2. VESICULAR TRAFFIC, SECRETION, AND ENDOCYTOSIS

In the previous chapter we explored how proteins are targeted to and translocated across the membranes of different intracellular organelles. In this chapter we turn our attention to the mechanisms that allow soluble and membrane proteins synthesized on the rough ER to move to their final destinations via the secretory pathway. A single unifying principle governs all protein trafficking in the secretory pathway: transport of membrane and soluble proteins from one membrane-bounded compartment to another is mediated by transport vesicles that collect “cargo” proteins in buds arising from the membrane of one compartment and then deliver these cargo proteins to the next compartment by fusing with the membrane of that compartment. Once newly synthesized proteins are incorporated into the ER lumen or membrane, they can be packaged into anterograde (forward-moving) transport vesicles. These vesicles fuse with each other to form a flattened membrane-bounded compartment known as the cis-Golgi cisterna. Certain proteins, mainly ER-localized proteins, are retrieved from the cis-Golgi to the ER via a different set of retrograde (backward-moving) transport vesicles. A new cis-Golgi cisterna with its cargo of proteins physically moves from the cis position (nearest the ER) to the trans position (farthest from the ER), successively becoming first a medial-Golgi cisterna and then a trans-Golgi cisterna. This process, known as cisternal progression, does not involve the budding off and fusion of anterograde transport vesicles. During cisternal progression, enzymes and other Golgi-resident proteins are constantly being retrieved from later to earlier Golgi cisternae by retrograde transport vesicles, thereby remaining localized to the cis-, medial-, or trans-Golgi cisternae. Proteins in the secretory pathway that are destined for compartments other than the ER or Golgi eventually reach a complex network of membranes and vesicles termed the trans-Golgi network (TGN). From this major branch point in the secretory pathway, a protein can be loaded into one of at least three different kinds of vesicles. After budding from the trans-Golgi network, (1) the first type of vesicle immediately moves to and fuses with the plasma membrane, releasing its contents by exocytosis. In all cell types, at least some proteins are loaded into such vesicles and secreted continuously in this manner. Examples of proteins released by such constitutive (or continuous) secretion include collagen by fibroblasts, serum proteins by hepatocytes, and antibodies by activated B lymphocytes. (2) The second type of vesicle to bud from the trans-Golgi network, known as secretory vesicles, are stored inside the cell until a signal for exocytosis causes release of their contents at the plasma membrane. Among the proteins released by such regulated secretion are peptide hormones (e.g., insulin, glucagon, ACTH) from various endocrine cells, precursors of digestive enzymes from pancreatic acinar cells, milk proteins from the mammary gland, and neurotransmitters from neurons. (3) The third type of vesicle that buds from the trans-Golgi network is directed to the lysosome, an organelle responsible for the intracellular degradation of macromolecules, and to lysosome-like storage organelles in certain cells. Secretory proteins destined for lysosomes first are transported by vesicles from the trans-Golgi network to a compartment usually called the late endosome; proteins then are transferred to the lysosome by a mechanism that is not well understood but may involve direct fusion of the endosome with the lysosomal membrane. Soluble proteins delivered by this pathway include lysosomal digestive enzymes (e.g., proteases, glycosidases, and phosphatases) and membrane proteins (e.g., V-class proton pump) that pump H⁺ from the cytosol into the acidic lumen of the endosome and lysosome. As we will see, some of the specific protein-processing and –sorting events that take place within these organelles depend on their low luminal pH. The endosome also functions in the endocytic pathway in which vesicles bud from the plasma membrane bringing membrane proteins and their bound ligands into the cell. After being internalized by endocytosis, some proteins are
transported to lysosomes, while others are recycled back to the cell surface. Endocytosis is a way for cells to take up nutrients that are in macromolecular form—for example, cholesterol in the form of lipoprotein particles and iron complexed with the serum protein transferrin. Endocytosis also can function as a regulatory mechanism to decrease signaling activity by withdrawing receptors for a particular signaling molecule from the cell surface.

