

Endocytosis and intracellular sorting of receptor tyrosine kinases

Alexander Sorokin

Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262, USA

Received 5/29/98 Accepted 5/8/98

TABLE OF CONTENTS

1. Abstract
2. Introduction: Receptor-mediated endocytosis
3. Endocytosis of growth factor receptors: down-regulation
4. Internalization
 - 4.1. Receptor recruitment into clathrin-coated pits
 - 4.2. Molecular mechanisms of internalization
5. Intracellular sorting
 - 5.1. Pathway through endosomes
 - 5.2. Kinetics of intracellular trafficking: recycling versus degradation
 - 5.3. Possible mechanisms of receptor sorting in endosomes
6. Role of endocytosis in growth factor signaling
7. Perspectives
8. Acknowledgments
9. References

1. ABSTRACT

Binding of the peptide growth factors (GF) on their surface receptors triggers rapid endocytosis of ligand-receptor complexes via clathrin-coated pits. Internalized receptors pass through the endosomal compartment where they are efficiently sorted to the lysosomal degradation pathway. Accelerated internalization and degradation of GF receptors leads to dramatic down-regulation of the receptors. Hence, we discuss the pathways, molecular mechanisms and the biological role of the GF-induced receptor endocytosis and lysosomal targeting. The endocytosis of epidermal growth factor (EGF) receptor is employed as a model system to describe the role of clathrin, clathrin adaptors and other proteins in the recruitment of receptors into coated pits and in the endosomal sorting process.

2. INTRODUCTION: RECEPTOR-MEDIATED ENDOCYTOSIS

Many macromolecules enter into the cell by the process of receptor-mediated endocytosis (1). The ligand-receptor complexes formed on the cell surface can be selectively recruited into clathrin-coated pits, small areas of the plasma membrane covered with the clathrin coat from the cytoplasmic side of the membrane (2). Coated regions of the membrane provide a mechanical basis for rapid vesicle budding as they can invaginate inward and capture the membrane to pinch off vesicles. Clathrin-coated pits and vesicles contain two major structural components: clathrin itself and adaptor protein complexes, APs. Clathrin consists of three copies each of heavy chain (~190 kD) and light chain (~23-27 kD), forming a three-legged structure called a triskelion. Clathrin triskelions are the assembly units of the polygonal lattice. Clathrin assembles into coats on the cytoplasmic side of the plasma membrane by

interacting with its adaptor complex AP-2. AP-2 is heterotetramer consisting of two large (~100 kD) alpha and beta-2 subunits, one medium mu-2 (50 kD) and one small delta-sigma subunit (17 kD). In addition, several other proteins that play a regulatory role are found in coated pits.

The endocytic vesicles fuse with specialized membrane organelles known as endosomes which deliver the receptors and their ligands to various intracellular destinations. Internalized molecules can be either recycled back from endosomes to the plasma membrane and participate in several rounds of endocytosis, or sequestered in endosomes for a long time, or transported to lysosomes or other organelles. Here, for simplicity, we classify endosomes into early, intermediate and late endosomes according to the time of appearance of the endocytic markers in these compartments after internalization. The intravesicular pH drops along the endocytic pathway, from 6.0-6.5 in early endosomes to 4.5-5.5 in late endosomes and lysosomes, which causes dissociation of many ligand-receptor complexes (3). The mechanisms of endosomal sorting and protein coats involved in trafficking of the ligand-receptor complexes through endosomal compartments remain obscure.

3. ENDOCYTOSIS OF GROWTH FACTOR RECEPTORS: DOWN-REGULATION

For many receptors, ligand binding does not affect endocytosis and recycling, and, therefore, the level of expression. However, for receptors which transduce signals across membrane, such as receptors for polypeptide growth factors (GFs) or G protein-coupled receptors, endocytosis

Endocytosis of growth factor receptors

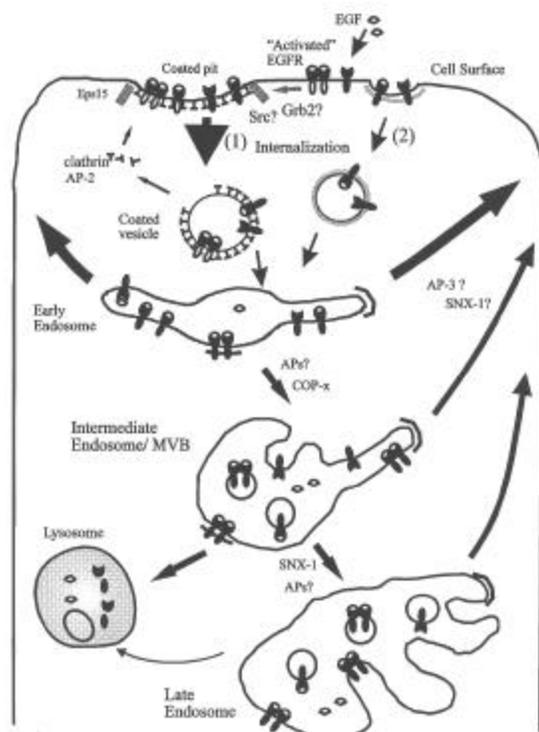


Figure 1. Pathways of internalization and intracellular sorting of GF receptors. Two pathways of endocytosis of EGF receptors are indicated: (1) high-affinity saturable pathway via coated pits (internalization rate constant $k_e = 0.2-0.4$, capacity is typically less than 50,000 receptors) which is utilized by EGF-activated receptors (EGFR); (2) non-saturable clathrin-independent pathway with slow kinetics ($k_e=0.02-0.10$) used by unoccupied and occupied EGF when the first pathway is overwhelmed. Clathrin, AP-2 and eps15 are indicated as components of the clathrin coat. The coats involved in the second pathway are not known. Both clathrin-dependent and independent internalization pathways lead receptors to the early endosomal compartments. Black arrows show recycling of occupied and unoccupied receptors from endosomes. The relative width of arrows indicates that the recycling is much slow from intermediate and late endosomes than from the early compartment. Putative endosomal sorting and coat proteins are indicated as AP-3; APs?, unidentified AP-like complexes; SNX-1 (sorting nexin-1), COP-X (coatamer (COP-I)-like proteins).

and subsequent sorting of the internalized receptors to the lysosome-degradation pathway is dramatically accelerated by the ligand. The elevated endocytosis leads to reduction of the surface and total cellular receptor pools, a phenomenon called ligand-induced "down-regulation" of the receptors.

The endocytosis of GF receptors with intrinsic tyrosine kinase activity remains the classical model system to study mechanisms of down-regulation. The specificity of down-regulation for the GF and other signaling receptors suggests that GF-induced endocytosis may play an

important role in the control of GF signaling. However, the molecular mechanisms by which ligand triggers receptor endocytosis and by which endocytosis modulate signal transduction process are only poorly understood. In this review, the receptor-mediated endocytosis of receptor tyrosine kinases will be described with the focus on the mechanisms that are unique for GF receptors. A considerable amount of work has been performed using receptors for epidermal growth factor (EGF) and insulin (for review see ref. 4). We will build our discussion around endocytosis of the EGF receptor for two reasons. First, studies of this receptor have produced most of the original data on which the current model of GF receptor endocytosis is based. Secondly, insulin-induced down-regulation of the insulin receptor is typically much less dramatic than that of EGF and many other GF receptors.

