tion between the hole and the jet has proven to be somewhat controversial. Part of the problem is that several distinct mechanisms may be operating simultaneously. Electromagnetic energy may not only be transferred directly from the hole to the jet; there may also be an indirect transfer, with the jets being connected magnetically to the gas that swirls around just outside the black hole's event horizon (the "point of no return" from a black hole). In this case, the extracted energy can still be charged to the black hole because the gas is forced onto lower energy orbits by the magnetic field. When we balance the books after the gas crosses the event horizon, we find that the hole has a lower mass than if there had been no magnetic action.

It is this indirect process that Koide *et al.* are simulating. The study provides the most complete numerical calculation to date that demonstrates the extraction of energy from a spinning black hole. The numerical challenge is considerable. Not only do the equations of magnetohydrodynamics have to be solved, but this must be done within the framework of general relativity, where clocks appear to tick more slowly close to the event horizon. As a consequence, the calculation can only be followed for at most a couple of orbits and the system never reaches an equilibrium configuration.

Much remains to be explored numerically (the calculations are intractable analytically). We need to understand how much power is removed directly and indirectly from the black hole and compare this with the magnetic power released by the disk. In addition, Koide *et al.*'s axisymmetric calculation may hide serious instabilities associated with twisting magnetic field lines, rather like what happens when you twist a rope under tension. It will also be interesting to see whether the outflow evolves to a steady state or is intrinsically episodic. The necessary computing power is now available, but more robust algorithms must be developed to address these questions.

Observational astronomers are homing in on black holes, especially at x-ray wavelengths, and the next generation of space satellites, in tandem with major upgrades to existing radio telescopes, should transform our view of black holes over the next few years. Electromagnetic effects around black holes may turn out to play an important role in gamma-ray bursts (*10*) and "galactic microquasars" (*11*) and are similar to the processes that underlie pulsar wind nebulae such as the famous Crab Nebulae (*12*).

Much attention has been paid, in recent years, to the quest to reconcile quantum mechanics with gravity. The observational considerations above also motivate a more modest theoretical program to unify classical electromagnetism and gravity.

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PERSPECTIVES: CELL BIOLOGY

A Pit Stop at the ER

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When ligands such as hormones, growth factors, or neurotransmitters bind to their cell surface receptors, a signal transduction cascade is initiated. Although signaling pathways activated by ligand are well understood, less is known about how these pathways are switched off. In general, "off" switches are spatially separated from "on" switches, providing a necessary time delay during which the biological response takes place. The off switch for activated receptor tyrosine kinases (RTKs) has three parts: dissociation of the activating ligand, removal of phosphate groups (dephosphorylation) from tyrosine residues of the RTK (where the signaling machinery is assembled), and degradation of both ligand and receptor. The imaging study by Haj *et al.* (*1*) on page 1708 of this issue provides an important and unexpected insight into the journey taken by an activated RTK as it goes through the steps to be switched off. The authors found that after internalization (endocytosis) of RTKs from the cell surface, these receptors travel to the endoplasmic reticulum (ER) where the protein tyrosine phosphatase PTP1B resides. This enzyme removes phosphate groups from tyrosine residues in the cytoplasmic domain of RTKs, which then continue on to their final destination, the lysosomes (see the figure).

Ligand-activated receptors are internalized at a rate that is about 10-fold greater than that for receptors not occupied by ligand (*2*). The enhanced endocytosis rate for epidermal growth factor receptor (EGFR) requires both intrinsic tyrosine kinase activity and endocytic sequence "codes" located in the cytoplasmic domain of the receptor. Subsequent trafficking of endocytosed receptors to lysosomes also depends on specific receptor sequence codes (*3*). Endocytosis removes RTKs from further exposure to ligands in the extracellular milieu. The endocytic vesicles (endosomes) containing RTK cargo are progressively acidified so that the receptors become dissociated from their ligands. The differential pH sensitivity for dissociation of EGF from EGFR versus dissociation of transforming growth factor–α from EGFR accounts for the different signal strengths of these two ligands (*4*).

