The 56th Annual Meeting and Symposium of the Society of General Physiologists

** Trafficking of Transporters**

(Organized by Ron R. Kopito and William N. Green)

In addition to their direct catalytic role, the proper function of transport proteins depends on multiple cellular processes to ensure: that the protein is folded and assembled into its correct conformation; that misfolded proteins are degraded; that the protein is targeted to its correct location within the cell; that the copy number of the protein is maintained by regulated synthesis and turnover; and that any other proteins involved in the transport process, whether they be subunits in a macromolecular complex or proteins in other membrane domains, are present in sufficient number and in the correct location(s). The importance of membrane protein processing and targeting for normal cellular and body function has become abundantly clear over the past decade, as an increasing number of genetic “transport” diseases, with cystic fibrosis being the prototypical example, turn out to be due to disrupted protein trafficking (compare M. Aridor and L.A. Hannan. 2000. Traffic. 1:836–851 and 3:781–790). Likewise, a remarkable generality of the underlying processes is emerging—in the sense that the targeting signals and trafficking machinery are conserved evolutionarily and among different cells.

The recent advances in elucidating the mechanisms underlying protein trafficking were the focus of the 56th Annual Meeting of the Society of General Physiologists, which took place in Woods Hole, MA, September 4–7, 2002. Ron R. Kopito from Stanford University and William N. Green from the University of Chicago organized the symposium on Trafficking of Transporters, which highlighted the recent progress that has taken place in this important field. With 150 participants, and 82 invited and poster presentations covering a broad range of topics, the meeting was lively and the major issues were put in perspective.

Intracellular protein traffic occurs by vesicular transport between different organelar membrane compartments as well as between organelar membranes and the plasma membrane (Fig. 1).

After their initial synthesis and assembly in the endoplasmic reticulum (ER), membrane proteins are passed on to the Golgi stack, from which they may be retrieved back to the ER. The main route, however, is through the Golgi stack on to the plasma membrane, where the proteins’ destinies are determined by specific targeting signals. Once in the plasma membrane, the proteins are retrieved by endocytosis to the endocytic recycling compartment from which they may be returned to the plasma membrane—either directly or via the Golgi stack—or targeted for destruction in the lysosomes. Because membrane protein trafficking occurs by vesicular transport, many questions relating to the protein trafficking eventually become questions about the mechanisms underlying the sequestration of proteins into the appropriate population of transport vesicles.

Two key steps in protein trafficking are export from the ER and passage through, and processing by, the Golgi stack. Jennifer Lippincott-Schwartz (National Institutes of Health) summarized work on vesicular traffic within the Golgi stack—and between the Golgi stack and the ER or the plasma membrane. Using a combination of green fluorescent protein (GFP)-labeled vesicular stomatitis virus glycoprotein (VSVG) and temperature-dependent release from the ER, it is possible to monitor the passage of proteins from the ER through
the Golgi to the plasma membrane using high-resolution optical microscopy. At 40°C, the VSVG-GFP is localized in the ER; at 32°C, the protein is released and moves to the plasma membrane. Thus, it is possible to load the ER with VSVG-GFP, follow the protein movement through the Golgi to the plasma membrane, and determine the kinetics of the transport, which proceeds as a directed wave front in which the proteins move to the Golgi and then on to the plasma membrane (with rate constants of ~3% per min, which do not vary as a function of VSVG-GFP concentration). The rate constant for protein retrieval from the plasma membrane is ~10-fold less than the rate constant for delivery, such that the steady-state protein surface concentration in the plasma membrane will be 10-fold higher than in the Golgi—and the plasma membrane area in fact expands as the protein-containing vesicles are inserted into membrane.

Though the Golgi stack is polarized, with proteins entering the cis stack and exiting at the trans stack, the passage of VSVG-GFP from the ER to the plasma membrane does not provide support for a gradual passage through the Golgi stack. Indeed, there are currently several models for the passage of protein through the Golgi stack (Fig. 2).

