Immunity, Vol. 11, 391–398, October, 1999, Copyright ©1999 by Cell Press

Intracellular Protein Traffic in Lymphocytes: "How Do I Get THERE from HERE?"

Review

Paul A. Roche*
Experimental Immunology Branch
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20892

Many of the problems facing immunologists today are exactly the same as the problems facing cell biologists today. Well, actually, they might not be exactly the same, since immunologists might like to know how their favorite proteins move from point A to point B in a lymphocyte, whereas many cell biologists would be happy to know how any protein moves from point A to point B in a lymphocyte. In the past 10 years there have been incredible advances in our understanding of the basic processes of protein transport in cells, and we (as immunologists) should use this information to our advantage to better understand specific immunological processes. In this review I will attempt to cover our current understanding of the basic principles and key players regulating protein transport, which is defined as the movement of proteins from their point of synthesis to their sites of action.

A Quick Trip Though the Cell

Proteins enter the secretory/biosynthetic pathway in a specialized organelle called the endoplasmic reticulum (ER), where they fold and acquire transport competence (Figure 1). Upon exiting the ER, proteins enter the stacks of the Golgi apparatus, which contain numerous enzymes that modify glycoproteins. After the Golgi apparatus, proteins enter the *trans*-Golgi network (TGN), where they are packaged into vesicles that are destined for (1) the plasma membrane (along what is called the constitutive secretory pathway), (2) endosomes (along the endosomal transport pathway), or (3) secretory granules (along the regulated secretory pathway).

Just as there is a distinct cellular machinery to transport proteins from the cell interior outward, there is also intracellular machinery to bring material from the outside of the cell inward (Figure 1). From the plasma membrane, material enters the cell using one of a variety of endocytic pathways. Internalized material often passes sequentially from early endosomes to late endosomes and, finally, to the highly proteolytic lysosome. In some cases, however, internalized cargo recycles from early endosomes back to the plasma membrane, as typified by the transferrin cycle. Interestingly, there is significant interplay between the secretory (i.e., biosynthetic) pathway and the endocytic pathway, as traffic routes exist that allow proteins traversing the secretory pathway to enter into endosomes, highlighting the complexity of these processes.

Where It All Begins: The Cytosol

Perhaps the most important compartment along the secretory pathway in all eukaryotic cells is the cytosol. Despite the fact that protein synthesis is often said to occur "in" the ER, the cytosol is in fact the initial site of synthesis of most cellular proteins. Protein translation is initiated on cytosolic ribosomes and in some cases the first protein sequence to emerge from the ribosome contains a short hydrophobic N-terminal signal sequence (Rapoport et al., 1996). For soluble proteins destined for secretion (such as cytokines) and most resident plasma membrane proteins (such as T cell receptor subunits), the nascent polypeptide chain is then recognized by a protein complex called the signal recognition particle (SRP; Figure 2, step 1). This interaction results in a lag in mRNA translation, during which time the nascent polypeptide/ribosome/SRP complex docks onto the ER membrane (step 2). Following ER docking, protein translation resumes, and the remainder of the polypeptide is cotranslationally inserted through an aqueous channel into the ER lumen termed the translocon (step 3). In most cases the signal sequence is cleaved off by a signal peptidase present in the ER lumen (step 4), although in some cases the signal sequence is not removed. Signal sequence cleavage, together with the presence or absence of additional hydrophobic transmembrane domain(s) in the polypeptide, determines whether or not a given protein will traffic through the cell as a soluble protein or as an integral membrane protein (Hegde and Lingappa, 1997). It should be emphasized that the ER-translocation process is not only utilized by proteins destined for secretion or localization in the plasma membrane but is also used by most, if not all, membrane proteins located within the ER itself, the Golgi apparatus, and endosomal compartments.

While most proteins access the secretory pathway through the aqueous channel in the ER membrane, certain viruses have adapted a system to use this aqueous channel to their advantage. For example, the US2 and US11 proteins of cytomegalovirus function by binding to MHC class I heavy chains in the ER membrane. This interaction ultimately results in the dislocation of membrane-anchored MHC class I molecules out of the ER through the aqueous channel of the translocon into the cytosol, where the heavy chains are rapidly degraded by the multicatalytic proteosome complex (Wiertz et al., 1996). The net effect of this is that cytomegalovirus inhibits surface expression of MHC class I molecules, thereby providing the virus with an opportunity to evade immune surveillance by exploiting the cell's own protein trafficking machinery.

Vesicle Transport: Back and Forth

Following translocation into the ER, most polypeptides are folded by the action of resident ER chaperones like calnexin and BiP, and many assemble into multisubunit protein complexes and are ready to move on through the cell. But how? Although it is still a matter of heated debate, many investigators believe that traffic through the secretory pathway is mediated by the packaging of membrane and proteins into transport vesicles (Rothman and Wieland, 1996). These vesicles are not preexisting entities but are derived from the membrane of a

