Protein Kinases: Starting a Molecular Systems View of Endocytosis

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Annu. Rev. Cell Dev. Biol. 2008. 24:501–23

First published online as a Review in Advance on July 3, 2008

The *Annual Review of Cell and Developmental Biology* is online at cellbio.annualreviews.org

This article's doi:
10.1146/annurev.cellbio.041008.145637

1081-0706/08/1110-0501$20.00

Key Words

membrane trafficking, phosphorylation, signal transduction, complexity, nonlinear systems, genetical physics

Abstract

The field of endocytosis is in strong need of formal biophysical modeling and mathematical analysis. At the same time, endocytosis must be much better integrated into cellular physiology to understand the former's complex behavior in such a wide range of phenotypic variations. Furthermore, the concept that endocytosis provides the space-time for signal transduction can now be experimentally addressed. In this review, we discuss these principles and argue for a systematic and top-down approach to study the endocytic membrane system. We provide a summary of published observations on protein kinases regulating endocytic machinery components and discuss global unbiased approaches to further map out kinase regulatory networks. In particular, protein phosphorylation is at the heart of controlling the physical properties of endocytosis and of integrating these physical properties into the signal transduction networks of the cell to allow a fine-tuned response to the continuously varying physiological conditions of a cell.

Contents

COMPLEXITY IN THE ENDOCYTIC MEMBRANE SYSTEM

The endocytic membrane system in mammalian cells is complex. The basic steps of membrane trafficking—cargo recruitment, vesicle formation, vesicle transport, vesicle docking, and vesicle fusion—are a concerted series of events that involves many different proteins and lipids (Gruenberg 2001, McNiven & Thompson 2006, Miaczynska & Zerial 2002, Soldati & Schliwa 2006). Although the general principle of endocytosis is always the same, there is not one particular series of molecular events that always applies. Recent progress in the field of endocytosis is rapidly dismissing our textbook view. Not only is the contribution of clathrin-dependent processes compared with alternative endocytic routes debated in numerous instances, but also the definition of clathrin-mediated endocytosis finds itself on loose ground. The canonical clathrin-mediated route now appears to have different variants, which can make use of different adaptors, different GTPases, and different trafficking itineraries and can bypass the canonical early endosome (Lakadamyali et al. 2006, Schmid & McMahon 2007). Also, recent work on *Listeria monocytogenes* entry has shown that clathrin can assemble into very large lattices that appear to support a form of phagocytosis (Veiga & Cossart 2005). The more these differences are studied, the more it becomes clear that only clathrin is the common factor. Thus, although the term clathrin-dependent endocytosis incorporates all these variants, it is hard to maintain the view that from a functional perspective, these are all just one route. Similar controversies are programmed to arise (if they have not already arisen) for terms such as caveolae-mediated, fluid phase, or macropinocytosis (Lajoie & Nabi 2007, Mayor & Pagano 2007).

The above-discussed impasse in the field of endocytosis is probably exemplary for several impasses in molecular cell biology and should be blamed on our highly reductionist, molecular, and deterministic approach to these problems in a time when global and unbiased comparisons were not possible. All pathways in cell biology will likely have to be redefined with unbiased global and quantitative methods, revealing a standardized set of rules and definitions (Kirschner 2005, Mogilner et al. 2006). In one sense, we can compare this transition in biology with that in chemistry approximately 150 years ago. At that time, alchemy (reluctantly) gave way to modern-day chemistry, founded on a

standardized set of rules and definitions discovered by Antoine Lavoisier, who started to meticulously weigh metals, gases, liquids, and all kinds of materials and chemicals without any a priori hypothesis of a periodic table (Lavoisier 1789). He might have believed in finding something fundamental in the data, but there was no scientific foundation for that belief. This probably seems boring to many modern-day scientists, but from the accurate study of a comprehensive set of measurements, a systematic pattern was discovered.

We will thus have to go through a phase of painstakingly measuring, in an unbiased manner, as many relevant properties of cellular systems as possible. Essential for this phase will be methods to quantify large populations of single molecules and single particles; to quantify large populations of individual cells (eventually within tissues); and to quantify functional roles of whole genomes (of which the protein-encoding part is just a fraction), of whole proteomes (including the enormous complexity of posttranslational modifications), and of the interactions between them. One can predict that when genome-wide functional analysis of endocytosis in mammalian cells becomes more accurate and quantitative and can incorporate quantitative properties of all vesicles in a cell and all cells in a cell population, we might be astonished by the number of differences between the internalization of two ligands that both use clathrin-dependent endocytosis. Nevertheless, when this is done for a dozen of such ligands, the data might reveal certain patterns, some of which we have no notion of today. We will find functional modules of cellular components, which can be linked to functional groups of physical properties that constitute certain design principles of a vesicle pattern (Milo et al. 2002). Moreover, we might find that ligands will fall into groups of pathways assembled from similar functional modules of molecular components and similar physical design principles. Such information, when quantitative, will allow us to create a set of formal and standardized rules with which to define the properties of the endocytic membrane system in human cells. This phase may be expensive, take a long time, and require a large and complex infrastructure. However, this concept can be applied to smaller sets of genes, proteins, and physical properties (Pelkmans et al. 2005). The iterative process of thinking about the principal components of the endocytic membrane system on which to focus first to reveal the data and to use those data to think about how to expand the initial focus will reveal sets of rules and definitions better and quicker and will optimize them along the way.

DEFINITION OF COMPLEXITY

A complex system is a system composed of interconnected parts that as a whole displays properties not obvious from the individual parts (Adami 2002, Ricard 2003). This makes every biological system with some nonlinear properties (like a simple feedback loop) complex. The field of complex systems theory adds that a system is complex when there are difficulties with its bottom-up formal modeling and simulation. For systems biology, this means that the system cannot be accurately modeled by a set of deterministic equations (for instance, differential equations) (Huang & Wikswo 2006). It is often argued that this is because the system's components, their concentrations, and their ways of interaction are not (yet) known. But there are fundamental nondeterministic properties (such as stochastic behavior) that, when amplified or dampened in nonlinear ways, make nonstatistical models inappropriate. One may regard this basic property as the uncertainty principle in biology. The biological uncertainty principle seems particularly relevant for dynamic systems that consist of many different components and interactions that span several orders of magnitude on the space-time scale. It is likely that a crucial system such as endocytosis has many built-in mechanisms to deal with this uncertainty, but this remains to be experimentally addressed.

WHY IS THE ENDOCYTIC MEMBRANE SYSTEM IN HUMAN CELLS SO COMPLEX?

The complexity of endocytosis is a specific trait of cells from multicellular organisms. There is a large increase in complexity from *Saccharomyces cerevisiae* to *Caenorhabditis elegans* and *Drosophila melanogaster* to *Homo sapiens* (Jekely 2007, Toret

STRUCTURAL VERSUS SYSTEMS BIOLOGY IN ENDOCYTOSIS

For certain aspects of endocytosis, structural biologists have visualized functional modules with a remarkable degree of resolution. Often, structural biology is seen as the ultimate foundation on which to attempt bottom-up modeling of biological systems. The molecular structure itself is a model, either an average of many possible conformations or a specific, trapped conformation that allows the growth of a crystal. The biological uncertainty principle will point out that one averaged or one specific structure will not be able to account for the complexity of the system. Thus, systematic structural biology using nuclear magnetic resonance (NMR) or X-ray crystallography on isolated components will allow us to reveal basic structural rules that are generally applied in endocytosis (e.g., the clathrin cage or matricity) (Fotin et al. 2004, Schmid & McMahon 2007), whereas single-particle or single-molecule methods, such as cryoelectron and optical microscopy with nanometer resolution, will be necessary to reveal structural variation principles that underlie systems behavior.

& Drubin 2006). Whereas the complexity of one or two core endocytic routes, and their core machinery, appeared earlier in evolution and is conserved through evolution, the particular properties of endocytosis in human cells appeared late.

In a multicellular organism, the endocytic membrane system needs to demonstrate extreme plasticity (Kennedy & Ehlers 2006, Mostov et al. 2003). It needs to transcytose massive amounts of liquid in epithelial cells of the renal duct, to transport vesicles over very long distances in neurons (Rodman et al. 1990, Südhof 2004), to relocalize specific membrane components for cell polarization and migration, and to shift from a sampling state to an antigen-presenting state during the maturation of dendritic cells (Le Roy & Wrana 2005). In these different functions, the endocytic membrane system displays very different properties. Sometimes the vesicles are of a very narrow size range and always contain the same amount of cargo (neurons) (Voglmaier & Edwards 2007), sometimes the endocytic organelles reshape into long tubular structures that fuse with the cell surface (maturing dendritic cells) (Kleijmeer et al. 2001), and sometimes the endosomes are located at the leading or ruffling edge of a cell (migrating cells) (Rappoport & Simon 2003). Also, within an individual cell, the endocytic membrane system can display very different behavior, depending on extrinsically and intrinsically varying conditions. Recycling of membrane components is blocked during mitosis when the cells round up and overshoots the normal activity when mitosis is completed and cells spread out again (Boucrot & Kirchhausen 2007). Fluid-phase endocytosis is regulated as a function of the metabolic state and size of a cell (Hennig et al. 2006), and the activity of raft-mediated endocytosis seems to depend on the adhesive state of cells (Echarri & Del Pozo 2006, Pelkmans 2005). Molecular biology tends to explain differences in organelle behavior by the existence of cell-type- or cell-state-specific proteins. Indeed, there is tissue-, cell-type-, and cell-state-specific expression of proteins that are part of the endocytic machinery. It is, however, unclear if the behavior of the endocytic system in one cell can be changed into that of another cell by just expressing these specific proteins.

