In all cellular life forms, membranes establish the physical barrier that prevents random mixing of molecules. Mechanisms must exist, however, for the transport of select molecules across membranes, and in the case of eukaryotes, for the transport of select molecules from one membrane-bound organelle to another. These types of transport reactions are executed by cellular proteins that either span membranes to create selective pores or bind to the cytosolic surfaces of membranes to create transport vesicles.

Cellular machineries dedicated to establishing membrane transport mechanisms evolved early in life and have maintained a relatively high degree of conservation. As with any biological system, however, the business of membrane transport is not a perfect one, so mechanisms must also exist to eliminate defective components, be they proteins or whole organelles. Moreover, these systems are vulnerable to pathogens that, themselves, have evolved mechanisms to exploit or inhibit transport activities for dubious purposes.

The theme of this issue of Current Opinion in Cell Biology is the movement of macromolecules across and between membranes, the mechanisms that ensure the fidelity of these systems, and their pathogenic abuse.

**Threading through the eye of a needle**

Membranes prevent the uncontrolled flux of molecules between cellular compartments as well as between the cytoplasm and the extracellular space. At the same time, polypeptides need to be transported across these membranes in order to supply organelles with newly synthesized proteins. Moreover, peptides need to be removed from compartments in the course of quality control and signaling processes.

Mitochondria import more than 99% of their proteins from the cytoplasm from which they are separated by two membranes. Only a small subset of mitochondrial-encoded, inner membrane proteins is synthesized on membrane-associated ribosomes in the mitochondrial matrix. Recent years have led to the discovery of new components of the protein transport machineries, new signals in precursors as well as unexpected new import pathways. Becker and colleagues review here the recent insights into the mechanisms by which the mitochondrial translocase complexes in the inner and outer membranes mediate the insertion of membrane proteins and how they cooperate with each other during this process.

Similar to mitochondria, chloroplasts have to import most proteins from the cytosol. However, chloroplasts display a more complex set of membranes consisting of outer and inner envelope and the thylakoid membrane system. Kessler and Schnell focus in their review on the regulation of chloroplast
protein import and how this allows to adapt this organelle to the cellular developmental and physiological changes.

The Sec translocase is evolutionarily conserved from bacteria (SecYEG) to higher eukaryotes (Sec61 complex). This protein complex mediates protein export across the bacterial membrane and represents the entry gate into the endoplasmic reticulum, respectively. In their review article Mandon and colleagues highlight recent advances on the function of the Sec translocase. On the basis of structural information on the SecYEG complex together with biochemical studies, exciting new mechanistic insights into the transport processes by which this complex mediates protein translocation across and into membranes have been obtained.

Besides polypeptides a large variety of short peptides are transported across cellular membranes. Abele and Tampé take us through the mechanisms by which ABC transport machineries mediate the intracellular transport of peptides. They emphasize recent advances on the role of ABC transporters in the presentation of antigens by MHC I molecules and in the removal of degradation products from cellular compartments.

**The good into the pot, the bad into the crop**

Intracellular protein quality control mechanisms are required to protect cells from misfolded nonfunctional proteins. In some cases chaperones prevent the misfolding of newly synthesized polypeptide chains and assist in the process of protein folding in order to prevent misfolding. In cases where protein misfolding has occurred the nonfunctional protein has to be degraded to protect the cell and to recycle amino acids. In the case of the endoplasmic reticulum, through which all secreted proteins have to pass, malformed proteins are subjected to ER-associated degradation (ERAD). Brodsky and Wojcikiewicz describe here how proteins of the ER are recognized and subjected to cytoplasmic degradation by the proteasome. The fast development of this research field and the discovery of an increasing number of ERAD substrates has concomitantly led to the identification of specific accessory factors that may prevent or promote substrate degradation. These specificity factors are the focus of this review.

Similar to the quality control mechanisms that need to ensure functionality of cellular proteins, eukaryotic cells also need to protect themselves from damaged or unnecessary organelles. In fact, during metabolic adaptation such as nutrient starvation or upon damage of cellular organelles a process termed autophagy mediates the removal of organelles. Several mechanistically distinct autophagy subtypes exist that eventually lead to the uptake of organelles or parts of organelles into the vacuole/lysosome. Farré and coworkers discuss the current view on how peroxisomes, mitochondria, ER, and parts of the nucleus are degraded and provide a comprehensive overview on the components and mechanisms underlying these processes.