**SLIDE 2 Molecular Mechanisms of Vesicular Traffic**

Small membrane-bounded vesicles that transport proteins from one organelle to another are common elements in the secretory and endocytic pathways. These vesicles bud from the membrane of a particular “parent” (donor) organelle and fuse with the membrane of a particular “target” (destination) organelle. Although each step in the secretory and endocytic pathways employs a different type of vesicle, each of the different vesicular transport steps is simply a variation on a common theme. In this section we explore that common theme, the basic mechanisms underlying vesicle budding and fusion. The budding of vesicles from their parent membrane is driven by the polymerization of soluble protein complexes onto the membrane to form a proteinaceous vesicle coat. Interactions between the cytosolic portions of integral membrane proteins and the vesicle coat gather the appropriate cargo proteins into the forming vesicle. Thus the coat not only adds curvature to the membrane to form a vesicle but also acts as the filter to determine which proteins are admitted into the vesicle. The integral membrane proteins in a budding vesicle include v-SNAREs, which are crucial to eventual fusion of the vesicle with the correct target membrane. Shortly after formation of a vesicle is completed, the coat is shed exposing its v-SNARE proteins. The specific joining of v-SNAREs in the vesicle membrane with cognate t-SNAREs in the target membrane brings the membranes into close apposition, allowing the two bilayers to fuse.

Three types of coated vesicles have been characterized, each with a different type of protein coat. Each type of vesicle, named for its primary coat proteins, transports cargo proteins from particular parent organelles to particular destination organelles: (1) COPII vesicles transport proteins from the rough ER to the Golgi. (2) COPI vesicles mainly transport proteins in the retrograde direction between Golgi cisternae and from the cis-Golgi back to the rough ER. (3) Clathrin vesicles transport proteins from the plasma membrane (cell surface) and the trans-Golgi network to late endosomes. Researchers have not yet identified the coat proteins surrounding the vesicles that move proteins from the trans-Golgi to the plasma membrane during either constitutive or regulated secretion.

In order for transport vesicles to move specific proteins from one compartment to the next, vesicle buds must be able to discriminate among potential membrane and soluble cargo proteins, accepting only those cargo proteins that should advance to the next compartment and excluding those that should remain as residents in the donor compartment. In addition to sculpting the curvature of a donor membrane, the vesicle coat also functions in selecting specific proteins as cargo. The primary mechanism by which the vesicle coat selects cargo molecules is by directly binding to specific sequences, or sorting signals, in the cytosolic portion of membrane cargo proteins. Soluble proteins within the lumen of parent organelles can in turn be selected by binding to the luminal domains of certain membrane cargo proteins, which act as receptors for luminal cargo proteins.

Shortly after a vesicle buds off from the donor membrane, the vesicle coat disassembles to uncover a vesicle-specific membrane protein, a v-SNARE. Likewise, each type of target membrane in a cell contains t-SNARE membrane proteins. After docking of a vesicle on its target (destination)
membrane, the interaction of cognate SNAREs brings the two membranes close enough together that they can fuse. One of the best-understood examples of SNARE-mediated fusion occurs during exocytosis of secreted proteins. In this case, the v-SNARE, known as VAMP (vesicle-associated membrane protein), is incorporated into secretory vesicles as they bud from the trans-Golgi network. The t-SNAREs are syntaxin, an integral membrane protein in the plasma membrane, and SNAP-25, which is attached to the plasma membrane by a hydrophobic lipid anchor.

**SLIDE 3** Receptor-Mediated Endocytosis and the Sorting of Internalized Proteins
In previous sections we have explored the main pathways whereby secretory and membrane proteins synthesized on the rough ER are delivered to the cell surface or other destinations. Cells also can internalize materials from their surroundings and sort these to particular destinations. A few cell types (e.g., macrophages) can take up whole bacteria and other large particles by phagocytosis, a nonselective actin-mediated process in which extensions of the plasma membrane envelop the ingested material, forming large vesicles called phagosomes. In contrast, all eukaryotic cells continually engage in endocytosis, a process in which a small region of the plasma membrane invaginates to form a membrane-limited vesicle about 0.05–0.1 μm in diameter. In one form of endocytosis, called pinocytosis, small droplets of extracellular fluid and any material dissolved in it are nonspecifically taken up. Our focus in this section, however, is on receptor-mediated endocytosis in which a specific receptor on the cell surface binds tightly to an extracellular macromolecular ligand that it recognizes; the plasma-membrane region containing the receptor-ligand complex then buds inward and pinches off, becoming a transport vesicle. Among the common macromolecules that vertebrate cells internalize by receptor-mediated endocytosis are cholesterol-containing particles called low-density lipoprotein (LDL); the iron-binding protein transferrin; many protein hormones (e.g., insulin); and certain glycoproteins.