There is also a fundamental need for complexity in systems like endocytosis. Complexity makes dynamic systems robust, permits them to evolve, allows them to oscillate or be noisy (by applying negative-feedback loops) or stable (by applying positive-feedback loops), and can contain built-in adaptation principles (e.g. bistability, criticality) that result in different behavior of the system when a few key parameters are changed (Kholodenko 2006, Shinar et al. 2007). The emergent properties that arise from such changes may appear to the molecular biologist as a completely different system with different molecular requirements. But it is the complexity itself that allows one system consisting of one set of components to behave differently under different conditions (Balazsi & Oltvai 2005, Mayo et al. 2006).

This complexity brings us to an alternative principle of how plasticity of the endocytic membrane system might be achieved. Perhaps

it is the particular topology of certain regulatory circuits within the endocytic membrane system that leads to a certain behavior (Kashtan et al. 2004). If so, the endocytic membrane system must have all properties intrinsically built-in. We can then imagine why the endocytic membrane system in any cell from a multicellular organism is so complex: It must have the intrinsic ability to demonstrate a wide range of different states and behaviors. This possibility remains to be empirically addressed, but we must be prepared for the prospect that in large-scale perturbation screens, all these different behaviors can emerge, even in a population of simple, nonpolarizing, nondifferentiating tissue culture cells.

ENDOCYTOSIS: SPACE-TIME FOR SIGNAL TRANSDUCTION

Another reason why the endocytic membrane system is so complex is its essential role in cell signaling. Here we discuss how the endocytic membrane system might regulate the space-time in which an input signal (extracellular and intracellular) is transduced, processed, and translated in the cell.

A variety of sensors continuously monitor the physiological status of the cell and the extracellular environment. Well-known examples are sensors that measure energy status, nutrient status, ion concentrations, levels of oxysterols, or the amount of cell stretching (Cota et al. 2006, Janowski et al. 1996). Many sensors consist of components that are associated with or span cellular membranes, such as integrins, ion channels, growth hormone and cytokine receptors, Toll-like receptors, lipid sensors on endosomes, pH sensors (e.g., the vacuolar ATPase), or redox-potential sensors (Chang et al. 2006, Morgan et al. 2007). By movement of these sensors between cellular compartments, membrane trafficking, and endocytosis in particular, will have a major impact on the sensing capability of a cell.

Endocytosis also plays important roles in signal transduction and processing (Miaczynska et al. 2004, von Zastrow & Sorkin 2007). Receptor kinases and other membrane-associated kinases, but also signaling adaptors and signaling GTPases, can be localized to specific membrane vesicles and compartments in the cell. Such molecules can be brought into close proximity to each other by vesicle transport and membrane fusion, can be separated from each other by sorting and membrane fission, or can be inactivated or degraded by the endocytic membrane system. Not only can receptor kinases be targeted for degradation by transport to the lysosome, but the actual process of invaginating vesicles into the lumen of late endosomes (multivesicular bodies), and their regulated backfusion or degradation, provides the cell with a mechanism to target cytosolic proteins for temporary inactivation (they are shielded from the cytosol) or degradation independent of the proteasome (Hurley & Emr 2006, van der Goot & Gruenberg 2006).

Endocytosis is also essentially involved in signal translation. The eventual outcome of many signal-processing events involves the relocalization of membrane components. For

DEFINITIONS IN SIGNAL TRANSDUCTION

The field of signal processing defines the following components of a signaling system: the primary signal, the sensor, the signal transducer, the acceptor, and the effector. This applies equally well to signal transduction in human cells. Signals are diverse and can be a sterol, a growth hormone, protons, calcium, ADP:ATP ratio, or a stretched integrin. Sensors can be (*a*) a cytosolic kinase that becomes activated when the concentration of cAMP changes or (*b*) a hormone receptor on the cell surface. Transducers often are also kinases but can be GTPases or ubiquitin ligases. These are activated by the sensors and transfer information to a downstream acceptor. Various transducers normally pass on a signal in complex ways. An acceptor receives the signal and activates a response to the signal via an effector. The specific topology of the network by which such information is received, transduced, and accepted can achieve complex signal integration and translation. Many separate signals can be integrated to create one output that is short- or long-lasting, that oscillates, or that is binary, and a simple signal can be translated into a complex output that may have everlasting consequences for the cell (such as irreversible cell differentiation).

example, the response might be to internalize integrins or stably assemble them in adhesion complexes on the surface, or to internalize neurotransmitter receptors or glucose channels or to accumulate them on the surface. Other cellular responses might be to migrate in one particular direction, to grow in size, or to round up during the mitotic cycle, which all need massive relocation of the surface membrane.

Thus, the endocytic membrane system lies at the very heart of signal transduction, processing, and translation. It is then likely that part of the complexity of the endocytic membrane system has evolved to incorporate specific properties that allow the system to play this central role. This picture would predict that the endocytic machinery provides many points of interaction with protein kinases (and other molecules) that sense and transduce cellular signals.

The above discussion raises another point. If the endocytic membrane system defines to a significant extent the actual space-time in which the regulation of signaling networks takes place, standard reaction-diffusion diagrams will not be appropriate for modeling signal processing. The interaction between signaling molecules will not be dictated by diffusion but by membrane dynamics, will not be able to rely on a homogeneous concentration in the cell, and will concern only a few active molecules. The collective behavior of vesicles, tubules, and organelles does not display a random walk, nor does it display consistent active motion (Holcman & Triller 2006, Taflia & Holcman 2007). Time-lapse images of cells containing fluorescent vesicles and organelles reveal the notion that vesicles display a form of mixed behavior (Holcman & Triller 2006, Taflia & Holcman 2007). It is not clear which formal models can describe this type of behavior, but perhaps certain agent-based models, such as Brownian agents, may prove useful in the future.

Furthermore, at small scales, membranes provide a quasi-two-dimensional surface (Kholodenko et al. 2000). From surface chemistry, we know that chemical reactions on surfaces proceed according to different principles. But at larger scales, the three-dimensional structure of a vesicle or an organelle must be taken into account. Approximate simulations of complex shapes indicate that particle geometry can strongly influence reaction-diffusion kinetics (Sbalzarini et al. 2005), but formal rules still need to be discovered. It thus seems appropriate to assume that signaling that depends on the endocytic membrane system will behave according to physical rules that we do not yet know but that will be fundamentally different from standard reaction-diffusion kinetics. In **Figure 1**, we summarize these concepts.

If the endocytic membrane system constitutes the space-time for signal transduction reactions (at least to a certain extent), and signal transduction is able to change the properties of the endocytic membrane system, we deal with the situation that the actors (sensors, signal transducers, and acceptors) influence their own space-time (dynamic shapes, patterns and interactions of membranes, vesicles, and organelles), which in turn will influence the actors. In other words, the physical rules determining the diffusion-reaction kinetics of signaling components are influenced by the components themselves (**Figure 1**). This introduces an aspect of complexity that is usually not considered in systems biology. It is a type of feedforward or feedback loop, but the effect is on the space-time dimension in which the signaling reactions take place, which is fundamentally different from the kind of loops we usually consider in standard signal processing diagrams (**Figure 1**). Given the importance of dynamics of complex shapes in biology, this may be a fundamental principle of processes in living systems.

