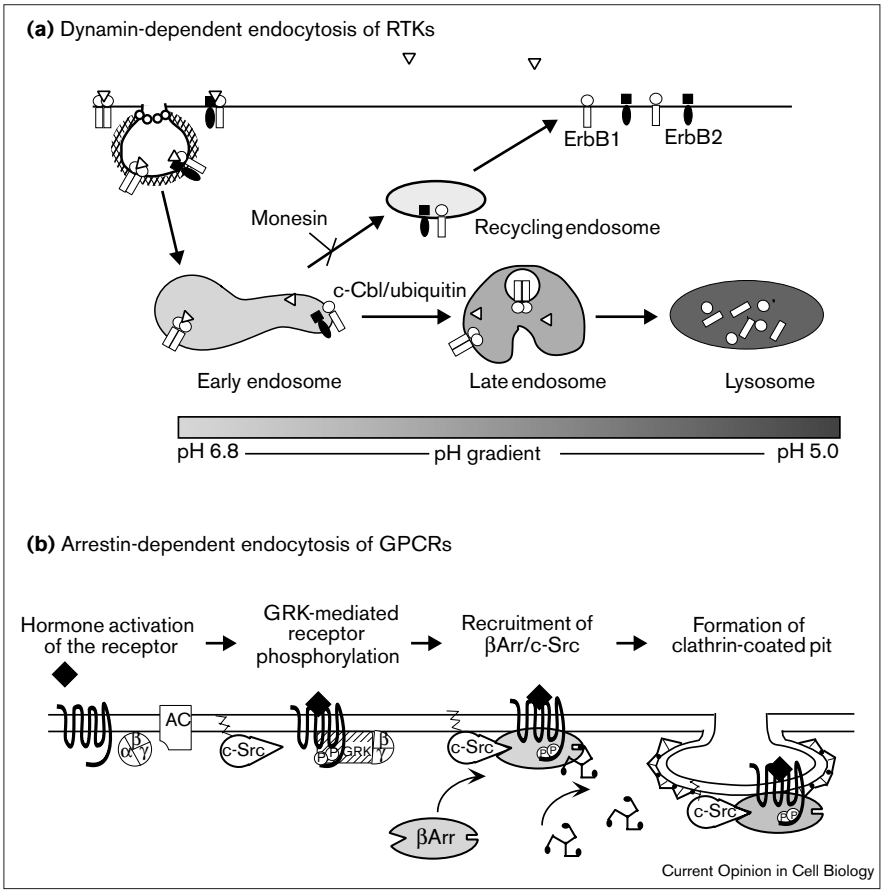
Signaling from within the endosome

The concept that activated receptors interact with downstream signaling molecules at discrete endocytic locations has been postulated for years [26,27]. Previously, testing this hypothesis was limited by the inability to trap an activated receptor at unique endocytic locales without significantly altering the receptor structure and/or impairing signaling pathways. Now, with a clearer understanding of the initial stages of endocytosis and receptor desensitization, less invasive cell biological methods for disrupting endocytosis have been developed. Thus, there has been a plethora of data examining receptor signaling prior to entry into the endocytic pathway.

The two most commonly used tools are dominant-negative constructs of the GTPase dynamin and the GPCR-binding protein arrestin. Overexpression of dominant inhibitory forms of dynamin (those that can not bind or hydrolyze GTP) blocks clathrin-mediated endocytosis [28], causing the retention of many, but not all, receptors at the cell surface. Overexpression of dominant-negative forms of arrestin [10,29•] specifically blocks the endocytosis of GPCR. Arrestin binds activated GPCRs after they become phosphorylated on serine residues within their carboxyl termini through a GPCR-kinase-mediated feedback loop. Arrestin binding prevents activated GPCR further interacting with heterotrimeric G proteins and transducing signals. In addition, arrestins serve as adapter molecules that target activated GPCR to endocytic coated pits [30]. Together, these methods have proven effective in a direct comparison of the signaling of receptors retained on the cell surface with that of those allowed to enter the endocytic pathway.