*E-mail: paul.roche@nih.gov

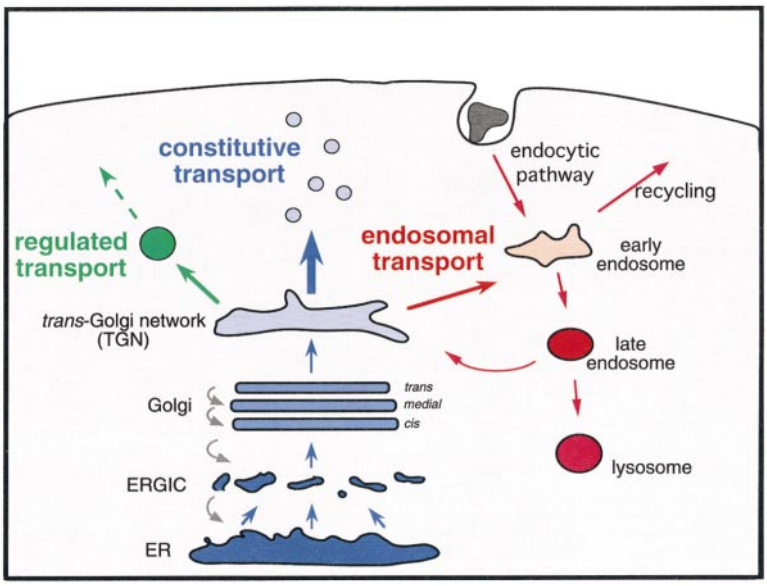

Figure 1. Pathways of Protein Traffic along the Secretory and Endocytic Pathways

Soluble proteins and membrane proteins are cotranslationally translocated into the ER. Following protein folding, they exit the ER in COPII-coated vesicles that fuse with the ER-Golgi intermediate compartment (ERGIC). The proteins then sequentially pass through the Golgi apparatus and arrive in the *trans*-Golgi network (TGN). In addition to forward (i.e., anterograde) transport (blue arrows), many proteins are retrieved back to the ER or early Golgi stacks by backward (i.e., retrograde) transport in COPI-coated vesicles (gray arrows). In the TGN, proteins are selectively packaged into (1) vesicles to be constitutively secreted (constitutive transport; blue arrows), (2) vesicles to be released upon cell stimulation (regulated transport; green arrows), or (3) vesicles destined for endosomes and/or lysosomes (endosomal transport; red arrows). From the extracellular space, molecules enter the cell by a variety of endocytosis/pinocytosis mechanisms (endocytic pathway). Following the fusion of endocytic vesicles with early endosomes, proteins may segregate to endosomes for recycling to the cell surface; alternatively, molecules may traffic along the endocytic pathway from early endosomes to late endosomes/prelysosomes and finally onto terminal lysosomes.

donor compartment. The process of vesicle generation is initiated by the addition of coat proteins, called coatomer (or COP), to the leaving face of a donor organelle, in this case the ER membrane (Figure 3, step 1). The addition of coat proteins lead to a deformation of the membrane (step 2), which ultimately reseals itself and pinches off, giving rise to a phospholipid bilayer–containing vesicle (step 3). Following vesicle dissociation from the donor membrane, the COP coat disassembles and the naked vesicle must find its target. In the case of anterograde (i.e., forward) transport, the ER-derived vesicle docks with the acceptor membrane of an organelle located between the ER and the *cis*-Golgi termed the ER/*cis*-Golgi intermediate compartment (ERGIC). Following the docking of the ER-derived vesicle with the ERGIC membrane (step 4), the membranes fuse and the cargo is out of the ER and well on its way to the Golgi apparatus (step 5).

From the ERGIC, proteins enter the *cis* face of the Golgi apparatus. For the past 15 years or so it has been believed that like ER-to-ERGIC transport, protein traffic through the distinct cisternae of the Golgi apparatus occurred by a transport vesicle–dependent mechanism (Rothman, 1994; Farquhar and Palade, 1998). In this static model of intra-Golgi traffic, the distinct stacks of the Golgi apparatus can be envisioned as fixed structures that receive and dispense cargo by small carrier vesicles. Very recently, however, compelling evidence in support of a more dynamic cisternal maturation model of intra-Golgi traffic has been obtained that suggests that anterograde traffic through the Golgi apparatus occurs by the sequential maturation of preformed Golgi cisternae (Bonfanti et al., 1998; Glick and Malhotra, 1998; Pelham, 1998). In this attractive model, a new *cis* cisternae is generated by the fusion of ER-derived vesicles, with a preexisting *cis*-cisternae becoming a *medial*-cisternae, a *medial*-cisternae becoming a *trans*-cisternae, and the *trans*-cisternae fragmenting into the vesicles

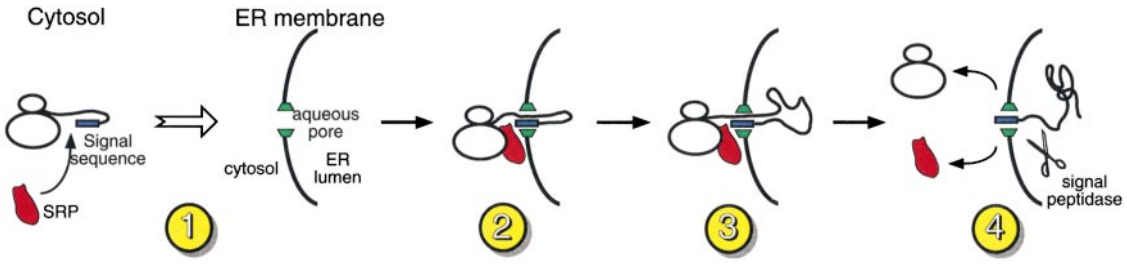

Figure 2. Protein Translocation into the ER

For soluble and membrane proteins destined to enter the secretory pathway, entry into the ER is initiated by the binding of cytosolic SRP to an exposed N-terminal hydrophobic signal sequence on the nascent polypeptide chain. The binding of SRP to the ribosome-bound polypeptide targets the complex to the ER-membrane (step 1), where the signal sequence is inserted into an aqueous translocation pore, or translocon (step 2). Protein synthesis on membrane-bound ribosomes continues (step 3), and once completed, the ribosome and SRP dissociate from the ER membrane (step 4). For soluble proteins and type I transmembrane proteins (which also posses a C-terminal transmembrane domain), the signal sequence is cleaved off by a lumenal signal peptidase, while failure to remove the signal sequence results in a transmembrane protein with a cytosolic N-terminal type II topology.