PROTEIN KINASES REGULATING ENDOCYTIC MACHINERY

The previous three sections laid out our ignorance of the integrated activities of membrane trafficking and signal transduction and the need to readjust our mindset for studying them. To a certain extent, however, there is a molecular

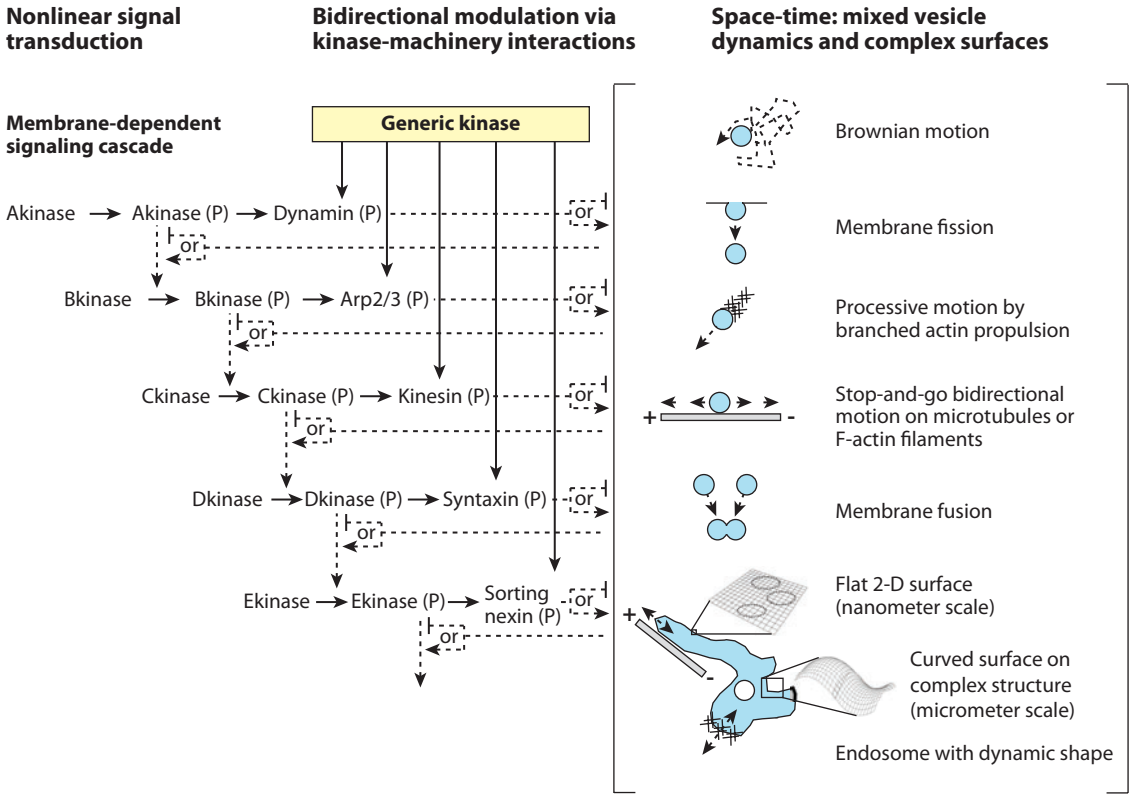


Figure 1

Endocytosis defines a flexible space-time for signal transduction. This conceptual overview shows a hypothetical membrane-dependent kinase phosphorylation cascade (*left*) in which each kinase of the cascade is localized somewhere in the endocytic membrane system. By phosphorylation of endocytic machinery components (a dynamin, Arp2/3, a kinesin, a syntaxin, a sorting nexin) along the way, the cascade will change (either increase or decrease) the dynamics of endocytic vesicles, fission and fusion reactions, and the shapes of endocytic organelles. This defines the space-time for the reaction-diffusion kinetics of signal transduction, creating a mechanism by which the cascade can regulate its own transduction kinetics (either negatively or positively). We can also imagine the existence of generic kinases that broadly influence the space-time of membrane-dependent signal transduction by regulating many endocytic machinery components. In this scenario, signal transduction reactions occur at steady state and do not require any specific input but will change their rate and direction when the dynamics of the endocytic membrane system are changed, resulting in specific signaling cascades.

foundation on which to build systems models of endocytosis, and protein phosphorylation takes a central role in this process. Phosphorylation allows the actors of signaling cascades (protein kinases) to control their space-time by regulating membrane deformation, fission, trafficking, docking, and fusion (the endocytic machinery). Phosphorylation is also a tangible observation that has been documented for many proteins and protein kinases for several decades. Therefore, we expect that a systematic inventory of these reactions will provide a first molecular foundation on which to further develop graphical models of signaling-endocytosis networks (Pelkmans et al. 2005). Although such models do not explain systems behavior, they do provide a useful way to navigate through the system's components and to think about how systems properties might emerge.

We collected all known direct phosphorylation reactions of endocytic machinery components assigned to specific protein kinases from the literature (**Supplemental Table 1**; follow the **Supplemental Material**

Supplemental Material

NETWORK TERMINOLOGY

In general, a network consists of nodes and edges. Nodes are discrete entities, genes, proteins, or metabolites. Edges are interactions between the nodes and can be of any type. Many biologists understand an interaction as something physical, such as a (non)covalent binding between the two nodes, a biochemical modification of one node by another (such as phosphorylation), or a biochemical transition (metabolic networks). Increasingly, one finds networks in which statistical correlations between two nodes are displayed as an interaction. Such networks can be derived from transcriptome profiling, in which the profile of mRNA abundance in a series of particular conditions or in particular tissues is used to correlate genes. Above a certain correlation threshold, a connection is drawn. In functional RNAi screens, when the phenotype is described by a quantitative multivariate expression or phenotype feature vector (statistically similar to a transcription profile), clusters of phenotypes (phenoclusters) can be made. These distances can be used as connections in a network, in which a link between two nodes indicates that they have similar loss-of-function phenotypes.

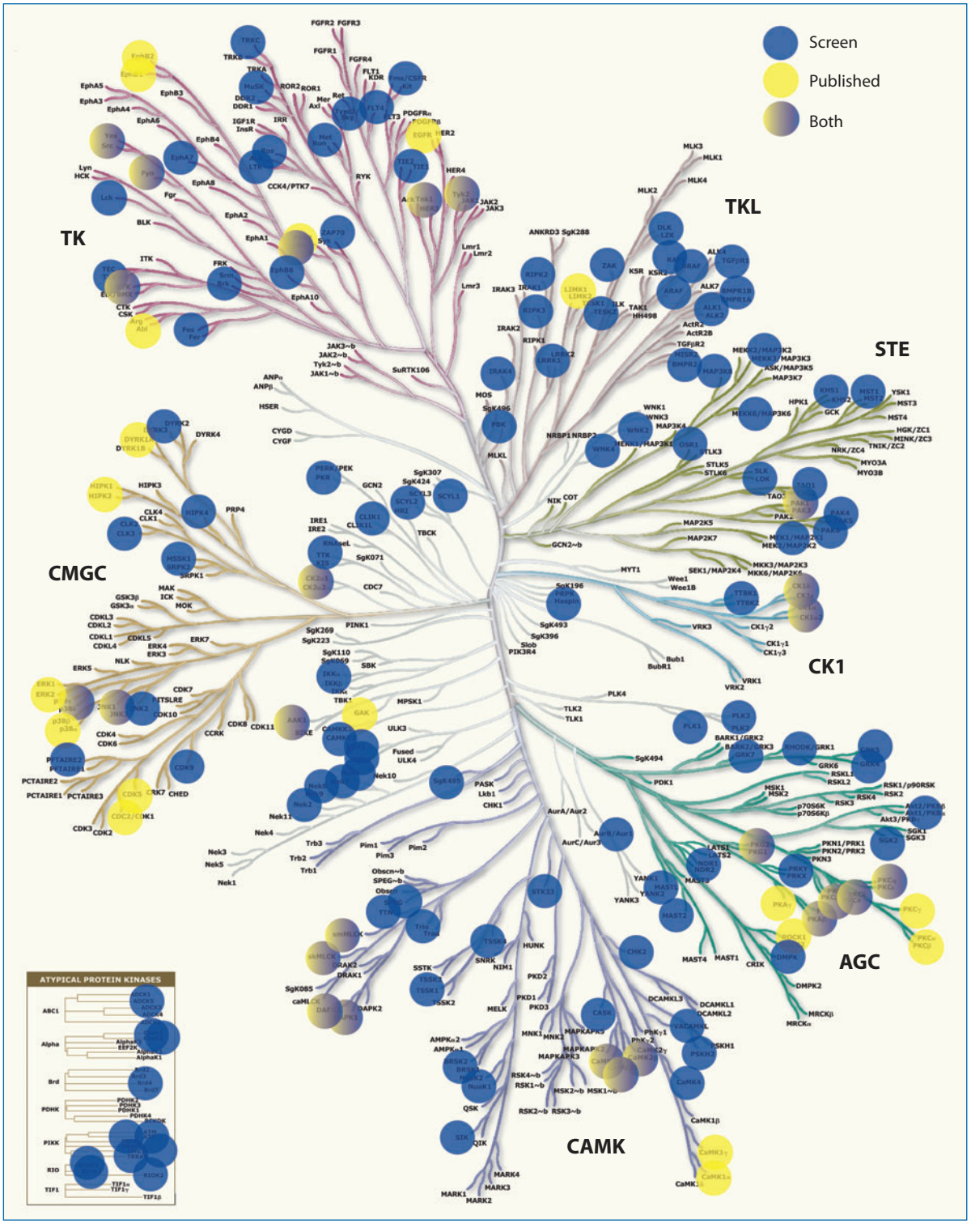
link from the Annual Reviews home page at **http://www.annualreviews.org**) and annotated those kinases onto the protein kinome tree (**Figure 2**). There is not one specific class of protein kinases that phosphorylates endocytic machinery. Rather, the kinases are distributed throughout the kinome tree. This suggests that the diversity of the protein kinome and the diversity of the endocytic membrane system have coevolved. The yeast protein kinome consists of 130 kinases, compared with 518 kinases in humans (Manning et al. 2002). We can imagine that as the demands increased for both endocytosis and signal transduction to measure, process, integrate, and react to more diverse signals, both systems grew in complexity and became intertwined, whereby the number and extent of interactions between protein kinases and endocytic machinery components increased. In addition, evolution may have favored the endocytic membrane system to become a flexible space-time scaffold for signal transduction, providing advantages for complex signal processing tasks in cells of multicellular organisms that are yet to be discovered.

