Get Off My Back! Rapid Receptor Internalization through Circular Dorsal Ruffles

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Abstract

Internalization and subsequent trafficking of receptor tyrosine kinases (RTKs) play an important role in the modulation of growth factor–stimulated signaling events that affect different cellular processes, from cell growth and mitosis to motility and invasion. The intracellular transport of these receptors has traditionally been viewed as being initiated via clathrin-coated pits. However, nonclathrin pathways have been implicated as well, although these remain poorly understood. Most recently, the formation of dynamic, transient endocytic structures termed circular dorsal ruffles or “dorsal waves” have been reported to selectively sequester and internalize a large percentage of a specific RTK from the surface of growth factor–stimulated cells. This process is dependent on dynamin and cortactin, two endocytic proteins that are also associated with the actin cytoskeleton, whereas it is independent of traditional coat proteins, such as clathrin and caveolin. Additionally, dorsal wave formation requires the participation and remodeling of a dynamic actin cytoskeleton. Most importantly, the formation of these structures may be less frequent in tumor cells and thereby have significant effects on receptor signaling and cell growth. (Cancer Res 2006; 66(23): 11094-6)

Selective Internalization of Receptor Tyrosine Kinases

Receptor tyrosine kinases (RTKs), such as epidermal growth factor (EGF) receptors (EGFRs), platelet-derived growth factor (PDGF) receptors (PDGFRs), and hepatocyte growth factor receptors (HGF/c-Met), are internalized via an elaborate clathrin-based machinery that assembles at the plasma membrane. A variety of clathrin adaptors provide a physical bridge between the clathrin coat and cytoplasmic tail of an activated receptor, resulting in receptor sequestration into a forming vesicle. Understanding the regulated and sequential assembly of this vast array of coat, adaptor, scaffolding, and cytoskeletal proteins dedicated to the sequestration and internalization of specific activated receptors at the plasma membrane at the exclusion of many other dormant receptors has been a major focus of cell biologists. Why are so many proteins needed for this task and how do they interact in a regulated and precise manner? How is targeting to distinct endocytic compartments facilitated and are different vesicle trafficking pathways selectively involved in specific downstream signaling events?

It has become evident that the clathrin-dependent endocytic pathway may be only one mechanism of several that are used to rapidly internalize RTKs. Indeed, over 20 years ago, Haigler et al. (1, 2) proposed that activated EGFRs can be internalized via a clathrin-independent mechanism. Using ferritin-conjugated EGF in A431 epidermoid carcinoma cells, it was found that some ferritin-EGF particles localized to regions of the plasma membrane lacking the characteristic features of clathrin-mediated endocytosis. Until recently, these potential clathrin-independent mechanisms have remained elusive.

Caveolae, small, flask-shaped endocytic structures containing the coat protein caveolin, have been implicated as a non-clathrin-based mechanism to bind, sequester, and internalize RTKs. Indeed, EGFRs and PDGFRs have been found to occupy caveolin-rich domains of the plasma membrane (3–6). Interestingly, expression of caveolin-1 was found to inhibit the activation of EGFRs and PDGFRs. Thus, it was suggested that inactive receptors reside in these caveolin-rich domains and, upon activation, they translocate to and are internalized by clathrin-coated pits (3). As a recent extension of this, Sigismund et al. (7) have observed two distinct endocytic compartments that differentially internalize EGFRs depending on the concentrations of EGF ligand applied to cells. At low concentrations of EGF (defined as 1.5 ng/mL by the authors), endocytosis of EGFRs was clathrin-dependent, whereas at high concentrations of EGF (20-100 ng/mL), a simultaneously occurring, clathrin-independent mode emerged. The clathrin-independent mode was dependent on EGFRs being modified by ubiquitin and a single ubiquitin moiety was enough to route receptors to this pathway.

