sequence is clipped off during secretion. Such proteins exist in a pre-form and mature form. The pre-form is what is made from the *m*-RNA, but the actual active form is the mature form. The pre-form is the signal sequence plus the mature form.

In procaryotes secretion of proteins occurs through the cytoplasmic membrane. In *E. coli* and most gram-negative bacteria the outer membrane blocks release of the secreted protein into the extracellular compartment. In gram-positive cells secreted proteins readily pass the cell wall into the extracellular compartment. Whether a protein product is retained in a cell or released has a major impact on bioprocess design.

In eucaryotic cells proteins are released by two pathways. Both involve *exocytosis,* where *transport vesicles* fuse with the plasma membrane and release their contents. Transport vesicles mediate the transport of proteins and other chemicals from the endoplasmic reticulum (ER) to the Golgi apparatus and from the Golgi apparatus to other membrane-enclosed compartments. Such vesicles bud from a membrane and enclose an aqueous solution with specific proteins, lipids, or other compounds. In the secretory pathway vesicles, carrying proteins bud from the ER, enter the *cis* face of the Golgi apparatus, exit the Golgi *trans* face, and then fuse with the plasma membrane. Only proteins with a signal sequence are processed in the ER to enter the secretory pathway.

Two pathways exist. One is the *constitutive exocytosis pathway,* which operates at all times and delivers lipids and proteins to the plasma membrane. The second is the *regulated exocytosis pathway,* which typically is in specialized secretory cells. These cells secrete proteins or other chemicals only in response to specific chemical signals.

Other modifications to proteins can take place, particularly in higher eucaryotic cells. These modifications involve the addition of nonamino acid components (for example, sugars and lipids) and *phosphorylation*. *Glycosylation* refers to the addition of sugars. These modifications can be quite complex and are important considerations in the choice of host organisms for the production of proteins. A bioprocess engineer must be aware that many proteins are subject to extensive processing after the initial polypeptide chain is made.

A particularly important aspect of posttranslational processing is *N-linked glycosylation*. The glycosylation pattern can serve to target the protein to a particular compartment or to control its degradation and removal from the organism. For therapeutic proteins injected into the human body these issues are critical ones. A protein product may be ineffective if the N-linked glycosylation pattern is not humanlike, as the protein may not reach the target tissue or may be cleared (i.e., removed) from the body before it exerts the desired action. Further, undesirable immunogenic responses can occur if a protein has a nonhumanlike pattern. Thus, the glycoform of a protein product is a key issue in bioprocesses to make therapeutic proteins (see Chapter 14).

The process of N-linked glycosylation occurs *only* in eucaryotic cells and involves both the ER and Golgi. Thus, the use of procaryotic cells, such as *E. coli*, to serve as hosts for expression of human therapeutic proteins is limited to those proteins where N-linked glycosylation is not present or unimportant. However, not all eucaryotic cells produce proteins with humanlike, N-linked glycosylation. For example, yeasts, lower fungi, and insect cells often produce partially processed products. Even mammalian cells (including human cells) will show altered patterns of glycosylation when cultured in bioreactors, and these patterns can shift upon scale-up in bioreactor size.

The process of N-linked glycosylation is depicted in Fig. 4.8. The pattern shown is "typical," and many variants are possible. The natural proteins in the human body usually