We created in **Figure 3** a network of these protein kinases and the endocytic machinery components they phosphorylate. We included 40 kinases and 70 endocytic machinery components with a total of 140 interactions between them. Our definition of endocytic machinery is arbitrary. For instance, the border between endocytic machinery and machinery regulating actin dynamics is not clearly definable. The network illustrates that protein phosphorylation regulates endocytic membrane trafficking at all levels. Protein phosphorylation occurs during adaptor recruitment to the membrane, coat formation, uncoating and membrane shaping (induction or stabilization of the correct curvature for endocytic carriers), the fission of membrane carriers, actin polymerization, transport along microtubules, vesicle docking at target membranes, and vesicle fusion.

Before describing each node and each edge in more detail, we must start with a word of caution. In particular, our understanding of the human kinome is tremendously biased toward a handful of protein kinases that are being actively studied in laboratories worldwide in primarily cancer-derived tissue culture cell lines (e.g., HeLa, A431). One argument is that these are also the important kinases of each human cell, but that statement has no empirical

Figure 2

The human kinome tree is annotated with all protein kinases found to directly phosphorylate endocytic machinery components (*yellow circles*) and all protein kinases identified in an RNAi phenotypic screen (*blue circles*) to give a phenotype in any or more of the image-based assays scoring for infectious virus entry of simian virus 40 (SV40) or vesicular stomatitis virus (VSV); internalization of transferrin, cholera toxin B, or low-density lipoprotein (LDL); and staining patterns of early endosome antigen 1 (EEA1), lysosomal-associated membrane protein 1 (LAMP1), or caveolin-1-GFP. Kinases described in the literature and also found in the screen are annotated with yellow-blue circles. The human kinome is provided courtesy of Cell Signaling Technology (**http://www.cellsignal.com**).



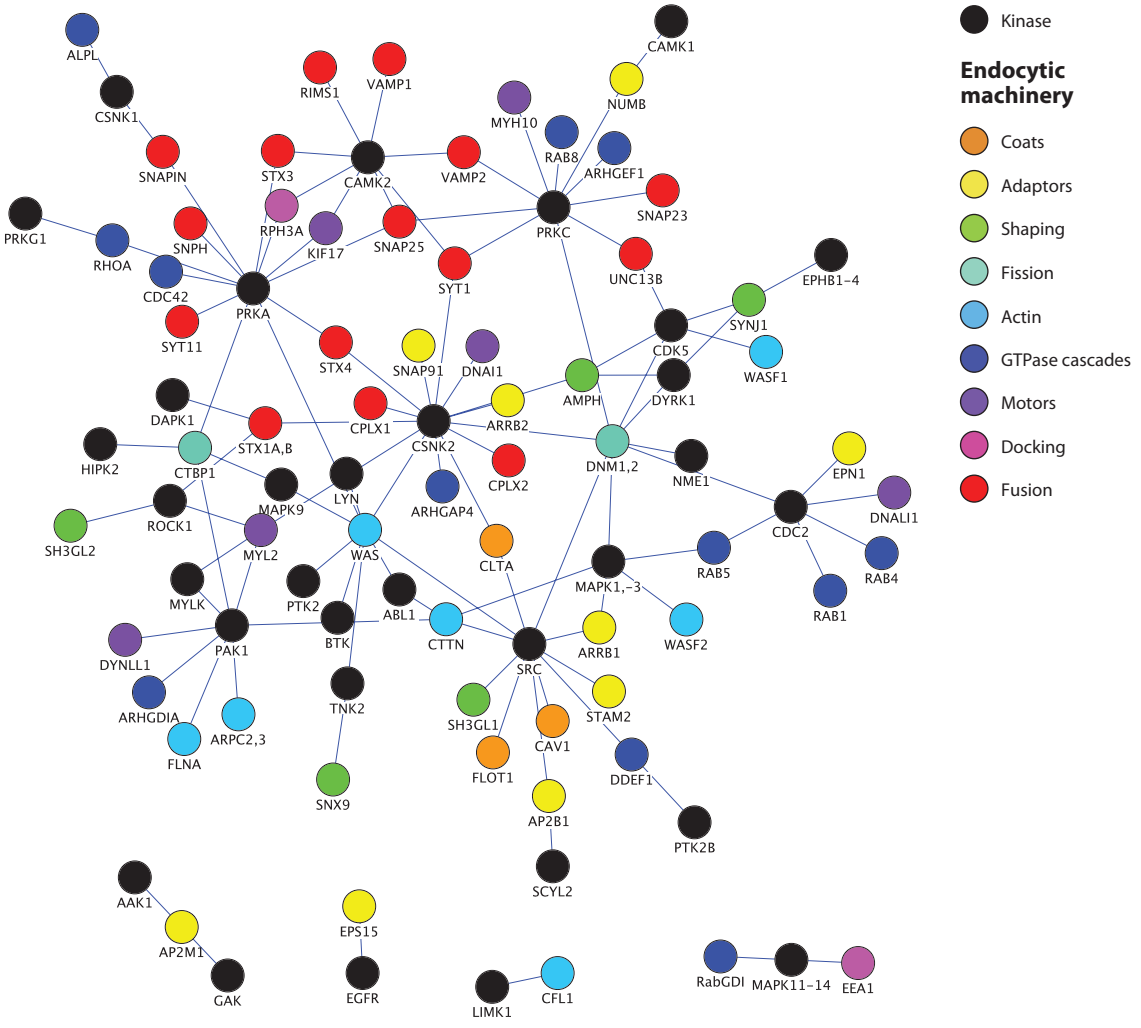


Figure 3

Network of 111 phosphorylation reactions between 32 protein kinases (isoforms have been fused to one kinase) (*black nodes*) and 61 endocytic machinery components (isoforms have been fused to one component) (*colored nodes*). Endocytic machinery is color-coded according to the functional module to which they belong.

foundation. Most of these heavily studied kinases were identified in forward genetic screens and behave well in biochemical assays. Only when human short interfering RNA (siRNA) libraries became available (in 2003) could we perform genome-wide unbiased analyses of protein kinases in human cells and empirically address the question as to which kinases, globally compared, are important regulators of the phenotypic properties of cellular processes. Furthermore, especially in cancer cells, the set of kinases regulating cellular processes might be very different from the set of kinases regulating these same processes in primary cells from a particular tissue.

With the above considerations in mind, we can analyze the network. Seven kinases act as major hubs in the network, having at least seven endocytic machinery components as direct substrates. These kinases are cell division cycle

2 (CDC2), c-Src (SRC), casein kinase 1 and 2 (CSNK1 and -2, respectively) [here treated as one kinase, protein kinase C (PRKC)], protein kinase A (PRKA), p21-activated kinase 1 (PAK1), and calcium/calmodulin-regulated kinase 2B (CAMK2). CDC2 primarily phosphorylates RabGTPases, which is believed to occur during the mitotic cycle. SRC is a heavily studied signaling kinase that is membrane anchored and associated with the plasma membrane and perhaps also with intracellular organelles. It phosphorylates components involved in early events of the endocytic membrane trafficking cycle, namely vesicle formation, fission, and actin-mediated propulsion. It thus appears that SRC can act as a general on switch for endocytic membrane trafficking, downstream from growth factor receptors and integrins, which activate SRC. Casein kinase has a very broad range of substrates and is usually considered to be a nonspecific switch that modulates structural properties of many different types of proteins. It is activated by many receptor signals (e.g., growth factor receptors, cadherins) during mitosis. PRKC has been studied extensively in membrane trafficking and endocytosis; it has 10 isoforms, which we here, for sake of simplicity, collectively treat as one kinase. PRKC regulates fission, fusion, and the RabGTPase cycle. Many signals can activate PRKC, but most prominently among these are activated G protein–coupled receptors (GPCRs) and growth-factor receptors. PRKA regulates membrane fission, microtubule transport, actin propulsion, and membrane fusion. It is activated by cAMP downstream of GPCRs and has an important role in nutrient signaling. PAK1 receives signals from growth factor receptors, GPCRs, and integrins on the cell surface and is a well-known hub in the regulation of the actin cytoskeleton and microtubule-dependent transport. It is essential for ruffle formation, cup closure, and internalization of the membrane carriers during macropinocytosis. In our network, it regulates actin-binding and -branching proteins, molecular motors, membrane fission, and RhoGTPases. The last hub is CAMK2, which is activated by calmodulin as soon as intracellular Ca^{2+} concentrations rise. CAMK2 regulates primarily membrane fusion. The effect of intracellular Ca^{2+} on membrane fusion has been extensively studied for synaptic vesicles, where the arrival of an electrical impulse at the synapse leads to immediate opening of Ca^{2+} channels and immediate fusion of numerous synaptic vesicles already docked on the synapse membrane. Ca^{2+}-regulated fusion is not a specific characteristic of membrane trafficking in synapses but is seen in any cell type. This is an example of an element of the endocytic membrane system that is generally built-in but used more predominantly in a specific cell type (e.g., neurons). In **Figure 4**, we created a simplified hierarchical network to illustrate that, by mere consideration of these seven kinase hubs, cellular physiology is already linked in complex networks to the endocytic machinery modules.