4. INTERNALIZATION

4.1. Receptor recruitment into clathrin-coated pits

One group of receptors (class I) are clustered in coated pits and rapidly internalized regardless of the ligand occupancy. The well-known examples of this type are the transferrin and mannose-6-phosphate receptors. Accumulation of signaling receptors, for example EGF receptors, in coated pits is ligand-dependent (class II) (5). In the absence of EGF, EGF receptors are diffusely distributed at the cell surface (5, 6). A small pool of unoccupied receptors can be seen in coated pits (5, 7). The relative size of this pool appears to be dependent on cell type and level of receptor expression (6-8). Quantitative studies of the distribution of EGF receptors in coated and uncoated regions of the plasma membrane are difficult because the presence of receptors in coated pits is transient and its amount is very low. Several reports, however, demonstrated rapid aggregation and significant accumulation of EGF receptors in coated pits upon EGF binding (5, 6, 8-10) (figure 1).

An individual coated pit can package more than one type of receptor (11). Conformational rearrangements of the clathrin lattice result in deep invagination of the coated pit and fission of the coated endocytic vesicle containing cargo proteins. Clathrin and associated proteins must then return back to the plasma membrane to re-assemble coated pits (figure 1). Several stages of the coated pit cycle require energy, physiological temperature, and are regulated by dynamin and other GTP-binding proteins. Importantly, whereas late steps of coated vesicle formation are common for all types of receptors, receptor recruitment into coated pits appears to be the rate-limiting and ligand-dependent step of the internalization of GF receptors (10, 12).

EGF-receptor complexes can be seen in plasma membrane invaginations not covered with clathrin coat in A-431 cells (5, 13). Although the possibility exists that clathrin has dissociated during fixation and other sample preparation procedures, it has been proposed that EGF receptors can be internalized by clathrin-independent pathway in some cells. This notion is supported by the analysis of EGF endocytosis kinetics data (see below). The mechanism of clathrin-independent pathway and the nature

Endocytosis of growth factor receptors

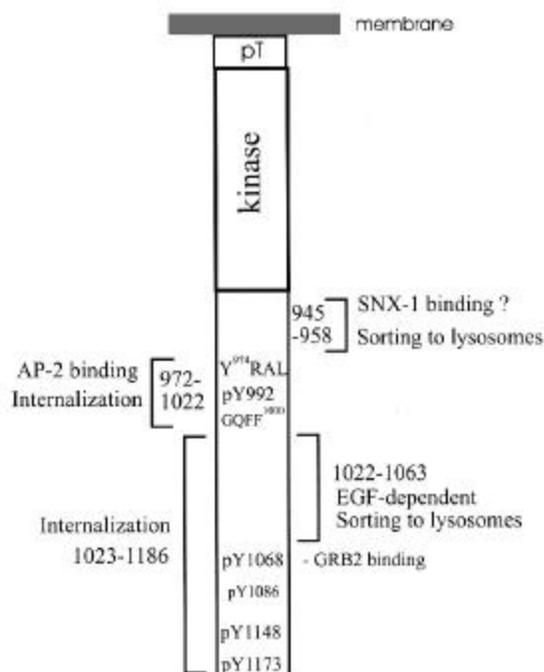


Figure 2. Trafficking signals in the cytoplasmic domain of the EGF receptor. The EGF receptor is a 170-kDa glycoprotein that is composed of three major regions: an extracellular ligand binding domain, a hydrophobic transmembrane region and a cytoplasmic domain. The extracellular and transmembrane domains are important for EGF-induced dimerization of the receptor. Cytoplasmic domain consists of conserved kinase domain located between two regulatory regions: juxtamembrane and carboxyl-terminal domains containing multiple serine, threonine and tyrosine phosphorylation sites (phosphorylated by the receptor kinase itself). Phosphorylated residues regulate receptor kinase activity and provide binding sites for other proteins. Depicted are the regions of the EGF receptor that have been implicated in the regulation of internalization and lysosomal targeting. Major tyrosine phosphorylation sites are indicated as *pY*, whereas serine and threonine phosphorylation is indicated as *pS* and *pT*. Two putative internalization motifs are Y⁹⁷⁴RAL and GGQFF¹⁰⁰⁰. The first sequence has been also shown to serve as the major AP-2 binding site in the EGF receptor.

of non-clathrin coats at the plasma membrane are unknown. EGF receptors can bind to caveolae (14), which may serve as the mechanism of clathrin-independent uptake.

4.2. Molecular mechanisms of internalization

Although endocytosis of some receptors can occur in the absence of functional coated pits, clathrin-dependent endocytosis is the most efficient and fastest pathway. Receptors are recruited to coated pits efficiently and selectively because the coat proteins can recognize “endocytic codes” or “internalization signals”, the sequence motifs in the cytoplasmic domains of the receptors (15). Among such signals the motifs based on a tyrosine residue

and a hydrophobic residue in +3 position are the most well characterized (15). Studies with constitutively-internalized class I receptor revealed that the receptor-recognition protein in coated pits is the μ -2 subunit of AP-2 (16). Our recent studies using yeast two-hybrid system showed that EGF receptors can also bind μ -2 subunit of AP-2 (Sorkina, T, Bild, A., Tebar, F., Sorkin, A.; submitted for publication).

The EGF receptor interaction with AP-2 has been demonstrated by several *in vivo* and *in vitro* techniques (17, 18). However, it is not formally proven whether this interaction is important and sufficient for EGF-induced recruitment of receptors into coated pits. In fact, functional testing *in vivo* showed that EGFRs lacking the major AP-2 binding site can be internalized via clathrin-dependent mechanism (19, 20). Moreover, co-immunoprecipitation with AP-2 has been reported exclusively for receptors of the EGF receptor family (21, 22). The interactions of other GF receptors with AP-2 have not been shown in any type of experimental assays. Furthermore, our studies revealed that co-immunoprecipitation of EGF receptors and AP-2 is due to their interaction after internalization in endosomes (Sorkina, T, Bild, A., Tebar, F., Sorkin, A.; submitted for publication). All these data leave the question of whether the EGF receptor/AP-2 interaction is important for coated pit endocytosis, unresolved.

Several peptide sequences of the carboxyl-terminus of EGF receptor are capable of supporting rapid endocytosis and potentially binding AP-2 (23, 24). These sequences are analogous to the coated pit localization motifs (endocytic codes) found in class I receptors and contain tyrosine or phenylalanine residues (figure 2). It is not clear, however, which of these endocytic codes function in native, full-length EGF receptor, which of them are cryptic and exposed only in truncated receptor mutants, and whether different motifs can be involved in distinct pathways of internalization. Interestingly, that the tyrosine-containing motif which is essential for internalization of c-Fms receptor was found in its juxtamembrane region (25). In platelet-derived growth factor (PDGF) receptor beta, the hydrophobic region downstream of kinase domain has been implicated in the control of internalization (26). However, the kinase activity of the receptor mutants lacking this region is also severely impaired, and therefore, the direct involvement of the region in the interaction with the endocytic machinery is unlikely.

