This Supporting File contains additional information and discussion related to Biosynthesis of Wall Components at the Plasma Membrane, β1,3-glucan. The subheadings used in the main text are retained, and new subheadings are underlined.

**Fks family of β1,3-glucan synthases:**

Identification of Fks1, Fks2, and Fks3. Fks1 (Cwh53/Etg1/Gsc1/Pbr1) was identified in screens for hypersensitivity to the calcineurin inhibitors FKS06 and cyclosporin A and to CFW, for resistance to echinocandin and papulocandin, and following purification of β1,3-glucan synthase activity (reviewed by Orlean, 1997 and Lesage and Bussey, 2006). Cross-hybridization with FKS1 and copurification with Fks1 led to identification of Fks2/Gsc2, which is 88% identical to Fks1 (Inoue et al. 1995; Mazur et al. 1995). The S. cerevisiae proteome also contains Fks3, which is 55% identical to Fks1 and Fks2 (Dijkgraaf et al. 2002). The Fks proteins are assigned to GT Family 48, and a strong case can be made for them being processive β1,3-glucan synthases themselves, although roles as glucan exporters cannot yet be excluded (Mazur et al. 1995; Dijkgraaf et al. 2002; Lesage and Bussey, 2006).

**Functional domains of Fks1.** Fks1 is predicted to have an N-terminal cytoplasmic domain of some 300 amino acids that is followed by six transmembrane helices, a second cytoplasmic domain of about 600 amino acids, then 10 transmembrane helices (Inoue et al. 1995; Mazur et al. 1995; Qadota et al. 1996; Dijkgraaf et al. 2002; Okada et al. 2010). Three functional domains have been distinguished (Okada et al. 2010). Amino acids important for β1,3 glucan synthesis in vivo are located in the first cytoplasmic domain. Mutations here have little impact on in vitro activity and do not affect the protein’s interaction with Rho1, but cells have a lowered β1,3 glucon content. Mutations in the second cytoplasmic domain that lie close to the C-terminus of the sixth helix lead to a loss of cell polarity as well as defects in endocytosis, but have little effect on in vitro and in vivo b-glucan synthesis, and this part of Fks1 may interact with factors involved in cell polarity (Okada et al. 2010). Mutations in Fks1 in residues more distal to the sixth helix lead to low in vitro glucan synthase activity and large decreases in in vivo incorporation of [14C]glucose into β1,3 glucan, suggesting that if Fks1 is a synthase, this part of the protein contains the catalytic site (Dijkgraaf et al. 2002; Okada et al. 2010).

**Fatty acid elongases and phytosphingosine and Fks1 function.** The ER-localized fatty acid elongase Elo2/Gns1 may impact Fks1 at the level of that organelle, because gns1 mutants, isolated on account of their resistance to a papulocandin analogue, have very low in vitro β1,3-glucan synthase activity (el-Sherbeini and Clemas, 1995) and accumulate...
phytosphingosine in the ER membrane (Abe et al. 2001). Phytosphingosine inhibits β1,3 glucan synthase in vitro, leading to the idea that this sphingolipid synthetic intermediate is a negative regulator of β1,3-glucan synthesis at the level of the ER (Abe et al. 2001).

**Roles of the Fks proteins in β1,3-glucan synthesis**

Roles of Fks3 and Fks3 in sporulation. Fks2 is important in sporulation because fks2Δfks2Δ diploids have a severe defect in this process (Mazur et al. 1995; Huang et al. 2005), and form disorganized ascospore walls with lower relative amounts of hexose in their alkali-insoluble fraction and a lower alkali soluble β1,3-glucan content (Ishihara et al. 2007). Homozygous fks3Δfks3Δ diploids also form abnormal spores, indicating a role for the third Fks homologue in ascopore wall formation, but showed no alteration in the distribution of hexoses between alkali soluble- and insoluble fractions (Ishihara et al. 2007). However, the walls of ascospores formed in diploids lacking both Fks2 and Fks3 were more disorganized than those of ascospores made by fks2Δfks2Δ diploids (Ishihara et al. 2007). Expression of FKS2 or FKS1 under the control of the FKS2 promoter, but not the FKS1 promoter, corrected the sporulation defect of homozygous fks1Δfks2Δ diploids, suggesting that the function of Fks2 in sporulating diploids resembles that of Fks1 in vegetative cells. In contrast, overexpression of FKS3 did not suppress the phenotype of fks2Δ spores, and FKS1 or FKS2 overexpression does not correct the defect in fks3Δ spores, indicating Fks3’s function in sporulation does not overlap with that of Fks2. It was proposed that Fks2 is primarily responsible for synthesis of β1,3-glucan in the ascospore wall, and that Fks3, rather than functioning as a synthase, modulates glucan synthesis by interacting with glucan synthase regulators such as Rho1 (Ishihara et al. 2007).