Focus on “EGF receptor downregulation depends on a trafficking motif in the distal tyrosine kinase domain”

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ON BINDING OF GROWTH FACTORS, growth factor receptors in the plasma membrane can trigger a variety of intracellular signaling pathways, promoting responses ranging from differentiation to proliferation and cell migration (21). A principal mechanism for modulating cellular responses to growth factors is control of their accessibility to receptors by dynamically regulating receptor distribution between plasma and intracellular membrane compartments. The trafficking of the epidermal growth factor (EGF) receptor, a member of the ErbB family of protein-tyrosine kinases (21), has been perhaps the most extensively studied of any of the growth factor receptors. Binding of EGF to EGF receptor initiates the EGF-mediated intracellular signaling cascade (21) while concomitantly initiating the rapid clearance of EGF-EGF receptor complexes from the plasma membrane to endosomes by clathrin-mediated endocytosis (17, 19). Unlike other types of receptors such as nutritional receptors (e.g., transferrin receptors) that are rapidly recycled to the plasma membrane after release of ligand in endosomes (10), the EGF-EGF receptor complex is routed to lysosomes for degradation (1). This phenomenon is referred to receptor downregulation, and it is a key element in controlling the magnitude and duration of EGF signaling.

Although a pool of EGF receptors may recycle constitutively, endocytosis of EGF receptors is enhanced by EGF binding and the resulting increase in its intrinsic receptor kinase activity (22). Work by Opresko et al. (14) with different EGF receptor mutants lacking the conserved tyrosine kinase domain but retaining distal carboxy terminus sequences has extended this proposed model of ligand-dependent EGF receptor phosphorylation and internalization, suggesting that receptor phosphorylation elicits a conformational change responsible for unmasking of cryptic targeting signals within the cytoplasmic domain that cue endocytosis and lysosomal targeting.

What sorts of targeting signals might cue EGF receptor internalization and downregulation? The increased availability of protein sequence information has led to the identification of different amino acid motifs present in the cytoplasmic tails of a variety of membrane receptors of all types (signaling, nutritional, housekeeping) that interact with components of the membrane trafficking machinery to promote selective targeting to specific membrane compartments. These motifs include those containing tyrosine residues such as NPXY (X representing any amino acid; Refs. 4, 6) and YXX\phi (\phi representing a bulky hydrophobic amino acid; Refs. 5, 6), as well as those containing LL, with or without a cluster of acidic amino acid residues (acidic cluster; Refs. 3, 6, 7, 15). Other motifs in the cytoplasmic tails of receptors have been identified that specify interactions with regulatory elements such as the ubiquitination machinery (16, 20).

Several sequences within the EGF receptor cytoplasmic tail have been implicated in the lysosomal targeting of ligand-occupied EGF receptor that is critical for its downregulation, including the tyrosine-based sorting signal YLVI, which resembles the YXX\phi lysosomal sorting signal identified for the resident lysosomal protein, LAMP1 (5). In the current article in focus (Ref. 6a; see p. C420 in this issue), Jones et al. present data substantiating the involvement of this tyrosine-based sorting signal, \textsuperscript{954}YLVI, in the targeting of human EGF receptor to lysosomes. These investigators generated two mutant human EGF receptors by replacing the \textsuperscript{954}YL with either AA or VD and then examined the consequences of these replacements to EGF receptor endocytosis and lysosomal targeting in transiently or stably transfected HEK 293 cells, which have extremely low endogenous levels of EGF receptor. Fluorescence detection of wild-type and mutant EGF receptors with Texas red EGF in transiently transfected cells revealed that the patterns of cell surface distribution and internalization to early endosomes (marked by rab5-GFP) were comparable. However, biochemical analyses revealed that these mutations significantly impaired EGF-induced downregulation of EGF receptor without affecting EGF-induced substrate phosphor-
ylation. Further analysis in cell lines stably expressing wild-type and mutant EGF receptors substantiated the observations from transiently transfected cells, showing that the mutations completely blocked EGF-induced EGF receptor downregulation. Furthermore, immunofluorescence microscopy revealed that wild-type but not mutant EGF receptors could be colocalized with LAMP1 after exposure to EGF for 60 min.