Kinetics analysis of EGF endocytosis revealed that the specific internalization rate constant of EGF internalization was several fold higher at low than at high EGF concentrations (27). Mathematical modeling of these data led to the proposal that there are two pathways of internalization of EGF receptors: a rapid saturable pathway used by a limited number of EGF-activated receptors, and a five to ten times slower non-saturable endocytosis. The latter is employed by the unoccupied receptors or by EGF-occupied receptors when the rapid pathway is saturated (12). It has been also postulated that whereas the saturable pathway involves clathrin-coated pits, the slow pathway is clathrin-independent (12).

Endocytosis of growth factor receptors

Low capacity of high-affinity EGF-induced receptor internalization suggested that unique mechanisms may regulate this pathway. It has been hypothesized that proteins other than AP-2, expressed in limited amounts in the cell, control rapid pathway (10, 12, 28, 29). These proteins are not yet identified. The study on MDCK cells demonstrated that the microinjection of *src*-homology domains 2 (SH2) domains of GRB2 adapter protein blocks endocytosis of the EGF receptor (30). This finding is, however, difficult to reconcile with the previous results of kinetics analysis of receptor mutants (23, 24, 31, 32). For instance, mutational removal of all phosphorylation sites of the EGF receptor, including GRB2 binding, did not block EGF endocytosis (24, 32). Another putative player in EGFR internalization is the cytoplasmic tyrosine kinase c-Src that can be activated in some cell types by EGF (33).

An EGF receptor phosphorylation substrate, eps15, has been also implicated in EGF receptor endocytosis (34). Eps15 is located in plasma membrane coated pits and is associated with the γ -subunit of AP-2 (35, 36). However, eps15 appears to play an important role in a general endocytic machinery and is not specific for growth factor internalization (37).

Intensive studies were directed to map regions of the receptor molecule that are critical for internalization. The intracellular domain of the EGF receptor has been shown to be essential for endocytosis (figure 2). Mutational inactivation of the receptor kinase resulted in significant reduction of the internalization rate (38-42). Kinase-negative receptors were capable of internalization with a moderate speed that did not depend on EGF concentration (non-saturable pathway), suggesting that kinase activity controls the saturable pathway of internalization. Kinase activity is also necessary for the maximum rapid internalization of PDGF (43), macrophage colony-stimulating factor (m-CSF) (c-Fms receptor) (25), fibroblast growth factor (FGF) (44) and *c-kit* receptors (45). Receptor tyrosine kinases are known to be autophosphorylated but it is unlikely that kinase activity is required only for receptor autophosphorylation. For instance, an EGF receptor deletion mutant lacking all autophosphorylation sites, was internalized in kinase-dependent manner (24, 32). It is possible that tyrosine phosphorylation of an unidentified substrate of the receptor kinase is necessary for the receptor internalization through rapid saturable pathway.

Rapid internalization of EGF also requires multiple tyrosine phosphorylation of the receptor (31, 42, 46), although the role of autophosphorylation is not clear. The experiments with partially truncated receptor mutants (24) suggested that tyrosine phosphorylation *per se* is not essential for the internalization of EGF receptors. However, tyrosine phosphorylation of the full-length receptor may be needed to support conformational changes that expose endocytic codes. In PDGF receptor beta the phosphorylation site in the juxta membrane region, tyrosine 579, was found to be involved in the control of internalization (47). Phosphorylation of the c-Fms and *c-kit* receptors is not important for internalization (25, 45, 48)

whereas the phosphorylation site of the FGF receptor is involved in its endocytosis (44). It is possible that observed effects of mutations of autophosphorylation sites of FGF and PDGF receptors on endocytosis reflect the necessity of phosphorylation-dependent conformational changes rather than the direct involvement of SH2-containing proteins.

In summary, rapid saturable pathway of GF endocytosis is controlled by endocytic codes, receptor kinase activity and in some cases by receptor phosphorylation. The endocytic codes presumably interact with AP-2 although other mechanisms might be involved. The nature of saturability is unknown. Receptors that lack carboxyl-terminal tail do not interact with AP-2 (49) and undergo internalization through the slow and non-saturable pathway of constitutive clathrin-independent endocytosis (figure 1).

5. INTRACELLULAR SORTING

5.1. Pathway through endosomes

After internalization, EGF and EGF receptors begin their passage through the endosomal compartments (figure 1). Clathrin-coated vesicles first fuse with early endosomes, which requires uncoating of the vesicle. Early endosomes are the tubular-vesicular compartments often connected into networks and located at the cell periphery. EGF-receptor complexes can be seen in early endosomes within 2-5 min of EGF-induced internalization at 37°C (5, 6, 50, 51). After 10-15 min receptors begin to accumulate in large tubular-vesicular endosomes located in the perinuclear area, often surrounding the centriole (5, 6, 9, 51). On conventional electron microscopic sections, perinuclear endosomes frequently appear as multivesicular bodies (MVB) because they contain internal vesicles (5, 6, 9, 51). MVBs are likely to correspond to the intermediate and late endosomes. The transition delivery of receptors from intermediate to late endosomes/lysosomes is highly-temperature dependent as it is blocked at 16-20°C (51-53). The late endosomes serve as the last destination of molecules sorted to the lysosomal pathway, and often referred to as prelysosomal compartment (54). Although the appearance of EGF receptors in late endosomes can be monitored by subcellular fractionation in density gradients, the colocalization of the EGF and mannose-6-phosphate receptors (a marker of late endosomes) has not been shown morphologically (55). Moreover, the direct fusion of MVB containing EGF receptors with lysosomes has been recently demonstrated (53). Thus, the role of late endosomes in intracellular sorting of EGF receptors is unclear.

The degradation of EGF and other GFs as well as their receptors begins after 20-30 min of continuous endocytosis (43, 56, 57), suggesting that the proteolysis occurs in MVB/late endosomes which contain functionally active enzymes. However, the complete degradation of EGF and its receptors is thought to occur in mature lysosomes. EGF and EGF receptors become detectable in lysosomes after 30-60 min of internalization but in some cells can be seen in MVBs for several hours (51). The products of partial proteolysis of the EGF receptor are