Not surprisingly, there are some signaling pathways that are completely unaffected or enhanced by retaining activated receptors at the cell surface. These include the most proximal events in receptor signaling, such as the intramolecular kinase activity of RTKs, activation of heterotrimeric G proteins,

Figure 2



Endocytosis of signaling receptors. **(a)** Differential endocytic trafficking of homodimeric and heterodimeric ErbB1 and ErbB2 RTKs. Ligand binding induces receptor dimerization and targeting to clathrin-coated pits, which pinch off in a dynamin-dependent manner. The coated vesicles form, then shed their clathrin coats and fuse with the mildly acidic early endosome, where the RTKs are sorted for recycling or degradation. Ligands dissociate from ErbB2-containing dimers, which are then sorted on a monesin-sensitive step to the recycling endosome and returned to the cell surface. ErbB1 dimers retain bound ligand until encountering the lower pH or later endosomal compartments. Within endosomes, ErbB1 dimers associate with c-Cbl, are ubiquitinated and targeted for degradation in the lysosome. **(b)** Formation of the β_2AR–β-arrestin–c-Src complex as a mechanism to form the β_2AR-containing endosome. Upon hormone stimulation, the α and βγ subunits of the activated heterotrimeric G protein dissociate to cause activation of downstream effectors such as adenylyl cyclase (AC). The βγ subunits facilitate the G-protein-coupled receptor kinase (GRK)-mediated phosphorylation of ligand-bound β_2AR. β-Arrestin (βArr) binds both the phosphorylated β_2AR and c-Src, causing formation of the β_2AR–β-arrestin–c-Src complex at the plasma membrane. The β-arrestin interaction with clathrin targets the β_2AR to coated pits. Its subsequent internalization is a prerequisite for signaling to the ERK 1/2 pathway.

phosphorylation of insulin receptor substrate-1 (IRS-1) by the insulin receptor, regulation of adenylyl cyclase activity and stimulation of phospholipase Cγ. Given the plasma membrane location and/or the kinetics of activation of these effectors, it seems unlikely that receptor endocytosis would be a prerequisite for their activity.

More intriguing are those events that are attenuated when receptor endocytosis is inhibited — namely the activation of phosphatidyl inositol 3-kinase (PI3-K) and ERK 1/2 [31–36]. In the case of insulin receptor signaling, PI3-K associates with IRS-1 in an insulin-dependent manner as a prerequisite for signaling. When insulin receptor endocytosis is blocked by dominant-negative dynamin, there is a significant reduction in insulin-dependent PI3-K activity, despite full tyrosine phosphorylation of the insulin receptor and IRS-1. The change in kinase activity is reflected in a corresponding decrease in the amount of p85 regulatory subunit of PI3-K associated with IRS-1 [31].

Although there are a number of cell surface receptors that require endocytosis for ERK 1/2 activation, there are an equal number that do not (Table 1). One possible explanation is that the need for endocytosis is receptor-specific and that the role of endocytosis in ERK 1/2 activation may be a mechanism through which signal specificity is conferred. These results are reminiscent of the differential effects on ras signaling through MAP kinase observed upon caveolae disruption (see above) and suggest that multiple mechanisms exist to spatially regulate the MAP kinase signaling pathway.

Interestingly, it has been reported that dominant inhibitory dynamin decreases μ opiod receptor mediated activation of ERK 1/2 in HEK293 cells independent of an effect on the endocytosis of the μ opiod receptor [37]. It is possible this is a consequence of dynamin's role in regulating an intermediate signaling protein whose endocytosis is required for ERK 1/2 activation. Alternatively, these data may suggest a second role for dynamin, in addition to regulating endocytosis. However, expression of dominant-negative dynamin has no effect on ERK 1/2 activation by the α_2 adrenergic receptor, which, like the μ opiod receptor, couples to $G\alpha_{i/o}$ guanine-nucleotide-binding proteins and is internalized in a dynamin-independent manner [38].

Taken together, this selective inhibition of signaling pathways suggests that an activated receptor and requisite

Table 1

Consequences of inhibited endocytosis on ERK activity.

Receptor	Endocytic block	Cell line	ERK activity	References
Receptor tyrosine kinase				
EGFR	Dynamin	HeLa	↓ 50%	[35]
IR	Dynamin	H4IIE	↓ 50%	[31]
G-protein-coupled receptors				
α_{2A}AR	Dynamin*	COS-1, HEK293	No change	[33,38]
α_{2B}AR	Dynamin	COS-1, HEK293	No change	[33,38]
α_{2C}AR	Dynamin	COS-1	No change	[33]
βAR	Dynamin, arrestin	HEK293	↓ 60%	[32,45]
δ opiod	Dynamin	Cos-7, HEK293	↓ 80%	[46]
μ opiod	Dynamin	HEK293	↓ 80%	[46]
κ opiod	Dynamin, arrestin	CHO	No change	[47]
	Dynamin	HEK293	↓ 80%	[46]
$5HT_{1A}$ Serotonin receptor	Dynamin, arrestin	HEK293	↓ 50%	[34]
LPA	Dynamin, arrestin	HEK293	↓ 50–80%	[32]
CXCR2	Dynamin	HEK293	No change	[48]

*Endocytosis is dynamin independent.

signaling molecules are not sufficient to direct appropriate cellular responses — endocytosis is also an essential component.

