MEDICINE

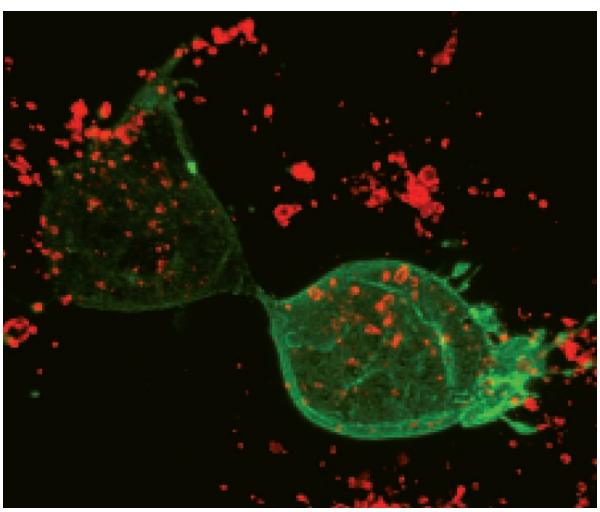
Membrane traffic en route to cancer

The spatial organization of internal membranes influences receptor signaling and disease

By **Shawn M. Ferguson**

Binding of growth factors to cell surface receptors initiates complex intracellular signaling cascades that promote cell growth, survival, and proliferation (*1*). The membrane composition and spatial arrangement of intracellular organelles represents a critical network that influences the transduction of such signals. This close relationship blurs the boundaries of classifying proteins into simple categories such as signaling and membrane trafficking. The challenge of maintaining such strict distinctions is illustrated on page 211 of this issue by Wheeler *et al.* (*2*), who identify oncogenic functions for Rab35, a protein involved in regulating endosomal membrane traffic (*3*).

The excessive activity of growth factor receptor signaling pathways is a pathogenic mechanism in many forms of cancer (*4*). This has motivated major efforts to understand the mechanisms that propagate such signals within cells and how modulating these pathways might yield therapeutic benefits. It is increasingly appreciated that intracellular signaling pathways rarely rely on random, diffusion-mediated encounters between signal transduction proteins but instead depend heavily on intracellular membranes to help concentrate and organize signaling modules in specific subcellular locations.



Membrane bound. Growth factor receptors like PDGFRα (green) localize to the cell membrane in the absence of their ligand.

Wheeler *et al.* identified the new function for Rab35 in a genetic screen for novel regulators of Akt [also known as protein kinase B (PKB)], a protein kinase that integrates multiple signals in the class 1 phosphatidylinositol 3-kinase (PI3K) signaling pathway (*1*). Growth factor receptor stimulation recruits PI3K from the cytosol to the plasma membrane (either through direct interaction with the receptor or via protein adaptors). Here, it can phosphorylate the 3 position of the inositol ring of phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$] (a lipid that is predominantly found in the plasma membrane) to yield $PI(3,4,5)P_3$. Multiple signaling proteins contain pleckstrin homology (PH) domains with selective affinity for binding to $PI(3,4,5)P_3$ or $PI(3,4)P_2$ [produced by the action of 5-phosphatases on $PI(3,4,5)P_3$] and are thus recruited to the plasma membrane in response to synthesis of this lipid.

Membrane recruitment via PH domain–$PI(3,4,5)P_3$ interactions promotes downstream signaling by controlling both the local concentration and activity of signaling proteins, such as phosphoinositide-dependent protein kinase 1 (PDK1), Akt, and mechanistic target of rapamycin complex 2 [mTORC2 (Sin1 subunit)] (*5*, *6*). Upon their plasma membrane recruitment and activation, PDK1 and mTORC2 both phosphorylate distinct sites on Akt that modulate its ability to engage diverse downstream targets that promote cell growth, proliferation, and survival (*1*).

Wheeler *et al.* took advantage of this synergistic activation of Akt by PI3K-dependent signals to perform a lentiviral short hairpin RNA screen for genes that regulate PI3K activity. This strategy identified Rab35 as important for supporting normal growth factor–induced Akt phosphorylation and activation. The existence of predicted activating Rab35 mutations in cancer sequence databases provided a further rationale for investigating the contributions of Rab35 to the PI3K-Akt signaling pathway.