We have identified circular dorsal ruffles (CDRs) as another mode by which cells can internalize significant amounts of EGFRs (8). CDRs have been observed by many different groups over the years in a variety of distinct cell types following stimulation with growth factors, such as EGF, PDGF, and HGF (9). These structures have several names, most recently being termed “dorsal waves,” based on their propagation along the dorsal plasma membrane (10). Most often, these dynamic structures are studied with regard to F-actin regulation and membrane ruffling in response to EGF, PDGF, or HGF rather than membrane and receptor trafficking. However, we have observed that the formation of CDRs is dependent on the synergistic interactions of the large GTPase dynamin and its binding partner cortactin (10, 11). In addition, proteins such as the small GTPases Rac, Ras, and Ralβ; the non-RTKs c-Abl and c-Src; and the lipid and serine-threonine kinases phosphatidylinositol 3-kinase and p21-activated kinase-1; as well as other regulatory components, are required for CDR formation (9).

Table 1 lists numerous proteins in CDRs, their functions, and their interaction with other endocytic proteins. These proteins are involved in the formation and regulation of CDRs, providing new insights into the mechanisms of receptor internalization in the context of cellular processes such as growth, proliferation, and invasion.

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vesicle formation and trafficking (20, 21).

involved in sensing and inducing membrane curvature during dynamin and contains a Bin-Amphiphysin-Rvs domain, a domain tubulation and vesicle trafficking at CDRs, as Tuba directly binds to an intriguing result with regard to the mechanism of membrane trafficking, Kovacs et al. (19) recently showed a role for CDRs; because of space limitations, the proteins corresponding to previous studies (12–18) are not further discussed here. These proteins perform numerous functions, ranging from the regulation of actin to GTPase signaling and membrane trafficking. The proteins corresponding to previous studies (12–18) are not discussed.

References to the primary studies that identified their roles in CDRs; because of space limitations, the proteins corresponding to previous studies (12–18) are not further discussed here. These proteins may participate in signaling pathways that regulate actin and membrane dynamics involved not only in membrane ruffling, but also in membrane trafficking. Consistent with a role for CDR in membrane trafficking, Kovacs et al. (19) recently showed a role for the multidomain scaffolding protein Tuba in dorsal ruffling. This is an intriguing result with regard to the mechanism of membrane tubulation and vesicle trafficking at CDRs, as Tuba directly binds to dynamin and contains a Bin-Amphiphysin-Rvs domain, a domain involved in sensing and inducing membrane curvature during vesicle formation and trafficking (20, 21).

As reported in the April issue of Cancer Research (8), a remarkable feature of CDRs is a capacity to clear the dorsal plasma membrane of EGF-stimulated cells of significant levels of EGFRs were selectively sequestered within nascent CDRs (Fig. 1A and B). These concentric structures progressed inward to form a compact mass of EGFR-containing membrane tubules that generated many endosomes (Fig. 1C and D). The levels of receptor internalized by these structures were not trivial, removing up to 50% to 60% of total surface EGFR in just 10 to 20 minutes, no small accomplishment. Second, transmission electron microscopy of thick sections taken from stimulated cells revealed that the site of wave formation was transformed into a tubular-reticular network of membranes that sometimes contained electron dense striations like those seen for dynamin on invaginating clathrin-coated pits. Third, wave-based sequestration was observed to be specific for the receptor type that had been activated by ligand. For example, activated EGFRs accumulated in forming waves at the exclusion of transferrin receptors and other nonactivated RTKs, such as PDGFRs. Finally, this sequestration/internalization process was found to be independent of conventional coat-adaptor complexes such as clathrin, AP2, and caveolin. These findings may affect the way at least some cell biologists view mechanisms by which cells internalize growth factor receptors. Mainly, RTK internalization and down-regulation does not occur only through the formation of numerous individual clathrin-coated pits or caveolae, but also en masse through large, dynamic CDRs.