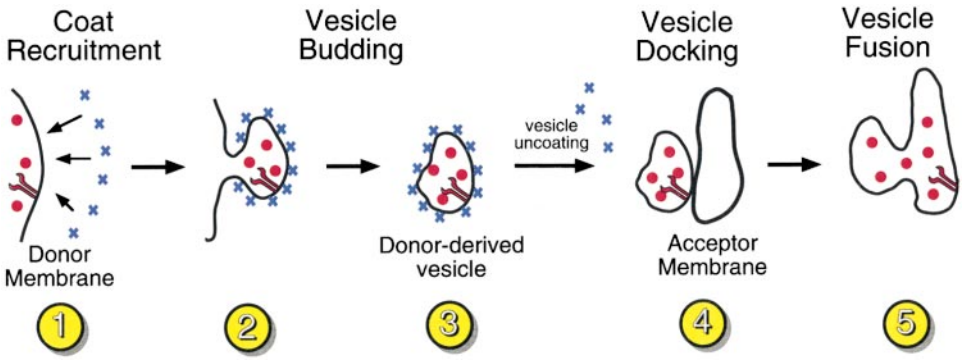

Figure 3. Mechanism of Vesicle Budding, Docking, and Fusion

The formation of transport vesicles is initiated by the recruitment of coat proteins such as COPII to a donor membrane (step 1). The addition of coat proteins deforms the membrane to generate a coated bud (step 2) that finally pinches off to give rise to a donor membrane–derived coated vesicle (step 3). Encapsulated inside or on the membrane of this vesicle is a sampling of the cargo proteins from the donor organelle. Following uncoating, the vesicle docks with an appropriate acceptor membrane (step 4) to which it ultimately fuses (step 5), thereby transferring soluble and transmembrane protein cargo from the donor membrane to the target (acceptor) membrane.

that give rise to the various transport pathways out of the Golgi. This model can explain both recent and ancient observations of intra-Golgi traffic and is currently being hotly investigated and even more hotly debated. Regardless of whether the precise mechanism of intra-Golgi transport occurs by vesicles docking and fusing with discreet Golgi membranes or by maturation of individual Golgi cisternae, proponents of either model generally agree that in a late Golgi compartment, proteins are once again sorted into transport vesicles that traffic to and eventually fuse with a variety of target membranes, thereby keeping things moving along the secretory pathway.

ER Retention and COPs

An important and often confusing reality of cell biology is that protein transport in the forward (anterograde) direction coexists with protein transport in the backward (retrograde) direction. There is perhaps no better example of this than in the description of the molecular events leading to the retention of various resident proteins in the ER (such as calnexin and BiP). Whereas it was originally thought that these proteins possessed retention signals that tethered them to the ER and excluded their packaging into anterograde-destined vesicles, it is now clear that these proteins do in fact leave the ER and enter the ERGIC (Pelham, 1998). However, unlike most proteins that proceed forward to the Golgi apparatus from the ERGIC, this class of proteins is recycled back to the ER from the ERGIC in retrograde-directed vesicles that possess a coat termed COPI, thereby leading to their concentration in the ER proper (see Figure 1). Although this mechanism of ER retention is generally conserved from yeast to man, there do appear to be exceptions that may be necessary for proper cell function. For example, in immature thymocytes the resident ER chaperone calnexin escapes this retrograde transport step and traffics to the plasma membrane (Wiest et al., 1995), demonstrating that the mechanism(s) of ER retention can be modified for particular cell types.

Retrograde traffic is not unique to the ERGIC–ER pathway. As mentioned above, the cisternal progression model of intra-Golgi transport proposes that stable Golgi compartments do not exist in cells, yet Golgi-specific enzymes, such as the *cis*-Golgi glycosidase mannosidase I, do in fact reside almost exclusively in distinct regions of the Golgi apparatus. To explain this apparent paradox, it has been proposed that cisternal maturation of the Golgi stacks in the *anterograde* direction is accompanied by vesicle-mediated *retrograde* traffic, thereby retrieving specific intra-Golgi residents to distinct locations. Indeed, just as COPII-coated vesicles traffic from the ER to the ERGIC, COPI-coated vesicles mediate retrograde traffic through the Golgi apparatus (Pelham, 1998). In addition, it has recently been shown that even resident late Golgi enzymes can recycle back to the ER, only to once again traffic in an anterograde manner toward the late Golgi (Cole et al., 1998). These examples highlight the dynamic regulation of protein traffic in the early secretory pathway.

SNARE Pairs

One of the most intriguing aspects of the study of protein traffic in cells is attempting to understand how any given vesicle in the cytosol knows where to go next. This is where the so-called SNARE and Rab proteins come into play. SNAREs are integral membrane proteins with the majority of their protein–protein interaction domains present in the cytosol, and it has been almost ten years since these proteins were identified as being central players in synaptic vesicle exocytosis (Rothman and Wieland, 1996). Since that time, it has become increasingly clear that SNAREs play significant roles in many different membrane docking and fusion events in diverse cell types (Scheller, 1995; Rothman and Wieland, 1996; Pfeffer, 1999). For example, the SNARE protein SNAP-23 is essential for compound exocytosis in mast cells, a process that involves multiple secretory granule fusion events followed by massive exocytosis (Guo et al., 1998). On the vesicle itself is a protein called a vesicle SNARE, or v-SNARE, while on the target membrane is a target SNARE, or t-SNARE (Figure 4). In its simplest form, the SNARE hypothesis proposes that a vesicle with a particular v-SNARE only interacts with a target

