Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

Reciprocal Regulation of Endocytosis and Metabolism

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The cellular uptake of many nutrients and micronutrients governs both their cellular availability and their systemic homeostasis. The cellular rate of nutrient or ion uptake (e.g., glucose, Fe^{3+}, K^{+}) or efflux (e.g., Na^{+}) is governed by a complement of membrane transporters and receptors that show dynamic localization at both the plasma membrane and defined intracellular membrane compartments. Regulation of the rate and mechanism of endocytosis controls the amounts of these proteins on the cell surface, which in many cases determines nutrient uptake or secretion. Moreover, the metabolic action of diverse hormones is initiated upon binding to surface receptors that then undergo regulated endocytosis and show distinct signaling patterns once internalized. Here, we examine how the endocytosis of nutrient transporters and carriers as well as signaling receptors governs cellular metabolism and thereby systemic (whole-body) metabolite homeostasis.

Interactions between the cell and its environment obligatorily involve events at the plasma membrane. Cell-surface proteins mediate nutrient uptake, product release, and the sensing of environmental changes, including signals from other cells. Appropriate sensing and response to extracellular cues is essential for the individual cell's survival and for the coordinated cellular behavior in multicellular organisms. Accordingly, maintenance and dynamics of membrane proteins are fundamental mechanisms of cellular homeostasis and survival.

Most plasma membrane proteins are in defined equilibria with intracellular endosomal compartments, such that the amount of a given protein at the plasma membrane is determined by the balance of its endocytosis and its recycling back to the cell surface from endosomes and other intracellular compartments (Fig. 1). Changes in the kinetics of membrane protein traffic acutely affect the levels of individual proteins at the cell surface and thereby impact how cells intake nutrients, sense the environment, and respond to external cues.

Selective molecular mechanisms trigger traffic of plasma membrane proteins through endomembranes. Among them, ubiquitination and phosphorylation stand out as they can directly target the cargo proteins. Ubiquitination is the covalent attachment of the 76-amino acid polypeptide ubiquitin to the ε-amino group of specific lysine residues (reviewed by Miranda

Editors: Sandra L. Schmid, Alexander Sorkin, and Marino Zerial
Additional Perspectives on Endocytosis available at www.cshperspectives.org

 doi: 10.1101/cshperspect.a016964
Cite this article as *Cold Spring Harb Perspect Biol* 2014;6:a016964

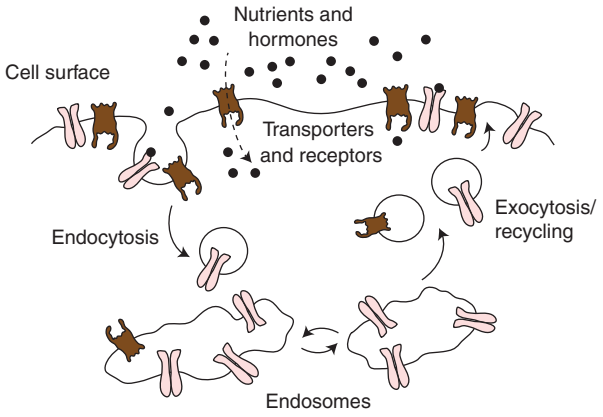

Figure 1. Dynamic regulation of the cell-surface content of membrane proteins. Integral membrane proteins found at the cell surface are dynamically localized to the plasma membrane. The amount of any of these proteins at the cell surface is the result of the balance of exocytosis or recycling of vesicles containing that protein from intracellular membrane compartments and the endocytosis of the protein from the cell surface. Regulation of either the rate of exocytosis or endocytosis results in alteration of the cell-surface content of a given protein.

and Sorkin 2007; and see also Piper et al. 2014). Ubiquitination of cell-surface proteins is the principal mechanism of control of endocytosis in yeast (MacGurn et al. 2012), whereas in mammals, additional molecular mechanisms regulate the endocytosis of cell-surface proteins, including alterations in conformation that impact interaction with other proteins, and as mentioned, phosphorylation. Each of these modifications can either enhance or reduce the rates internalization, recycling, or degradation of specific proteins, highlighting the complexity of the regulation of endomembrane traffic. The intricate mechanisms that underlie the reciprocal regulation of endocytosis and metabolism are beginning to be understood. Here we discuss the endocytosis mechanisms in the regulation of cellular intake or efflux of iron, cholesterol, Na^+, and glucose, and in the regulation of receptor signaling relevant to metabolism.

ENDOCYTOSIS IS REQUIRED FOR THE UPTAKE OF CIRCULATING NUTRIENT CARRIERS

Regulation of Iron Homeostasis by Transferrin Internalization

Ferric iron (Fe^{3+}) is an essential nutrient that is carried in the blood by transferrin (Tf), a high-affinity iron chelating glycoprotein made by the liver (Gkouvatsos et al. 2012). Endocytosis of diferric Tf is the principal mechanism for cellular Fe^{3+} uptake. Transferrin receptor 1 (TfR1), a homodimeric type II transmembrane protein that binds Tf-Fe_2^{3+} with nanomolar affinity, is the primary receptor mediating cellular uptake (Fig. 2). The Tf-Fe_2^{3+}:TfR1 complex is internalized by clathrin-mediated endocytosis (CME) (Dautry-Varsat et al. 1983; Klausner et al. 1983). At the acidic luminal pH of endosomes (pH 5.5–6.0), Fe^{3+} is released from Tf, reduced, and transported to the cytosol. Apo-Tf (iron-free) has high affinity for TfR1 at acidic pH and therefore remains bound to TfR1 in endosomes. The Apo-Tf:TfR1 complex returns to the plasma membrane through mildly acidic, endocytic recycling compartments. Apo-Tf has an approximate ~500-fold lower affinity for TfR1 at neutral pH and it is therefore released from TfR1 following the fusion of recycling vesicles with the plasma membrane (where it is exposed to extracellular milieu of pH 7.4). The released apo-Tf is free to bind Fe^{3+} in the blood and mediate additional Fe^{3+} delivery upon binding to TfR1.

TfR1 constitutively cycles between the plasma membrane and endosomes (Watts 1985; Gironès and Davis 1989). Internalization of TfR1 is mediated by a cytoplasmic YTRF-based motif

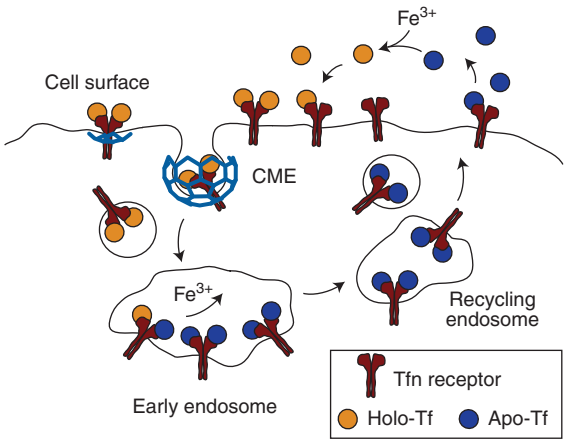

Figure 2. The uptake of iron through endocytosis and recycling of transferrin. Iron-bound transferrin (holo-Tf) binds to the transferrin receptor (TfR) at the cell surface. TfR constitutively internalizes via clathrin-mediated endocytosis. Arrival of the Tf/TfR complex to the acidic environment of endosomes results in dissociation of iron from Tf. This is followed by recycling of the apo-Tf/TfR complex to the cell surface, where apo-Tf dissociates from TfR. The iron is thereby retained intracellularly, and Tf and TfR are able to participate in additional rounds of iron uptake.

associating with the μ-subunit of the AP2 clathrin adaptin complex (Jing et al. 1990; McGraw and Maxfield 1990; Ohno et al. 1995; Owen and Evans 1998). Mutations in YTRF reduce the internalization rate constant of the TfR1 and correspondingly reduce the rate of Fe^{3+} intracellular accumulation, revealing that internalization of the TfR1 is rate limiting for Fe^{3+} uptake (McGraw and Maxfield 1990; Pytowski et al. 1995).