MODULES OF ENDOCYTIC MACHINERY COMPONENTS REGULATED BY PHOSPHORYLATION

We next separated the phosphorylation networks surrounding and interconnecting each functional module of endocytic machinery components for more detailed discussion (**Figure 5**). We do not embark here on detailed descriptions of the mechanics by which these modules operate. For that, we refer to a series of excellent reviews (Cai et al. 2007, D'Souza-Schorey & Chavrier 2006, Farsad & De Camilli 2003, Jahn & Scheller 2006, McNiven & Thompson 2006, Miaczynska & Zerial 2002, Robinson 2004, Roth 2007, Soldati & Schliwa 2006). The purpose here is to create an informational network of all known phosphorylation reactions. In most cases, it is not yet understood how reversible phosphorylation can change the mechanics of the endocytic machinery. For the few cases in which more detailed insights have been obtained, we refer to the original work and their reviews (Henderson & Conner 2007, Mace et al. 2005, Yarar et al. 2007).

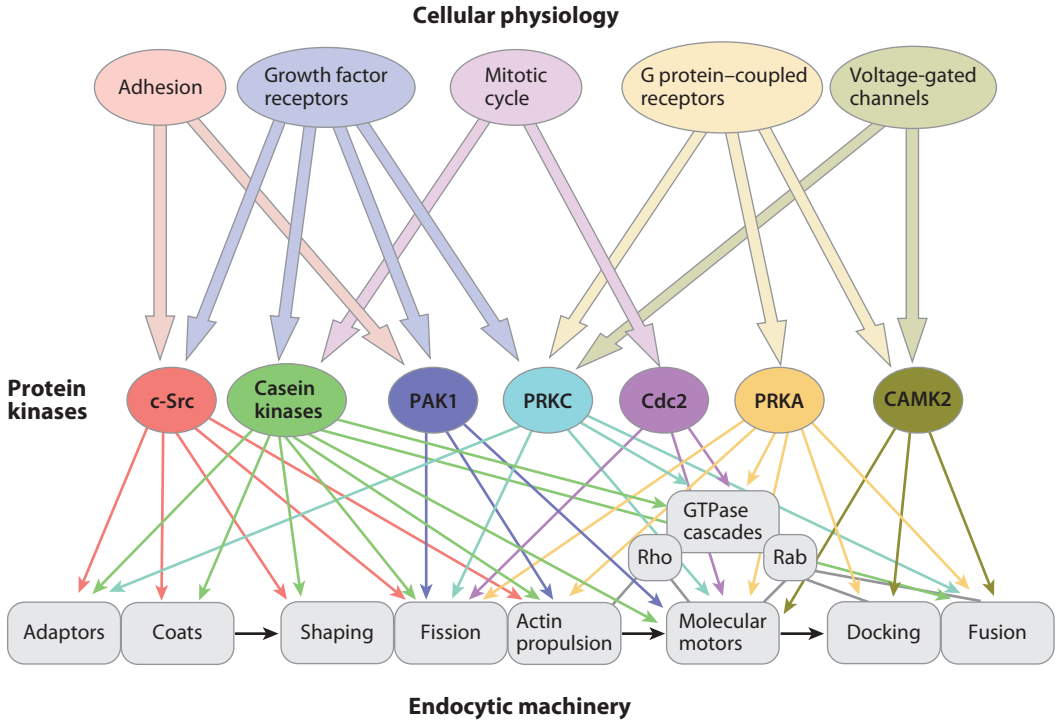


Figure 4

Hierarchical representation of a simplified view on how cell physiology, primarily via G protein–coupled receptors (GPCRs), calcium channels, growth factor receptors, adhesion sensors, and sensors of the mitotic cycle control the endocytic machinery via seven major protein kinase hubs. c-Src controls vesicle formation. Casein kinases and protein kinase C (PRKC) control all steps of the endocytic membrane trafficking cycle. p21-activated kinase 1 (PAK1) and cell division cycle 2 (CDC2) control membrane fission, cytoskeleton-dependent transport, and GTPase cascades. Protein kinase A (PRKA) and calcium/calmodulin-regulated kinase 2 (CAMK2) control fission, cytoskeleton-dependent transport, and fusion of membrane vesicles.

Adaptor Module

Components of the adaptor complexes AP1 and AP2, as well as the adaptor proteins AP180 and the β arrestins, are phosphorylated. Cyclin G–associated kinase (GAK)/auxillin phosphorylates AP1 and AP2, whereas the adaptor-associated kinase 1 (AAK1) phosphorylates just AP2 (Conner & Schmid 2002, Korolchuk & Banting 2002, Smythe 2002). The major hub SRC phosphorylates β arrestins (Fessart et al. 2007). Phosphorylation of adaptors regulates adaptor recruitment to cargo (β arrestins are usually recruited to GPCRs, thereby bridging them with clathrin) and the (dis)assembly of coat components on the adaptors (Langer et al. 2007). How phosphorylation is timed with the cycle of coat assembly and disassembly is less well understood. SRC becomes activated downstream of GPCRs, but how GAK/auxillin and AAK1 become activated or if they are constitutively active needs to be investigated.

Coat Module

We here include clathrin, caveolin, and flotillin coats, even though these coats function by very different principles (Bauer & Pelkmans 2006, Frick et al. 2007, Kirchhausen 2000, Parton & Simons 2007). It can just as well be argued that caveolin and flotillin belong to the membrane-shaping module discussed next. Coat proteins

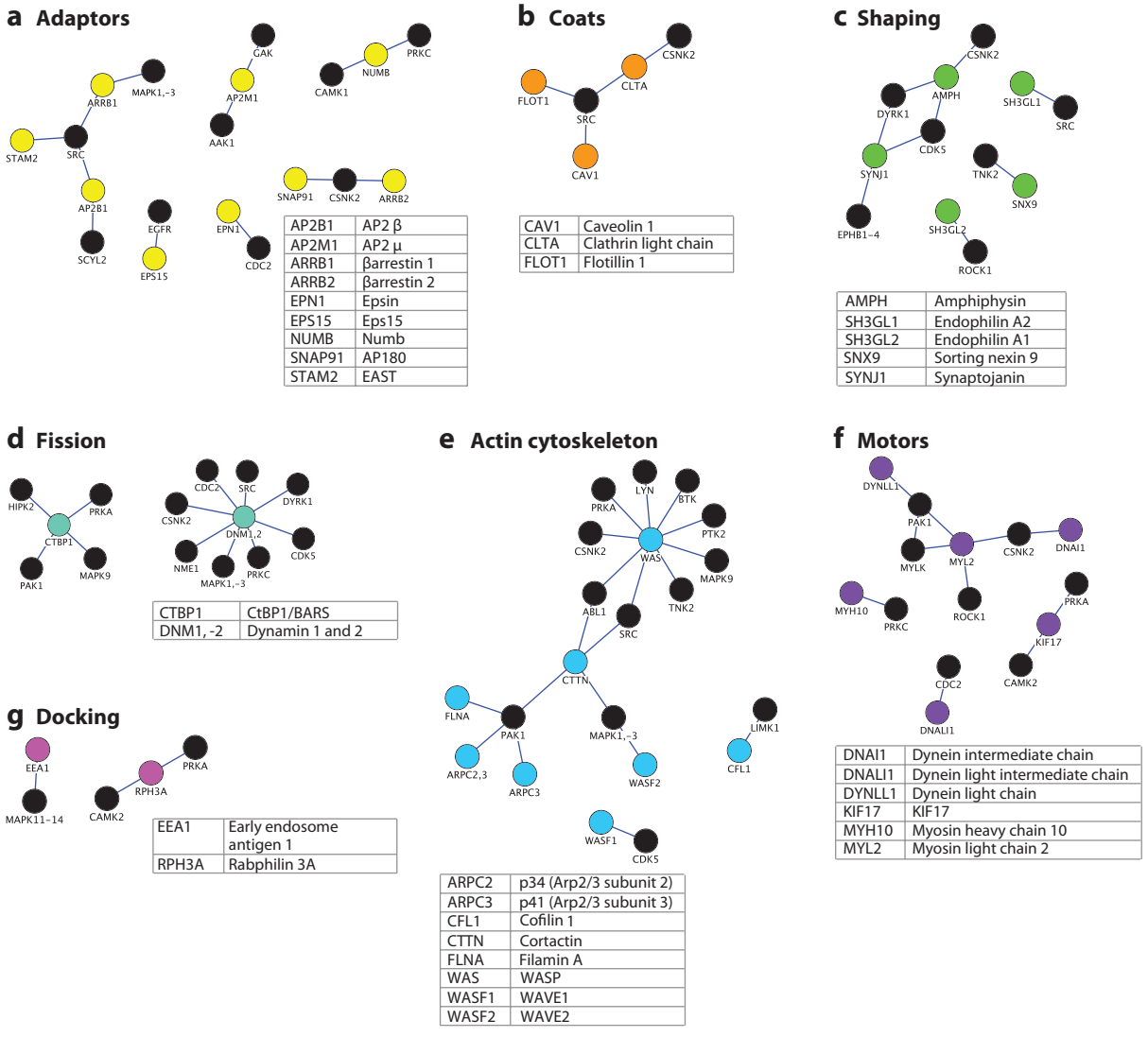
are directly phosphorylated by the two major hubs CSNK2 and SRC. SRC, which phosphorylates tyrosine 14 of caveolin, may play a role in the internalization of caveolae (Li et al. 1996). However, this reaction may also be linked to regulating cell adhesion, a prominent role of SRC in the cell. SRC-caveolin phosphorylation has roles in signal transduction (Williams & Lisanti 2004) and may regulate the caveolin-mediated scaffolding or transport of certain molecules and lipids important for cell adhesion (Echarri & Del Pozo 2006). Why SRC phosphorylates flotillin is completely unclear, but such phosphorylation may have a similar purpose as phosphorylation of caveolin, given that both proteins have similar topologies and may share a similar structural organization in scaffolding lipid raft components (Neumann-Giesen et al. 2007). The purpose of phosphorylating clathrin by SRC (Wilde et al. 1999) or by CSNK2 (Bar-Zvi & Branton 1986) is also not clear. SRC may transduce signals from growth factor receptors or integrins to clathrin, which may contribute to the initiation of clathrin coat formation around the receptor. CSNK2 is the major kinase activity associated with clathrin-coated vesicles and can phosphorylate many proteins on these vesicles. It is inactive when the clathrin coat is polymerized and becomes active as soon as uncoating starts (Korolchuk & Banting 2002). Additionally, COPI-coat components are known to be phosphorylated (Sheff et al. 1996), but to date no specific kinases have been identified.

