Precursors and Carrier Lipids

This Supporting File contains additional information related to Precursors and Carrier Lipids. 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.

Sugar nucleotides

Regulation of glucosamine supply and chitin levels. Glucosamine supply is highly regulated and impacts chitin levels, which increase in response to mating pheromones and cell wall stress. Expression of GFA1 and AGM1 is upregulated upon treatment of MATa cells with α-factor (Watzele and Tanner, 1989; Hoffman et al. 1994), and is accompanied by an increase in chitin deposition (Schekman and Brawley, 1979; Orlean et al. 1985). The cell wall stress-induced increase in chitin synthesis (Popolo et al. 1997; Dallies et al. 1998; Kapteyn et al. 1999; see Wall Composition and Architecture) is also accompanied by elevated GFA1 expression (Terashima et al. 2000; Lagorce et al. 2002; Bulik et al. 2003). Elevation of glucosamine levels by other means also elicits increased chitin synthesis, for chitin levels are correlated with levels of expression of GFA1 itself (Lagorce et al. 2002; Bulik et al. 2003), and exogenous glucosamine also leads to increased chitin synthesis (Bulik et al. 2003). However, Bulik et al. (2003) found that chitin formation was not proportional to UDP-GlcNAc concentration. These observations led to the conclusion that chitin synthesis is proportional to Gfa1 activity but that additional factors, for example a glucosamine metabolite or Gfa1 itself, must modulate chitin levels (Bulik et al. 2003). It is also formally possible that additional chitin is in a soluble or intracellular form and not detected in cell wall analyses.

Dolichol and dolichol phosphate sugars

Dolichol phosphate synthesis: Rer2 and Srt1. Biosynthesis of dolichol starts with the extension of trans farnesyl-PP by successive addition of cis-isoprene units by the homologous cis-prenyltransferases Rer2 and Srt1 (Sato et al. 1999; Schenk et al. 2001b). Rer2 is the dominant activity and makes dolichols with 10-14 isoprene units, whereas dolichols made by Srt1 in cells lacking Rer2 contain 19-22 isoprenes, like mammals. rer2Δ strains have severe defects in growth and in N- and O-glycosylation, and SRT1 is a high-copy suppressor of rer2 mutants (Sato et al. 1999). The rer2Δ srt1Δ double null is inviable (Sato et al. 1999). Rer2 and Srt1 both behave as peripheral membrane proteins (Sato et al. 2001; Schenk et al. 2001b), but Rer2 is localized to the ER membrane, whereas Srt1 is detected in “lipid particles” (Sato et al. 2001).
**Dfg10.** Dfg10 has a steroid 5α reductase domain, and is responsible for much of the activity that reduces the α-isoprene unit of polyprenol activity. Both dfg10-100 transposon insertion mutants and dfg10Δ strains underglycosylate carboxypeptidase Y to the same extent, and dolichol levels are decreased by 70% in dfg10-100 cells, with a corresponding increase in unsaturated polyprenol (Cantagrel *et al.* 2010). The biosynthetic origin of the residual dolichol is not known.

**Membrane organization of Sec59 dolichol kinase.** Sec59 is a multispansion membrane protein whose CTP-binding site is oriented towards the cytoplasm (Shridas and Waechter, 2006).

**Dolichol chain length specificity of yeast glycosyltransferases and flippases.** The enzymes that act after Rer2 and Srt1 can use shorter chain dolichols. Thus, the growth and glycosylation defects of rer2Δ cells can be complemented by expression of the *E. coli* cis-isoprenyltransferase, which generates C55 isoprenoids, or of the *Giardia* homologue, which makes C55-60 (Rush *et al.* 2010; Grabinska *et al.* 2010). The native glycosyltransferases and flippases must therefore also be able to use shorter chain dolichols as substrates.

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**Dol-P-Man and Dol-P-Glc synthesis:**

**Relationship between Dpm1 and Alg5.** Alg5 and Dpm1 are most similar in their N-terminal halves, which contain their GT-A superfamily domain, but diverge in their C-terminal halves. Both are likely to catalyze their reactions at the cytoplasmic face of the ER membrane.

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**Literature Cited**