In the cisternal progression/maturation model, the cisternae form de novo at the cis face of the stack and cargo then moves up through the stack by anterograde vesicular transport, whereas retained proteins move down by retrograde transport such that protein traffic through the stack resembles movement on a conveyor belt (and protein efflux from the Golgi stack can be described by a linear function of time). Eventually, the cargo reaches the trans compartment where the stack disassembles and the cargo moves on to the plasma membrane. In the vectorial model, the cisternae again form at the cis face, and proteins destined for the plasma membrane move forward by anterograde vesicular transport to the trans compartment and then to the plasma membrane (and protein efflux from the Golgi stack can be described by a multiexponential time course). In the well-mixed (or steady-state) model, the proteins again move by vesicular transport; but in contrast to the two preceding models, the exchange of material between the different stacks is fast, and the notion of cisternal maturation begins to break down in
the sense that the Golgi stack has become a single, well-mixed compartment (and protein efflux from the Golgi stack can be described by a single exponential time course).

To distinguish between three models, cells first were "labeled" uniformly with GFP-labeled proteins and then photo-bleached to eliminate all fluorescence from outside of the Golgi stack. Thus, it becomes possible to distinguish among the models by following the time course of protein exit from the Golgi stack. Results with several different GFP-labeled proteins (VSVG, collagen, and a signal sequence) were consistent with the well-mixed (or steady-state) model. To ensure that this is not an artifact of the photo-bleaching method an alternative method was developed in which cells were transfected with photo-activatable GFP-labeled proteins, such that one could activate only the GFP-labeled proteins in the Golgi stack. Again, the results were consistent with the notion that the Golgi stack constitutes a single well-mixed compartment (the steady-state model)—in which the mixing is complete within ~5 min.

That is, the Golgi stack appears to be a remarkably dynamic organelle. This conclusion reinforces previous work on cells treated with brefeldin A (BFA), which inactivates the GTPase ARF1 and thereby dissociates coatomer proteins from the COPI vesicles that underlie protein trafficking from the ER to the Golgi stack. When cells are treated with BFA, the Golgi stack appears to “dissolve” into the ER in about 8 min, which suggests that the Golgi stack’s integrity is maintained by vesicular export from the ER.

Forward traffic from the Golgi stack to the plasma membrane was investigated using yellow fluorescent protein (YFP) to label with galatosyltransferase (GalT), a Golgi-resident protein, as well as VSVG-GFP. Thus, it is possible to show that the VSVG-GFP–containing vesicles that move the protein to the plasma membrane are depleted of Golgi proteins—and that the budding occurs from VSVG-GFP–enriched membrane domains that are large enough to be visible in the optical microscope. This result suggests that the sorting of the VSVG-GFP into their transport vesicles could be mediated by partitioning between membrane domains of different thickness—with the plasma membrane-destined proteins preferring the thicker domains (c.f. Bretscher, M.S., and S. Munro. 1993. Science. 261:1280–1281). Consistent with this notion, VSVG mutants with shortened trans-membrane domains remain localized to the Golgi stack.

Though the GFP labeling could alter key aspect of a protein’s trafficking, the above results show that the Golgi stack is more dynamic than previously recognized. Given the dynamics of the protein traffic between (and within) the key compartments within the cell, it becomes necessary to have stringent control systems in place to ensure that integral membrane proteins reach their proper destination.

The oligomeric organization of many membrane proteins provides for a rich source of such trafficking control mechanisms. L.Y. Jan (University of California, San Francisco) summarized work on inward rectifier channels (Kir), which occur as homotetramers, heterotetramers, and heterooctamers (together with the sulfonyl urea receptor, SUR). G-protein–regulated channels (GirK), for example, are heterotetramers; the ATP-sensitive channels (KATP) are heterooctamers. KATP channels are important regulators of insulin secretion, and mutations in either the Kir or the SUR subunits, which disrupt Kir-SUR subunit interactions, cause unchecked insulin secretion because the KATP channels are not delivered to the plasma membrane. When expressed in Xenopus oocytes, KATP channels are expressed on the surface membrane only when both the Kir and SUR subunit messages are present. Homotetrameric Kir complexes can form potassium channels; but the Kir subunit has an Arg-Lys-Arg ER retention signal, which is “masked” by the SUR subunit such that the octamer can move to the Golgi stack and on to the plasma membrane. If the Arg-Lys-Arg retention signal is mutated, to abolish ER retention, there is constitutive transfer of the tetrameric Kir complex to the plasma membrane—and the channels are insensitive to sulfonylureas. Similar “masking” of ER retention signals may be a very general phenomenon, as members of the PDZ family of scaffolding proteins can bind to a diverse group of channel subunits and thereby promote their exit from the ER. This “masking” can be achieved even by phosphorylating two Ser residues (that are canonical protein kinase C substrates) adjacent to the ER retention signal.