Membrane-Shaping Module

We included in this module those BAR domain–containing proteins that are known to be phosphorylated, namely amphiphysin (AMPH) and endophilin A1 and A2 (SH3GL1 and SH3GL2). Sorting nexin 9 (SNX9) is a BAR domain–containing sorting nexin that links vesicle shaping to the actin cytoskeleton (Yarar et al. 2007). Synaptojanin 1 (SYNJ1) is a phosphoinositide lipid phosphatase involved in the uncoating of clathrin-coated vesicles (Slepnev et al. 1998). Besides the major hubs SRC and casein kinase, we find here dual-specificity tyrosine phosphorylation–regulated kinase 1 (DYRK1), CDK5, tyrosine kinase nonreceptor 2 (TNK2), and Rho kinase 1 (ROCK1). DYRK1 and CDK5 both phosphorylate AMPH and SYNJ1. DYRK1-dependent phosphorylation of AMPH is probably involved in the formation of synaptic vesicles, and DYRK1 deletion mutants of *D. melanogaster* have brain developmental defects (minibrain mutant) (Dierssen & de Lagran 2006). CDK5-mediated phosphorylation of AMPH regulates its binding to the membrane (Liang et al. 2007). CDK5 and ephrin receptor B phosphorylation of SYNJ1 inhibits the binding to endophilin and its inositol 5-phosphatase activity (Irie et al. 2005, Lee et al. 2004). Phosphorylation of endophilin by ROCK1 regulates binding to CIN85 (Cbl-interacting protein of 85 kDa) and blocks epidermal growth factor receptor (EGFR) endocytosis (Kaneko et al. 2005).

Membrane Fission Module

In this module we list dynamin 1 and 2 as well as C-terminal binding protein1/BFA-induced ADP-ribosylated substrate (CtBP1/BARS) (Corda et al. 2006, Praefcke & McMahon 2004). The latter is not characterized to the extent as dynamins but has a role late in the formation of macropinosomes and, most likely, in the actual closure of the macropinocytic cup (Liberali et al. 2008). For the dynamins, we again find the major hubs SRC and casein kinase as well as DYRK1 and CDK5. Four additional kinases phosphorylate dynamins, the major hubs PRKC and CDC2, nonmetastatic protein 1 (NME1), and mitogen-activated protein kinase (MAPK)1. If and how these diverse phosphorylation reactions change the activity of dynamins or their recruitment to membranes remain to be investigated. CtBP1/BARS is phosphorylated by the major hubs PAK1 and PRKA and by MAPK9 and homeodomain-interacting protein kinase 2 (HIPK2). PAK1 regulates closure of the macropinocytic cup via CtBP1/BARS (Liberali et al. 2008). Given that PAK1 has an important



AP2B1	AP2 β
AP2M1	AP2 μ
ARRB1	βarrestin 1
ARRB2	βarrestin 2
EPN1	Epsin
EPS15	Eps15
NUMB	Numb
SNAP91	AP180
STAM2	EAST

CAV1	Caveolin 1
CLTA	Clathrin light chain
FLOT1	Flotillin 1

AMPH	Amphiphysin
SH3GL1	Endophilin A2
SH3GL2	Endophilin A1
SNX9	Sorting nexin 9
SYNJ1	Synaptojanin

CTBP1	CtBP1/BARS
DNM1, -2	Dynamin 1 and 2

ARPC2	p34 (Arp2/3 subunit 2)
ARPC3	p41 (Arp2/3 subunit 3)
CFL1	Cofilin 1
CTTN	Cortactin
FLNA	Filamin A
WAS	WASP
WASF1	WAVE1
WASF2	WAVE2

DNAI1	Dynein intermediate chain
DNALI1	Dynein light intermediate chain
DYNLL1	Dynein light chain
KIF17	KIF17
MYH10	Myosin heavy chain 10
MYL2	Myosin light chain 2

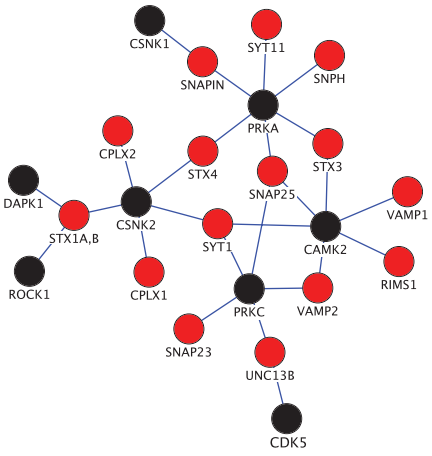
EEA1	Early endosome antigen 1
RPH3A	Rabphilin 3A

Figure 5

Modules of endocytic machinery components and protein kinases. Specific phosphorylation networks of each module are depicted. Endocytic machinery components are denoted by colored nodes, whereas protein kinases are denoted by black nodes.

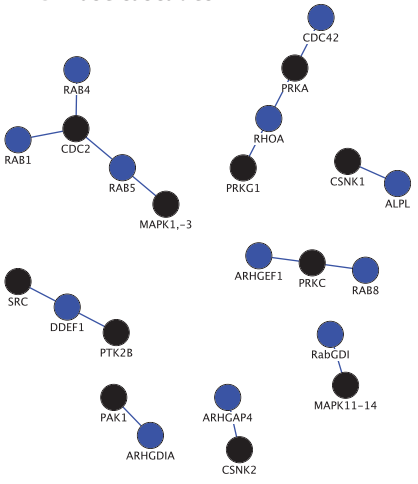
role in regulating both F-actin assembly and anchoring to the membrane as well as microtubule motor activity, it emerges as a central regulator of macropinocytosis. MAPK9 and HIPK2 phosphorylation targets CtBP1/BARS for degradation (Wang et al. 2006, Zhang et al. 2005), and PRKA changes the interactions of CtBP1/BARS with other proteins (Dammer & Sewer 2008). It is interesting to note that the kinases regulating dynamin activity are all different from the kinases regulating CtBP1/BARS activity, indicating that these two types of membrane fission are downstream of different signal transduction cascades and therefore likely have nonoverlapping roles in membrane trafficking.

h Fusion



CPLX1	Synaphin2
CPLX2	Synaphin1
RIMS1	Rim; Rab3-interacting protein
SNAP23	SNAP23
SNAP25	SNAP25
SNAPIN	Snapin
SNPH	Syntaphilin
STX1A, -B	Syntaxin 1A and B
STX3	Syntaxin3
STX4	Syntaxin4
SYT11	Synaptotagmin 12
UNC13B	Munc-18
VAMP1	VAMP1
VAMP2	VAMP2

i GTPase cascades



ALPL	HOPS
ARHGAP4	p115 ARHGAP4
ARHGDIA	RhoGDI
ARHGEF1	p115RhoGEF
CDC42	Cdc42
DDEF1	ASAP1
RabGDI	GDI 1 and 2
RAB1	All Rab1 isoforms
RAB4	All Rab4 isoforms
RAB5	All Rab5 isoforms
RAB8	All Rab8 isoforms
RHOA	RhoA

