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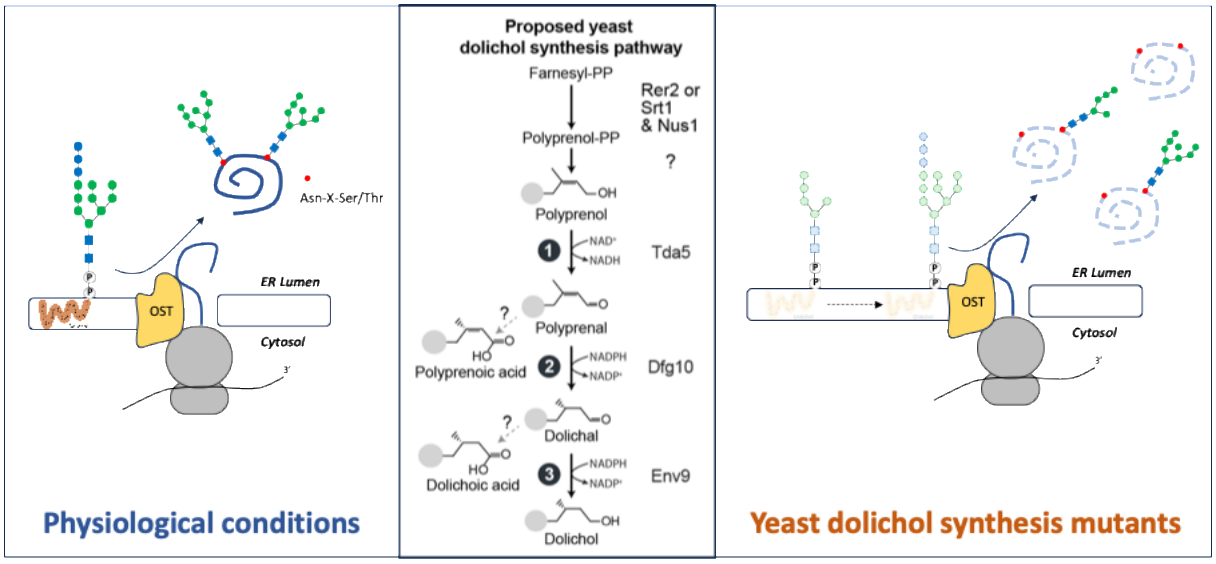
Decipher the lipidic secrets of N-glycosylation

Abstract

The availability of dolichol, a long-chain polyisoprenoid alcohol, is a rate-limiting factor for protein glycosylation, a fundamental post-translational modification essential for eukaryotic cell viability. Decades of glycobiology research had identified the reduction of polyprenol into dolichol as a definitive late-stage fundamental step in the pathway. This conversion was catalyzed by specific polyprenol reductases: SRD5A3 in mammals and its ortholog Dfg10 in yeast. Recent discoveries led to a complete and unexpected revision of the dolichol biosynthesis pathway. The overarching objective of this Mizutani project was to fully dissect the molecular mechanisms by which deficiencies in DHRSX/Env9, SRD5A3/Dfg10, and Tda5 impair dolichol synthesis and precipitate glycosylation abnormalities—ultimately clarifying why dolichol biosynthesis requires this specific metabolic detour. By employing engineered KO yeast cells, glycomics approaches and metabolic labeling to unravel ER N-glycosylation, we achieved two pivotal breakthroughs that fundamentally refine our understanding of this pathway: (**i**) the functional reassignment of the *Saccharomyces cerevisiae* protein Dfg10 as a polyprenal reductase and the true ortholog of human SRD5A3; and (**ii**) the discovery that the dual catalytic role of human DHRSX is partitioned between two previously uncharacterized yeast enzymes, Env9 and Tda5. We found that yeast Env9 catalyzes the reduction of dolichal to dolichol while Tda5 would be involved in the conversion of polyprenol to polyprenal. Accordingly, deletion of *ENV9* and *TDA5* caused polyisoprenoid intermediates to accumulate and immature lipid-linked oligosaccharides to be transferred onto nascent proteins. These changes were found more profound in *DFG10*-deficient yeast cells. Consequently, N-glycosylation was disrupted, leading to varying degrees of underglycosylation and altered cell wall α-mannan content, revealing a critical sensitivity of yeast mannan biosynthesis to the quality of nascent N-linked glycans



Yeast dolichol biosynthesis pathway: Impacts of yeast dolichol synthesis mutants on LLO, N-glycan fidelity and site occupancy.