But the regulation is even more elaborate. Though it generally is believed that any well-folded protein will leave the ER, distinct ER export signals have been identified in the carboxy-terminal cytoplasmic loop of some Kir channel subunits. Some GirK channel subunits, for example, have a Phe-Cys-Tyr-Glu-Asn-Glu export signal in their carboxy-terminal cytoplasmic loop, which controls ER export and thus the surface expression of the channels. In contrast to the ER retention signals, however, the export signal does not appear to impose absolute control on channel trafficking; they cause a shift in the distribution of the channel subunits among different intracellular compartments.

The role of scaffolding proteins in regulating protein targeting was also examined in several other presentations. W.N. Green (University of Chicago) described the control of AMPA and NMDA receptor trafficking by synapse-associated proteins (SAPs), which are a family of membrane-associated guanylate kinases (MAGUKs). SAP97 interacts with both AMPA and NMDA subunits
(GluR1 and NR2B, respectively), but the trafficking consequences of the SAP97-AMPA or SAP97-NMDA interactions are quite different: SAP97 promotes AMPA receptor trafficking to the plasma membrane, whereas it inhibits the surface expression of NMDA receptors. Results that, again, emphasize the complexity and multiplicity of the regulatory mechanisms. V. Bennett (Duke University Medical Center) described the varied functions of ankyrin in organizing ion channels (in both organellar and plasma membranes), ion pumps and exchangers, and cell adhesion molecules. Disrupting these interactions have, not unexpectedly, serious consequences; but rather unexpected, disruption in Ankyrin-B function is the basis for the type 4 Long QT syndrome. The sequence of events leading to the disrupted cardiac excitability is quite complex, as it involves an initial mistargeting of the Na⁺K⁺-ATPase to the plasma membrane, with a consequent increase in intracellular [Na⁺], which in turn increases intracellular [Ca²⁺] (cytoplasmic and in the sarcoplasmic reticulum) to cause increased contractility and eventually extrasystoles.

P. Shrager (University of Rochester) focused on the organization of the node of Ranvier. Using lysophosphatidylcholine-induced demyelination, it is possible to examine the channel distribution in the nodal and internodal membranes. The sodium channel density in the internodal regions is only ~4% of that in the node; but the membrane area is 1,000-fold higher, so by far the greater number of channels are found in the internodal membrane. The potassium channels are found almost exclusively in the internodal membrane. The demarcation between nodal and internodal membrane domains is sharp, a feature that persists after demyelination. The close packing of the sodium channels is mediated by contactin, a member of the immunoglobulin superfAMILY with homology to the β₂ sodium channel subunit. When new nodes appear, they tend to be ectopic and rather diffuse; but they become “firmed up” as the Schwann cells expand. A process that depends not only on the presence of contactin but also of sodium channel β₁ subunits. That is, more or less normal sodium channel activity can be obtained by expressing only the α subunit; but the current density increases, due to closer packing of the channels, when the β₁ and contactin (or maybe β₂) subunits also are expressed.

T.H. Stevens (University of Oregon) described the assembly and trafficking of vacuolar ATPases. These proton pumps, which are conserved from yeast to plants and mammals, are composed of two complexes: the bilayer-spanning Vₒ complex, which is composed of five different subunits that assemble in the ER, and the catalytic V₁ complex, which is composed of eight different subunits that assemble in the cytoplasm. The assembly of the Vₒ complex depends on ER-resident “assembly factors”, which also are important for ATPase exit from the ER, but the Vₒ complex does not exit the ER unless it has bound the V₁ complex. A result that, again, may be due to the “masking” of ER retention signals—in the Vₒ complex or in associated proteins—by the V₁ complex.

