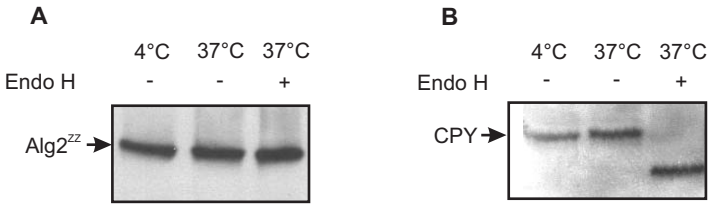
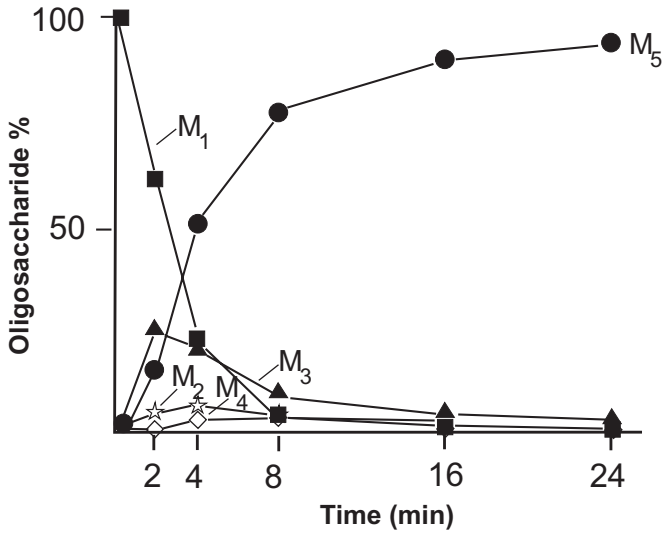
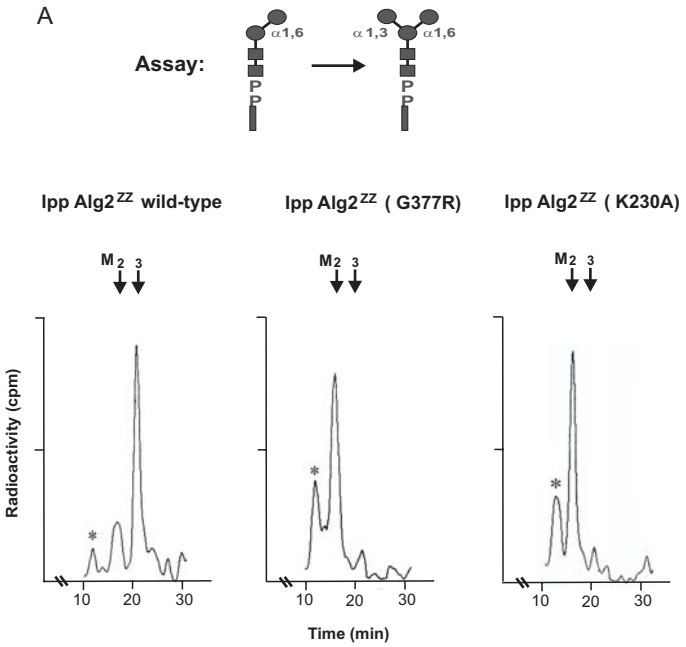
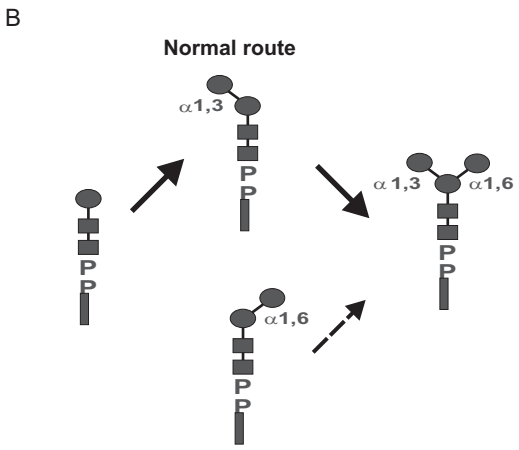


Supplemental Fig. 1



Supplemental Fig. 2





Supplemental Fig. 3

SUPPLEMENTAL FIGURES

SUPPLEMENTAL FIGURE 1. **Alg2 has three potential *N*-glycosylation sites, but is not *N*-glycosylated.** *A*, membranes were isolated from cells expressing Alg2 C-terminally tagged with Protein A (ZZ). Membranes were incubated without (-) (lanes 1 and 2), or with (+) endoH for 3 h (lane 3) at the temperatures indicated. Alg2 was then analyzed by Western blot. *B*, as a control, to monitor glycan release, the soluble fraction was also incubated under the same conditions, and carboxypeptidase Y (CPY) was analyzed (lanes 4-6) with anti-CPY antiserum.

SUPPLEMENTAL FIGURE 2. **Time dependence of lipid-linked oligosaccharide formation.** Solubilized enzyme was incubated for the times indicated using assay 1, as described in Experimental Procedures. Glycolipid fraction was extracted and analyzed by thin layer chromatography on silica gel60-coated plates (Merck), developed in chloroform/methanol/water 65:25:4 (v/v/v). Radioactivity was monitored and quantified by phosphorimaging.

SUPPLEMENTAL FIGURE 3. **Elongation of Man(1,6)Man-GlcNAc$_2$-PP-Dol is defective in *alg2-1* (G377R) and *alg2*(K230A).** *A*, Man(1,6)Man-GlcNAc$_2$-PP-Dol, obtained by limited digestion of [^{3}H]Man$_5$GlcNAc$_2$-PP-Dol with jack bean mannosidase, according to (21), was used as acceptor and incubated with immunoprecipitated Alg2^{ZZ} as enzyme source under the conditions of assay I. Whereas wild-type Alg2 was able to elongate to Man$_3$GlcNAc$_2$-PP-Dol (left image), Alg2 with a G377R (middle image) and K230A (right image) mutation was inactive. The *asterisks* indicate Man$_1$GlcNAc$_2$-PP-Dol impurity of the acceptor substrate. *B*, pathway of Man$_3$GlcNAc$_2$-PP-Dol formation. The normal route employed is from α1,3- to α1,6-linked mannose. *In vitro*, Alg2 seems to have a somewhat relaxed specificity.