Although both constitutive endocytosis and recycling of TfR1 are key elements controlling cellular Fe^{3+} accumulation by maintaining the appropriate steady-state level of TfR1 at the plasma membrane, the regulation of TfR1 traffic (endocytosis and/or recycling) is not a major mode for the modulation of cellular of Fe^{3+} accumulation. Rather, regulation of iron accumulation is controlled at the level of TfR1 mRNA stability (Rouault 2006). In low Fe^{3+} conditions there is increased TfR1 mRNA stability and elevated total TfR1 protein that brings about a steady-state increase of TfR1 at the plasma membrane and augmented capacity for Tf uptake. Conversely, with elevated Fe^{3+} there is reduced TfR1 translation. In addition, increased Fe^{3+} results in TfR1 ubiquitination, which directs the receptor to degradation in lysosomes (Tachiyama et al. 2011). Together, these mechanisms control less Fe^{3+} accumulation upon elevated cellular Fe^{3+}.

Whole body iron homeostasis is achieved by the balance of TfR1-mediated uptake from duodenal enterocytes (dietary iron) and macrophages (iron recycled from erythrocytes) to the plasma via the ferroportin (FPN)-dependent iron transport (Donovan et al. 2005). The amount of FPN at the surface of these cells determines the rate of iron export to plasma. Hepcidin, a liver-produced, 27-amino acid hormone, controls iron transport from enterocytes and macrophages into the plasma by inhibiting FPN. Hepcidin binds FPN inducing its ubiquitylation-dependent endocytosis (Nemeth et al. 2004; Qiao et al. 2012). Internalized FPN is targeted to lysosomes for degradation. Although the molecular details of the ubiquitin-based FPN internalization and down-regulation have not been fully elucidated, it is clear that hepcidin regulation of FPN endocytosis is essential for iron homeostasis, because mutations that affect hepcidin-FPN binding or FPN ubiquitylation cause iron overload diseases in animal models and humans (De Gobbi et al. 2002). Thus, reg-

ulation of FPN endocytosis is a physiologically important example of the acute regulation of a nutrient carrier by endocytosis.

Low-Density Lipoprotein Receptor Endocytosis Facilitates Cellular Cholesterol Uptake

Cellular cholesterol uptake occurs through binding of low-density lipoprotein (LDL) particles by the LDL receptor (LDLR), followed by receptor endocytosis. LDLR binds apo-B100, which is present in a single copy within each LDL particle (Jeon and Blacklow 2005; Goldstein and Brown 2009). Upon internalization, the lower endosomal pH causes dissociation of LDL from its receptor. LDLR recycles back to the cell surface (Brown et al. 1983), whereas LDL continues to lysosomes, where cholesterol is released and rerouted. Cholesterol uptake by this mechanism also suppresses endogenous cholesterol biosynthesis.

LDLR internalization occurs via CME (Jeon and Blacklow 2005; Goldstein and Brown 2009). Early studies identified aromatic-based endocytic sorting motifs on LDLR (Davis et al. 1987) and the ^{802}FDNPVY^{807} sequence was later shown to act as a recognition motif for the CME adaptor protein ARH (Traub 2003). Interestingly, LDLR endocytosis by CME requires the endocytic adaptor proteins ARH or Dab2, which are dispensable for internalization of other CME cargo such as TfR1 (He et al. 2002; Maurer and Cooper 2006).

LDL particles are ~22 nm in diameter, making each LDL-LDLR complex significantly larger than most CME cargo. Expression of a chimeric protein harboring the cytoplasmic region of LDLR results in ARH- and Dab2-dependent changes in the size of clathrin-coated pits, even in the absence of LDL (Mettlen et al. 2010). This indicates that incorporation of LDLR-ARH/Dab2 complexes into endocytic structures may "customize" CME to accommodate these larger cargoes.

LDLR also contributes to the internalization of other lipoproteins, including very-low-density lipoproteins (VLDL). This raises the question of how the internalization of a single receptor may regulate the unique homeostasis of distinct lipoproteins. Interestingly, LDL uptake but not that of VLDL by LDLR involves ARH *S*-nitrosylation at C199 and C286 (Zhao et al. 2013), required for ARH interaction with AP2. This suggests a mechanism by which LDLR-dependent cellular internalization of LDL can be separately regulated from that of VLDL, thereby defining a mechanism by which the systemic metabolism of these different lipoproteins can be distinctly regulated.

Strikingly, of the nearly 1000 mutations in LDLR linked to genetic hypercholesterolemia (Leigh et al. 2008), several impair LDLR internalization but not LDL binding (Hobbs et al. 1990). In particular, one of the first monogenetic mutations identified in LDLR leading to hypercholesterolemia was Y807C; thus mutated LDLR shows markedly lower recruitment of LDLR to clathrin-coated pits and virtually ablated LDLR endocytosis (Davis et al. 1986). Moreover, mutations in ARH that impair LDLR internalization also lead to autosomal-recessive hypercholesterolemia (Soutar et al. 2003). Collectively, these studies reveal a critical contribution of LDLR endocytosis to systemic cholesterol homeostasis (Bonfleur et al. 2010).

ENDOCYTOSIS LIMITS GLUCOSE UPTAKE AND Na^+ EFFLUX THROUGH MEMBRANE TRANSPORTERS

In contrast to the cellular internalization of iron and cholesterol carriers described above, other nutrients and micronutrients are taken up directly through membrane-bound transporters straight into the cytosol. Efflux also occurs directly from the cytosol to the extracellular milieu across membrane transporters. The rate of nutrient or micronutrient uptake/efflux is largely determined by the permanence of their cognate transporters at the cell surface, which is fundamentally controlled by the balance of their endocytosis and recycling.

GLUT Endocytosis Limits the Rate of Cellular Glucose Uptake

A family of facilitative glucose transporters, GLUTs, is responsible for the transport of glu-

cose from the blood into cells. The 14 different GLUTs vary in their tissue expression and intrinsic transport parameters such as turnover number (Mueckler and Thorens 2013). Here we discuss the contribution of endocytosis of two of them, GLUT1 and GLUT4, to the regulation of glucose transport. Notably, each of these transporters cycles differentially within the cell, and indeed their rates of endocytosis differ substantially: Whereas GLUT4 is rapidly removed from the cell surface to be stored in still poorly described but highly dynamic storage vesicles, GLUT1 has a longer permanence at the cell surface. Recent studies have discovered that the endocytosis of each of these transporters is exquisitely regulated. Brief examples are presented next.