Endocytosis of growth factor receptors

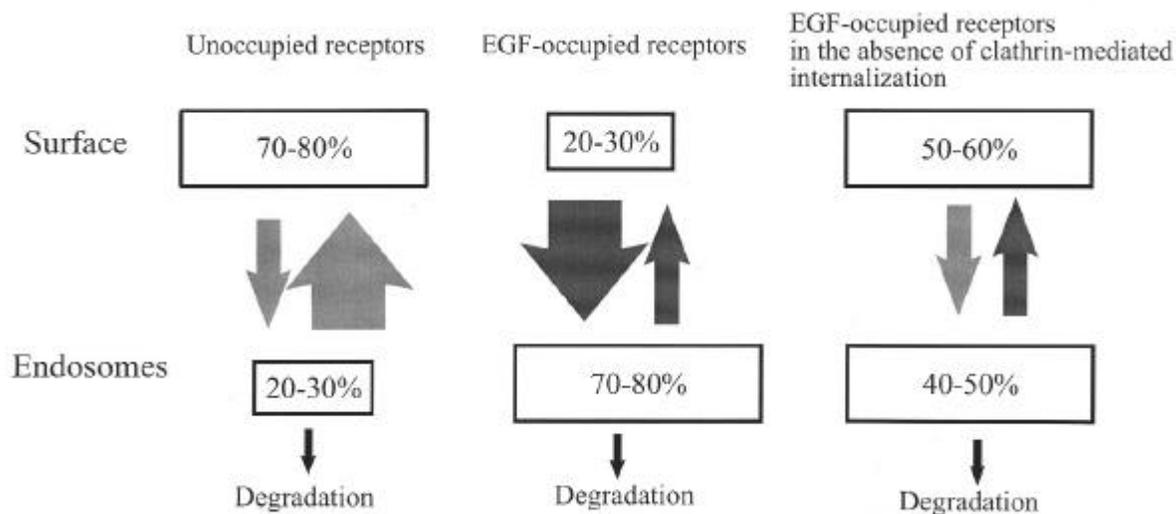


Figure 3. GF-induced changes in the rate parameters of the trafficking and subcellular distribution of the receptors. The relative values of the specific rate constants of internalization, recycling and degradation were averaged from several studies of EGF receptor endocytosis (12, 29, 31, 42, 66, 73) and expressed as the widths of arrows. The model is applicable to the cells expressing a moderate physiological amount of EGF receptors (not more than 100,000 per cell) and assumes no saturation of internalization and degradation systems. In this model we propose that EGF binding accelerates receptor internalization by activating the rapid pathway and reduces recycling of the receptors by retaining occupied receptors in endosomes and preventing them from recycling. The resulting accumulation of 70-80% of occupied receptors in endosomes after 20-30 min of continuous ligand-induced endocytosis leads to the increased apparent degradation rate and down-regulation of receptors without any change in the specific rate constant of degradation. When clathrin-dependent pathway is blocked (for instance, by expression of dominant-negative mutant of dynamin (85), EGFR down-regulation is partial.

difficult to detect in lysosomes, presumably because they are very rapidly degraded to low molecular weight peptides (58, 59).

Several lines of evidence indicate that despite an acidic environment in endosomes the release of EGF from the receptor is insignificant until the late stages of endocytosis, and that there remains a large pool of endosomal EGF-receptor complexes (60-62). This is in agreement with the common localization of EGF and EGF receptor throughout the endocytic pathway (51, 63) (figure1). Similarly, a pool of intact PDGF-receptor (64) and NGF-Trk receptor complexes (65) can be detected in endosomes. Thus, internalized GF receptors maintain their ligand-dependent dimerization and phosphorylation status during trafficking through the endosomal compartments.

5.1. Kinetics of intracellular trafficking: recycling versus degradation

The relative values of rate parameters of endocytic trafficking of occupied and unoccupied EGF receptors are compared in figure 3. In the absence of EGF, EGF receptors are slowly internalized and rapidly recycled which results in accumulation of the receptors at the cell surface (29). As described above EGF accelerates receptor internalization. EGF-receptor complexes can also rapidly recycle after internalization (41, 52, 66). The overall rate of recycling of EGF-occupied receptors is, however, 2-3 times slower than recycling of unoccupied receptors(66, 67). It

can be estimated that after each round of endocytosis about 20-30% of internalized EGF-receptor complexes are trapped in endosomes and sorted to the degradation pathway, whereas only 5-10% of unoccupied receptors escape recycling. Given the low internalization rate and small pool of endocytosed receptors in the absence of EGF, the apparent degradation rate of unoccupied receptors is very low, and the loss of receptors is compensated for by biosynthesis. EGF binding reduces the recycling rate and, therefore, increases the degradation/recycling rate ratio that determines the fate of receptor population after endocytosis. Reduced recycling together with EGF-accelerated internalization result in the significant elevation of the overall degradation of receptors which leads to receptor down-regulation.

The importance of ligand binding for the lysosomal targeting and down-regulation of internalized receptors can be illustrated by the example of the differential effect of EGF and transforming growth factor alpha (TGFalpha) on endocytosis of their common EGF receptor. TGFalpha binding to the EGF receptor is much more pH-sensitive than binding of EGF to the receptor and, probably, most of TGFalpha dissociates from the receptor at pH 5.5-6.0 in early and intermediate endosomes (68, 69). This dissociation allows rapid recycling of ligand-free receptors and reduces overall receptor degradation. Consequently, TGFalpha causes much weaker down-regulating effect on EGF receptors compared to that of EGF (68, 69).

5.3. Possible mechanisms of receptor sorting in endosomes

Recycling of EGF-receptor complexes is partially inhibited at 18°C when receptors are accumulated in MVB/intermediate endosomes, suggesting that recycling does occur from these compartments (52). This “late” recycling pathway is, however, much slower than recycling from early endosomes. In general, the deeper receptors advance along the endocytic pathway, the slower the overall rate and the smaller the extent of receptor recycling.

The reduced recycling correlates with elevated sorting to the degradation pathway. To understand the mechanisms of the retention of EGF-receptor complexes in “maturing” endosomes during endocytosis, the pathways of intracellular sorting of EGF receptors have been compared with those of transferrin receptors which are targeted to lysosomes very insignificantly (9, 50). Morphological analysis revealed that in early endosomes and MVB EGF receptors tend to accumulate in vesicular parts of the compartments (figure 1), whereas transferrin receptors are mostly located in tubular parts (9). Moreover, in MVB-like endosomes, EGF receptors are preferentially associated with the internal vesicular structures, whereas transferrin receptors are distributed mainly in the outer membrane and tubular parts of endosomes. On the basis of these observations, the following model of endosomal sorting has been proposed (9, 70). Receptors located in the tubular portions of endosomes are constitutively recycled. In contrast, a pool of EGF receptors are trapped (retained) in the vesicular parts and subsequently become incorporated into internal vesicles of MVBs. According to this model, receptor packaging into internal vesicles prevents their recycling and leads to their retention in MVB/late endosomes and subsequent exposure to proteolytic enzymes after endosome-lysosome fusion.

The molecular mechanisms of the selective sorting of occupied EGF receptors to the internal membranes of endosomes are unknown. It has been hypothesized that EGF-induced activation of receptor kinase and tyrosine phosphorylation of annexin I in MVBs is important for inclusion of EGF receptors in internal vesicles of MVBs (71, 72). However, the data that receptor mutants lacking an ATP-binding site or an entire kinase domain are degraded as fast as wild-type receptors, have questioned the direct involvement of tyrosine kinase activity in the intracellular sorting of EGF receptors (23, 29, 66, 73). Recent reports (23, 74) suggested that carboxyl-terminal regions of the receptor between residues 945-958 and 1022-1063 (figure 2) are necessary for the receptor degradation. Interestingly, kinetic studies revealed that the lysosomal sorting (or retention) pathway of EGF receptors is also saturable (73), indicating that either the proteins responsible for sorting or the pool of EGF receptors competent for lysosomal sorting are limited. Whereas degradation of large concentration of endosomal EGF receptors did not depend on the receptor kinase activity, the saturable pathway is apparently controlled by the receptor kinase (74).