Protein kinases	
AAK1	AAK1
ABL1	c-ABL
BTK	Btk
CAMK1	Ca^{2+}/calmodulin-dependent protein kinase 1
CAMK2	Ca^{2+}/calmodulin-dependent protein kinase 2
CDC2	cdc2
CDK5	cdk5
CSNK1	casein kinase 1 (yeast yck3)
CSNK2	All casein kinase 2 isoforms
DAPK1	DAP kinase
DYRK1	Dyrk1a and Dyrk1b
EGFR	EGFR
EPHB1–4	EphrinB-EphB1, -B2, -B3, and -B4
GAK	Cyclin G associated kinase/auxilin 2
HIPK2	HIPK2
LIMK1	LIM kinase
LYN	Lyn
MAPK1, -3	ERK1 and -2
MAPK11–14	Stress-activated MAPK p38 α, β, and Δ
MAPK9	Jnk
PAK1	Pak1
PRKA	All protein kinase A isoforms
PRKC	All protein kinase C isoforms
PRKG1	PKG
PTK2	Fak
PTK2B	Fyk2
ROCK1	Rho kinase
SCYL2	CVAK104
SRC	c-Src
TNK2	Activated Cdc42-associated kinase-2 (ACK2)

Figure 5

(*Continued*)

Actin Cytoskeleton Module

In this module, we include only those actin-binding, -nucleating, or -modulating proteins that have been functionally implicated in endocytosis and are phosphorylated by specific kinases. A protein with many known phosphorylations is N-WASP (WAS), which activates the Arp2/3 complex to initiate the formation of branched actin networks (Takenawa & Suetsugu 2007). WAVE1 (WASF1) and WAVE2 (WASF2), which have similar roles as N-WASP (Takenawa & Suetsugu 2007), are also included. The module also contains three actin-binding proteins: cortactin (CTTN), filamin A, and cofilin1. Besides the major kinase hubs SRC, which phosphorylates N-WASP and CTTN, and CSNK2, which phosphorylates N-WASP, we find here several other kinases that also phosphorylate other modules. These kinases are the hub PAK1—which phosphorylates the Arp2/3 complex, filamin A, and CTTN—and MAPK1 and -3, (Erk1/2), which phosphorylate CTTN and WAVE1.

We also find here the major hub PRKA as well as CDK5, both known to phosphorylate N-WASP. The module furthermore consists of Abelson cytoplasmic tyrosine kinase 1 (ABL1), which regulates CTTN and N-WASP, as well as MAPK9, the SRC family tyrosine kinase LYN, TNK2 (activated Cdc42-associated kinase), PTK2 (focal adhesion kinase), and Bruton's tyrosine kinase (BTK), all known to phosphorylate WASP. Finally, we include LIM kinase 1, which phosphorylates cofilin1.

Molecular-Motors Module

The following molecular motors with roles in endocytosis are known to be phosphorylated by specific kinases: (*a*) three subunits of the dynein motor complex, the dynein light chain 1 (DYNLL1), the dynein light intermediate chain 1 (DNALI1), and the dynein intermediate chain 1 (DNAI1); (*b*) myosin motor 2 heavy chain 10 (MYH10) and myosin light chain 2 (MYL2); and (*c*) the kinesin KIF17. The kinases responsible for this are the hubs CDC2, CSNK2, PAK1, PRKA, PRKC, and CAMK2 as well as the nonhubs myosin light chain kinase (MYLK) and ROCK1. A set of five kinases coordinates the activity of MYL2 and the dynein complex. PAK1 and CSNK2 are interesting because they regulate both a component of the dynein complex and myosin 2. For many endocytic events, myosin and dynein motors need to act sequentially to switch from movement on F-actin to movement on microtubules. Sequential phosphorylation of the responsible motor may establish this switch.

Membrane Docking Module

Not much is known about phosphorylation in membrane vesicle docking. Only two known docking (or tethering) proteins, namely early endosome antigen 1 (EEA1) and rabphilin 3A (RPH3A), are phosphorylated by specific kinases. EEA1 is phosphorylated by the stress response kinase p38 (MAPK11, -13, -14), and RPH3A by PRKA and CAMK2. EEA1 is phosphorylated in its FYVE domain, and this may be important for the recruitment of EEA1 to PI(3)P-enriched membranes, such as the early endosome (Mace et al. 2005).

Membrane Fusion Module

The molecular mechanisms of membrane fusion are well characterized, and the role of phosphorylation has been studied extensively. Several kinases regulate multiple proteins involved in membrane fusion. In this module we find the hubs CAMK2, casein kinase, PRKC, and PRKA. CAMK2 phosphorylates syntaxin 3 (STX3), two vesicle-associated membrane proteins (VAMP1, -2), soluble NSF attachment protein 25 (SNAP25), synaptotagmin 1, and RIM (RIMS1) or Rab3-interacting protein. CSNK1 and CSNK2 phosphorylate a SNAP-interacting protein (SNAPIN), two syntaxins (STX1A and -4), synaphin 1 and 2 (CPLX1 and -2), and synaptotagmin. PRKC phosphorylates synaptotagmin, SNAP23 and -25, and UNC13B (also known as MUNC18). PRKA phosphorylates two syntaxins (STX3 and -4), SNAP25, SNAPIN, synaptotagmin 12, and syntaphilin. Kinases with few substrates in this module are death-associated protein kinase 1 (DAPK1) and ROCK1, which both phosphorylate syntaxin 1a.

GTPase Cascade Module

This module is not assigned to a specific step in the formation, transport, or fusion steps of a membrane vesicle. RabGTPases regulate several aspects of this cycle and have various components of these modules as their effectors. The GTPase cycle does however receive input from other sources, which can change its cycle time and, as a consequence, the activity of membrane traffic. Many of the above-mentioned kinases modulate the GTPase cascade. The general hubs casein kinase and SRC are found, but not as prominently as CDC2. CDC2 phosphorylates several RabGTPases (Rab1, Rab4, Rab5) during mitosis. Other hubs found here are PRKA and PRKC. PRKA is linked specifically to the RhoGTPases RhoA and Cdc42, which

play (distant) roles in endocytosis by regulating the cytoskeleton. PRKC has been specifically linked to Rab8 and a Rho GDP/GTP exchange factor (ARHGEF1). Interestingly, we also find two major MAPKs: p38 (MAPK11, -13, -14) and Erk1/2 (MAPK1, -3). p38 is the major transducer of stress response signaling in the cell and regulates Rab GDP dissociation inhibitor (GDI). Erk1/2, the major bottleneck in transducing growth factor signals to cell proliferation, phosphorylates Rab5.

UNBIASED GLOBAL APPROACHES TO LINK ENDOCYTOSIS TO KINASE REGULATORY NETWORKS OF THE CELL

The previous section demonstrates that all modules of the endocytic machinery are highly interconnected by protein kinases and that certain modules are enriched in phosphorylation reactions. These are membrane fission, the GTPase cascade, membrane fusion, and actin-mediated propulsion (in particular N-WASP).

However, if an unbiased analysis of protein phosphorylation of all these modules were available, the picture would probably look quite different. The first step toward such a global view was taken several years ago by systematically silencing each kinase of the human kinome using siRNA and studying how this affects a variety of properties of the endocytic membrane system (Pelkmans et al. 2005). This study included the infectious entry of simian virus 40 through caveolae/raft-mediated endocytosis; the infectious entry of vesicular stomatitis virus through clathrin-mediated endocytosis; and 23 parameters of the endocytic membrane system describing internalization patterns of fluorescent transferrin, of cholera toxin subunit B, and of low-density lipoprotein and the intracellular distributions of early endosomes, of late endosomes, and of caveolin-1. Because it was the first study of its kind, and inherent to any high-throughput study, some experimental noise as well as false-positive and false-negative observations must be expected. The rapid technological development in this field, including better siRNA libraries and advanced computational methods for quantifying and classifying loss-of-function phenotypes (Kittler et al. 2007, Lamprecht et al. 2007, Pepperkok & Ellenberg 2006, Reimers & Carey 2006, Root et al. 2006), will allow much improved global and unbiased analysis of the endocytic membrane system in the future. The importance of this approach for creating a set of formal and standardized rules with which to define the properties of the endocytic membrane system justifies a large investment by the cell biology community to make these methods more mature, well adapted to the problems of membrane trafficking, and more mainstream.