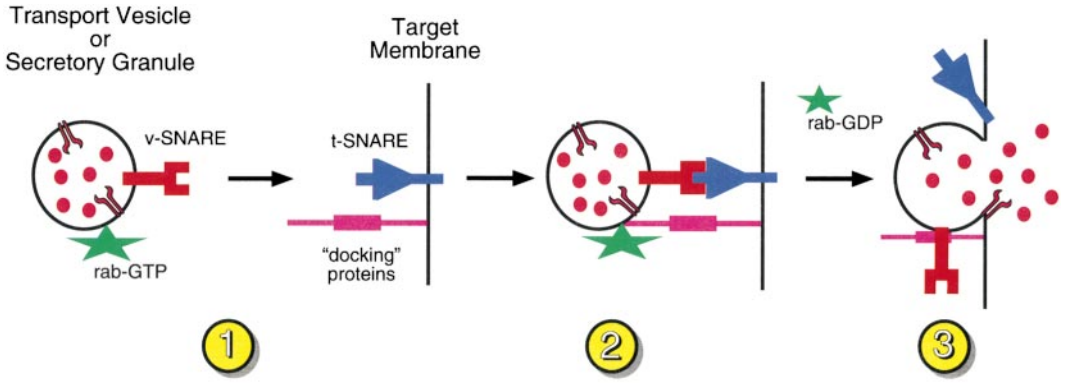

Figure 4. Regulation of Vesicle Docking and Fusion with Target Membranes

Transport vesicles or regulated secretory granules are believed to find appropriate target membranes by the interactions of vesicle-associated v-SNARE proteins with target membrane–associated t-SNARE proteins. Following translocation to an appropriate target membrane (step 1), the vesicle is tethered to the membrane by a poorly defined complex of Rab GTPases, various vesicle docking proteins, and perhaps SNARE proteins (step 2). After vesicle docking, the opposing membranes fuse with each other (step 3), a process that may be mediated directly by the SNARE complex itself. The specific pairing of unique v-SNAREs and t-SNAREs, together with the specific association of distinct Rab-GTPases with distinct membranes, likely regulates the specificity of vesicle docking and fusion reactions throughout the cell.

membrane with a particular t-SNARE, thereby providing the specificity for a particular transport reaction.

Despite the fact that dozens of v- and t-SNAREs have been identified and many of them reside on distinct intracellular membranes, in only a few cases has a particular v-SNARE/t-SNARE combination been shown to be essential for a given transport step. This may reflect the fact that there are more SNAREs to be identified, or, alternatively, that other proteins regulate the interaction of these SNAREs (Pfeffer, 1999). Indeed, the cytosolic Rab proteins, members of the Ras superfamily of small GTPases, are known regulators of SNARE assembly (Søgaard et al., 1994). Furthermore, it is important to draw a distinction between vesicle *docking* with a target membrane and vesicle *fusion* with that membrane, distinct processes that utilize distinct proteins for their function. In fact, it is likely that the SNAREs function primarily in the vesicle fusion reaction, and that other proteins such as Rabs or other Rab-interacting proteins recruit and/or serve as docking proteins that tether vesicles with target membranes (Schimmoller et al., 1998; Pfeffer, 1999). Since there are currently more than 30 cloned Rab proteins, many of which reside on distinct membrane compartments, it could take some time to untangle the mechanism by which a particular Rab/SNARE combination functions to regulate vesicle trafficking reactions.

The TGN: Traffic COP of the Cell

The TGN is a collection of cisternae, tubular extensions, and associated vesicles that is distinct from the Golgi apparatus. Like the ERGIC, the TGN is a major protein-sorting station in cells, and for immune cell biologists this is where things get particularly interesting. In the TGN, proteins are sorted into distinct vesicles and tubular structures destined for distinct compartments (see Figure 1). Since the majority of proteins in the secretory pathway are destined for the plasma membrane, these proteins are packaged into TGN-derived vesicles and tubules and follow the so-called *constitutive* secretory pathway to the cell surface. Examples of proteins that follow this pathway are MHC class I molecules, T cell receptors, integrins, and secreted cytokines. Another class of proteins are packaged into vesicles and tubules that specifically traffic to the endosome–lysosome system from the TGN following the *endosomal* transport pathway, with prime examples being lysosomal hydrolases, MHC class II molecules, and antigen-processing accessory molecules like HLA-DM. Still other molecules are packaged into secretory granules at the TGN that are only released from the cells following stimulation, thus giving rise to the *regulated* secretory pathway.

As mentioned above, most molecules are transported to the cell surface via the constitutive secretory pathway. Unlike the coats of ER- or Golgi-derived vesicles or vesicles destined for the endocytic pathway (see below), the actual coat composition of TGN-derived transport vesicles and tubules remains a mystery. From an immunological point of view, the process of constitutive secretion is vitally important but relatively unremarkable. However, traffic from the TGN to the endocytic pathway or into secretory granules is very relevant for immune system cells, and it is useful to consider each of these in turn.

TGN-to-Lysosome Traffic

Perhaps nowhere is the distinction between immunology and cell biology more blurred than in our examination of the mechanism of MHC class II–restricted antigen processing and presentation. From the discovery almost 20 years ago that antigens presented by class II molecules were proteolytically processed in lysosomes (Ziegler and Unanue, 1982) to recent work using fluorescent GFP-tagged class II molecules to study class II traffic in living cells (Wubbolts et al., 1996), the field of class II–restricted antigen processing and presentation has become a paradigm to study lysosome biogenesis and function.