An endosomal protein called sorting nexin, SNX-1 has been recently discovered based on its ability to

interact with the cytoplasmic domain of the EGF receptor in yeast two-hybrid system (75). Overexpression of SNX-1 leads to down-regulation of the EGF receptor but not other GF receptors suggesting that SNX-1 plays an important role in the specific targeting of EGF receptors to the degradation pathway. PDGF-dependent activation of phosphatidylinositol-3-kinase has been implicated in regulation of the endosomal sorting of the PDGF receptor (76). However, this kinase appears to play role in some universal steps of endosomal sorting and is not specific for GF endocytosis. The protein coats and adaptor molecules involved in endosomal sorting of GF receptors are unknown. Recently, clathrin coats containing adaptor complex AP-3 have been identified in the early endosomes of A-431 cells (77). It is attractive to propose that these coats are involved in recycling of receptors as they contain transferrin receptors (78).

6. ROLE OF ENDOCYTOSIS IN GROWTH FACTOR SIGNALING

GF-induced receptor down-regulation is thought to serve as feedback attenuation of mitogenic and transforming receptor signaling (79). The main argument to support this notion is the observation of an elevated mitogenic, transforming and tumorigenic activity of the EGF receptor mutant that is internalized very inefficiently (80). In physiological systems endocytosis can negatively regulate signaling by at least two ways. First, ligand-induced down-regulation leads to significant reduction of the total receptor pool in the cell. Second, extensive endocytosis may lead to depletion of GF from the extracellular fluid (79). The saturability of the specific endocytic pathway of internalization and lysosomal sorting has important implication in respect of regulation of signaling by endocytosis. Cells that express moderate levels of GF receptors can balance GF signaling by down-regulating GF receptors. In contrast, cells that overexpress GF receptors are incapable of efficient down-regulation of these receptors because the endocytic machinery is overwhelmed and can not handle the large amount of activated GF receptors. In results, a large pool of active GF receptors is maintained in such cells for the long periods of time in the presence of GF, which leads to extensive and unbalanced GF signaling. An example of such system are several types of breast and squamous carcinoma cells that overexpress EGF receptors (81).

Another conceivable consequence of endocytosis for GF signaling is the possibility of signaling from endosomes. Studies on perfused rat liver and tissue culture cells demonstrated that EGF remains bound to the receptor during endocytosis, and that receptors remain dimerized, tyrosine phosphorylated, active as kinase and associated with the signal transduction proteins in endosomes (61, 62, 82, 83). Similarly, the existence of active PDGF receptors β in endosomes has been proposed (64). In neurons, there is a physical distance between the site of initial activation of Trk receptor tyrosine kinase by the nerve growth factor (NGF) in the nerve terminal and the site of action in the cell body. It has been proposed that activated Trk is transported to the cell body in specialized endosomes where it preserves kinase activity (65). Thus, “signaling” endosomes may be important in

Endocytosis of growth factor receptors

mediating a variety of early and late signaling events in GF-stimulated cells.

An important limitation of the signaling from endosomes is the sequestration of receptors into internal vesicles of MVB/intermediate/late endosomes. While the carboxyl-terminal domain of the receptors located in the limited endosomal membrane is accessible to kinase substrates and stably interacting proteins, most receptors are accumulated in the internal vesicles within several minutes of continuous endocytosis and cannot be involved in signaling. Whether internal vesicles are physically separated from the limited membrane of endosomes is not clear. Large perinuclear endosomes appear on frozen sections as the tubular-vesicular structures of complex morphology rather than round-shaped MVB seen on the conventional Epon sections (51). It can be proposed that at least some of the vesicle profiles are the deep invaginations of the limited membrane of the endosome.

Despite the possibility of signaling from endosomes, the existence of endosome-specific signals is yet to be demonstrated. The importance of endocytosis for activation of the individual signal transduction pathways has been demonstrated in HeLa cells in which clathrin-dependent endocytosis of EGF receptors was inhibited by overexpression of the dominant-negative mutant dynamin (84). The EGF-induced DNA synthesis was not affected in the absence of endocytosis whereas the expression of mutant dynamin abolished the MAP (ERK) kinase activation by EGF. How receptor-mediated endocytosis controls MAP-kinase activation remains to be determined.

7. PERSPECTIVES

We have discussed pathways, kinetics, possible molecular mechanisms and role in signaling machinery of the receptor-mediated endocytosis of GFs. Although several molecules that interact with EGF receptors and might be involved in endocytic trafficking of these receptors have emerged, the mechanism and the role of these interactions needs further investigation. It is important to determine whether the same or similar proteins control endocytosis of other GF receptors. Certainly, new components of endocytic machineries that are specific for signaling receptors will be discovered. Heterologous expression of sorting proteins may allow the manipulation of the levels and activities of GF receptors in cancerous cells. On the other hand, sequence motifs within GF receptors that are important for their trafficking should be mapped to allow structure-function analysis of receptor interaction with the sorting proteins. Finally, we predict that the issue of relationships between endocytosis and cell signaling will be in a focus of future investigation.

8. ACKNOWLEDGEMENTS

I thank Royston Carter for critical reading of the manuscript. Supported by NIH grant DK46817.

9. REFERENCES

1. Goldstein, J. L., Brown, M. S., Anderson, R. G. W.,

Russel, D. W. & Schneider, W. J.: Receptor-mediated endocytosis: concepts emerging from the LDL receptor system. *Annu. Rev. Cell. Biol.* 1, 1-19 (1985)

2. Schmid, S.: Clathrin-coated vesicle formation and protein sorting: An integrated process. *Annu. Rev. Biochem.* 66, 511-548 (1997)

3. Gruenberg, J. & Maxfield, F.: Membrane transport in the endocytic pathway. *Curr. Opin. Cell Biol.* 7, 552-63 (1995)

4. Carpentier, J.-L.: Insulin receptor internalization: molecular mechanisms and pathophysiological implications. *Diabetologia* 37, S117-S24 (1994)

5. Haigler, H. T., McKanna, J. A. & Cohen, S.: Direct visualization of the binding and internalization of a ferritin conjugate of epidermal growth factor in human carcinoma cells A431. *J. Cell Biol.* 81, 382-95 (1979)

6. Beguinot, L., Lyall, R. M., Willingham, M. C. & Pastan, I.: Down-regulation of the epidermal growth factor receptor in KB cells is due to receptor internalization and subsequent degradation in lysosomes. *Proc. Natl. Acad. Sci. USA* 81, 2384-8 (1984)