The Rab family of small guanosine triphosphatases (GTPases) comprises more than 60 different members that are best characterized for their roles in regulating numerous distinct intracellular membrane trafficking reactions (*7*). Rab35 has been specifically implicated in physiological processes that depend on recycling of proteins from early endosomes (*3*). Spatial control of Rab35 function is conferred by the Connecdenn/DENND1 family of guanine nucleotide exchange factors (GEFs) that promote the loading of Rab35 with GTP (*3*, *8*). Through interactions with clathrin and its adaptor, AP-2, at plasma membrane clathrin-coated pits, these GEFs ensure the presence of activated Rab35 on early endosomes (*3*) (see the figure). At these endosomes, Rab35 interacts with specific effectors involved in the actin cytoskeleton, lipid metabolism, and protein trafficking to ensure the recycling of multiple receptors and cell adhesion proteins back to the plasma membrane, so as to limit their delivery to and degradation by lysosomes (*3*). Although several GTPase-activating proteins (GAPs) have been identified that limit Rab35 function (*3*), the underlying mechanisms that direct their spatial and temporal specificity are less well understood.

Wheeler *et al.* demonstrated that loss of Rab35 impairs growth factor–stimulated activation of the PI3K-Akt signaling pathway. Conversely, expression of Rab35 mutants that favor the active, GTP-bound, state (including those identified in human cancers) promoted PI3K activity and protected cells against growth factor deprivation by maintaining a high basal level of Akt activation. Through an elegant series of experiments, the actions of constitutively active Rab35 were placed upstream of PI3K in the signaling pathway. In fact, the GTP-bound form of Rab35 copurified with PI3K, suggesting a function that is spatially close to PI3K. This observation brings to mind the critical role for the Ras GTPase in supporting PI3K signaling in development and cancer (*9*). Rab5 has also been shown to interact with PI3K and stimulate its activity (*10*), thus raising mechanistic questions about how the actions of these different GTPases toward PI3K relate to one another.

CREDIT: WHEELER *ET AL.*

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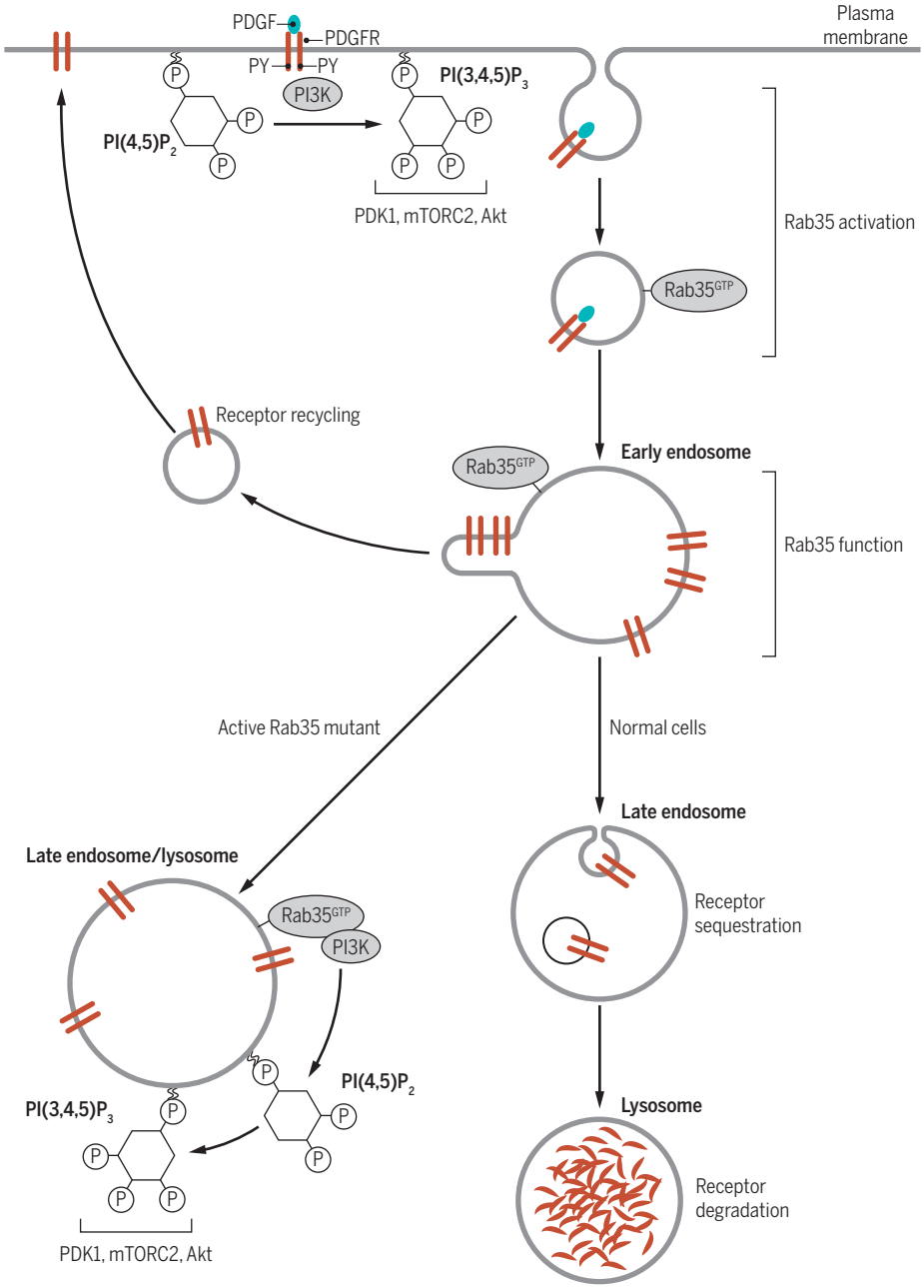