**CDRs and Cancer**

One might predict that formation of cellular structures, such as CDRs, that mediate the internalization of large numbers of activated RTKs could have profound effects on cell growth and transformation. Indeed, different endocytic pathways resulting in either rapid (1-2 minutes) or delayed (several minutes) internalization of RTKs are likely to influence cell signaling and lead to differential outcomes from the same stimulus, such as a more migratory behavior as opposed to cell growth. It is interesting to note that fewer CDRs seem to form in tumor cells than in some normal nonneoplastic cells. For example, we have observed that pancreatic and prostate tumor cell lines (BxPC3, PC3, HPAF, and PANC-1) form fewer waves (<5-10%) compared with mouse fibroblasts and primary human fibroblasts (>60%). Thus, an inability of tumor cells to form CDRs and rapidly clear RTKs from their surface for degradation, normally dampening signaling, may lead to unchecked growth. A statistical analysis comparing the formation of CDRs in response to different growth factor receptor ligands in normal versus tumor cells needs to be done to support this model.

Regarding cell migration, by sequestering activated EGFRs into CDRs, it is possible that a cell may establish polarity toward an EGF gradient. In support of this, experiments using local delivery of EGF via a microneedle showed that CDRs tend to form more proximal to the source. It has been proposed that CDRs may also contribute to matrix degradation and cell migration three dimensionally (22), an important process during tumor cell invasion. Seugetsu and colleagues found that matrix metalloprotease-2 localizes to CDRs, that mediate the internalization of large numbers of activated RTKs such as PDGFRs. Finally, this sequestration/internalization process was found to be independent of conventional coat-adaptor complexes such as clathrin, AP2, and caveolin. These findings may affect the way at least some cell biologists view mechanisms by which cells internalize growth factor receptors. Mainly, RTK internalization and down-regulation does not occur only through the formation of numerous individual clathrin-coated pits or caveolae, but also en masse through large, dynamic CDRs.

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<table>
<thead>
<tr>
<th>Class</th>
<th>Protein</th>
<th>Function</th>
<th>References</th>
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<tr>
<td>Cytoskeleton</td>
<td>Cortactin</td>
<td>F-actin regulation</td>
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<td></td>
<td>Dynamin</td>
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<td></td>
<td>Gelsolin</td>
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<td>Tuba</td>
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<td></td>
<td>Eps8</td>
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<td></td>
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<td></td>
<td>Paladin</td>
<td>F-actin binding, adherence</td>
<td>(22)</td>
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NOTE: Many proteins have been localized to, and shown to affect, CDR formation. These include cytoskeletal, GTPase, kinase, and adhesion/matrix proteins. This is not a comprehensive list, but rather an abbreviated list of proteins that represent the range of those identified in CDRs. These proteins perform numerous functions, ranging from the regulation of actin to GTPase signaling and membrane trafficking. The proteins corresponding to previous studies (12–18) are not discussed.
We now know that CDRs are composed of many cytoskeletal and signaling proteins, which are rapidly assembled and disassembled in just minutes after ligand addition. Currently, however, the mechanics of exactly how these structures form and selectively sequester and internalize a specific class of activated RTKs are largely undefined. The formation and release of clathrin-coated pits at the cell surface and their role in receptor down-regulation have been topics of intense study for the past three decades. Now, it seems that part of our efforts should be dedicated toward better understanding the mechanisms behind this parallel, CDR-mediated pathway.

**References**


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**Figure 1.** CDR formation and some key components. A. PANC-1 pancreatic adenocarcinoma cell expressing EGFR-GFP shows rapid stimulation of EGFR-GFP (arrows and box). B, the boxed region in (A) at high magnification. EGFR-GFP is sequestered within the CDR (arrows). C, after ~15 minutes, this CDR has progressed to a point where it is now a compact mass of tubules and vesicles that contain EGFR-GFP (arrows). This endocytic body remains dynamic and often extends tubules that vesiculate. D, after ~25 minutes, the mass has decreased in size, indicating active trafficking. Tubules (arrows) and vesicles formed from the mass (arrowheads) are also visible.
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