7. Gorden, P., Carpentier, J.-L., Cohen, S. & Orci, L.: Epidermal growth factor: Morphological demonstration of binding internalization and lysosomal association in human fibroblasts. *Proc. Natl. Acad. Sci. USA* 75, 5025-9 (1978)

8. Hanover, J. A., Willingham, M. C. & Pastan, I.: Kinetics of transit of transferrin and epidermal growth factor through clathrin-coated membranes. *Cell* 39, 283-93 (1984)

9. Hopkins, C. R. & Trowbridge, I. S.: Internalization and processing of transferrin and the transferrin receptor in human carcinoma A-431 cells. *J. Cell Biol.* 97, 508-21 (1983)

10. Lamaze, C., Baba, T., Redelmeier, T. E. & Schmid, S. L.: Recruitment of epidermal growth factor and transferrin receptors into coated pits *in vitro*: differing biochemical requirements. *Mol. Biol. Cell* 4, 715-27 (1993)

11. Carpentier, J.-L., Gorden, P., Anderson, R. G. W., Brown, M. S., Cohen, S. & Orci, L.: Co-localization of 125I-epidermal growth factor and ferritin-low density lipoprotein in coated pits: A quantitative electron microscopic study in normal and mutant human fibroblasts. *J. Cell Biol.* 95, 73-7 (1982)

12. Lund, K. A., Opresko, L. K., Starbuck, C., Walsh, B. J. & Wiley, H. S.: Quantitative analysis of the endocytic system involved in hormone-induced receptor internalization. *J. Biol. Chem.* 265, 15713-3723 (1990)

13. Hopkins, C. R., Miller, K. & Beardmore, J. M.: Receptor-mediated endocytosis of transferrin and epidermal growth factor receptors: a comparison of constitutive and ligand-induced uptake. *J. Cell Science* –

Endocytosis of growth factor receptors

Suppl. 3, 173-86 (1985)

14. Couet, J., Sargiacomo, M. & Lisanti, M. P.: Interaction of a receptor tyrosine kinase, EGF-R, with caveolins. *J. Biol. Chem.* 272, 30429-38 (1997)

15. Kirchhausen, T., Bonifacino, J. S. & Riezman, H.: Linking cargo to vesicle formation: receptor tail interactions with coat proteins. *Curr. Opin. Cell Biol.* 9, 488-95 (1997)

16. Ohno, H., Stewart, J., Fournier, M. C., Bosshart, H., Rhee, I., Miyatake, S., Saito, T., Gallusser, A., Kirchhausen, T. & Bonifacino, J. S.: Interaction of tyrosine-based sorting signals with clathrin-associated proteins. *Science* 269, 1872-5 (1995)

17. Sorkin, A. & Carpenter, G.: Interaction of activated EGF receptors with coated pit adaptors. *Science* 261, 612-5 (1993)

18. Nesterov, A., Kurten, R. C. & Gill, G. N.: Association of Epidermal Growth Factor Receptors with Coated Pit Adaptors via a Tyrosine Phosphorylation-regulated Mechanism. *J. Biol. Chem.* 270, 6320-7 (1995)

19. Nesterov, A., Wiley, H. S. & Gill, G. N.: Ligand-induced endocytosis of epidermal growth factor receptors that are defective in binding clathrin adaptors. *Proc. Natl. Acad. Sci. USA* 92, 8719-23 (1995)

20. Sorkin, A., Mazzotti, M., Sorkina, T., Scotto, L. & Beguinot, L.: Epidermal growth factor interaction with clathrin adaptors is mediated by the Tyr974-containing internalization motif. *J. Biol. Chem.* 271, 13377-84 (1996)

21. Gilboa, L., Ben-Levy, R., Yarden, Y. & Henis, Y. I.: Roles for a cytoplasmic tyrosine and tyrosine kinase activity in the interactions of Neu receptors with coated pits. *J. Biol. Chem.* 270, 7061-7 (1995)

22. Baulida, J., Kraus, M. H., Alimandi, M., Di Fiore, P. P. & Carpenter, G.: All ErbB Receptors Other Than the Epidermal Growth Factor Receptor Are Endocytosis Impaired. *J. Biol. Chem.* 271, 5251-7 (1996)

23. Opreko, L. K., Chang, C.-P., Will, B. H., Burke, P. M., Gill, G. N. & Wiley, H. S.: Endocytosis and lysosomal targeting of epidermal growth factor receptors are mediated by distinct sequences independent of the tyrosine kinase domain. *J. Biol. Chem.* 270, 4325-33 (1995)

24. Chang, C.-P., Lazar, C. S., Walsh, B. J., Komuro, M., Collawn, J. F., Kuhn, L. A., Tainer, J. A., Trowbridge, I. S., Farquhar, M. G., Rosenfeld, M. G., Wiley, H. S. & Gill, G. N.: Ligand-induced internalization of the epidermal growth factor receptor is mediated by multiple endocytic codes analogous to the tyrosine motif found in constitutively internalized receptors. *J. Biol. Chem.* 268, 19312-20 (1993)

25. Myles, G. M., Brandt, C. S., Carlberg, K. & Rohrschneider, L. R.: Tyrosine 569 in the c-Fms

Juxtamembrane Domain Is Essential for Kinase Activity and Macrophage Colony-Stimulating Factor-Dependent Internalization. *Mol. Cell. Biol.* 14, 4843-54 (1994)

26. Mori, S., Claesson-Welsh, L. & Heldin, C.-H.: Identification of a Hydrophobic Region in the Carboxyl Terminus of the Platelet-derived Growth Factor α -Receptor Which Is Important for Ligand-mediated Endocytosis. *J. Biol. Chem.* 266, 21158-64 (1991)

27. Wiley, H. S.: Anomalous Binding of Epidermal Growth Factor to A431 Cells Is Due to the Effect of High Receptor Densities and a Saturable Endocytic System. *J. Cell Biol.* 107, 801-10 (1988)

28. Lamaze, C. & Schmid, S. L.: Recruitment of Epidermal Growth Factor Receptors into Coated Pits Requires Their Activated Tyrosine Kinase. *J. Cell Biol.* 129, 47-54 (1995)

29. Wiley, H. S., Herbst, J. J., Walsh, B. J., Lauffenberger, D. A., Rosenfeld, M. G. & Gill, G. N.: The role of Tyrosine Kinase Activity in Endocytosis, Compartmentalization and Down-regulation of the Epidermal Growth Factor Receptor. *J. Biol. Chem.* 266, 11083-94 (1991)

30. Wong, Z. & Moran, M. F.: Requirement for the adapter protein GRB2 in EGF receptor endocytosis. *Science* 272, 1935-9 (1996)

31. Sorkin, A., Helin, K., Waters, C. M., Carpenter, G. & Beguinot, L.: Multiple autophosphorylation sites of the epidermal growth factor receptor are essential for receptor kinase activity and internalization. *J. Biol. Chem.* 267, 8672-8 (1992)

32. Chang, C.-P., Kao, J. P. Y., Lazar, C. S., Walsh, B. J., Wells, A., Wiley, H. S., Gill, G. N. & Rosenfeld, M. G.: Ligand-induced internalization and increased cell calcium are Mediated via distinct structural elements in the carboxyl terminus of the epidermal growth factor receptor. *J. Biol. Chem.* 266, 23467-70 (1991)