Membrane traffic control. Activated receptor tyrosine kinase growth factor receptors (including PDGFR) bind ligand and recruit PI3K to the plasma membrane to trigger the conversion of $PI(4,5)P_2$ to $PI(3,4,5)P_3$, which recruits and activates PDK1, mTORC2, and Akt (**top middle**); PY, phospho-tyrosine. Activated receptors are also subject to internalization by both clathrin-dependent and independent pathways (**top right**). The clathrin-mediated endocytosis machinery also activates Rab35, an important regulator of recycling from early endosomes. Receptors that are not recycled (**top left**) at early endosomes are subject to sequestration on the intraluminal vesicles of late endosomes and subsequently degraded in lysosomes (**bottom right**). The study by Wheeler *et al.* suggests an alternative role for late endosomes or lysosomes as sites of PDGFR signaling in cells that express active Rab35 mutants (**bottom left**).

ILLUSTRATION: P. HUEY/*SCIENCE*

Of multiple growth factor receptors examined, the platelet-derived growth factor receptor (PDGFR) pathway was found to be particularly sensitive to Rab35 activity, and PDGFR itself was constitutively activated in cells expressing active Rab35 mutants. In contrast to the canonical role for PDGFR-PI3K signaling at the plasma membrane and Rab35 actions early in the endocytic pathway, active Rab35 mutants and the PDGFR were surprisingly both enriched at lysosomes when coexpressed, regardless of whether cells had been stimulated with PDGF (see the figure). Meanwhile, in the absence of constitutively active Rab35, PDGFR accumulated at lysosomes only after acute PDGF treatment. Thus, the localization of PDGFR to lysosomes in Rab35 mutant cells is consistent with Rab35's persistently activated state.

Lysosomal localization of growth factor receptors after stimulation is normally linked to their sorting into intraluminal vesicles and subsequent degradation. The lysosome thus seems like an unlikely site for PDGFR to signal via the PI3K pathway. However, despite the old-fashioned view that lysosomes are simply the "garbage can" of the cell, they are now increasingly appreciated as an important signaling platform for the mTORC1 pathway (*11*, *12*). Even though such endosomal membranes have not traditionally been viewed as sites that contain appreciable levels of $PI(4,5)P_2$ (the preferred substrate of PI3K), recent studies support the existence of a pool of $PI(4,5)P_2$ on endosomal membranes that regulates growth factor receptor sorting and degradation (*13*). In this light, a novel role for the lysosome in PDGFR-PI3K signaling is an intriguing possibility that warrants further investigation.

This new study raises questions about the roles played by Rab proteins in cancer. In parallel, another recent study revealed a critical role for Rab13 in the invasive behavior of epithelial cells, with implications for cancer metastasis (*14*). Collectively, these advances point to the importance of better understanding the relationships between endocytic membrane traffic, the PI3K signaling pathway, and the aberrant properties of cancer cells. Such studies also raise questions about how the membrane architecture that is sculpted by membrane trafficking reactions may have a broader impact on other signaling pathways in health and disease. ■

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