GLUT1

GLUT1, the most widely expressed GLUT isoform, mediates glucose uptake into most cell types. Glucose uptake through GLUT1 is regulated by changes in the individual transporter efficiency ("transport activity," such as by intracellular ATP levels) (Blodgett et al. 2007) and in the number of transporters at the plasma membrane. Here we focus on the latter mechanism as a means to regulate glucose flux.

In hematopoietic cells, cytokines (e.g., interleukin 3) promote glucose uptake by raising the amount of GLUT1 at the plasma membrane (Kan et al. 1994; Rathmell et al. 2001). This increase is achieved via cytokine-induced inhibition of GLUT1 internalization from the membrane and stimulation of GLUT1 translocation from intracellular compartments to the membrane (Fig. 3) (Wieman et al. 2007; Wofford et al. 2008). Upon cytokine withdrawal, GLUT1 internalization is accelerated and recycling to the plasma membrane inhibited, resulting in an acute, net reduction of GLUT1 at the plasma membrane and a corresponding drop in glucose

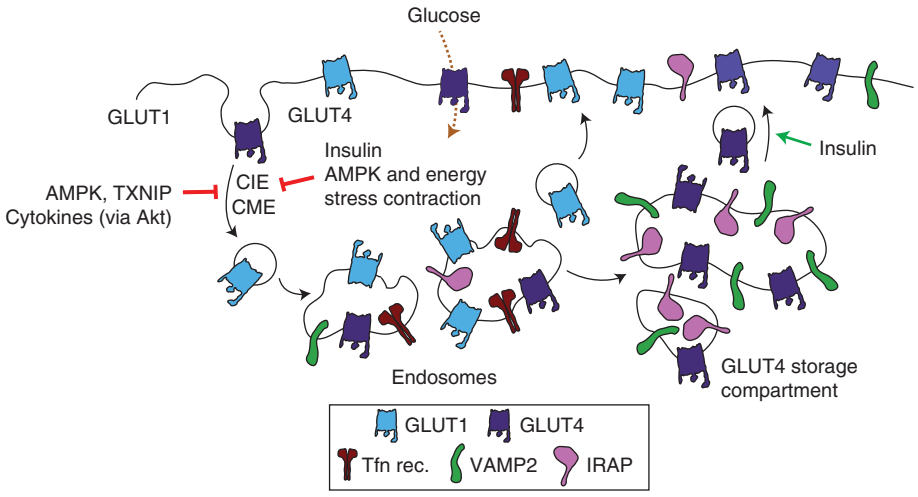

Figure 3. Control of glucose uptake by regulation of GLUT1 and GLUT4 endocytosis. Glucose transport from the extracellular milieu to the cytoplasm occurs selectively by glucose transporters (GLUTs) present at the cell surface. GLUT4 endocytosis occurs through both clathrin-mediated (CME) as well as clathrin-independent (CIE) endocytosis. The cell-surface content, and hence the rate of glucose uptake by GLUT1 and GLUT4, is regulated by alterations in the endocytosis of these glucose transporters, as well as the rates of recycling. Moreover, GLUT1 and GLUT4 undergo distinct intracellular sorting, with ~50% of GLUT4 residing in a specialized storage compartment. Shown are some of the stimuli and intracellular signals that reduce the endocytosis of GLUT1 and GLUT4 or increase transporter recycling and thereby increase the rate of cellular glucose uptake. Also shown are the insulin-responsive aminopeptidase (IRAP) and VAMP2, which are enriched in the GLUT4 storage compartment and the transferrin receptor (Tfn rec.), which is not, and is instead found largely in recycling endosomes.

transport. The molecular mechanism for cytokine-induced changes in GLUT1 traffic has not been fully described. It is known that it occurs downstream from the Akt kinase, because enforced expression of constitutively active Akt prevents enhanced GLUT1 internalization and reduced recycling upon growth factor withdrawal (Wieman et al. 2007).

In a different physiological context, cellular energy deprivation enacts an emergency response that increases glucose uptake. This is in part mediated by an increase in surface GLUT1, which is in the short run mediated by inhibition of its constitutive endocytosis, and in the long run by enhanced GLUT1 synthesis. The elements participating in the acute response were recently found to involve the arrestin-domain-containing protein (ARRDC), thioredoxin-interacting protein (TXNIP). TXNIP expression correlates with glucose transport and its knockdown in adipose and muscle cells lowered glucose uptake (Parikh et al. 2007). TXNIP affects GLUT1 traffic (Wu et al. 2013), by forming a complex that promotes its internalized through clathrin-coated pits. Although ARRDCs are involved in the ubiquitination of membrane proteins (MacGurn et al. 2012), ubiquitination of GLUT1 has not been reported, and it is not known if TXNIP controls GLUT1 endocytosis by ubiquitination of another protein. The TXNIP-induced drop in surface GLUT1 reduces glucose transport.

TXNIP transcription is under the regulation of a number of different glucose-sensing transcription complexes, CHREBP-Mlx and MondoA-Mlx, such that TXNIP mRNA levels increase with increasing glucose uptake. Because TXNIP has an inhibitory effect on glucose uptake, the glucose-stimulated increased TXNIP mRNA provides a negative-feedback loop for GLUT1-mediated glucose uptake. In addition, TXNIP phosphorylation by AMP-dependent protein kinase (AMPK) promotes its degradation (Wu et al. 2013). AMPK is activated when the AMP/ATP ratio increases, therefore AMPK-induced degradation of TXNIP will result in elevated plasma membrane GLUT1 as a consequence of reduced internalization, and a corresponding increase of glucose uptake and ATP generation ensue. Further, TXNIP also reduces GLUT1 transcription, contributing to the long-term reduction in glucose uptake.