The results of this first study showed that protein kinase silencing has widespread effects on the endocytic membrane system. The 140 protein kinases found to have a loss-of-function phenotype in any of the parameters studied are also annotated on the kinome tree in **Figure 2**. Of the 32 published kinases regulating endocytic machinery components, 26 had a loss-of-function phenotype in the screen. Importantly, several of these were actually found to regulate endocytic machinery components after the RNAi screen was completed (see **Supplemental Table 1**), thus independently validating several observations. It can be expected that a number of the protein kinases found in a phenotypic screen do not directly phosphorylate endocytic machinery components but have a role more upstream in signal transduction to the endocytic machinery. This hypothesis can be tested in a network in which one adds a layer of protein kinases that are upstream of the protein kinases directly phosphorylating the endocytic machinery (**Figure 6**). The information to construct such a network was derived from STRING (**http://string.embl.de**), a protein association network that quantitatively integrates predicted and known physical and functional interaction data from various sources. In this complex graph, we can now link more than half of the protein kinases identified in the screen to endocytic machinery components. There is no doubt that more extensive bioinformatics

Supplemental Material

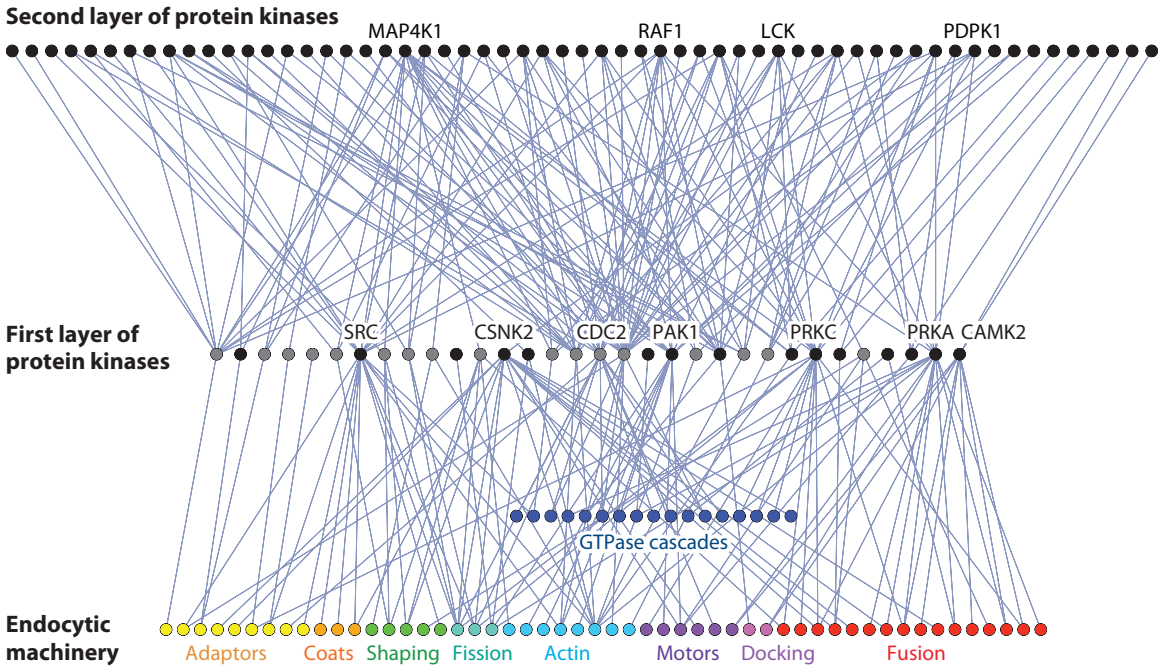


Figure 6

Integration of endocytic machinery components and the protein kinases that directly phosphorylate these components into larger kinase signaling networks of the cell. The hierarchical network consists of 69 endocytic machinery components from **Figure 3**, the first layer consists of 32 protein kinases from **Figure 3**, and the peripheral layer consists of 59 protein kinases that interact with the first layer. The major hubs of the first layer, SRC, CSNK2, CDC2, PAK1, PRKC, PRKA, and CAMK2, as well as four examples of upstream kinases in the peripheral layer (MAP4K1, RAF1, LCK, and PDPK1) are indicated. With this bioinformatics approach, we can link 74 out of 131 kinases (56%) found in a comprehensive image-based RNAi screen to interact either directly (15), or via one intermediate kinase node (59), with the endocytic machinery.

analysis, combined with advanced statistics and network modeling, will give a much better analysis of these results. It is therefore imperative that the field of endocytosis also embrace this discipline (Alon 2007, Sharan & Ideker 2006, Zaidel-Bar et al. 2007). It will be essential to validate these networks, to improve them, and to further expand them using quantitative proteomics. In particular, phosphoproteomics (Bodenmiller et al. 2007) combined with RNAi of protein kinases will be ideally suited to map out globally the cellular substrates of protein kinases and to delineate which endocytic machinery components are phosphorylated by which kinases. Combined with protein-protein interaction networks derived from quantitative proteomics and systematic pull-down experiments (Aebersold & Mann 2003), we can obtain the biochemical architecture of kinase-endocytosis networks. We will then be able to understand how cell adhesion signaling controls caveolae/raft-mediated endocytosis; how nutrient sensing controls clathrin-mediated endocytosis (Galvez et al. 2007); and how mitogenic signaling, calcium signaling, and the actin cytoskeleton coordinate the activities of the two endoytic routes (Pelkmans et al. 2005).

CONCLUSIONS AND OUTLOOK

We must stress that we ignore the importance of lipids in this review. There is no doubt that the heterogeneity of lipid organization in membranes, the particular use of phosphoinositides, and their reversible phosphorylation to create membrane domains and recruit specific

endocytic machinery to membranes as well as lipid second messengers are crucial elements of the endocytic membrane system (Di Paolo & De Camilli 2006). Cellular signaling regulates many lipid kinases and phosphatases, hydrolases, carbohydrate transferases, flippases, pumps, and nonvesicular transferases between compartments, which strongly influences the behavior of membranes (Di Paolo & De Camilli 2006). This topic deserves a review of its own.

If we have the ambition to create a physical explanation of the behavior of the endocytic membrane system, we need to fill in an enormous conceptual and informational gap, requiring both top-down and bottom-up approaches. In the top-down approach, we must attempt to model and predict the activity of endocytic pathways and their phenotypic diversity by identifying patterns in large sets of measurements from individual cells and vesicles. Image-based screens of individual cells in whole cell populations combined with novel image analysis methods and advanced statistical and mathematical analysis tools will be essential. Eventually, this may lead to the discovery of formal rules that describe basic principles of the endocytic membrane system, which is required to separate nondeterministic aspects from deterministic aspects of the system. Only for the latter will bottom-up approaches be useful. Meanwhile, we must identify the principal proteins, enzymes, lipids, and metabolites responsible for this deterministic behavior and delineate the tightly controlled interaction schemes between them. We have already come a long way to identifying these molecules, and the various-omics disciplines will make this list more complete. Systematic analysis of the effect of silencing, inactivating, or overexpressing these molecules will allow us to link these molecules to the physical principles underlying phenotypic complexity of endocytosis. We here coin the term genetical physics for this approach. Genetical physics links genetic perturbations (e.g., by RNAi) to specific parameters and degrees of freedom of the physical principles underlying the cellular system under scrutiny.

Genetical physics: a new discipline that quantitatively links genetic perturbations to degrees of freedom in a formal, physical model of the biological system of interest

For accurate deterministic bottom-up models, we will have to determine the abundances and turnover of all these molecules, measure all the association and dissociation constants between these molecules, and ascertain how these variables are regulated over time. However, the biological uncertainty principle (due to small and noisy numbers of interacting components and unpredictable interaction kinetics) will prevent fully deterministic descriptions at this level. This last aim therefore lies in the very far future for a complex system such as endocytic membrane trafficking, and we may have to ask ourselves to what extent we should pursue the bottom-up approach. If we can identify predictive physical principles and link them to sets of individual molecules without having a complete biochemical model of how these molecules interact, they may be remarkably sufficient in explaining the roles of the endocytic membrane system in cellular physiology.

SUMMARY POINTS

1. Protein kinases play an essential role in regulating the endocytic membrane system.
2. Protein kinases are the interface between the endocytic machinery and the physiological status of the cell.
3. Protein kinases allow the endocytic machinery to respond to changing demands from the cell and thereby ensure that the appropriate cellular phenotype is established or maintained.

4. The endocytic membrane system in turn plays an essential role in signal transduction and provides a flexible and changeable space-time for signal transduction reactions.
5. Signal transduction and the endocytic membrane system have most likely coevolved into the complex and integrated system that we observe in mammalian cells today.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank all members of the laboratory for stimulating discussions. L.P. is supported by the Swiss National Science Foundation, SystemsX.ch, the European Union, and the ETH Zurich. P.L. is a long-term fellow of the Federation of European Biochemical Societies (FEBS), and P.R. is a long-term fellow of the European Molecular Biology Organization (EMBO).

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These two discussions by Kholodenko and colleagues nicely lay out the concept that membranes define a space-time for signal transduction that is different from standard reaction-diffusion kinetics.

Indicates that the canonical endocytic pathway must be considered as a population of vesicles that display specific heterogeneity in the kinetics of internalization and transport of cargo.

Demonstrates the importance of the open-source mentality in the life sciences to share image-processing tools. CellProfiler is based on MatLab and provides an easy-to-use graphical user interface enabling every biologist to do computational image processing.

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By applying computer simulation of diffusion in complex-shaped membranes (the endoplasmic reticulum), the authors showed that organelle shape has strong influences on diffusion at the macroscopic scale.

Discusses protein-protein interaction network approaches to dissect mechanisms of clathrin-coated pit assembly and internalization. Introduced the concept of matricity.

Important theoretical considerations of how cellular compartmentalization will affect Brownian motion.

Annual Review of Cell and Developmental Biology

Volume 24, 2008

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Errata

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