Misfolded proteins may cause disease, and it is necessary for cells to maintain a tight quality control. In addition to the control mechanisms that are inherent in the interdependent export retention signals in oligomeric proteins, the ER exerts an alternate control that identifies misfolded proteins within the ER. R.R. Kopito (Stanford University) gave a brief overview of diseases of transport protein misfolding, where cystic fibrosis is the prime example. Another example is the heterodimeric T cell receptor (TCR). Using influenza hemagglutinin (HA)-TCR chimeras, and introducing positively charged residues into the bilayer-spanning segments, it is possible to identify two sequential quality control checkpoints, one being the cytoplasmic proteasomes and the other the lysosomes. But are these protein-degrading mechanisms always quality control mechanisms? R.Y. Hampton (University of California, San Diego) showed how HMG-CoA reductase (HMGR) undergoes controlled ubiquitin-dependent degradation in the cytoplasm in response to changes in the cellular demand for sterol pathway products, which would suggest that the quality control checkpoints may serve multiple functions, including feedback control of metabolic pathways. The mechanism underlying this ubiquitin-dependent protein degradation was discussed further by T. Sommer (Max-Delbrück-Center for Molecular Medicine, Berlin). Ubiquitination turns out to be a late step in ER-associated protein degradation (ERAD), in which the first step is the identification of misfolded proteins in the ER lumen. These proteins are then targeted to the translocon, which normally catalyzes the cotranslational movement of integral membrane and secretory proteins across the ER membrane, and transported back into the cytosol where they are ubiquitinated and thereby targeted for proteolysis by the cytosolic 26S proteasomes.

Eventually all proteins are degraded, but short-term regulation of protein function often occurs by vesicular transport to, and from, the relevant membrane compartment (usually the plasma membrane). More generally, these transport events also serve to maintain normal cell structure and constitutive function. As would be surmised by the Symposium’s title, these exocytotic/ endocytotic trafficking events were the focus of many presentations. A particularly intriguing problem is protein trafficking in polarized cells, such as epithelia and neurons, where there needs to be distinct targeting signals to different membrane domains. E. Rodriguez-Boulan (Weill Cornell Medical College) briefly empha-
sized the generality of these targeting processes, where proteins destined for the basolateral membrane (in polarized epithelia) use similar targeting signals as the proteins destined for the soma and dendrites do (in neurons). Conversely, proteins destined for either the apical membrane (in epithelia) or the axonal membrane (in neurons) use similar targeting signals. Basolateral targeting is due to specific cytoplasmic sequence motifs, which may vary depending on the context in which they occur. Apical targeting may be due to raft anchoring/association, glycosylation, and in some cases also to cytoplasmic sequence motifs. To further understand the underlying processes, high-resolution confocal and evanescent wave microscopy was used to examine the trafficking in nonpolarized and polarized epithelial cells. In nonpolarized cells, post-Golgi transport intermediates (PGTs) carrying proteins destined for either the apical or the basolateral membranes are found throughout the cytoplasm and both types of transport vesicles can fuse with the basal plasma membrane. When the cells polarize, the PGTs carrying apical and basolateral membrane proteins become more restricted in their distribution, and now the two types of PGTs fuse only with their intended target membranes. The transfer of the vesicles form the trans-Golgi stack to the plasma membrane occurred along microtubule and actin cytoskeleton, which again serves as a targeting mechanism because microtubule disruption causes proteins that normally are destined for the apical membrane to fuse with the basolateral membrane.

Membrane protein targeting in neurons was addressed by G. Banker (University of Oregon). Different cargo (either somato-dendritic or axonal proteins) are packaged into different vesicles that then are transported to their respective destinations along microtubular cytoskeleton. These proteins are handled very differently, however. Proteins targeted to the axon can be found in both axons and dendrites, but they are retained only in the axons. This nonspecific initial delivery implies that there must be a highly selective fusion/retention mechanism. Proteins targeted to the dendrites cannot be found in the axons. The bidirectional movement (in the dendrites) of transport vesicles loaded with axonal proteins indicate that the vesicles interact with at least two different motors, so as to allow movement in both directions along the microtubules, which provides for yet another level of control.

