File S8
β1,6-Glucan

This Supporting File contains additional information and discussion related to β1,6-Glucan. 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.

Proteins involved in β1,6-glucan assembly

ER proteins:

_Fungus-specific ER chaperones required for β1,6-glucan synthesis:_

Evidence for the chaperone function of Rot1, Big1, and Keg1 in β1,6-glucan synthesis, Rot1, Big1, and Keg1, which do not resemble known carbohydrate-active enzymes, seem unlikely to catalyze formation of β1,6-glucan (Lesage and Bussey, 2006). Rather, they seem to function as ER chaperones with varying degrees of importance for the stability of proteins involved in β1,6-glucan synthesis, and in some cases, they may cooperate. Observations supporting this notion, and indicating a relationship to Kre5, are as follows. Analyses of levels of β1,6-glucan synthesis-related proteins in a rot1-Ts mutant indicate that Kre6 has the strongest dependence on Rot1 for stability, although Kre5 and Big1 show appreciable dependence as well (Takeuchi et al. 2008). Keg1, a protein essential for growth in osmotically supported medium, physically interacts with Kre6 in the ER membrane, and a keg1-Ts mutant is suppressed at high copy by ROT1, though not BIG1; however, a physical interaction between Keg1 and Rot1 could not be detected (Nakamata et al. 2007). Because the big1Δ rot1Δ double mutant has the same growth rate as each single mutant, it was suggested that Rot1 and Big1 impact β1,6-glucan synthesis in the same way, and possibly function in the same compartment or even in a complex (Machi et al. 2004). However, although rot1, big1, and kre5 mutations individually all lower β1,6-glucan levels to the same extent, the kre5 big1 double mutant, but apparently not a kre5 rot1 strain (Lesage and Bussey, 2006), shows a reduced growth rate and lowered β1,6-glucan content compared with each single mutant, suggesting the function of Rot1 is partly distinct from that of Kre5 (Azuma et al. 2002; Lesage and Bussey, 2006). Indeed, the non-conditional rot1-1 mutant shows a synthetic growth and N-glycosylation defect in combination with ost3Δ (though not ost6Δ), as well as a partial defect in O-mannosylation of the chitinase Cts1, indicating a wider role for Rot1 in glycosylation (Pasikowska et al. 2012).

More widely distributed secretory pathway proteins:

Kre6 and Skn1:
Localization and transport of Kre6. Recent studies indicate that much of Kre6 is ER-localized, where it interacts with Keg1, but Kre6 is also detectable in secretory vesicles and at the plasma membrane at sites of polarized growth (Nakamata et al. 2007; Kurita et al. 2011). In addition to Kre6’s lumenal domain, the protein’s cytoplasmic tail is important for Kre6’s function in β1,6-glucan assembly and its transport to the plasma membrane (Li et al. 2002; Kurita et al. 2011). A truncated form of Kre6 lacking its 230 N-terminal amino acids failed to be localized to the plasma membrane, and did not correct the β1,6-glucan synthetic defect of kre6Δ, although it appeared stable (Kurita et al. 2011). It was concluded that transport of Kre6 to the plasma membrane is necessary for the protein to fulfill its role in β1,6-glucan synthesis (Kurita et al. 2002). Localization of Skn1 has not been explored in detail.

Skn1 and plant defensin resistance. skn1Δ, but not kre6Δ strains, are defective in M(IP)2C synthesis and resistant to a plant defensin that interacts with this sphingolipid to exert its antifungal activity (Thevissen et al. 2005). Defensin-susceptibility is unconnected with cellular β1,6-glucan content because other β1,6-glucan synthesis mutants are defensin-sensitive (Thevissen et al. 2005).

Plasma membrane protein Kre1:

Kre1 as receptor for K1 killer toxin. Membrane anchored Kre1 has an additional role as receptor for K1 killer toxin. Spheroplasts of kre1Δ cells are resistant to this toxin, but expression of the C-terminal 63 amino acids of Kre1 was sufficient to make spheroplasts, but not intact cells, toxin sensitive again, leading to the proposal that Kre1’s GPI-modified C-terminus serves as the membrane receptor for K1 toxin after initial toxin binding to β1,6-glucan (Breinig et al. 2002).

Literature Cited