GLUT4

GLUT4 expression is mostly limited to muscle and adipose cells (Mueckler and Thorens 2013) and is responsible for most of the glucose retrieval from blood following a meal. In the fasted state (between meals) glucose transport into adipose and muscle cells is low because 90%–95% of GLUT4 is sequestered intracellularly (Li et al. 2001; Huang and Czech 2007). Insulin, which increases in the blood upon feeding, stimulates a rapid (within minutes) redistribution of GLUT4 from intracellular compartments to the plasma membrane, causing a concomitant surge in glucose transport into these tissues (Foley et al. 2011; Rowland et al. 2011). The elevated rate of glucose transport thus achieved is behind the return of blood glucose levels back to prefeeding levels within 1–2 h (Ferrannini et al. 1985). The redistribution of GLUT4 to the plasma membrane is reversible. When blood insulin levels wane, GLUT4 redistributes back from the plasma membrane to intracellular compartments. Unlike intracellular GLUT1, which transits only through recycling endosomes, GLUT4 is unique in its sorting out of recycling endosomes into the TGN and a specific "GLUT4 storage" compartment. The nature of this compartment is highly debated, but it is apparent that it excludes certain proteins such as GLUT1 and most of the TfR1, whereas it is enriched in proteins such as VAMP2 and IRAP (Fig. 3) (Jedrychowski et al. 2010; Foley et al. 2011; Stöckli et al. 2011; Leto and Saltiel 2012). Most studies agree that the GLUT4 storage compartment is distinct from the TGN although it contains proteins such as Syntaxin-6 and -16, and these may contribute to its sorting or functionality. Sorting to this selective compartment, unique to fat and muscle cells, may prevent GLUT4 from degradation and may account for its strikingly long half-life (nearly 48 h). The localization of this unique compartment is also poorly defined, in part owing to spatial constraints in adipose and mature

muscle cells, and to its dynamic nature. However, there is general agreement that GLUT4 sequestration is essential for its appropriate response to insulin and hence defining the full complement of signals dictating the dynamic retention of GLUT4 in intracellular stores is of utmost physiological importance.

GLUT4 Internalization in the Basal State

GLUT4 is a very long-lived protein; hence, each molecule can undergo numerous cycles of internalization and recycling. The pronounced intracellular GLUT4 sequestration in unstimulated fat and muscle cells is owing to its rapid internalization and slow recycling. GLUT4 contains sequences that determine its endocytosis and intracellular sorting. When ectopically expressed in fibroblast-like cells, both the phenylalanine-based motif FQQI on the GLUT4 amino-terminal cytoplasmic domain and a dileucine motif on its carboxy-terminal cytoplasmic domain function as internalization motifs (Corvera et al. 1994; Garippa et al. 1994, 1996; Verhey et al. 1995). These motifs suffice to promote internalization when transferred to other proteins, and belong to the two major families of membrane protein sorting signals: aromatic-based motifs and dileucine-based motifs (Seaman 2008; see also Traub and Bonifacino 2013). However, the aromatic-based motif on GLUT4 has a phenylalanine instead of the more common tyrosine. Substitution of the endogenous phenylalanine for tyrosine in the GLUT4 motif promotes faster (2×) internalization of the transporter (Garippa et al. 1996; Blot and McGraw 2006). Similarly phenylalanine-based motifs on other proteins promote a slower internalization compared with tyrosine-based motifs (e.g., McGraw and Maxfield 1990). The behavior of GLUT4 in fibroblast-like cells is consistent with its internalization by a clathrin/AP2-dependent pathway, although the mechanism of internalization of ectopically expressed GLUT4 endocytosis in these cells has not been rigorously investigated. In contrast, more recent studies in 3T3-L1 adipocytes reveal that, in the basal state, GLUT4 is internalized by a cholesterol-dependent, nystatin-sensitive pathway that is independent of AP2 or any of the known GLUT4 membrane protein traffic motifs (Blot and McGraw 2006). In unstimulated L6 muscle cells, GLUT4 internalizes via both a clathrin-dependent and a cholesterol and dynamin-dependent but caveolin-independent mechanism (Antonescu et al. 2008a, 2009). Kinetically, GLUT4 endocytosis differs from that of the TfR1 or the receptor for α2-macroglobulin (Habtemichael et al. 2011), markers of CME and non-CME, noncaveolar internalization.

GLUT4 Internalization in Response to Insulin

In muscle and fat cells the predominant effect of insulin is enhanced recycling (i.e., re-exocytosis) of GLUT4 to the plasma membrane (Li et al. 2001; Karylowski et al. 2004; Martin et al. 2006; Blodgett et al. 2007; Antonescu et al. 2008b, 2008a; Ishikura et al. 2010), and this is also the case in skeletal muscle (Karlsson et al. 2009). In adipocytes, insulin also slows down GLUT4 internalization (Fig. 3) (Czech and Buxton 1993; Blot and McGraw 2006), but this does not occur in either cardiomyocytes (Yang and Holman 2005) or L6 muscle cells (Li et al. 2001; Antonescu et al. 2008a, 2009) (although a report to the contrary exists [Fazakerley et al. 2010]). Once internalized, insulin enhances the rate of GLUT4 transit through recycling endosomes (Foster et al. 2001). Although the magnitude of the effect of insulin on recycling is greater than on internalization, both changes contribute significantly to the net redistribution of GLUT4 to the plasma membrane. This may be one reason why the insulin-dependent net gain in surface GLUT4—and its consequent glucose uptake—is larger in adipocytes than in muscle or heart cells. The molecular basis of insulin-induced reduction in GLUT4 internalization in adipocytes is beginning to be understood. Under this stimulus, the FQQI motif functions as the main GLUT4 internalization motif, promoting uptake via an AP2-dependent mechanism (Blot and McGraw 2006). Thus, the data suggest that the GLUT4 internalization route varies between insulin-stimulated and -unstimulated adipocytes (respectively, AP2-depen-

dent and -independent). How this change in route is achieved is unknown. By comparison, GLUT4 internalizes through both clathrin-dependent and clathrin-independent routes in both basal and insulin-stimulated muscle cells, and insulin does not appear to change the proportion or rate of internalization of either route (Antonescu et al. 2008a, 2009). GLUT4 internalization appears to require the F-BAR-domain-containing protein CIP4, perhaps to cause membrane curvature for formation of endocytic vesicles (Hartig et al. 2009; Feng et al. 2010).

What is the significance of the different internalization routes? Although this is still an open question, one can make certain testable predictions: If the cholesterol-dependent route is faster than the FQQI-dependent one, the insulin-dependent shift to clathrin/AP2-mediated internalization through the FQQI motif in adipocytes might allow for longer permanence at the plasma membrane of each transporter—to enact more rounds of glucose transport—before being internalized. This would be facilitated by the slower internalization conferred to GLUT4 by the FAAI motif (compared with the canonical YQQI motif). The slower internalization would be compounded by the gain in transporters at the membrane resulting from increased recycling. In muscle, the clathrin-dependent pathway operating in the basal state might confer a slower internalization that could be advantageous for its physiological needs.

GLUT4 Internalization in Response to Energy Demand

Processes that increase energy demand require an adaptive increase in carbon source to sustain cellular viability (Cartee et al. 1991). This adaptive response in other cells is ascribed to GLUT1 (see above), but muscle largely depends on GLUT4 for influx of glucose. In contrast to insulin stimulation, energy status signaling leads to a marked slowdown of GLUT4 endocytosis in muscle cells. Metabolic stress resulting from treatment of cardiomyocytes with metformin or oligomycin (Yang and Holman 2005) or of L6 muscle cells with 2,4-dinitrophenol (DNP) (Antonescu et al. 2008a) slows the rate of GLUT4 endocytosis (Fig. 3). DNP causes an initial and abrupt drop in cellular ATP with concomitant activation of AMPK; within minutes, intracellular ATP levels bounce back, coincident with the AMPK-dependent increase in cell surface GLUT4 elicited by DNP (Klip et al. 2009). Thus, AMPK reduces GLUT4 endocytosis to enhance GLUT4 permanence at the membrane. Indeed, activation of AMPK by treatment of L6 muscle cells with the AMPK-activator AICAR alone (Fazakerley et al. 2010) or AICAR in combination with exogenous activation of protein kinase C (Antonescu et al. 2008a) lowered the rate of GLUT4 endocytosis. Other examples of reduced GLUT4 endocytosis upon exposure of energy stressors include the response of adipocytes to dimethyl sulfoxide (Berenguer et al. 2011), rosiglitazone (Velebit et al. 2011), metformin (Yang and Holman 2006), and chronic insulin (Pryor et al. 2000).