In their study, Haj and co-workers address the spatial and temporal features of RTK dephosphorylation. They engineered immortalized mouse fibroblasts, derived from embryos lacking the tyrosine phosphatase PTP1B, to express a mutant phosphatase in which Asp^{181} is replaced by an Ala residue, resulting in an enzyme that traps RTK substrate. Dephosphorylation involves formation of a cysteinyl phosphate intermediate (generated by attack of the active-site Cys on the phosphorus atom) followed by cleavage of the P-O bond by the acidic residue Asp^{181} (*5*). Thus, the Asp^{181} to Ala transition creates a mutant phosphatase that favors formation of stable RTK-phosphatase complexes. The authors needed to use this mutant phosphatase because the turnover of wild-type PTP1B is too great to allow visualization of the interaction between the enzyme and RTKs. Haj *et al.* used fluorescence resonance energy transfer (FRET) to visualize the interactions of ligand-activated EGFR and platelet-derived growth factor receptor (PDGFR) with mutant PTP1B. It had been presumed that PTP1B interacts with EGFR only during EGFR biosynthesis, because PTP1B is localized on the cytoplasmic face of the ER (through a hydrophobic carboxyl-terminal anchor sequence). Haj *et al.* first verified the ER localization of wild-type and mutant PTP1B and then discovered that mutant PTP1B clustered in specific regions of the ER after growth factor stimulation of cells. Direct enzyme-receptor interactions were visualized by tagging EGFR and PDGFR with green fluores-

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cent protein and mutant PTP1B with sulfoindocyanine (Cy3)–conjugated monoclonal antibodies. The time course of receptor-phosphatase interaction after ligand addition was consistent with the known time course for dephosphorylation of RTKs in endosomes (*6*). The authors calculated that a high percentage of endocytosed RTKs became associated with mutant PTB1B. Thus, endocytosed RTKs seem to be routed close to the ER where resident PTP1B removes phosphates from RTK tyrosines. The dephosphorylation step releases signaling proteins attached to tyrosine phosphates and these proteins are then recycled. After this pit stop at the ER, endocytic vesicles containing dephosphorylated RTKs continue their journey to the lysosomes where their receptor cargo is finally degraded (see the figure).

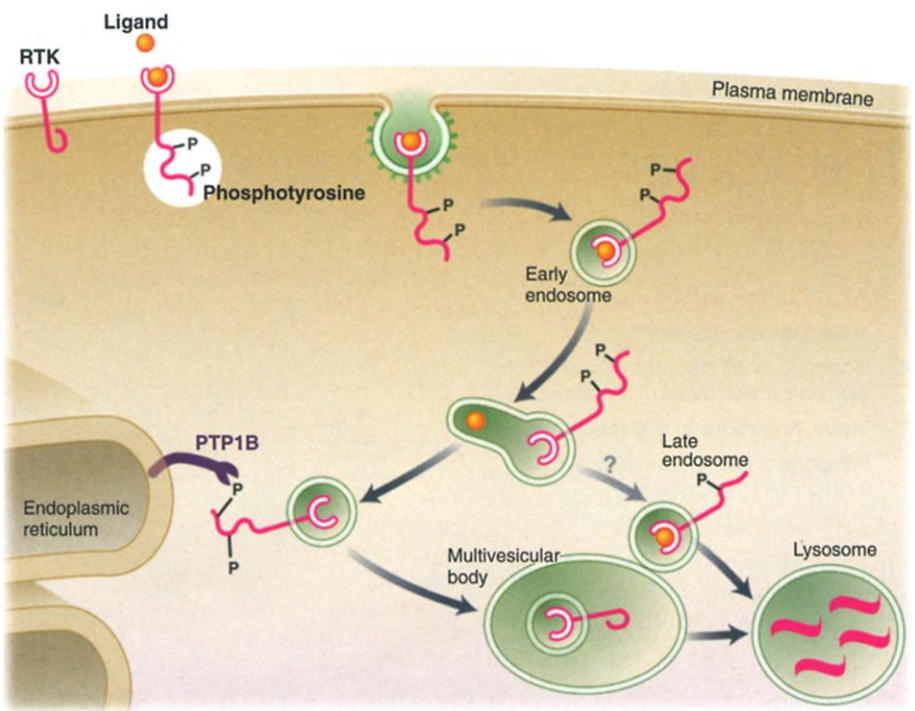
PTP1B localized in the ER may not be the only enzyme that dephosphorylates endocytosed RTKs. A phosphatidylinositol (PI) kinase, hVps34, induces endosomes containing EGFR to form multivesicular bodies (MVBs) through inward vesiculation (*7*). Interestingly, blocking inward vesiculation of endosomes with the PI 3-kinase inhibitor wortmannin does not impair delivery of EGFR to lysosomes, but does impair tyrosine dephosphorylation of EGFR and other cellular proteins. Inclusion of endosmes in MVBs thus favors EGFR dephosphorylation and presumably prevents other cellular enzymes from gaining access to RTKs.