33. Ware, M. F., Tice, D. A., Parsons, S. J. & Lauffenburger, D. A.: Overexpression of cellular Src in fibroblasts enhances endocytic internalization of epidermal growth factor receptor. *J. Biol. Chem.* 272, 30185-90 (1997)

34. van Delft, S., Schumacher, C., Hage, W., Verkleij, A. J. & van Bergen en Henegouwen, P. M. P.: Association and colocalization of Eps15 with adaptor protein-2 and clathrin. *J. Cell Biol.* 136, 811-21 (1997)

35. Benmerah, A., Gagnon, J., Begue, B., Megarbane, B., Dautry-Varsat, A. & Cerf-Bensussan, N.: The tyrosine kinase substrate eps15 is constitutively associated with the plasma membrane adaptor AP-2. *J. Cell Biol.* 131, 1831-8 (1995)

36. Tebar, F., Sorkina, T., Sorkin, A., Ericsson, M. & Kirchhausen, T.: Eps15 is a component of clathrin-coated

Endocytosis of growth factor receptors

- pits and vesicles and is located at the rim of coated pits. *J. Biol. Chem.* 271, 28727-30 (1996)
37. Di Fiore, P. P., Pelicci, P. G. & Sorkin, A.: EH: A novel protein-protein interaction domain potentially involved in intracellular sorting. *Trends Biochem. Sci.* 22, 411-413 (1997)
38. Glenney, J. R., Jr., Chen, W. S., Lazar, C. S., Walton, G. M., Zokas, L. M., Rosenfeld, M. G. & Gill, G. N.: Ligand-induced endocytosis of the EGF receptor is blocked by mutational inactivation and by microinjection of anti-phosphotyrosine antibodies. *Cell* 52, 675-84 (1988)
39. Chen, W. S., Lazar, C. S., Lund, K. A., Welsh, J. B., Chang, C. P., Walton, G. M., Der, C. J., Wiley, H. S., Gill, G. N. & Rosenfeld, M. G.: Functional Independence of the Epidermal Growth Factor Receptor From a Domain Required For Ligand-Induced Internalization and Calcium Regulation. *Cell* 59, 33-43 (1989)
40. Countaway, J. L., Nairn, A. C. & Davis, R. J.: Mechanism of Desensitization of the Epidermal Growth Factor Receptor Protein-Tyrosine Kinase. *J. Biol. Chem.* 267, 1129-40 (1992)
41. Felder, S., LaVin, J., Ullrich, A. & Schlessinger, J.: Kinetics of Binding, Endocytosis, and Recycling of EGF receptor mutants. *J. Cell Biol.* 117, 203-12 (1992)
42. Sorkin, A., Waters, C. M., Overholser, K. A. & Carpenter, G.: Multiple autophosphorylation site mutations of the epidermal growth factor receptor. *J. Biol. Chem.* 266, 8355-62 (1991)
43. Sorkin, A., Westermark, B., Heldin, C.-H. & Claesson-Welsh, L.: Effect of receptor kinase inactivation on the rate of internalization and degradation of PDGF and the PDGF beta receptor. *J. Cell Biol.* 112, 469-78 (1991)
44. Sorokin, A., Mohammadi, M., Huang, J. & Schlessinger, J.: Internalization of Fibroblast Growth Factor Receptor Is Inhibited by a Point Mutation at Tyrosine 766. *J. Biol. Chem.* 269, 17056-61 (1994)
45. Yee, N. S., Hsiao, C.-W. M., Serve, H., Vosseller, K. & Besmer, P.: Mechanism of down-regulation of c-kit receptor. *J. Biol. Chem.* 269, 31991-8 (1994)
46. Helin, K. & Beguinot, L.: Internalization and down-regulation of the human epidermal growth factor receptor are regulated by the carboxyl-terminal tyrosines. *J. Biol. Chem.* 266, 8363-8 (1991)
47. Mori, S., Ronnstrand, L., Claesson-Welsh, L. & Heldin, C.-H.: A Tyrosine Residue In the Juxtamembrane Segment of the Platelet-Derived Growth Factor beta-Receptor Is Critical For Ligand-Mediated Endocytosis. *J. Biol. Chem.* 269, 4917-21 (1994)
48. Carlberg, K., Tapley, P., Haystead, C. & Rohrschneider, L.: The role of kinase activity and the kinase insert region in ligand-induced internalization and degradation of the c-fms protein. *EMBO. J.* 4, 877-83 (1991)
49. Sorkin, A., McKinsey, T., Shih, W., Kirchhausen, T. & Carpenter, G.: Stoichiometric interaction of the epidermal growth factor receptor with the clathrin-associated protein complex AP-2. *J. Biol. Chem.* 270, 619-25 (1995)
50. Hopkins, C. R., Gibson, A., Shipman, M. & Miller, K.: Movement of internalized ligand-receptor complexes along a continuous endosomal reticulum. *Nature* 346, 335-9 (1990)
51. Miller, K., Beardmore, J., Kanety, H., Schlessinger, J. & Hopkins, C. R.: Localization of epidermal growth factor (EGF) receptor within the endosome of EGF-stimulated epidermoid carcinoma (A431) cells. *J. Cell Biol.* 102, 500-9 (1986)
52. Sorkin, A., Krolenko, S., Kudrjavtceva, N., Lazebnik, J., Teslenko, L., Soderquist, A. M. & Nikolsky, N.: Recycling of epidermal growth factor-receptor complexes in A431 cells: identification of dual pathways. *J. Cell Biol.* 112, 55-63 (1991)
53. Futter, C. E., Pearse, A., Hewlett, L. J. & Hopkins, C. R.: Multivesicular endosomes containing EGF-EGF receptor complexes mature and fuse directly with lysosomes. *J. Cell Biol.* 132, 1011-24 (1996)
54. Griffiths, G. & Gruenberg, J.: The arguments for pre-existing early and late endosomes. *Trends Cell Biol.* 1, 5-9 (1991)
55. Hirst, J., Futter, C. E. & Hopkins, C.: The kinetics of mannose 6-phosphate receptor trafficking in the endocytosis pathway in HEp-2 cells: The receptor enters and rapidly leaves multivesicular endosomes without accumulating in prelysosomal compartment. *Mol. Biol. Cell* 9, 809-16 (1998)
56. Carpenter, G. & Cohen, S.: ¹²⁵I-Labeled human epidermal growth factor: binding internalization, and degradation in human fibroblasts. *J. Cell Biol.* 71, 159-71 (1976)
57. Heldin, C.-H., Wasteson, A. & Westermark, B.: Interaction of platelet-derived growth factor with its fibroblast receptor. *J. Biol. Chem.* 257, 4216-21 (1982)
58. Stoscheck, C. M. & Carpenter, G.: Characterization of the metabolic turnover of epidermal growth factor receptor protein in A-431 cells. *J. Cell Physiol.* 120, 296-302 (1984)
59. Stoscheck, C. M. & Carpenter, G.: "Down-regulation" of EGF receptors: Direct demonstration of receptor degradation in human fibroblasts. *J. Cell Biol.* 98, 1048-53 (1984)
60. Sorkin, A., Teslenko, L. & Nikolsky, N.: The endocytosis of epidermal growth factor in A431 cells: a pH of microenvironment and the dynamics of receptor complexes dissociation. *Exp. Cell Res.* 175, 192-205 (1988)
61. Sorkin, A. & Carpenter, G.: Dimerization of internalized growth factor receptors. *J. Biol. Chem.* 266, 23453-60 (1991)
62. Lai, W. H., Cameron, P. H., Doherty II, J.-J., Posner, B. I. & Bergeron, J. J. M.: Ligand-mediated autophosphorylation activity of the epidermal growth factor receptor during internalization. *J. Cell Biol.* 109, 2751-0 (1989)