**Content exchange by transport vesicles**

Contents are exchanged between organelles largely (though not exclusively) by the process of vesicular transport, whereby a membrane-enclosed vesicle buds from one organelle and fuses with another. In principle, this process seems a simple one, yet it features many layers of regulation, with both protein and lipid components involved. Structural studies of the protein coats that shape vesicles have contributed much toward our understanding of vesicular transport, but the molecular basis defining the site of coat protein assembly remains somewhat elusive. Moreover, comparatively less is known about the mechanisms ensuring that vesicles, once detached from their membrane of origin, attach to the membrane of their correct destination. Spang reviews all of these issues in the context of the vesicular shuttle routes linking the endoplasmic reticulum and the Golgi; Guo and colleagues focus specifically on the mechanism that tethers Golgi-derived secretory vesicles with their target, the plasma membrane, while Merz and colleagues focus on membrane tethering in the endolysosomal system.

The ability of most cellular organelles to execute their characteristic functions depends on their export and import capabilities. This reliance means that few vesicular transport routes can be completely disabled without lethality. Dell’Angelica describes an exception to this dependence, as vesicular transport to lysosome-related organelles involves a conserved mechanism that is, nevertheless, nonessential, but disruption of this pathway in humans manifests itself in abnormalities ranging from albinism to prolonged bleeding.

Traditionally, the paradigm of vesicular transport has been studied in the context of the secretory and endocytic pathways. McBride and colleagues review the recent discovery of transport vesicle formation from mitochondria and delivery of these vesicles to peroxisomes. This unexpected finding has revealed that the relationship between mitochondria and peroxisomes is broader than anticipated, and the mechanism driving this process likely has origins tracing back before mitochondria diverged from bacteria.

Most vesicular transport pathways feature membrane-enclosed vesicles that travel through the cytoplasm to reach their destination. Transport vesicles that bud away from the cytoplasm also exist and must rely on mechanisms for their creation that are fundamentally distinct from the coat protein complexes involved in cytoplasmic transport vesicle production. Hanson and colleagues describe recent advances in understanding how one class of these unconventional transport vesicles might be
formed at endosomal multivesicular bodies, giving rise to lumenal vesicles that are destined for delivery to the lysosome. Simons and Raposo consider a related class of transport vesicles known as exosomes, which travel in the extracellular milieu between cells. Factors that control the biogenesis of exosomes are reviewed as well as their targets, which are largely dictated by the types of cells in which they are formed.

Breaching the membrane barrier
Pathogens have evolved varied mechanisms by which they move into and out of host cells. Many times, these strategies involve components produced by the pathogen that take advantage of normal cellular activities. Some of the best-known exemplars of this behavior are viruses. Gruenberg reviews viral mechanisms of entry that specifically exploit the dynamics of endosomes and describes how their discoveries have revealed properties of endosomes not previously appreciated. Bacteria are also experts at circumventing obstacles, and one of the most startling means they employ is the formation of aqueous pores in the lipid bilayer. This topic is covered by van der Goot and colleagues, who also describe how the same effect is achieved by certain eukaryotic cells and the consequences of this type of injury to the plasma membrane.

Nuclear envelope and endoplasmic reticulum
The endoplasmic reticulum forms a continuous network within eukaryotic cells that consists of structurally distinct regions, which can be discriminated microscopically. Accordingly, three regions can be defined based on their morphology: firstly, the nuclear envelope, a double membrane layer that is continuous with the endoplasmic reticulum; secondly, a network of peripheral endoplasmic reticulum tubules; and thirdly, peripheral endoplasmic reticulum sheets. English and coworkers address in their review the current ideas on how these regions are shaped and maintained in a cell. What are the factors that shape the ER and how is this shape altered during mitosis are central questions of the field that are highlighted here by the authors and also the question as to how the ER connects to other cellular membranes.

The endoplasmic reticulum forms the nuclear envelope and faces the nucleoplasm with its inner nuclear membrane and the cytoplasm with its outer nuclear membrane. Nuclear pore complexes are the gates spanning this double membrane to allow the transport of small molecules, proteins, and RNA between the cytosol and the nucleoplasm. Nuclear pore complexes are large symmetric channel-forming protein complexes with up to 70 MDa size. Fernandez-Martinez and Rout describe here the structure of this complicated assembly and discuss how nuclear pore complexes mediate the transport of molecules across the nuclear envelope. Moreover, they address the current view of the processes by which this complex protein structure is assembled, disassembled, and turned over.