How does the cell direct endosomes containing RTKs toward the ER, coax them to form MVBs, and then direct the MVBs to fuse with lysosomes? Sequence codes within the RTK cytoplasmic domain are required for these steps, implying that proteins directing vesicle trafficking recognize these codes (in the same way that the μ subunit of the clathrin adaptor protein 2 recognizes endocytic sequence codes) (*8*). The addition of ubiquitin groups (ubiquitination) to RTKs and other cargo receptors in endosomes contributes additional information that enhances their degradation in lysosomes. The E3 ubiquitin ligase c-Cbl, part of the ubiquitin pathway of protein degradation, enhances the breakdown of internalized EGFR (*9*). Both viral and mutant oncogenic forms of c-Cbl interfere with this process and enhance recycling of EGFR back to the cell surface. Thus, c-Cbl–mediated ubiquitination of EGFR provides additional sequence information that enhances trafficking of this RTK to lysosomes.

Monoubiquitination rather than polyubiquitination adds targeting information to endosomal proteins destined for degradation in lysosomes (*10*). Ubiquitination, which may affect endocytosis of receptors, is required for sorting endosomal proteins into MVBs (*11*). In yeast, a complex of three proteins—the endosomal sorting complex required for transport (ESCRT-1)—is required for sorting ubiquitinated proteins into MVBs (*12*). The yeast protein Vps23, which contains a ubiquitin-conjugating enzyme-like domain that lacks an active-site Cys residue, recognizes the ubiquitin moiety. After targeting proteins to MVBs, ubiquitin is removed and recycled (*13*). Cells lacking the mammalian ortholog of Vps23, TSG101, cannot effectively downregulate EGFR (*14*).

In addition to ubiquitin degradation signals that are recognized by the sorting machinery, endosomal phosphatidylinositol (PtdIns) lipids are modified by PI kinases and phosphatases to create docking sites for proteins that coat endosomes. PI kinases provide vesicle membrane docking sites for proteins containing PH, FYVE, and PX domains (*15*). The product of PI 3-kinase, PtdIns-3-P, is best characterized in early endosomes where it is recognized by the FYVE domains in EEA1 and Hrs proteins and by PX domains in SNX2 and SNX3 proteins and in yeast proteins (*16*). Multiple phosphorylated isomers of PtdIns also may serve as targeting signals at other locations within the endocytic sorting pathway. The family of sorting nexins containing PX domains (there are 17 in the human genome) is also likely to be important for vesicle trafficking (*17*).

The Haj *et al.* study provides the latest road map for the journey of endocytosed RTKs. It will be a challenge to identify the proteins directing RTKs in endosomes along the correct route, resulting in their dephosphorylation and subsequent degradation. Molecular complexes containing proteins that recognize intrinsic and ubiquitinated cargo signal sequences and phosphorylated PtdIns are likely to be essential components of the sorting machinery, ensuring RTKs an orderly journey through the vesicle-trafficking maze.



Switching off RTKs. Binding of ligand to RTKs at the cell surface results in receptor activation and signal transduction. Activated RTKs are subsequently internalized (endocytosed) in clathrin-coated pits. Progressive acidification of endocytic vesicles (endosomes) results in dissociation of receptors from their ligands. Endosomes then travel to the ER where the tyrosine phosphatase PTP1B resides (*1*). PTP1B efficiently removes phosphates from tyrosine residues of RTKs, further inactivating these receptors. Endosomes containing dephosphorylated RTKs are then routed to lysosomes where degradation occurs. Inward vesiculation of endosomes into MVBs en route to lysosomes further sequesters RTKs from substrates in the cell cytoplasm and may serve to remove any remaining tyrosine phosphates.

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