In physiological conditions, muscle contraction vigorously uses ATP that must be replenished by glucose influx. Contraction increases surface GLUT4 levels in skeletal muscle (Douen et al. 1990; Goodyear et al. 1991) and this occurs through both AMPK- and Ca^{2+}-dependent signals. Interestingly, in noncontracting muscle cells, a depolarization-induced influx of Ca^{2+} suffices to reduce GLUT4 internalization, and this response is independent of AMPK availability (Wijesekara et al. 2006). Moreover, a calcium ionophore, ionomycin, can slow down the rate of GLUT4 endocytosis and promote GLUT4 exocytosis, but only the latter effect is AMPK-dependent (Q Li, P Bilan, W Niu, et al., unpubl.). The exocytic increase in surface GLUT4 in exercised muscle is also AMPK dependent (Lauritzen et al. 2010). Intriguingly, the AICAR had only a minor effect on GLUT4 exocytosis in skeletal muscle (Karlsson et al. 2009), suggesting that AMPK may instead act on the endocytic step and/or that it may act in concert with other physiological signals to increase surface GLUT4 during muscle contraction. Hence, AMPK regulates GLUT4 endocytosis in conditions of energy demand (e.g., DNP treatment) and GLUT4 exocytosis in response to Ca^{2+}-dependent signals. Collectively, these

studies show that signals elicited by alterations in energy status and during skeletal muscle contraction converge to regulate the rates of GLUT4 endocytosis and exocytosis, thereby enhancing intake of energy-providing glucose.

Na/K-ATPase Endocytosis Controls Multiple Metabolic Functions

In all mammalian cells, an electrochemical gradient—creating a voltage difference that is typically negative with regards to the external milieu—ensures proper movement of ions and regulation of cell volume. At the core of the maintenance of this electrochemical Na^+ gradient lays the Na^+/K^+-ATPase or Na^+/K^+ pump. By expending one ATP molecule per cycle of efflux of three intracellular Na^+ ions and influx of two extracellular K^+ ions, the pump ensures rapid riddance of any Na^+ ions that increase in the cytosol as a result of numerous Na^+-dependent influx processes. Such preceding influx processes are key for lowering intracellular Ca^{2+} (Na^+/Ca^{2+} exchange), Na^+-coupled amino acid transport, Na^+-coupled glucose uptake in epithelial cells, and for a diversity of Na^+, K^+ and Cl^- transport processes. In fact, the Na^+/K^+ pump is the main, if not only, mechanism of Na^+ efflux from most cells. In parallel, the pump returns K^+ to cells that release or leak this ion in the course of their electrical activity, such as during action potentials in neurons, skeletal, smooth and heart muscles, and neuroendocrine cells. Another fundamental function of the pump is the translocation of Na^+ from one side of epithelia to the other, that along with obligatory anion movement generates the osmotic gradient that drives water absorption or resorption.

With this tall order, it is not surprising that the activity of the Na^+/K^+ pump is rapidly and exquisitely regulated. This is achieved by changes in both the activity and the availability of the pump at the surface of the cell in question.

Structural Properties of the Na^+/K^+ Pump

The pump consists of a heterodimer of one catalytic α subunit and one stabilizing, highly glycosylated β subunit. The 110-kDa α subunit has 10 transmembrane segments, whereas the 31.5 kDa β subunit crosses the membrane once. The αβ heterodimer is the stable form, as changes in copy number of the α subunit require concomitant changes in the β subunit to create functional units. Depending on the cell type, about 800,000 to 30 million pump copies are present at the cell surface at any given time. Abnormal changes in the copy number of pump units occur in several pathologies such as heart failure and hypertension associated with excessive renal Na^+ resorption. The functional pump involves one of four isoforms of the catalytic α subunit and one of any of the three isoforms of the stabilizing β subunit. The gene transcription and translation control of each of these subunits is tissue specific and differentially regulated by diverse physiological conditions, as is the catalytic activity of the α subunit. On top of that, the regulation of pump availability at the cell membrane through exocytosis and endocytosis is a fundamental physiological control mechanism.

Pump Regulation by Exocytosis

The increase in pump surface exposure is best exemplified by the increase in surface α2 and β1 subunits that occurs in skeletal muscle in response to insulin (Hundal et al. 1992; Ewart and Klip 1995; Benziane and Chibalin 2008). This rapid up-regulation of surface pump number occurs independently of biosynthetic changes and although typically ascribed to translocation to the membrane, the endocytic and exocytic arms of pump traffic in response to insulin have not been directly measured. A rapid increase in surface pump units is vital to manage the rapid uptake of dietary K^+ (along with the Na^+, K^+, Cl^- cotransporter) and importantly handles the elimination of cytosolic Na^+ excess that results from Na^+-coupled amino acid uptake and Na^+/H^+ exchange promoted by the hormone. A similar up-regulation occurs in the contracting skeletal muscle fiber. In the central nervous system, the pump is up-regulated on demand to mediate K^+ reuptake and rids neurons of Na^+ gained during electrical activity.

Na^+/K^+-Pump Endocytosis Triggered by CO_2 and Hypoxia in the Lung

Changes in CO_2 and O_2 tension occur continuously in the lung, and when these changes are extreme, fluid movement needs to be regulated. This is enacted primarily through changes in the availability of the Na^+/K^+ pump at the alveolar cell surface, as the pump is the driving force for ion-accompanying fluid movement (Bertorello and Sznajder 2005). Elevations in CO_2 (hypercapnia) and reductions in O_2 (hypoxia) demand an adaptive reduction in Na^+ flux through the cells, and this is achieved by reducing the number of Na^+/K^+ pumps at the cell surface, through their rapid endocytosis. In fact, the endocytosis of the pump in alveolar epithelial cells controls whole body CO_2 clearance and alveolar fluid resorption. The reduction in pump units at the cell surface also curbs ATP utilization, given the high energetic demand imposed by pump activity (up to 30% of the alveolar cell ATP). This reduction is beneficial in conditions of hypoxia. However, endocytosis of the pump can result in decreased alveolar fluid clearance.