Endocytosis of growth factor receptors

63. Carpentier, J. L., White, M. F., Orci, L. & Kahn, C. R.: Direct visualization of the phosphorylated epidermal growth factor receptor during its internalization in A-431 cells. *J. Cell Biol.* 105, 2751-62 (1987)
64. Sorkin, A., Eriksson, A., Heldin, C.-H., Westermark, B. & Claesson-Welsh, L.: Pool of Ligand-bound Platelet-derived Growth Factor α -Receptors Remain Activated and Tyrosine-Phosphorylated After Internalization. *J. Cell Physiol.* 156, 373-82 (1993)
65. Grimes, M. L., Zhou, J., Beattie, E. C., Yuen, E. C., Hall, D. E., Valletta, J. S., Topp, K. S., LaVail, J. H., Bunnett, N. W. & Mobley, W. C.: Endocytosis of activated TrkA: evidence that nerve growth factor induces formation of signaling endosomes. *J. Neuroscience* 16, 7950-64 (1996)
66. Herbst, J. J., Opresko, L. K., Walsh, B. J., Lauffenberger, D. A. & Wiley, H. S.: Regulation of postendocytic trafficking of the epidermal growth factor receptor through endosomal retention. *J. Biol. Chem.* 269, 12865-73 (1994)
67. Sorkin, A. & Waters, C. M.: Endocytosis of Growth Factor Receptors. *BioEssays* 15, 375-82 (1993)
68. Ebner, R. & Derynck, R.: Epidermal growth factor and transforming growth factor- α : differential intracellular routing and processing of ligand-receptor complexes. *Cell Reg.* 2, 599-612 (1991)
69. French, A. R., Tadaki, D. K., S.K., N. & Lauffenberger, D. A.: Intracellular trafficking of epidermal growth factor family ligands is directly influenced by the pH sensitivity of the receptor/ligand interaction. *J. Biol. Chem.* 270, 4334-40 (1995)
70. Hopkins, C. R.: Selective membrane protein trafficking: vectorial flow and filter. *Trends Biochem. Sci.* 17, 27-32 (1992)
71. Felder, S., Miller, K., Moehren, G., Ullrich, A., Schlessinger, J. & Hopkins, C. R.: Kinase activity controls the sorting epidermal growth factor receptor within the multivesicular body. *Cell* 61, 623-34 (1990)
72. Futter, C. E., Felder, S., Schlessinger, J., Ullrich, A. & Hopkins, C. R.: Annexin I is phosphorylated in the multivesicular body during the processing of the epidermal growth factor receptor. *J. Cell Biol.* 120, 77-83 (1993)
73. French, A. R., Sudlow, G. P., Wiley, H. S. & Lauffenberger, D. A.: Postendocytic trafficking of epidermal growth factor-receptor complexes is mediated through saturable and specific endosomal interactions. *J. Biol. Chem.* 269, 15749-55 (1994)
74. Kornilova, E., Sorkina, T., Beguinot, L. & Sorkin, A.: Lysosomal targeting of epidermal growth factor receptors via a kinase-dependent pathway is mediated by the receptor carboxyl-terminal residues 1022-1163. *J. Biol. Chem.* 271, 30340-6 (1996)
75. Kurten, R. C., Cadena, D. L. & Gill, G. N.: Enhanced degradation of EGF receptors by a sorting nexin, SNX-1. *Science* 272, 1008-10 (1996)
76. Joly, M., Kazlauskas, A., Fay, F. S. & Corvera, S.: Distribution of PDGF receptor trafficking by mutation of its PI-3 kinase binding sites. *Science* 263, 684-7 (1994)
77. Dell'Angelica, E. C., Klumperman, J., Stoorvogel, W. & Bonifacino, J. S.: Association of the AP-3 adaptor complex with clathrin. *Science* 280, 431-4 (1998)
78. Stoorvogel, W., Oorschot, V. & Geuze, H. J.: A novel class of clathrin-coated vesicles budding from endosomes. *J. Cell Biol.* 132, 21-33 (1996)
79. Starbuck, C. & Lauffenberger, D. A.: Mathematical Model for the Effects of Epidermal growth Factor Receptor Trafficking Dynamics on Fibroblast Proliferation Responses. *Biotechnol. Prog.* 8, 132-43 (1992)
80. Wells, A., Welsh, J. B., Lazar, C. S., Wiley, H. S., Gill, G. N. & Rosenfeld, M. G.: Ligand-Induced Transformation By a Noninternalizing Epidermal Growth Factor Receptor. *Science* 247, 962-4 (1990)
81. Di Fiore, P. P. & Kraus, M. H.: Mechanisms involving an expanding erbB/EGF receptor family of tyrosine kinases in human neoplasia. *Cancer Treat. Res.* 61, 139-60 (1992)
82. Di Guglielmo, G. M., Baass, P. C., Ou, W.-J., Posner, B. & Bergeron, J. J. M.: Compartmentalization of SHC, GRB2 and mSOS, and hyperphosphorylation of Raf-1 by EGF but not insulin in liver parenchyma. *EMBO. J.* 13, 4269-77 (1994)
83. Nesterov, A., Reshetnikova, G., Vinogradova, N. & Nikolsky, N.: Functional state of the epidermal growth factor-receptor complexes during internalization in A-431 cells. *Mol. Cell. Biol.* 10, 5011-4 (1990)
84. Vietra, A. V., Lamaze, C. & Schmid, S. L.: Control of EGF receptor signaling by clathrin-mediated endocytosis. *Science* 274, 2086-9 (1996)
85. Damke, H., Baba, T., Warnock, D. E. & Schmid, S. L.: Induction of mutant dynamin specifically blocks endocytic coated vesicle formation. *J. Cell Biol.* 127, 915-34 (1994)

Key words: Cell Biology; Mammalian Cells; Endocytosis; Intracellular Trafficking; Growth Factor Receptors

Send correspondence to: Dr Alexander Sorkin, Department of Pharmacology, University of Colorado Health Sciences Center, 4200 E. Ninth Avenue, Denver CO 80262, Tel: (303) 315-7252, Fax: (303) 315-7097, E-mail: alexander.sorkin@uchsc.edu