Signal transduction can regulate endocytosis

Although the role of the endocytic pathway in receptor signal transduction has only recently been appreciated, it has been known for some time that signaling receptors must be active for their endocytosis [39]. Our understanding of the role of receptor signaling in endocytosis has been limited to mutagenesis studies defining the receptor domains that are involved in recruitment to clathrin-coated pits and endocytosis. New studies using endocytosis-deficient cell lines have readdressed this mechanism and the nonreceptor tyrosine kinase c-Src has emerged as an important regulator of endocytosis.

The effect of the endocytic pathway on signaling and *vice versa* has been best characterized using β_2AR as a model. With the first observation of β_2AR internalization upon agonist stimulation came the hypothesis that this was strictly a mechanism by which activated receptors were removed from the cell surface and sequestered within the cell to prevent further activation of a downstream effector, namely adenylyl cyclase. This hypothesis continued to garner momentum with the observation of ligand-dependent phosphorylation and the subsequent association of β-arrestin to prevent further signaling to heterotrimeric G proteins.

More recent results have suggested an additional role for β-arrestin binding. In this work, Luttrell *et al.* [40••] demonstrate that agonist activation of β_2AR results in the formation of a β_2AR–β-arrestin–c-Src complex. This protein complex targets the receptor to a clathrin-coated pit (Figure 2b). It has been shown that kinase-inactive forms of c-Src can inhibit, and constitutively active forms of c-Src can enhance, β_2AR endocytosis [41]. Activation of both β_2AR and the LPA (lysophosphatidic acid) receptor leads to phosphorylation of dynamin, in the former case, through a c-Src-dependent process [41,42]. These data suggest that c-Src is an upstream regulator of dynamin function in β_2AR endocytosis. It has been shown that EGF-stimulated activation of c-Src leads to tyrosine phosphorylation of clathrin and that phosphorylation is required for the recruitment of clathrin to the membrane [43]. Taken together, these data strongly suggest a role for c-Src in receptor endocytosis — the question remains whether c-Src is a regulator of dynamin function or a recruiter of clathrin or both.

Another candidate protein for regulating β_2AR signaling is the ezrin–radixin–moesin (ERM)-binding phosphoprotein-50 (EBP50). EBP50 binds to the G-protein-regulated kinase 5 (GRK5)-phosphorylated cytoplasmic tail of β_2AR via a PDZ domain and to the cortical cytoskeleton through an ERM-binding domain. In HEK293 cells, the disruption of the β_2AR–EBP50–actin interaction results in diminished β_2AR recycling [44•]. Although EBP50 has not been shown to have a direct role in β_2AR signaling, its effects on β_2AR endocytic trafficking are likely to have important implications in signaling [44•], similar to those described above for ErbB family members.

Conclusions

The key to fully understanding the cell physiology mediated by cell surface receptors lies not only in the identification of downstream effectors but also in the exquisite spatial and temporal regulation of the interactions

with these effectors. Clearly, membrane trafficking plays an important role both in controlling the location of signaling interactions and in regulating the cellular degradation and recycling of the activated receptor. From a biomedical prospective, the identification of the sites and knowledge of the kinetics of receptor activation of downstream effectors provides an opportunity to design rational therapeutic strategies to manipulate a given signaling pathway.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Anderson RGW: **The caveolae membrane system.** *Annu Rev Biochem* 1998, **67**:199-225.

2. Galbiati F, Volonte D, Engelman JA, Watanabe G, Burk R, Pestell RG, Lisanti MP: **Targeted downregulation of caveolin-1 is sufficient to drive cell transformation and hyperactivate the p42/44 MAP kinase cascade.** *EMBO J* 1998, **17**:6633-6648.