MHC class II molecules traffic to prelysosomal antigen-processing compartments by the recognition of a

leucine-based sorting signal present in the class II–associated invariant chain cytosolic tail. This signal interacts with clathrin-associated adaptor molecules that link newly synthesized class II molecules to clathrin-coated vesicles on the TGN membrane (Kirchhausen et al., 1997). From the TGN these vesicles traffic to the endocytic pathway and fuse with endosomes essentially as described in Figure 3, thereby delivering their cargo from the biosynthetic pathway to the endocytic pathway. The TGN-to-endosome pathway is not used exclusively by class II molecules but is also used by lysosomal membrane proteins as well as the mannose 6–phosphate receptor (Traub and Kornfeld, 1997). This protein shuttles mannose 6–phosphate-tagged soluble enzymes like cathepsin D or granzymes to lysosomes or CTL granules. In a manner analogous to the retrograde transport pathways described above, the mannose 6–phosphate receptor recycles back to the TGN following delivery of its soluble cargo in the early/late endosomes, only to once again pick up ligand for delivery to the endocytic pathway all over again (see Figure 1).

As is the case with many proteins that traffic from the TGN to lysosome, it has been difficult to define the pathway used by MHC class II molecules to reach the endocytic pathway from the TGN without ambiguity. One possibility is that class II molecules traffic directly from the TGN to endosomes and then from endosomes to late endosomes/prelysosomes. This pathway has been championed by many and argues for a direct, intracellular transport pathway (Benaroch et al., 1995; Geuze, 1998). However, there are now numerous studies that reveal a pool of newly synthesized class II molecules at the plasma membrane that rapidly internalizes using the endocytic pathway, suggesting that class II molecules follow the default constitutive secretory pathway to the plasma membrane and then traffic to the endocytic pathway by endocytosis (Roche et al., 1993; Saudrais et al., 1998). Whichever pathway predominates, it is clear that class II molecules traffic to lysosomes from the TGN and that this trafficking pathway is essential for class II molecules to efficiently bind lysosome-derived antigenic peptides.

Within the realm of antigen processing and presentation, the localization and function of class II molecules in dendritic cells has recently received considerable attention (Mellman et al., 1998). Immature dendritic cells efficiently process antigen into peptide fragments but do not present them, as class II molecules reside primarily in intracellular lysosomal antigen-processing compartments in immature dendritic cells. During dendritic cell maturation, in which dendritic cells acquire the ability to present their processed antigen to T cells, class II molecules traffic from intracellular compartments to the plasma membrane, thereby allowing efficient antigen recognition by T cells. Although the mechanism leading to differential class II localization in dendritic cells has been attributed to differences in invariant chain processing between these cell types (Pierre and Mellman, 1998), it is possible that additional protein trafficking machinery is regulated during dendritic cell maturation, thereby facilitating protein trafficking in dendritic cells.

TGN-to-Granule Traffic

In many cell types, the lysosome is primarily a terminal degradative organelle that is critically important for cell survival. However, hemopoietic cells are unique in that they possess what have been termed secretory lysosomes (Page et al., 1998). In fact, microscopy studies have shown quite convincingly that classical lysosomes are not abundant in most hemopoietic cells. In general, these cells possess lysosomes that have been modified for that particular cells function. For example, CTL lysosomes contain granzymes, perforin, and other cytolytic agents, mast cell lysosomes contain histamine and other regulators of inflammation, and platelet lysosomes contain PDGF and other clotting and inflammatory factors.

The biogenesis of secretory granules is poorly understood, but it is likely that true secretory granules arise from immature secretory granules that are generated at the TGN (Tooze, 1998). Specific cargo is sequestered into regions of the TGN that will give rise to immature secretory granules, although how this occurs and how this cargo is diverted from the constitutive secretory pathway is unknown. It was originally proposed that the selective aggregation and condensation of specific proteins resulted in their incorporation into secretory granules. Recent studies have challenged this simple model, as there are examples of secretory granule cargo molecules that do not significantly aggregate in the TGN, giving rise to a variety of models to describe the mechanism by which secretory granule cargo specifically accumulates in what will eventually become a secretory granule (Tooze, 1998).

One Way Out: Exocytosis

Degranulation of the secretory lysosomes is triggered by the stimulation of cell surface receptors of hemopoietic cells, a process that results in regulated influx of calcium into the cell. In the same way that synaptic vesicle exocytosis is regulated by the interactions of cytosolic calcium with the SNARE machinery, there is recent data demonstrating an essential role of SNARE proteins in regulated exocytosis from the secretory lysosomes of hemopoietic cells. As mentioned above, perturbation of the intracellular SNARE SNAP-23 significantly inhibits mast cell exocytosis (Guo et al., 1998), and antisense depletion of the SNARE-interacting protein synaptotagmin II in mast cells also inhibits degranulation (Baram et al., 1999). Interestingly, overexpression of synaptotagmin II does not alter granule exocytosis but leads to secretion of conventional lysosomes from mast cells, suggesting that, as in neurons, organelle secretion in hemopoietic cells is regulated in part by vesicle–membrane interactions of SNARE proteins.

In addition to SNARE proteins, there has been considerable interest in the role of GTP and GTPases in regulated exocytosis. The introduction of nonhydrolyzeable analogs of GTP into permeabilized cells stimulates regulated exocytosis (Avery et al., 1999), although the molecular target of this drug in the exocytosis event has not been identified. The small GTPase Rab3 has also been implicated in regulated exocytosis from rat mast cells, but once again its precise role in stimulated secretion has not been proven. Since Rab GTPases are known regulators of SNARE assembly, it is possible that Rab3

stimulates exocytosis by potentiating SNARE assembly, although this too awaits further examination. Finally, heterotrimeric GTP-binding proteins have been implicated in regulated exocytosis, as activators or inhibitors of these proteins profoundly affected exocytosis in permeabilized mast cells, although once again the mechanism of action of these pharmacological agents on the exocytosis machinery awaits to be elucidated.