The lung preferentially expresses the α1 and β1 subunits. The endocytosis of pump subunits at the basolateral surface of primary and immortalized alveolar cells (e.g., human A549, rat ATII0) has been recorded using cell-surface biotinylation of proteins followed by pulldown with streptavidin beads. Pump endocytosis is promoted by CO_2 and by hypoxia, but also by thrombin (Vadász et al. 2005). The signals regulated this endocytic movement are only beginning to be identified, but hypercapnia activates ERK (extracellular signal-regulating kinase) and inhibiting ERK reduces pump endocytosis (Welch et al. 2010). On the other hand, hypoxia increases CaMKK-β (calcium/calmodulin-dependent protein kinase kinase 2) and ROS (reactive oxygen species), which activate AMPK (Vadász et al. 2008). Indeed, inhibiting either one of these inputs reduces hypoxia-induced pump endocytosis. It has been proposed that AMPK phosphorylates the atypical protein kinase C-ζ on Thr-410, activating it, and that this signaling pathway leads to phosphorylation of Ser-18 on the α1 subunit of the Na^+/K^+ pump (Gusarova et al. 2009). This is thought to lead to ubiquitylation of a lysine residue in the vicinity of Ser-18 as preamble to the pump endocytosis (Dada et al. 2007). This mechanism is partly reenacted in the kidney in response to elevated Na^+ in the basolateral milieu (see below).

Finally, mitochondrial ROS are generated in the course of hypoxia and these have been implicated in the endocytic regulation of the pump (Vadász et al. 2005), apparently through phosphorylation of the AP2 adaptin μ2 subunit. The responsible kinase remains to be identified, although AAK1 (adaptor-associated protein kinase 1) has been suggested (Chen et al. 2006).

Na^+/K^+-Pump Endocytosis Triggered by Na^+, Dopamine, and Cardiotonic Steroids in the Kidney

In the proximal tubule of the kidney, the Na^+/K^+ pump is the driving force for Na^+ and fluid recovery from lumen to blood (Na^+ resorption). The kidney expresses the α1- and β1-subunit isoforms that are abundant at the basolateral membrane, from where the dimer is endocytosed in response to several stimuli (Bertorello and Sznajder 2005).

The presence of elevated Na^+ in the blood and therefore at the peritubular interstitium of the proximal tubule demands a reduction in Na^+ resorption from the lumen. High levels of Na^+ cause release of dopamine, a natriuretic hormone that increases Na^+ excretion by diminishing its reabsorption, primarily in the proximal tubule. Dopamine is synthesized within the kidney from L-dopa, and both local and circulating dopamine binds and activates DA1 and DA2 receptors that generate cyclic AMP. Dopamine triggers Na^+/K^+-pump endocytosis at the basolateral membrane of kidney epithelial cells through three coincident events, documented in OK (opossum kidney) cells (Chibalin et al. 1999) and renal proximal tubule cells (Pedemonte et al. 2005):

1. Phosphorylation of Ser-18 on the α1 subunit of the Na^+/K^+ pump (Pedemonte et al.

2005), mediated by protein kinase C-ζ. This promotes association of dynamin-2 with the α subunit (Efendiev et al. 2002). Presumably, phosphorylation of dynamin-2 at position S848 limits basal Na^+/K^+-pump endocytosis and dopamine-regulated PP2 dephosphorylation of dynamin-2 S848 promotes Na^+/K^+-pump internalization (Efendiev et al. 2002).

2. Simultaneously, the α1 subunit is also phosphorylated on Y537, promoting binding of the p85 subunit of phosphatidylinositol-3-kinase (PI3K). Both phosphorylation events of α1 on Ser-18 and Y537 are required for its endocytosis (Doné et al. 2002). Binding of PI3K to the pump occurs via interaction of the SH3 domain of p85 with a proline-rich domain of the α1 subunit, a region of the pump distinct from that bound by AP2 (Yudowski et al. 2000). In fact, PI3K activity enhances association of AP2 with the α1 subunit (Yudowski et al. 2000). This results in formation of a complex of the Na^+/K^+ pump, AP2, clathrin, and dynamin-2 (Pedemonte et al. 2005).

3. Finally, the adaptin μ2 subunit is phosphorylated on T156 in response to dopamine and ROS (Chen et al. 2006), and μ2 binds directly to the YLEL motif on the main cytoplasmic loop of the Na^+/K^+ pump (Doné et al. 2002; Chen et al. 2006). The pump then apparently internalizes all the way to late endosomes (based on subcellular fractionation experiments) from whence it can recycle to the membrane (Chibalin et al. 1999).

In addition to this interesting model, other elements contribute to pump endocytosis in epithelial cells following activation of G-protein-coupled receptors (GPCRs) by dopamine, vasopressin, or adrenergic hormones. In COS cells (a monkey kidney cell line), Na^+/K^+-pump endocytosis involves its direct phosphorylation through GPCR-associated kinases 2 and 3 and binding of arrestin 2 and 3 (Kimura et al. 2007). In MDCK (Madin-Darby canine kidney) epithelial cells, inhibition of AMPK contributes to Na^+/K^+-pump endocytosis, through sparing phosphorylation of the Rab-GAP AS160 (thereby maintaining it in its GAP-active form) (Alves et al. 2010). This suggests that AS160, known to retain GLUT4 in intracellular stores, also retains the pump intracellularly, effectively preventing it from recycling to the cell surface.

Finally, additional to the Na^+-dependent release of dopamine that regulates the pump through GPCRs, basolateral Na^+ appears to cause release of cardiotonic steroids (endogenous, digitalis-like factors), which act on the proximal tubule cells, sending signals that promote Na^+/K^+-pump endocytosis (Bagrov et al. 2009). In cell cultures, these endogenous cardiotonic steroids are emulated by using ouabain (irrespective of its inhibitory action on pump activity). The ouabain-induced Na^+/K^+-pump endocytosis requires clathrin (Liu et al. 2004) although the same group also reported endocytosis through a cholesterol- and caveolin-1-dependent route (Liu et al. 2005). Clearly, more work is required to elucidate the endocytic route involved in the response to ouabain and, if more than one, it is paramount to identify which one is regulated, and how. Additional regulatory factors act on the pump, such as prostaglandins, reduce Na^+/K^+-pump activity in the collecting renal tubular cells, and it will be important to find if this effect is also mediated by pump endocytosis.

Collectively, the above examples illustrate the variety of stimuli that induce Na^+/K^+-pump endocytosis and the diverse intracellular mechanism that participate in this phenomenon.

RECIPROCAL REGULATION OF METABOLISM, ENDOCYTOSIS, AND RECEPTOR SIGNALING

Hormones and growth factors that are recognized by surface receptors have evolved mechanisms to signal differentially from the plasma membrane and endocytic locales (see Di Fiore and von Zastrow 2014). This applies particularly to receptor tyrosine kinases (RTKs) and GPCRs that impact on protein, lipid, and carbohydrate metabolism. Endocytosis is also a

mechanism to end defined signal transmission. Internalized receptors can then either recycle to the membrane or continue toward degradation. Receptor removal from the membrane, whether for recycling or degradation, can lead to ligand desensitization, whereas recycling affords resensitization. The internalization and postendocytic fate of the receptor-ligand complex governs the magnitude, duration, and specificity of receptor signaling (Sorkin and von Zastrow 2009; Kholodenko et al. 2010), and is tailored to conform to the physiological function of each hormone. Here we illustrate the endocytic regulation of metabolic signaling by the insulin, epidermal growth factor (EGF), β2-adrenergic, and D2 dopamine receptors.