Neurons raise some particularly interesting questions, as synaptic function depends on the proper apposition of presynaptic and postsynaptic membrane domains. Moreover, changes in synaptic strength depend on changes in protein trafficking to the relevant synapses. As shown by C. Rongo (Rutgers University), these questions can with advantage be examined in the nematode C. elegans, in which it is possible to follow the receptor localization and synapse organization in the live worm. Thus, it is possible to show that reorganization of the postsynaptic membrane is a CaMKInase II–dependent process involving PDZ-domain proteins, and use the wealth of genetic information that is available in C. elegans to define the interrelationships that exist between the pre- and postsynaptic domains. The organization of synaptic structures was also the topic of presentations by M. Sheng (Massachusetts Institute of Technology) and S. Burden (New York University School of Medicine). M. Sheng showed how the trafficking of the AMPA receptor is determined by subunit composition, with GluR2 subunits being expressed constitutively and the GluR1 subunits being inducible. Heterodimeric GluR2/GluR2 receptors behave like GluR1 receptors. The differences in expression are paralleled by differences in targeting, as GluR1 receptors initially are found to be distributed uniformly (i.e., also outside the synapses) over the postsynaptic membrane, for then to move to the synaptic regions, whereas GluR2 is expressed only in the synapses. But it remains unclear how the receptors “know” which synapse is being stimulated.

Lipid domains (lipid rafts) have long been implicated in protein trafficking, and M. Bagnat (Max-Planck-Institut für Molekulare Zellbiologie und Genetik, Dresden) summarized results on polarized protein traffic in the budding yeast Saccharomyces cerevisiae. The basic sorting mechanisms and signals are similar to those in epithelia, except that the raft domains are enriched in ergosterol (and sphingolipids) rather than cholesterol (as well as sphingolipids). Glycosphingolipidinositol (GPI)-anchored proteins, double-acylated proteins, and glycosylated proteins are targeted to raft domains, most other membrane proteins are targeted to nonraft membrane domains. The importance of the targeting to rafts was demonstrated in the case of the plasma H+–ATPase, which is mistracted to the vacuole by maneuvers that disrupt raft formation. Similarly, sorting-deficient ATPase mutants do not target to rafts—and go to the vacular membrane; but this missorting can be rescued by association with a peripheral membrane protein that causes ATPase clustering and targeting to raft domains. The stoichiometry and mechanism underlying this clustering-dependent raft association remain unknown. The yeast system is a particularly attractive system for investigating membrane protein targeting because cell polarization can be induced by pheromones, which trigger the mating response. Raft-associated proteins are targeted to the mating projection, and disruptions in either ergosterol or sphingolipid synthesis abolish this clustering, without altering the overall morphology of the pheromone-treated cells. Somewhat surprisingly, these mutants are able to mate, but preferentially with normal cells, which may suggest that normal mating
can proceed as long as just one of the partners is fusion competent.

A. Helenius (Eidgenössische Technische Hochschule, Zürich) described the reverse process, the retrieval of plasma membrane components to the endosomes. Two different pathways were described, using viral internalization as a model system. Many enveloped viruses, exemplified by the Semliki Forest Virus, enter cells by a clathrin-coated vesicle pathway, which is a canonical internalization path. Other viruses, exemplified by Simian virus 40 (SV40), enter cells by a clathrin-independent mechanism, which involves association with caveolae, tyrosine phosphorylation, actin reorganization, and eventually vesicle formation. These vesicles then appear to transfer their cargo to complex tubular membrane organelles, which have been termed caveosomes—preexisting caveolin-containing organelles distributed throughout the cytoplasm—which then move to the cargo to the ER. Caveosomes appear to be a new type of organelle, with a membrane lipid composition resembling that of plasma membrane rafts (being enriched in cholesterol and sphingolipids) and enriched in Src kinases; but, in contrast to other endocytic organelles, with a neutral intraorganellar pH. Though the normal function(s) of these organelles remain unknown, they might be involved in cholesterol recycling.