From the Outside In: Endocytosis

The endocytic pathway is distinct from the secretory pathway, with different proteins playing different roles, but the basic concepts of protein movement within the endosome system are remarkably similar to those of the secretory system. Foreign material, be they solvents, solutes, or particle matter, enter the cell by one of a number of endocytic processes (Mellman, 1996). Perhaps the best understood of these is receptor-mediated endocytosis, in which foreign material is brought into the cell bound to its plasma membrane receptor. In most cases this involves the interaction of the cytoplasmic tail of the receptor with an adaptor complex, which, appropriately enough, couples the receptor with the clathrin coat (Kirchhausen et al., 1997).

In a process that is analogous to that involving vesicle-mediated protein traffic out of the ER (Figure 3), endocytic vesicles uncoat, dock with, and eventually fuse with early endosomes. In receptor-mediated endocytosis, the coat protein is composed of clathrin, which links to the internalizing receptor cytoplasmic tail via clathrin-associated adaptor complexes. In some cases, the internalized ligand is sorted out of this compartment only to recycle back to the plasma membrane, as is the case for the transferrin receptor and, interestingly, for MHC class II $\alpha\beta$ dimers that internalize from the plasma membrane in the absence of the invariant chain (Pinet et al., 1995). In most other cases, however, the internalized ligand traffics sequentially from the early endosome to a late endosome/prelysosome and finally on to terminal lysosomes. For MHC class II molecules that traffic in association with the invariant chain, it is likely that a true lysosomal sorting signal is absent from the complex, as class II–peptide complexes tend to accumulate in prelysosomes rather than conventional lysosomes prior to cell surface arrival (Geuze, 1998).

Endocytosis and Lymphocyte Function

The internalization and degradation of protein antigens in so-called antigen-processing compartments illustrates well the fate of endocytosed proteins and the complexity within the endocytic pathway of antigen-presenting cells (Watts, 1997). Some foreign proteins are internalized by receptor-dependent pathways, such as antigen ligated to specific surface Ig molecules on B lymphocytes. Still other antigens enter the endocytic pathway by pinocytosis or macropinocytosis, while in some antigen-presenting cells phagocytosis is the pathway de jour. In each case, however, it is believed that the internalized cargo is delivered (once again by vesicle-mediated transport) to prelysosomal antigen-processing compartments, only to be degraded into antigenic peptides that ultimately associate with MHC class II molecules. While the pathways for transport out of the antigen-processing and peptide-loading compartment remain to be identified, it is intriguing to speculate that perhaps as in other hemopoietic cells, these compartments in antigen-presenting cells are secretory lysosomes, and we have only to discover the regulators of such a regulated exocytosis pathway.

The Flames of Hell: The Lysosome

Lysosomes are acidic, highly degradative organelles that contain numerous proteases, nucleases, glycosidases, lipases, and phosphatases. Therefore, it is not surprising that most internalized material is rapidly destroyed upon entering this harsh, proteolytic dispose-all of the cell. In addition to degrading internalized foreign material for the antigen-processing function of antigen-presenting cells, lysosomes may also degrade internalized cell surface proteins, and this function may be important for various cellular activities. For example, surface CD4 molecules can be internalized and degraded in lysosomes, a process that may be important for the conversion of immature $CD4^+CD8^+$ thymocytes into $CD4^-CD8^+$ T cells. Although the mechanism by which CD4 molecules are internalized in thymocytes has not been demonstrated, it is known that antigen stimulation of T cells leads to phosphorylation of the cytosolic domain of CD4, a process that results in the dissociation of $p56^{lck}$ from CD4 and ultimately leads to the internalization and lysosomal degradation of CD4 (Pelchen-Matthews et al., 1993). Like CD4, the expression of CTLA-4 on T cells is also regulated by internalization. However, in this case tyrosine phosphorylation regulates the interaction of the cytosolic domain of CTLA-4 with the clathrin adaptor protein AP2 at the plasma membrane, thereby coupling the cytosolic domain of CTLA-4 to the endocytosis and lysosomal degradation pathway (Shiratori et al., 1997). These are but two examples that highlight the importance of cell signaling and lysosomal proteolysis in normal lymphocyte biology.

Lysosomal proteolysis is also exploited by certain pathogens in an attempt to evade immune surveillance. The HIV gene product Nef couples surface CD4 to endosomal coat proteins, thereby promoting lysosomal degradation of internalized CD4 and resulting in the depletion of surface CD4 molecules from helper T cells (Piguet et al., 1999). Nef is also responsible for downregulation of MHC class I molecules from the surface of HIV-infected cells (Le Gall et al., 1998). Unlike the Nef-induced missorting of CD4, Nef is believed to couple surface class I molecules to the clathrin-dependent endocytosis machinery, resulting in the delivery of surface class I molecules to lysosomes for destruction. Despite likely differences in the molecular mechanism by which Nef is working in each of the cited examples, the net effect of Nef is to reduce the surface expression of important immune cell surface receptors, highlighting the virus' capacity to evade immune surveillance by exploiting the host cells capacity for endocytosis and lysosomal proteolysis.

Protein Traffic to Weird Places

Although the majority of this review focused on the trafficking of proteins to distinct sites using the conventional secretory pathway, there are many immunologically relevant proteins that target to membranes by unconventional pathways. Examples of such proteins

include the Src family of protein tyrosine kinases that bind to and phosphorylate the cytoplasmic domain of numerous substrates, most notable the CD3 components of the T cell receptor. Src kinases like p56^{lck} are synthesized on soluble, cytosolic ribosomes and do not enter the ER; these proteins associate with membranes by virtue of lipid modifications of their polypeptide chains (Resh, 1994). In most cases these kinases are fatty acylated while still on the ribosome, thereby enhancing the association of the resulting polypeptide chain with membranes. Subsequent posttranslational fatty acid modifications (such as palmitoylation) often result in the recruitment of these kinases to cholesterol-rich plasma membrane domains, termed rafts, where they exert their biological effects. Indeed, it has recently been shown that costimulation of T lymphocytes results in redistribution of these raft domains to sites of T cell receptor engagement (Viola et al., 1999), and it is possible that the reorganization of the plasma membrane rafts accompanies reversible palmitoylation of receptor tyrosine kinases like p56^{lck}, thereby providing a mechanism to concentrate molecules involved in T lymphocyte activation.