Regulation of Insulin Action by Insulin Receptor Endocytosis

Insulin, secreted by pancreatic β cells in response to ingested glucose (Rorsman and Renström 2003), binds to insulin receptors (IR) present on its main metabolic target tissues (liver, fat, muscle) as well as on epithelial, endothelial, and neuronal cells. Insulin binding to IR activates mitogenic signaling (e.g., Shc to Erk), gene expression (e.g., Foxo), and is a critical regulator of systemic carbohydrate and lipid metabolism (through Akt) (Saltiel and Kahn 2001). Insulin-dependent clearance of dietary glucose from blood involves the rapid mobilization of GLUT4 vesicles described above, which is regulated by the phosphatidylinositol-3-kinase to Akt axis that activates small G proteins directing vesicle traffic (Zaid et al. 2008; Foley et al. 2011).

Upon binding insulin, the IR rapidly internalizes via CME in rat liver cells (Pilch et al. 1983), 3T3-L1 adipocytes (Fan et al. 1982), mouse embryonic fibroblasts (Morcavallo et al. 2012), and CHO cells (Paccaud et al. 1992; Carpentier et al. 1993), as well as through nonclathrin mechanism(s) in rat adipocytes (Gustavsson et al. 1999; Fagerholm et al. 2009), endothelial cells (Wang et al. 2011), and mouse embryonic fibroblasts (Morcavallo et al. 2012). IR endocytosis is required for (1) transendothelial insulin transfer, (2) mitogenic and metabolic signaling, and (3) insulin degradation to terminate its action.

IR Endocytosis in Transendothelial Insulin Transport

Transendothelial insulin transport may represent the rate-limiting step of insulin action in vivo (Miles et al. 1995; Barrett et al. 2009), and in aorta endothelial cells occurs through a saturable mechanism involving caveolar internalization of insulin-IR complexes (Barrett et al. 2011). IR internalization and effective insulin transcytosis in endothelial cells determines insulin availability to muscle and fat cells. Surprisingly, the molecular underpinnings of this transendothelial transfer remain obscure, possibly due to suitable microvascular endothelial cell systems.

IR Endocytosis Controls Mitogenic but Not Metabolic Signaling

IR endocytosis is slow in skeletal muscle and fat cells, but fast in the liver. Upon internalization and dissociation from insulin in endosomes, the IR retains tyrosine kinase activity (Rosen et al. 1983; Klein et al. 1987) and activated insulin signaling intermediates are associated with endosomes in Fao hepatoma and rat liver cells (Backer et al. 1989; Balbis et al. 2000), adipocytes (Kublaoui et al. 1995), and skeletal muscle (Dombrowski et al. 2000). Interestingly, blocking IR internalization impairs insulin-stimulated Shc and Erk (but not Akt) phosphorylation in hepatoma and CHO cells (Ceresa et al. 1998; Hamer et al. 2002) but not in 3T3-L1 adipocytes (Kao et al. 1998). These findings suggest that IR endocytosis is required for activation of MAPK in a cell-selective manner, but is not required for Akt activation. In fact, inhibiting IR endocytosis does not prevent the Akt-dependent GLUT4 translocation, stimulation of glucose transport, or glycogen synthesis in adipocytes (Ceresa et al. 1998; Kao et al. 1998). Consistent with these differential requirements for IR endocytosis of the mitogenic versus metabolic facets insulin signaling, the Erk-dependent transcription of *c-fos* but not the Akt-dependent regulation of

the *glucokinase* gene by insulin was blocked by inhibition of IR endocytosis in INS1 cells (Uhles et al. 2007). Hence, the Akt-dependent metabolic facets of IR signaling do not require receptor endocytosis; however, IR endocytosis may provide negative-feedback regulation (Brännmark et al. 2010).

IR Internalization Leading to Insulin Degradation

Up to 50% of insulin secreted into the portal circulation is first degraded by the liver, a process that requires insulin binding to IR, receptor internalization, and insulin degradation in endosomes (Valera Mora et al. 2003). Once insulin reaches the peripheral circulation, its levels are again controlled by uptake and degradation in liver, in addition to regulation of insulin levels by uptake in the kidney, muscle, and fat (Valera Mora et al. 2003). IR endocytosis in the liver requires the cardioembryonic antigen-related cell adhesion molecule 1 (CEACAM1) (Poy et al. 2002), whereas in the kidney, insulin binds to megalin, and insulin-megalin complexes internalize via clathrin endocytosis that proceeds to lysosomal insulin degradation (Orlando et al. 1998). In all instances, including muscle and adipose cells, insulin dissociates from the IR in endosomes (Carpentier et al. 1986) and is rapidly degraded in by endosomal insulin-degrading enzymes (Fig. 4) (Doherty et al. 1990; Tundo et al. 2013). The IR is not degraded and is instead rapidly recycled to the membrane (Carpentier et al. 1986; Knutson 1991).

Inefficient Ligand-Stimulated IR Degradation

Compared with the IR, the EGF receptor (EGFR) is more efficiently degraded upon ligand binding and internalization. EGF stimulation activates several prosurvival and mitogenic signaling pathways (Huang et al. 2007). EGF remains bound to EGFR during endosomal transit, and this is required to cause EGFR ubiquitination leading to its lysosomal degradation (Acconcia et al. 2009), although some EGFR recycling also occurs (Fig. 4) (Sorkin and Goh 2009). Down-regulation of EGFR provides a limit to the duration of mitogenic signaling.

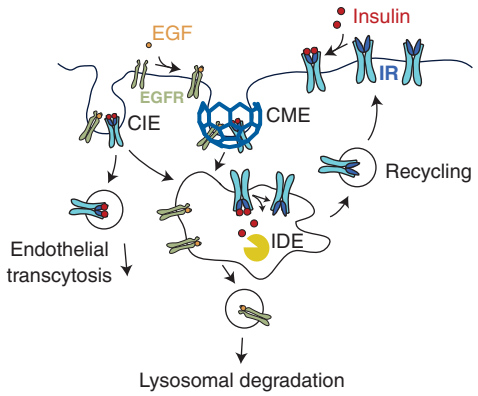

Figure 4. The internalization and recycling of the insulin receptor. Insulin binding to its receptor (IR) results in rapid internalization of the insulin-IR complex via either clathrin-mediated endocytosis (CME) or clathrin-independent endocytosis (CIE). On arrival within the acidic environment of endosomes, insulin dissociates from the IR. Insulin is degraded by insulin-degrading enzymes (IDE), whereas the IR undergoes efficient recycling to the PM. In contrast to other tissues, in endothelial cells insulin predominantly undergoes transcytosis without degradation. By comparison, the binding of epidermal growth factor (EGF) to its receptor (EGFR) results in rapid internalization leading largely to lysosomal degradation of the EGF-EGFR complex.