M.G. Caron (Duke University Medical Center) discussed the regulation of neurotransmitter transporters, Na+/Cl−-dependent cotransporters of dopamine, noradrenaline, serotonin, γ-NH₂-butyric acid, and glycine. These transporters have 12 putative α-helical transmembrane segments. They occur in two general classes: the vesicular transporters responsible for accumulating the neurotransmitter in the synaptic vesicles, which are relatively nonspecific, and the plasma membrane transporters responsible for the reuptake of neurotransmitters from the synaptic cleft, which are quite substrate specific. These transporters are oligomers (dimers), and oligomerization, which is mediated by PICK1—a member of the class 2 PDZ family of scaffolding proteins—is necessary for trafficking. Amphetamine, which binds to the monoamine transporters, inhibits transport activity by triggering transporter internalization via clathrin-coated pits. Transport activity is increased by phosphorylation and G-proteins, which then in turn activates transporter internalization—and perhaps also recycling. These transporters not only have a relatively complex transport mechanism, being Na+/Cl−-dependent cotransporters, they are also tightly organized and regulated transport and signaling entities. Regulation of activity by membrane recycling was also discussed by M. von Zastrow (University of California, San Francisco), who focused on β₂ adrenergic and δ opioid G-protein–coupled receptor (GPCR) regulation. Both GPCRs are recycled in response to agonist binding, but their subsequent fate is quite different, as the β₂ adrenergic GPCR is recycled, whereas the δ opioid GPCR is degraded. Their different fates can be traced to the last 10 amino acids in the β₂ adrenergic GPCR, which include a PDZ domain. The sorting in the endocytic recycling compartment involves differential, protein-dependent sorting into different membrane domains in the endosomes, and targeting to the lysosomes is ubiquitin dependent.

P. de Camilli (Yale University School of Medicine) summarized work on the role of phosphoinositides, and phosphoinositide turnover, in synaptic vesicle trafficking. The first hint that membrane phospholipids are involved in membrane trafficking was obtained 50 yr ago by Lowell and M. Hokin (1953. J. Biol. Chem. 203: 967–977), who showed that pancreatic secretion is associated with phosphorylation of membrane phospholipids. It later became apparent that only a minor fraction of the membrane phospholipids, the phosphoinositides, are involved in this turnover, but that it occurs in most secretory processes, including synaptic neurotransmitter release (and vesicle turnover) where the phosphoinositides serve as both structural and signaling molecules. The basic scheme is shown in Fig. 3.

During the maturation of synaptic vesicles, phosphatidylinositol (PI) is phosphorylated to PI(4)P by PI 4-kinase, an event that triggers vesicle translocation to the plasma membrane. At the plasma membrane, PI(4)P is further phosphorylated to PI(4,5)P₂, which somehow is important for fusion/exocytosis—presumably because it binds specifically to pleckstrin homology domains and thereby promotes protein–vesicle interactions. As the vesicle is retrieved, which involves the formation of a clathrin-coated pit, PI(4,5)P₂ serves to anchor the coat (as well as the dynamin that pinches the budding vesicle off from the plasma membrane) to the bilayer, which catalyzes the vesicle internalization. Other coat proteins,
amphiphysin and endophilin, which interact with dynamin, may contribute to the initial vesicle “budding” from the plasma membrane as these proteins in isolation can bind to liposomes and promote the formation of tubular structures. Dissolution of the clathrin coat, which is necessary for synaptic vesicle maturation, is initiated by synaptojanin 1, a polyphosphoinositide phosphatase that hydrolyzes PI(4,5)P₂ to PI(4)P and thereby facilitates dissolution of the clathrin coat. The importance of synaptojanin 1, and PI(4,5)P₂ hydrolysis, for synaptic vesicle turnover was demonstrated in synaptojanin knock-out animals, in which the PI(4,5)P₂ concentration and the density of coated pits are increased.

