Protein O-mannosylation is an evolutionarily conserved, essential posttranslational modification that impacts a variety of cellular processes in both fungi and mammals. It is initiated at the endoplasmic reticulum by a family of dolichyl phosphate mannose-dependent protein O-mannosyltransferaseswhich is conserved from yeast to humans. PMTs transfer mannose residues from dolichyl phosphate-D-mannose to protein seryl/threonyl residues. In fungi, secretory proteins are commonly mannosylated by protein O-mannosyltransferasein the ER, and subsequently glycosylated by several glycosyltransferases in the Golgi apparatus to form glycoproteins with diverse O-glycan structures. The evolutionarily conserved PMT family is classified into three subfamilies - PMT1, PMT2, and PMT4 - which mannosylate distinct target proteins. Members of the PMT1 subfamilyinteract with members of the PMT2 subfamily, with Pmt1p-Pmt2p and Pmt5p-Pmt3p heterodimers representing the predominant forms. Under certain conditions, Pmt1p can also interact with Pmt3p, and Pmt5p with Pmt2p. PMT2 and PMT3 are paralogs that arose from the whole genome duplication. Unlike the PMT1 and PMT2 subfamily members, the single member of the PMT4 subfamilyacts as a homodimeric complex. The Pmt1p-Pmt2p complex also functions in connection with the ERAD machinery and participates in ER protein quality control. PMTs are integral membrane proteins with two hydrophilic loopsfacing the ER lumen. Formation of dimeric PMT complexes is crucial for mannosyltransferase activity. Loop 1 contains an Asp-Glu motif that is highly conserved among PMTs. Single amino acid substitutions in this motif completely abolish activity of Pmt4p complexes, while both acidic residues need to be changed to eliminate activity of Pmt1p-Pmt2p complexes. Arg64, Glu78, Arg138 and Leu408 of Pmt1p are important for transferase activity, while Arg138 is also essential for complex formation with Pmt2p.In fungi the PMT family is highly redundant, and only the simultaneous deletion of PMT1/PMT2 and PMT4 subfamily members is lethal. pmt1 deletion mutants are highly sensitive to zymolyase. Strains bearing a pmt1 pmt2 double disruption show a severe growth defect. In cells lacking PMT4, Fus1p is not glycosylated and accumulates in late Golgi structures. Mutants lacking both functional Pmt2p and Pmt4p lyse as small-budded cells in the absence of osmotic stabilization, and treatment with mating pheromone causes pheromone-induced cell death. These phenotypes are partially suppressed by overexpression of upstream elements of the protein kinase C cell integrity pathway. Induction of Mpk1p/Slt2p tyrosine phosphorylation does not occur in pmt2 pmt4 double mutants during exposure to mating pheromone or elevated temperature. Further, Slg1p, Wsc2p, and Mid2p are aberrantly processed in pmt mutants. Adhesin subunit Aga1p and the alpha-agglutinin Ag1p are hypoglycosylated in cells lacking PMT1 and PMT2, with phenotypes manifested only in MATalpha cells for single mutants and in both cell types when both genes are absent. In Schizosaccharomyces pombe, lack of protein O-mannosyltransferase activity results in abnormal cell wall and septum formation, severely affecting cell morphology and cell-cell separation. PMTs are crucial for viability in mouse. In humans, O-mannosylation defects are associated with Walker-Warburg syndrome, a type of severe recessive congenital muscular dystrophy associated with defects in neuronal migration that produce complex brain and eye abnormalities. PMTs have also been identified in Ustilago maydis, Cryptococcus neoformans, Candida albicans, Aspergillus nidulans, Kluyveromyces lactis, and Trichoderma reesei, as well as Corynebacterium glutamicum. Because O-mannosylation of specific secretory proteins in C. albicans affects several virulence traits such as morphogenesis, adhesive properties, and antifungal resistance, PMTs can be considered as potential antifungal targets against human fungal pathogens. Similarly, O-mannosylation of specific secretory proteins of the bacterial pathogen Mycobacterium tuberculosis contributes significantly to virulence. PMTs are absent in green plants, and are therefore potential targets for antifungal drugs against phytopathogenic fungi.