

File S3

O-Mannosylation

This Supporting File contains additional information related to **Biosynthesis of Wall Components Along the Secretory Pathway, *O*-mannosylation**. 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.

Protein O-mannosyltransferases in the ER:

Substrate proteins for different Pmt complexes. Analyses of glycosylation of individual proteins in *pmtΔ* strains showed that Pmt1/Pmt2 complexes are primarily involved in O-mannosylation of Aga2, Bar1, Cts1, Kre9, and Pir2, whereas homodimeric Pmt4 modifies Axl2, Fus1, Gas1, Kex2 (Gentzsch and Tanner 1997; Ecker *et al.* 2003; Proszynski *et al.* 2004; Sanders *et al.* 1999). However, some proteins, including Mid2, the WSC proteins, and Ccw5, are modified by both complexes, although the Pmt1/Pmt2 and Pmt4/Pmt4 dimers modify different domains of these target proteins (Ecker *et al.* 2003; Lommel *et al.* 2004).

Mutations in substrate proteins can cause them to be O-mannosylated by a different PMT, and PMTs can also have a role in quality control of protein folding in the ER (see *N-glycan processing in the ER and glycoprotein quality control*). Thus, wild type Gas1 is normally O-mannosylated by Pmt4, whereas Gas1^{G291R}, a model misfolded protein, is hypermannosylated by Pmt1-Pmt2 as well as targeted to the HRD-ubiquitin ligase complex for degradation by the ERAD system (Hirayama *et al.* 2008; Goder and Melero, 2011). The latter, chaperone-like function of Pmt1-Pmt2 may be distinct from Pmt1-Pmt2's O-mannosyltransferase activity (Goder and Melero, 2011).

Extension and phosphorylation of O-linked manno-oligosaccharide chains:

Extension with α -linked mannoses. The Ser- or Thr-linked Man is extended with up to four α -linked Man that are added by GDP-Man-dependent Man-T of the Ktr1 and Mnn1 families (Lussier *et al.* 1999; **Figure 4** in main text). The contributions of these proteins was deduced from the sizes of the O-linked chains that accumulated in strains in which Man-T genes had been deleted singly or in different combinations. Transfer of the first two α 1,2-Man is carried out by Ktr1 sub-family members Ktr1, Ktr3, and Kre2, which have overlapping roles in the process, although Kre2 has the dominant role in addition of the second, α 1,2-Man (Lussier *et al.* 1997a). The major O-linked glycan made in the *ktr1Δ ktr3Δ kre2Δ* triple mutant consists of a single Man (Lussier *et al.* 1997a). Ktr1, Ktr3, and Kre2 are also involved in making α 1,2-branches to mannan outer chains (see *Mannan elaboration in the Golgi*).

Extension of the trisaccharide chain with one or two α 1,3-linked Man is the shared responsibility of Mnn1 family members Mnn1, Mnt2, and Mnt3, with Mnn1 having the major role in adding the fourth Man but Mnt2 and Mnt3 dominating when the fifth is added (Romero *et al.* 1999). Mnn1 also transfers Man to N-linked outer chains. The α 1,2 Man-T have been localized to the medial Golgi, and the Mnn1 α 1,3 Man-T to the medial and trans-Golgi (Graham *et al.* 1994). Because protein-bound O-mannosyl glycans pulse-labeled in mutants defective in ER to Golgi transport such as *sec12*, *sec18*, and *sec20* contain two, sometimes more mannoses, GDP-Man-dependent O-glycan extension can occur at the level of the ER (Haselbeck and Tanner, 1983; Zueco *et al.* 1986; D'Alessio *et al.* 2005). The process is independent of nucleotide sugar diphosphatases (see *Sugar nucleotide transport*; D'Alessio *et al.* 2005), but presumably mediated in the ER by Man-T *en route* to the Golgi.

Importance and function of O-mannosyl glycans:

Importance of O-mannosylation for function of specific proteins. Analyses of single and conditionally lethal double *pmt* mutants show that O-mannosylation can be important for function of individual O-mannosylated proteins. For example, *pmt4* Δ haploids show a unipolar, rather than the normal axial budding pattern, which is due to defective O-mannosylation and resulting instability and mislocalization of Axl2, which normally marks the axial budding site (Sanders *et al.* 1999). Pmt4-initiated O-mannosylation is also necessary for cell surface delivery of Fus1, because the unglycosylated protein accumulates in the late Golgi (Proszynski *et al.* 2004). Defects in Pmt4-dependent O-glycosylation of Msb2 (as well as N-glycosylation) of osmosensor Msb2 lead to activation of the filamentous growth signaling pathway (Yang *et al.* 2009). In this case, underglycosylation may unmask a domain that normally is exposed and makes interactions when the signaling pathway is activated legitimately. O-mannosylation of Wsc1, Wsc2, and Mid2 is necessary for these Type I membrane proteins to fulfill their functions as sensors that activate the CWI pathway. Underglycosylation of the CWI pathway-triggering mechanosensor Wsc1 in a *pmt4* Δ mutant eliminates the stiffness of this rod-like glycoprotein and abolishes its “nanospring” properties, impairing Wsc1’s function as a mechanosensor (Dupres *et al.* 2009). Further, in *pmt2* Δ *pmt4* Δ mutants, which, like CWI pathway mutants, require osmotic stabilization, deficient O-mannosylation results in incorrect proteolytic processing and instability of the sensors (Philip and Levin, 2001; Lommel *et al.* 2004).

Literature Cited

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