During N-linked glycosylation of proteins, oligosaccharide chains are assembled on the carrier molecule dolichyl pyrophosphate in the following order: 2 molecules of N-acetylglucosamine, 9 molecules of mannose, and 3 molecules of glucose. These 14-residue oligosaccharide cores are then transferred to asparagine residues on nascent polypeptide chains in the endoplasmic reticulum. As proteins progress through the Golgi apparatus, the oligosaccharide cores are modified by trimming and extension to generate a diverse array of glycosylated proteins.Together, Alg13p and Alg14p comprise a glycosyltransferasethat adds the second N-acetylglucosaminemoiety to the growing lipid-linked oligosaccharideon the cytosolic side of the endoplasmic reticulum.Alg13p, the catalytic subunit, lacks a recognizable transmembrane domain; its localization to the ER membrane requires interaction with the integral ER membrane protein Alg14p. Consistent with this model, overexpression of ALG13 or attenuation of ALG14 causes Alg13p to be partitioned into the cytoplasm. Cells in which ALG13 or ALG14 expression has been repressed exhibit slow growth and defective protein glycosylation and accumulate LLO's with one GlcNAc residue. Co-expression of the human homologs of ALG13 and ALG14 can complement deletion of either gene, but neither neither individual human gene can complement deletion of its yeast homolog, probably because the yeast and human proteins fail to interact with each other. ALG14 and ALG13 are homologous to separate proteins in Streptococcus pneumoniaeand to the N- and C-termini of E. coli MurG.