3. Engelman JA, Chu C, Lin A, Jo H, Ikezu T, Okamoto T, Kohtz DS, Lisanti MP: **Caveolin-mediated regulation of signaling along the p42/44 MAP kinase cascade *in vivo*.** *FEBS Lett* 1998, **428**:205-211.

4. Czarny M, Lavie Y, Fiucci G, Liscovitch M: **Localization of phospholipase D in detergent-insoluble, caveolin-rich membrane domains.** *J Biol Chem* 1999, **274**:2717-2724.

5. • Roy S, Luetterforst R, Harding A, Apolloni A, Etheridge M, Stang E, Rolls B, Hancock JF, Parton RG: **Dominant-negative caveolin inhibits H-ras function by disrupting cholesterol-rich plasma membrane domains.** *Nat Cell Biol* 1999, **1**:98-105.

The authors demonstrate that either dominant-negative caveolin or cholesterol depletion can specifically perturb one signaling pathway, but not another.

6. • Scheel J, Srinivasan J, Honnert U, Henske A, Kurzchalia TV: **Involvement of caveolin-1 in meiotic cell-cycle progression in *Caenorhabditis elegans*.** *Nat Cell Biol* 1999, **1**:127-129.

Using *C. elegans* as a model, this report shows the *in vivo* consequences of reduced caveolin expression or cholesterol depletion on MAP kinase signaling.

7. Engelman JA, Lee RJ, Karnezi A, Bearss DJ, Webster M, Siegel P, Muller WJ, Windle JJ, Pestell RG, Lisanti MP: **Reciprocal regulation of Neu tyrosine kinase activity and caveolin-1 protein expression *in vitro* and *in vivo*.** *J Biol Chem* 1998, **273**:20448-20455.

8. Ikonen E, Parton RG: **Caveolins and cellular cholesterol balance.** *Traffic* 2000, **1**:212-217.

9. DiGuglielmo GM, Drake PG, Baass PC, Authier F, Posner BI, Bergeron JJ: **Insulin receptor internalization and signalling.** *Mol Cell Biochem* 1998, **182**:59-63.

10. Ferguson SS, Dewney WE III, Colapietro A, Barak LS, Menard L, Caron MG: **Role of β-arrestin in mediating agonist-promoted G-protein-coupled receptor internalization.** *Science* 1996, **271**:363-366.

11. Reddy CC, Wells A, Lauffenburger DA: **Comparative mitogenic potencies of EGF and TGF alpha and their dependence on receptor-limitation versus ligand-limitation.** *Med Biol Eng Comput* 1998, **36**:499-507.

12. • Lenferink AEG, Pinkas-Kramarski R, van de Poll MLM, van Vugt MJH, Klapper LN, Tzahar E, Waterman H, Sela M, van Zoelan EJJ, Yarden Y: **Differential endocytic routing of homo- and hetero-dimeric ErbB tyrosine kinases confers signaling superiority to receptor heterodimers.** *EMBO J* 1998, **17**:3385-3397.

See annotation to [13•].

13. • Waterman H, Sabanai I, Geiger B, Yarden Y: **Alternative intracellular routing of ErbB receptors may determine signaling potency.** *J Biol Chem* 1998, **273**:13819-13827.

Through the use of cells that express various combinations of ErbB family members, this paper, together with [12•], provides a model that links differential endocytic trafficking of activated receptor tyrosine kinases with their mitogenic potential.

14. Sorkin A, DiFiore PP, Carpenter G: **The carboxyl terminus of epidermal growth factor/erbB2 chimerae is internalization impaired.** *Oncogene* 1993, **8**:3021-3028.

15. Baulinda J, Kraus MH, Alimandi M, DiFiore PP, Carpenter G: **All ErbB receptors other than the epidermal growth factor receptor are endocytosis impaired.** *J Biol Chem* 1996, **271**:5251-5257.

16. Levkowitz G, Klapper LN, Tzahar E, Freywald A, Sela M, Yarden Y: **Coupling of the c-Cbl protooncogene product to Erb-1/EGF-receptor but not to other ErbB proteins.** *Oncogene* 1996, **12**:1117-1125.

17. Yoon CH, Lee J, Jongeward GD, Sternberg PW: **Vulval induction during *Caenorhabditis elegans* development is mediated by LET-23, a homolog of the mammalian protooncogene c-cbl.** *Science* 1995, **269**:1102-1105.