Before this work, the 954YLVI site within the EGF receptor had been implicated in lysosomal targeting only indirectly. Mutagenesis studies previously demonstrated that a region located between residues 945 and 991 that included this motif was required for EGF receptor downregulation (14). Furthermore, a unique sorting nexin, SNX1, was identified through a yeast two-hybrid screen using the cytoplasmic tail of the EGF receptor as bait (11). The motif within the EGF receptor responsible for binding to SNX-1 was subsequently narrowed down to a 15-amino acid domain spanning residues 943–957 containing 954YLVI, whereas overexpression of SNX-1 in CV-1 cells significantly increased EGF receptor downregulation.

The work by Jones et al. (6a) provides the first direct evidence that this particular targeting motif confers lysosomal targeting of EGF receptor. However, two additional sorting motifs have been identified within the cytoplasmic tail region of the EGF receptor: an LL motif (679LL) and a c-Cbl recognition site for ubiquitination (1045Y). Like 954YLVI, mutagenesis studies of 679LL showed that this motif is essential for ligand-induced receptor degradation (9) and further suggested that this effect is associated with the ability of this motif to sequester EGF-EGF receptor complexes into multivesicular endosomes (8). Other investigations have implicated autophosphorylation of 1045Y in c-Cbl binding and phosphorylation, followed by recruitment of ubiquitin-activating and -conjugating enzymes that participate in receptor ubiquitination for enhanced degradation (13). As for 954YLVI and 679LL, mutation of 1045Y impairs EGF receptor downregulation (12).

By directly demonstrating the involvement of 954YLVI in lysosomal targeting of EGF receptor, this study by Jones et al. (6a) has raised several new questions. Clearly, a major issue is the relationship between these different sorting motifs present in the EGF receptor, each of which appears to be critical for EGF receptor downregulation. On the basis of their data and those of other investigators in this field, Jones et al. (6a) propose a novel but speculative model suggesting that these different motifs may act in a concerted manner. They suggest that ligand binding and the resultant autophosphorylation of 1045Y lead to rapid recruitment of c-Cbl that stabilizes and ubiquitinates the cytoplasmic region of the EGF receptor in such a way as to expose the 954YLVI and 679LL lysosomal targeting motifs. They then posit that these two domains may form a bipartite binding site for recruitment of SNX-1 and other factors responsible for escorting this complex to lysosomal compartments.

If these two motifs actually form a bipartite domain after receptor phosphorylation and c-Cbl-mediated ubiquitination, we would expect that this mechanism would be conserved at minimum across other species expressing the EGF receptor. Interestingly, the analysis by Jones et al. (6a) of the sequence conservation of 954YLVI reveals that it is highly conserved in EGF receptors from other species and in other ErbB family members. In contrast, the 679LL dileucine motif is not conserved in EGF receptors from other species and, furthermore, is not shared among other ErbB human family members. Other studies have suggested that one leucine within the LL motif can be replaced by isoleucine, valine, alanine, or methionine (6), although none of these “modified” LL motifs is apparent in the sequence alignment for the LL motif chosen by Jones et al. (6a) for nonhuman EGF receptors. It is also conceivable that a related hydrophobic motif such as the newly identified phenylalanine-isoleucine (FI) motif present in furin (18) may function in place of LL to form a bipartite binding site for SNX-based sorting machinery in other species. Alternatively, 679LL and 954YLVI may represent discrete sites for interaction of different membrane trafficking proteins that are each essential for accurate lysosomal transit.

Just as changes in EGF receptor expression levels have been linked to changes in cellular proliferation associated with cell transformation, modulation of cellular responsiveness to EGF can be achieved through changes in its trafficking. Recent work has shown that an oncopgenic form of c-Cbl, v-Cbl, is able to prevent EGF-induced EGF receptor downregulation, directing EGF receptor through recycling rather than degradative pathways (12). The adenosinergic protein E3–13.7 was recently localized to early and multivesicular endosomes in infected cells; this protein mediates the selective ligand-independent downregulation of EGF receptor by rerouting constitutively recycling receptor to lysosomes (2). Intriguingly, this protein appears to interact with a region of the EGF receptor containing the 679LL motif and itself contains a LL motif. Insights obtained from such models of viral pathogenesis as well as extension of EGF receptor trafficking work into additional physiological model systems may prove important in dissecting further intricacies of EGF receptor sorting.

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REFERENCES


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