Although the IR can undergo ubiquitination under some circumstances, this does not result in significant IR degradation (Kishi et al. 2007; Song et al. 2013). Insulin dissociation in endosomes is critical for IR recycling, as mutations of K460 in IR that impair insulin-IR endosomal dissociation lead to IR degradation and insulin resistance in vivo (Kadowaki et al. 1990). That the IR is rapidly recycled and not efficiently degraded ensures that insulin action is controlled by systemic insulin availability and not peripheral tissue IR down-regulation.

Hence, EGFR and IR differ in the extent to which RTK ubiquitination controls the endomembrane itinerary of the receptor. In addition to receptor ubiquitination by receptor-proximal signaling events, RTKs may also use receptor-distal signaling to broadly control the membrane traffic of numerous other proteins. A

recent study in yeast found that activation of mTOR resulted in control of ubiquitin ligase targeting by arrestin-related proteins (ARTs, homologous to mammalian ARRDCs), which in turn led to ubiquitination and internalization of a diverse group of cell-surface proteins (MacGurn et al. 2011). This work raises the possibility that activation of mTOR by RTKs in mammals may lead to robust control of the endomembrane traffic and function of a large number of cell-surface transporters and receptors.

Regulation of GPCR Signaling by Endocytosis

GPCRs regulate multiple aspects of cellular and systemic metabolism. GPCR agonists stimulate exchange of GDP for GTP on specific G_α subunits of heterotrimeric G proteins, activating signaling intermediates including G-protein-coupled receptor kinases (GRKs). GRKs enable binding of β-arrestins to GPCRs, which in turn down-regulate G-protein signaling and promote CME of the receptors (Shimokawa et al. 2010). Although GPCR activation of the mitogenic MAPK pathway begins at the cell surface, signaling continues within endosomes (Sorkin and von Zastrow 2009). Interestingly, adrenergic stimulation of Erk is reduced in cells with impaired β2-adrenergic receptor (β2-AR) endocytosis (Daaka et al. 1998). Hence, GPCR membrane traffic and signaling are intimately related, with specific signals emanating from distinct cellular locale(s). GPCRs show individual signatures of GRK-dependent GPCR phosphorylation, β-arrestin binding, and ubiquitylation. Here, we examine the control of metabolism by endocytosis of β2-AR and D2-dopamine receptor (D2DR), which contribute to the peripheral and central regulation of metabolism, respectively.

Adrenergic Control of Lipolysis Depends on β2-AR Endocytosis

Agonist stimulation of β2-AR in adipocytes causes release of fatty acids from triglyceride stores. Adrenergic stimulation elicits rapid β2-AR CME. β2-AR then undergoes Rab11-dependent recycling to the cell surface (Seachrist et al. 2000; Parent et al. 2009) with minimal receptor degradation (Tsao and von Zastrow 2000), although prolonged adrenergic stimulation causes β2-AR degradation (Moore et al. 1999). Importantly, impairment of β2-AR endocytosis by perturbation of Arf6 or dynamin selectively blunts adrenergic stimulation of lipolysis (Liu et al. 2010). Consistent with a physiological contribution of β2-AR endocytosis to systemic metabolism, polymorphisms of β2-AR (R16G and Q27E) with defective adrenergic desensitization (Dishy et al. 2001) are linked to differences in fat metabolism and obesity (Ukkola et al. 2000; Corbalan et al. 2002). Hence, β2-AR endocytosis is a critical controller of lipolysis, and suggests that either multiple cycles of β2-AR internalization and recycling or endosomal-specific β2-AR signaling are required for stimulation of lipolysis.

D2DR Endocytosis Controls Feeding and Energy Expenditure

D2DR is highly expressed in the striatum and nucleus accumbens regions of the brain, which regulate food intake (Stice et al. 2008; Beaulieu and Gainetdinov 2011). D2DR controls behavior associated with food intake and energy expenditure (Meguid et al. 2000). D2DR undergoes rapid internalization leading to desensitization following stimulation with dopamine or other ligands (Gainetdinov et al. 2004), and this occurs via clathrin-dependent (Paspalas et al. 2006) or -independent mechanisms (Vickery and von Zastrow 1999). Agonist-stimulated internalization of D2DR is GRK- and β-arrestin-independent, and hence involves a noncanonical endocytic mechanism for receptor desensitization, although β-arrestins do contribute to D2DR signaling (Namkung et al. 2009). The adaptor protein CIN85 (Shimokawa et al. 2010) as well as the BLOC-1 complex (Iizuka et al. 2007) are required for agonist-stimulated D2DR internalization, and subsequent recycling leading to D2DR resensitization occurs via a slow Rab-11-dependent pathway (Li et al. 2012). Importantly, mice lacking neuronal CIN85 that showed defective D2DR endocytosis in the striatum were lean despite having

elevated food intake (Shimokawa et al. 2010), suggesting that D2DR endocytosis may impact on energy expenditure (or may reflect action of other CIN85-dependent receptors). These results exemplify how endocytosis in neurons critically impacts on systemic metabolism.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

We have examined how the regulated endocytosis of nutrient carriers (e.g., TfR1, LDLR), transporters (GLUTs and Na/K-ATPase), and signaling receptors (IR, β2-AR, D2DR) facilitate or limit the function of these important regulators of systemic and cellular metabolism. For many of these examples, the regulation of endocytosis is well documented, and a critical physiological role for endocytosis in the homeostasis of various nutrients is emerging. Nonetheless, there is still much to learn about the molecular mechanisms regulating carrier, transporter, and receptor endocytosis within the context of metabolic homeostasis.

Furthermore, given the diversity of membrane proteins at the cell surface involved in the cellular uptake and systemic regulation of metabolites, it is surprising that relatively little is known about how the endocytosis of most transporters and receptors controls their function. Importantly, approximately half of the 590 human kinases (protein, lipid, and carbohydrate) are required for the normal endomembrane traffic of vesicular stomatitis virus (VSV) and/or Simian virus 40 (SV40), markers of clathrin-dependent and clathrin-independent endocytosis, respectively (Pelkmans et al. 2005). Indeed some of the kinases found to regulate endomembrane traffic are metabolic enzymes, such as *FN3KRP*, which controls protein modification by fructosamine (Pelkmans et al. 2005). This suggests that endomembrane traffic is tightly coupled to or controlled by a cell's metabolic state, and that we have only begun to understand this regulatory process. Furthermore, that mTOR was found to exert broad effects on the cell-surface membrane traffic of numerous transporters and receptors in yeast (MacGurn et al. 2011), and the central role of mTOR as a branched chain amino acid sensor (Tokunaga et al. 2004), suggests the existence of elaborate coordination of nutrient uptake, autophagy, and hormone signaling that we are only beginning to understand.

Future work should examine how the metabolic functions of the many cellular carriers, transporters, and receptors (e.g., lipid, nucleoside or amino acid transporters, other signaling receptors) are impacted by their endocytosis. Such information, along with a better understanding of the molecular underpinnings, will provide important insight into human metabolism and may generate novel corrective therapies for metabolic disease.

ACKNOWLEDGMENTS

C.N.A. is supported by operating grant No. 125854 from the Canadian Institutes of Health Research. A.K. is supported by Operating grant No. 3707 from the Canadian Institutes of Health Research. T.E.M. is supported by NIH RO1 DK52852.

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