File S5

Sugar nucleotide transport

This Supporting File contains additional information related to **Biosynthesis of Wall Components Along the Secretory Pathway, *Sugar nucleotide transport***. The subheadings used in the main text are retained, and new subheadings are underlined. Literature cited in this File but not In the main text is listed at the end of the File.

GDP-Man transport:

The GDP-Man transporter, Vrg4/Vig4. This protein forms homodimers (Abe *et al.* 1999; Gao and Dean, 2000), shows a wide distribution in the Golgi, and contains a GALNK motif involved in GDP-Man binding (Gao *et al.* 2001).

Gda1 and Ynd1. Evidence these proteins have partially overlapping functions is as follows. i) Deletion of either *GDA1* or *YND1* impacts mannosylation of N- and O-glycans, ii) high-level expression of *YND1* corrects some of *gda1Δ*'s glycosylation defects, and iii) *gda1Δ ynd1Δ* double mutants have a synthetic phenotype and show growth and cell wall defects (Gao *et al.* 1999). However, *gda1Δ ynd1Δ* double mutants are viable and capable of some mannosylation of N- and O-linked glycans, indicating that GDP-Man can enter the Golgi in their absence, and suggesting there may be a mechanism for GDP exit independent of GDP hydrolysis (D'Alessio *et al.* 2005).

GMP generated upon Man-P transfer to glycoproteins could also be a source of antiporter, but it is not a significant one because because the glycans made *gda1Δ* or *gda1Δ ynd1Δ* strains are not affected by disruption of *MNN4* or *MNN6* (Jigami and Odani, 1999; D'Alessio *et al.* 2005).

Other sugar nucleotide transport activities:

Transport activities for UDP-Glc, UDP-GlcNAc, and UDP-Gal also occur in *S. cerevisiae* (Roy *et al.* 1998; 2000 Castro *et al.* 1999), and there are eight further candidate transporters (Dean *et al.* 1997; Esther *et al.* 2008), a couple of which have been associated with these transport activities. Some of the transporters may have specificity for more than one sugar nucleotide. In the case of UDP-Glc, transport activity was present in the ER (Castro *et al.* 1999), but the responsible protein for that activity has yet to identified, although broad specificity Yea4 and Hut1 (see below) may transport UDP-Glc (Esther *et al.* 2008). One possible need for UDP-Glc transport into the ER might be for a glucosylation reaction at an early stage of β1,6-glucan assembly (**Section VI**). The Hut1 protein is a candidate for the UDP-Gal transporter (Kainuma *et al.* 2001), but whether that is Hut1's primary role *in vivo* is unclear because galactose has not been detected on *S. cerevisiae* glycans. Yea4 was characterized as an ER-localized UDP-GlcNAc transporter and its deletion impacts chitin synthesis (Roy *et al.* 2000; **Section V**). Of the other

transporter homologs, Hvg1 resembles Vrg4 most closely, but *hvgΔ* cells have neither a mannosylation nor a GDP-Man transport defect (Dean *et al.* 1997). The roles of the other proteins in sugar nucleotide transport, if any, is unknown. One or more transporters may supply the Golgi GlcNAc-T Gnt1 with its substrate (**Section IV.1.c.ii**).

Literature Cited

D'Alessio, C., Caramelo, J. J., Parodi, A. J., 2005 Absence of nucleoside diphosphatase activities in the yeast secretory pathway does not abolish nucleotide sugar-dependent protein glycosylation. *J. Biol. Chem.* **280:** 40417-40427.

Gao, X. D., Dean, N., 2000 Distinct protein domains of the yeast Golgi GDP-mannose transporter mediate oligomer assembly and export from the endoplasmic reticulum.
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Gao, X. D., Nishikawa, A., Dean, N., 2001 Identification of a conserved motif in the yeast Golgi GDP-mannose transporter required for binding to nucleotide sugar. *J. Biol. Chem.* **276:** 4424-4432.