In addition to protein trafficking from the cytosol to the plasma membrane, there is a particularly strange trafficking pathway that is relevant to immune system cells. The antiapoptotic protein Bcl-2 is an integral membrane protein that resides on the mitochondrial *outer* membrane, yet Bcl-2 does not insert into ER membranes like most membrane proteins and may not utilize vesicles for its trafficking to mitochondria. How then is Bcl-2 targeted to mitochondria? Bcl-2 possesses a transmembrane domain that is present at the extreme carboxy terminus of the polypeptide chain, and, like other C-terminal tail–anchored transmembrane proteins, Bcl-2 inserts into membranes posttranslationally, i.e., it inserts into membranes only after the synthesis of the polypeptide chain on cytosolic ribosomes is completed (Kutay et al., 1993). Although the signal for insertion of Bcl-2 into mitochondrial membranes has not been determined, manipulation of this event might lead to efficient ways of regulating cell death in immune system cells.

For many immunologists, the most important protein trafficking step in the cell is that used by receptor signaling molecules that move from the plasma membrane (or the cytosol) to the nucleus. Unfortunately, this is a very poorly understood and incredibly complicated problem. For example, it is well known that NF-κB only enters the nucleus following phosphorylation-induced degradation of its inhibitor IκB (Baldwin, 1996). Despite the fact that it took years to elucidate this elegant regulatory mechanism, we have yet to understand precisely how NF-κB or any other transcription factor enters the nucleus through the nuclear pore complex.

Epilogue

While I have tried to cover many of the basics of protein trafficking in the secretory and endocytic pathways in this brief review, there is also much that could not be addressed. For example, membrane movement in cells involves molecular motors such as dynein and kinesin, which direct traffic along microtubule tracts (Hirokawa, 1998). Similarly, although the specificity of many transport reactions has been attributed to protein–protein interactions involving SNAREs, Rabs, adaptors, and coat proteins, there is a growing appreciation and understanding of the role of protein–lipid and lipid–lipid interactions in all of these processes (Roth, 1999). Although we can certainly become overwhelmed with the enormous variety and complexity of these processes, elucidation of their molecular bases should lead to useful and simple generalizations.

References

Avery, J., Jahn, R., and Edwardson, J.M. (1999). Reconstitution of regulated exocytosis in cell-free systems: a critical appraisal. Annu. Rev. Physiol. *61*, 777–807.

Baldwin, A.S., Jr. (1996). The NF-κB and IκB proteins: new discoveries and insights. Annu. Rev. Immunol. *14*, 649–683.

Baram, D., Adachi, R., Medalia, O., Tuvim, M., Dickey, B.F., Mekori, Y.A., and Sagi-Eisenberg, R. (1999). Synaptotagmin II negatively regulates Ca^{2+}-triggered exocytosis of lysosomes in mast cells. J. Exp. Med. *189*, 1649–1658.

Benaroch, P., Yilla, M., Raposo, G., Ito, K., Miwa, K., Geuze, H.J., and Ploegh, H.L. (1995). How MHC class II molecules reach the endocytic pathway. EMBO J. *14*, 37–49.

Bonfanti, L., Mironov, A.A., Jr., Martinez-Menarguez, J.A., Martella, O., Fusella, A., Baldassarre, M., Buccione, R., Geuze, H.J., Mironov, A.A., and Luini, A. (1998). Procollagen traverses the Golgi stack without leaving the lumen of cisternae: evidence for cisternal maturation. Cell *95*, 993–1003.

Cole, N.B., Ellenberg, J., Song, J., DiEuliis, D., and Lippincott-Schwartz, J. (1998). Retrograde transport of Golgi-localized proteins to the ER. J. Cell Biol. *140*, 1–15.

Farquhar, M.G., and Palade, G.E. (1998). The Golgi apparatus: 100 years of progress and controversy. Trends. Cell Biol. *8*, 2–10.

Geuze, H.J. (1998). The role of endosomes and lysosomes in MHC class II functioning. Immunol. Today. *19*, 282–287.

Glick, B.S., and Malhotra, V. (1998). The curious status of the Golgi apparatus. Cell *95*, 883–889.

Guo, Z., Turner, C., and Castle, D. (1998). Relocation of the t-SNARE SNAP-23 from lamellipodia-like cell surface projections regulates compound exocytosis in mast cells. Cell *94*, 537–548.

Hegde, R.S., and Lingappa, V.R. (1997). Membrane protein biogenesis: regulated complexity at the endoplasmic reticulum. Cell *91*, 575–582.

Hirokawa, N. (1998). Kinesin and dynein superfamily proteins and the mechanism of organelle transport. Science *279*, 519–526.

Kirchhausen, T., Bonifacino, J.S., and Riezman, H. (1997). Linking cargo to vesicle formation: receptor tail interactions with coat proteins. Curr. Opin. Cell Biol. *9*, 488–495.

Kutay, U., Hartmann, E., and Rapoport, T. (1993). A class of membrane proteins with a C-terminal anchor. Trends Cell Biol. *3*, 72–75.

Le Gall, S., Erdtmann, L., Benichou, S., Berlioz-Torrent, C., Liu, L., Benarous, R., Heard, J.M., and Schwartz, O. (1998). Nef interacts with the μ subunit of clathrin adaptor complexes and reveals a cryptic sorting signal in MHC I molecules. Immunity. *8*, 483–495.