18. Levkowitz G, Waterman H, Zamir E, Kam Z, Oved S, Langdon WY, Beguinot L, Geiger B, Yarden Y: **c-Cbl/Sli-1 regulates ednocytic sorting and ubiquitination of the epidermal growth factor receptor.** *Genes Dev* 1998, **12**:3663-3674.

19. Waterman H, Lefkowitz G, Alory I, Yarden Y: **The RING finger of c-Cbl mediates desensitization of the epidermal growth factor receptor.** *J Biol Chem* 1999, **274**:22151-22154.

20. •• Joazeiro CAP, Wing SS, Huang H-K, Leverson JD, Hunter T, Liu Y-C: **The tyrosine kinase negative regulator c-Cbl is a RING-type, E2-dependent ubiquitin-protein ligase.** *Science* 1999, **286**:309-312.

This paper presents a novel mechanism of targeting cell surface receptors for ubiquitination.

21. Strous GJ, Govers R: **The ubiquitin-proteasome system and endocytosis.** *J Cell Sci* 1999, **112**:1417-1423.

22. • Wang Z, Zhang L, Yeung TK, Chen X: **Endocytosis deficiency of epidermal growth factor (EGF) receptor-ErbB2 heterodimers in response to EGF stimulation.** *Mol Biol Cell* 1999, **10**:1621-1636.

The authors provide evidence that sorting signals in the ErbB1 cytoplasmic domain direct its interactions with c-Cbl and its trafficking along the lysosomal degradation pathway.

23. Waterman H, Alroy I, Strano S, Seger R, Yarden Y: **The C-terminus of the kinase defective neuregulin receptor ErbB-3 confers mitogenic superiority and dictates endocytic routing.** *EMBO J* 1999, **18**:3348-3358.

24. Huang SH-J, Nagane M, Klingbeil CK, Line H, Nishikawa R, Ji X-D, Huang C-H, Gill GN, Wiley HS, Cavenee WK: **The enhanced tumorigenic activity of a mutant epidermal growth factor receptor common in human cancers is mediated by threshold levels of constitutive tyrosine phosphorylation and unattenuated signaling.** *J Biol Chem* 1997, **272**:2927-2935.

25. Liu E, Thor A, He M, Barcos BM, Ljung BM, Benz C: **The Her2(c-erbB-2) oncogene is frequently amplified in *in situ* carcinomas of the breast.** *Oncogene* 1992, **7**:1027-1032.

26. Khan MN, Baquiran G, Brule C, Burgess J, Foster B, Bergeron JJM, Posner BI: **Internalization and activation of the rat liver insulin receptor kinase *in vivo*.** *J Biol Chem* 1989, **264**:12931-12940.

27. Burgess JW, Wada I, Ling N, Khan MN, Bergeron JJM, Posner BI: **Decrease in β-subunit phosphotyrosine correlates with internalization and activation of the endosomal insulin recpetor kinase.** *J Biol Chem* 1992, **267**:10077-10086.

28. Damke H, Baba T, Warnock DE, Schmid SL: **Induction of mutant dynamin specifically blocks endocytic coated vesicle formation.** *J Cell Biol* 1994, **127**:915-934.

29. • Orsini MJ, Benovic JL: **Characterization of dominant negative arrestins that inhibit β_2-adrenergic receptor internalization by distinct mechanisms.** *J Biol Chem* 1998, **273**:34616-34622.

This paper characterizes arrestins and their specificity in blocking G-protein-coupled receptor endocytosis.

30. Goodman Jr, OB, Krupnick JG, Santini F, Gurevish VV, Penn RB, Gagnon AW, Keen JH, Benovic JL: **Beta-arresting acts as a clathrin adaptor in endocytosis of the beta2-adrenergic receptor.** *Nature* 1996, **383**:447-450.

31. Ceresa BP, Kao AW, Santeler SR, Pessin JE: **Regulation of insulin receptor signaling pathways by clathrin mediated endocytosis.** *Mol Cell Biol* 1998, **18**:3862-3870.

32. Daaka Y, Luttrell LM, Ahn S, Rocca GJD, Ferguson SSG, Caron MG, Lefkowitz RJ: **Essential role for G protein-coupled receptor endocytosis in the activation of mitogen-activated protein kinase.** *J Biol Chem* 1998, **273**:685-688.

33. DeGraff JL, Gagnon AW, Benovic JL, Orsini MJ: **Role of arrestins in endocytosis and signaling of α_2-adrenergic receptor subtypes.** *J Biol Chem* 1999, **274**:11253-11259.