The picture that emerges is that synaptic vesicle exocytosis and subsequent endocytosis is controlled by an elaborate protein machinery, which is under tight control, mediated by changes in the phosphorylation state of PI in a manner that depends on chemically specific binding of the phosphoinositides to their target protein domains. Moreover, in contrast to the conventional view that lipid–protein interactions exhibit little specificity, in this case the membrane recycling and its regulation depends on specific protein-phosphoinositide interactions.

The role of phosphoinositides for membrane trafficking was examined also by D.W. Hilgemann (University of Texas Southwestern Medical Center), who used membrane capacitance changes that show that maneuvers which are expected to change the phosphorylation state of PI alter the direction of membrane trafficking. In whole-cell voltage clamping, infusion of PI(4,5)P₂ into the cytoplasmic compartment causes membrane retrieval, as evident by a step-wise capacitance decrease suggestive of the endocytosis of plasma membrane–derived vesicles. When the PI(4,5)P₂ concentration subsequently is reduced, the capacitance increases in a step-wise pattern, thus proving a tantalizing real-time demonstration of the important of PI(4,5)P₂ in plasma membrane retrieval.

The final presentation was by the Symposium’s keynote Speaker, H.F. Lodish (Massachusetts Institute of Technology), who described the assembly and activation of the erythropoietin receptor (EpoR), a member of the cytokine receptor family. Binding of erythropoietin (Epo) to the EpoR in bone marrow progenitor cells is the critical first step in the production of red blood cells, and Epo secretion is controlled by a feedback mechanism that senses the tissue Po₂, which depends on the total mass of circulating red blood cells. As is the case for growth factor receptors, EpoR activation may involve an initial ligand-induced receptor dimerization and subsequent phosphorylation of receptor tyrosine residues, but recent evidence suggests that even the un-ligated apoform of the receptor may be a dimer, and that Epo binding induces a conformational change (rotation of the subunits relative to each other) in the dimeric receptor. In any case, the tyrosine kinase is not encoded in the EpoR, but is rather a member of the Janus family of tyrosine kinases (JAK), specifically JAK2. There are a total of eight tyrosine residues in the EpoR’s cytoplasmic domain; the phosphotyrosines serve as docking sites for the recruitment of SH2 domain–containing proteins, including PI(3)P kinase and STAT5, leading to their phosphorylation and activation, where STAT5 activation is likely to be key for Epo’s mitogenic activation of erythropoietic progenitor cells—and consequent production of red blood cells.

Surprisingly, JAK2 is not “just” important for initiating the Epo/EpoR signal transduction cascade, JAK2 is also important for proper targeting of the EpoR to the plasma membrane. When EpoR is synthesized, it is retained in the ER and degraded because it tends to fold improperly and therefore gets targeted for destruction by the ER quality control system(s). Proper folding is achieved when JAK2 binds to the juxtamembraneous cytoplasmic domain, such that JAK2 availability will determine the fate of newly synthesized EpoR, which provides for a new mode of control—early, in membrane trafficking, and late, in receptor activation—but the relevant JAK domains are different as EpoR trafficking is determined by JAK2’s noncatalytic NH₂-terminal domain, whereas EpoR activation is determined by JAK2’s catalytic domain. As an additional control element, both the apoform and Epo-liganded EpoR is rapidly internalized and degraded, such that the rate of EpoR trafficking to the plasma membrane becomes an effective control of the number of plasma membrane receptors.

EpoR activation, but not membrane trafficking, depends on a short, presumably α-helical, domain juxtamembraneous cytoplasmic segment. When one or more Ala residues are inserted into this segment, activity is killed with one, two, or four insertions; but activity is maintained with three insertions, which suggests that the membrane-spanning and juxtamembraneous segments are quite rigid—and that the activity is controlled by a rotating the cytoplasmic segments (and the associated JAK2 kinases) relative to each other. This result in turn implies that each JAK2 kinase does not phosphorylate the EpoR monomer to which it is not bound.

Altogether, these results provide an illuminating example of the increasingly blurred lines of demarcation between membrane trafficking and receptor activation. Together with the other presentations, membrane traffic really is moving at brisk pace, which puts demands not only on technological but also conceptual developments—promises that we have an exciting time ahead of us.

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Editor
The Journal of General Physiology