Mellman, I. (1996). Endocytosis and molecular sorting. Annu. Rev. Cell Dev. Biol. *12*, 575–625.

Mellman, I., Turley, S.J., and Steinman, R.M. (1998). Antigen processing for amateurs and professionals. Trends Cell Biol. *8*, 231–237.

Page, L.J., Darmon, A.J., Uellner, R., and Griffiths, G.M. (1998). L is for lytic granules: lysosomes that kill. Biochim. Biophys. Acta *1401*, 146–156.

Pelchen-Matthews, A., Parsons, I.J., and Marsh, M. (1993). Phorbol ester-induced downregulation of CD4 is a multistep process involving dissociation from p56lck, increased association with clathrin-coated pits, and altered endosomal sorting. J. Exp. Med. *178*, 1209–1222.

Pelham, H.R. (1998). Getting through the Golgi complex. Trends Cell Biol. *8*, 45–49.

Pfeffer, S.R. (1999). Transport-vesicle targeting: tethers before SNAREs. Nat. Cell Biol. *1*, E17–E22.

Pierre, P., and Mellman, I. (1998). Developmental regulation of invariant chain proteolysis controls MHC class II trafficking in mouse dendritic cells. Cell *93*, 1135–1145.

Piguet, V., Gu, F., Foti, M., Demaurex, N., Gruenberg, J., Carpentier, J.L., and Trono, D. (1999). Nef-induced CD4 degradation: a diacidic-based motif in Nef functions as a lysosomal targeting signal through the binding of β-COP in endosomes. Cell *97*, 63–73.

Pinet, V., Vergelli, M., Martin, R., Bakke, O., and Long, E.O. (1995). Antigen presentation mediated by recycling of surface HLA-DR molecules. Nature *375*, 603–606.

Rapoport, T.A., Jungnickel, B., and Kutay, U. (1996). Protein transport across the eukaryotic endoplasmic reticulum and bacterial inner membranes. Annu. Rev. Biochem. *65*, 271–303.

Resh, M.D. (1994). Myristylation and palmitylation of Src family members: the fats of the matter. Cell *76*, 411–413.

Roche, P.A., Teletski, C.L., Stang, E., Bakke, O., and Long, E.O. (1993). Cell surface HLA-DR-invariant chain complexes are targeted to endosomes by rapid internalization. Proc. Natl. Acad. Sci. USA *90*, 8581–8585.

Roth, M.G. (1999). Lipid regulators of membrane traffic through the Golgi complex. Trends Cell Biol. *9*, 174–179.

Rothman, J.E. (1994). Mechanisms of intracellular protein transport. Nature *372*, 55–63.

Rothman, J.E., and Wieland, F.T. (1996). Protein sorting by transport vesicles. Science *272*, 227–234.

Saudrais, C., Spehner, D., de la Salle, H., Bohbot, A., Cazenave, J.P., Goud, B., Hanau, D., and Salamero, J. (1998). Intracellular pathway for the generation of functional MHC class II peptide complexes in immature human dendritic cells. J. Immunol. *160*, 2597–2607.

Scheller, R.H. (1995). Membrane trafficking in the presynaptic nerve terminal. Neuron *14*, 893–897.

Schimmoller, F., Simon, I., and Pfeffer, S.R. (1998). Rab GTPases, directors of vesicle docking. J. Biol. Chem. *273*, 22161–22164.

Shiratori, T., Miyatake, S., Ohno, H., Nakaseko, C., Isono, K., Bonifacino, J.S., and Saito, T. (1997). Tyrosine phosphorylation controls internalization of CTLA-4 by regulating its interaction with clathrin-associated adaptor complex AP-2. Immunity. *6*, 583–589.

Søgaard, M., Tani, K., Ye, R.R., Geromanos, S., Tempst, P., Kirchhausen, T., Rothman, J.E., and Söllner, T. (1994). A rab protein is required for the assembly of SNARE complexes in the docking of transport vesicles. Cell *78*, 937–948.

Tooze, S.A. (1998). Biogenesis of secretory granules in the trans-Golgi network of neuroendocrine and endocrine cells. Biochim. Biophys. Acta *1404*, 231–244.

Traub, L.M., and Kornfeld, S. (1997). The trans-Golgi network: a late secretory sorting station. Curr. Opin. Cell Biol. *9*, 527–533.

Viola, A., Schroeder, S., Sakakibara, Y., and Lanzavecchia, A. (1999). T lymphocyte costimulation mediated by reorganization of membrane microdomains. Science *283*, 680–682.

Watts, C. (1997). Capture and processing of exogenous antigens for presentation on MHC molecules. Annu. Rev. Immunol. *15*, 821–850.

Wiertz, E.J., Tortorella, D., Bogyo, M., Yu, J., Mothes, W., Jones, T.R., Rapoport, T.A., and Ploegh, H.L. (1996). Sec61-mediated transfer of a membrane protein from the endoplasmic reticulum to the proteasome for destruction. Nature *384*, 432–438.

Wiest, D.L., Burgess, W.H., McKean, D., Kearse, K.P., and Singer, A. (1995). The molecular chaperone calnexin is expressed on the surface of immature thymocytes in association with clonotype-independent CD3 complexes. EMBO J. *14*, 3425–3433.

Wubbolts, R., Fernandez-Borja, M., Oomen, L., Verwoerd, D., Janssen, H., Calafat, J., Tulp, A., Dusseljee, S., and Neefjes, J. (1996). Direct vesicular transport of MHC class II molecules from lysosomal structures to the cell surface. J. Cell Biol. *135*, 611–622.

Ziegler, H.K., and Unanue, E.R. (1982). Decrease in macrophage antigen catabolism caused by ammonia and chloroquine is associated with inhibition of antigen presentation to T cells. Proc. Natl. Acad. Sci. USA *79*, 175–178.