34. Rocca GJD, Mukhin YV, Garnovskaya MN, Daaka Y, Clark GJ, Luttrell LM, Lefkowitz RJ, Raymond KR: **Serotonin 5-HT$_{1A}$ receptor-mediated Erk activation requires calcium/calmodulin-dependent receptor endocytosis.** *J Biol Chem* 1999, **274**:4749-4753.

35. Vieira AV, Lamaze C, Schmid SL: **Control of EGF receptor signaling by clatharin-mediated endocytosis.** *Science* 1996, **274**:2086-2089.

36. Vogler O, Nolte B, Voss M, Schmidt M, Jacobs KH, van Koppen CJ: **Regulation of muscarinic acetylcholine receptor sequestration and function by beta-arrestin.** *J Biol Chem* 1999, **274**:12333-12338.

37. Whistler JL, von Zastrow M: **Dissociation of functional roles of dynamin in receptor-mediated endocytosis and mitogenic signal transduction.** *J Biol Chem* 1999, **274**:24575-24578.

38. Schramm NL, Limbird LE: **Stimulation of mitogen-activated protein kinase by G protein-coupled α_2-adrenergic receptors does not require agonist-elicited endocytosis.** *J Biol Chem* 1999, **274**:24935-24940.

39. Carpenter G, Soler C, Baulida J, Beguinot L, Sorkin A: **Interaction of signaling and trafficking proteins with the carboxyterminus of the epidermal growth factor receptor.** *Ann NY Acad Sci* 1995, **7**:44-51.

40. •• Luttrell LM, Ferguson SSG, Daaka Y, Miller WE, Maudsley S, Rocca GJD, Lin F-T, Kawakatsu H, Owada K, Luttrell DK *et al.*: **β-Arrestin-dependent formation of β_2 adrenergic receptor-Src protein kinase complexes.** *Science* 1999, **283**:655-661.

This paper introduces a novel role for β-arrestin as a scaffolding protein that coordinates protein interactions involved in β_2 adrenergic receptor endocytosis.

41. Ahn S, Maudsley S, Luttrell LM, Lefkowitz RJ, Daaka Y: **Src-mediated tyrosine phosphorylation of dynamin is required for β_2-adrenergic receptor internalization and mitogen-activated protein kinase signaling.** *J Biol Chem* 1999, **274**:1185-1188.

42. Kranenburg O, Verlaan I, Moolenaar WH: **G_i-mediated tyrosine phosphorylation of Grb2 (growth-factor-receptor-bound protein 2) - bound dynamin-II by lysophosphatidic acid.** *Biochem J* 1999, **339**:11-14.

43. Wilde A, Beattie EC, Lem L, Tiefthof DA, Liu S-H, Mobley WC, Soriana P, Brodsky FM: **EGF receptor signaling stimulates SRC kinase phosphorylation of clathrin, influencing clathrin redistribution and EGF uptake.** *Cell* 1999, **96**:677-687.

44. • Cao TT, Deacon HW, Reczek D, Bretscher A, von Zastrow M: **A kinase-regulated PDZ-domain interaction controls endocytic sorting of the β_2-adrenergic receptor.** *Nature* 1999, **401**:286-290.

This paper identifies a new protein intermediate that stabilizes β_2-adrenergic receptor (β_2AR) interactions with the cell's cytoskeleton, suggesting a novel regulatory mechanism of β_2AR signaling.

45. Lin F-T, Miller WE, Luttrell LM, Lefkowitz RJ: **Feedback regulation of β-arrestin1 function by extracellular signal-regulated kinases.** *J Biol Chem* 1999, **274**:15971-15974.

46. Ignatova EG, Belcheva MM, Bohn LM, Neuman MC, Coscia CJ: **Requirement of receptor internalization for opiod stimulation of mitogen-activated protein kinase: biochemical and immunofluorescence confocal microscopic evidence.** *J Neurosci* 1999, **19**:56-63.

47. Li JG, Luo LY, Krupnick JG, Benovic JL, Liu-Chen LY: **U50,488H-induced internalization of the human κ-opiod receptor involves a β-arrestin- and dynamin-dependent mechanism.** *J Biol Chem* 1999, **274**:12087-12094.

48. Yang W, Wang D, Richmond A: **Role of clathrin-mediated endocytosis in CXCR2 sequestration, resensitization, and signal transduction.** *J Biol Chem* 1999, **274**:11328-11333.