View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by IBB PAS Repository

RESEARCH ARTICLE

Candida albicans; exploring glycosylation pathway in the search of targets for antimicrobial agents and yeast to hyphae transition.

Authors

Mateusz Juchimiuk, Anna Janik, Monika Niewiadomska, Urszula Lenart, Joanna Kruszewska, Grażyna Palamarczyk

Institute of Biochemistry and Biophysics; Polish Academy of Sciences

Correspondence

Grażyna Palamarczyk
Email: gp@ibb.waw.pl

Abstract

Microbial cell wall is mostly synthesized by the glycosylated proteins with the distinct enzymatic activity. In this review we have concentrated on the description of the certain steps of glycosylation and their effect on the cell wall integrity and yeast to hyphae transition, the process enhancing the pathogenic properties of *C.albicans*.

The glycoproteins play an invaluable role in *C. albicans* virulence and they modulate adhesive, invasive, morphogenetic and immune stimulating properties of the pathogen as well as its susceptibility to the antifungal agents. Therefore, understanding of *C. albicans* glycobiology might let us expand the arsenal in the war against fungal enemies. The early stages of N-, O-glycans and GPI-anchor synthesis requires dolichol - the lipid carrier of sugar residues. Diminished supply of dolichol causes series of defects in *C. albicans* cells, among which aberrant protein glycosylation is the most evident. Furthermore, the relations between the cell wall composition and integrity, resistance to some antifungal and cell wall disturbing agents and dolichol dependent glycosylation are observed. Moreover relevance of these reactions for the morphological differentiation of C.albicans is described.

Key words: C.albicans, dolichol, protein glycosylation, cell wall integrity

Copyright 2019 KEI Journals. All Rights Reserved

1, General characteristic of *Candida albicans*

C. albicans is an ascomycete fungus that normally lives commensally in the gastrointestinal tracts of warm-blooded animals. The 60-75% of human population (1,2) carries *C. albicans* asymptomatically on skin, mucosa, in the genitourinary and gastrointestinal tracts. Sometimes it affects healthy people causing superficial skin or mucous membranes infections. However, in immune-compromised patients the same yeast can colonize and invade host tissue and gain access to the blood stream. In patients with AIDS, immune-suppressed by cancer chemotherapy or seriously deficient in competing microflora (after antibiotic therapy) *C. albicans* can develop life-threatning candidaemia with mortality rate 30-50%. Prolonged stays in intensive care units, the development of more aggressive surgical techniques and the use of plastic catheters increases the number of candidiasis.

In consequence, *C. albicans* now represents an important agent of microbial septicaemia in hospitals in the US and European countries (3,4).

Candida albicans is an obligatory diploid which undergoes an unusual parasexual cycle, accounting for genomic instability (5). Additionally the fungus exhibits the non-conventional codon usage, where the CUG codon is decoded as serine, not leucine (6). A striking feature of *C. albicans* is polymorphism – the ability to grow in distinct forms (yeast, pseudohyphae, hyphae and chlamydospores). Moreover an epigenetic phenomenon of switching between the white and opaque cells related to mating ability was discovered (7) . Hyphal and pseudohyphal forms are considered as virulent and yeast to hyphae transition is thought to be required for *C. albicans* virulence, however the issue remains controversial due to the lack of clear evidence

2, The cell wall

Microbial cells are usually surrounded by the cell wall, a tough although dynamic structure which gives the shape and protects fungal cell from external stresses. As it is the most outward structure of the cell, the cell wall not only separates cytoplasm from the milieu but also is responsible for contacts with environment and sensing changing conditions. The cell wall has a layered structure, where inner layer composed of polysaccharides (β-glucans and chitin) forms an elastic three-dimensional scaffold which anchors proteins, usually glycoproteins.

Copyright 2019 KEI Journals. All Rights Reserved

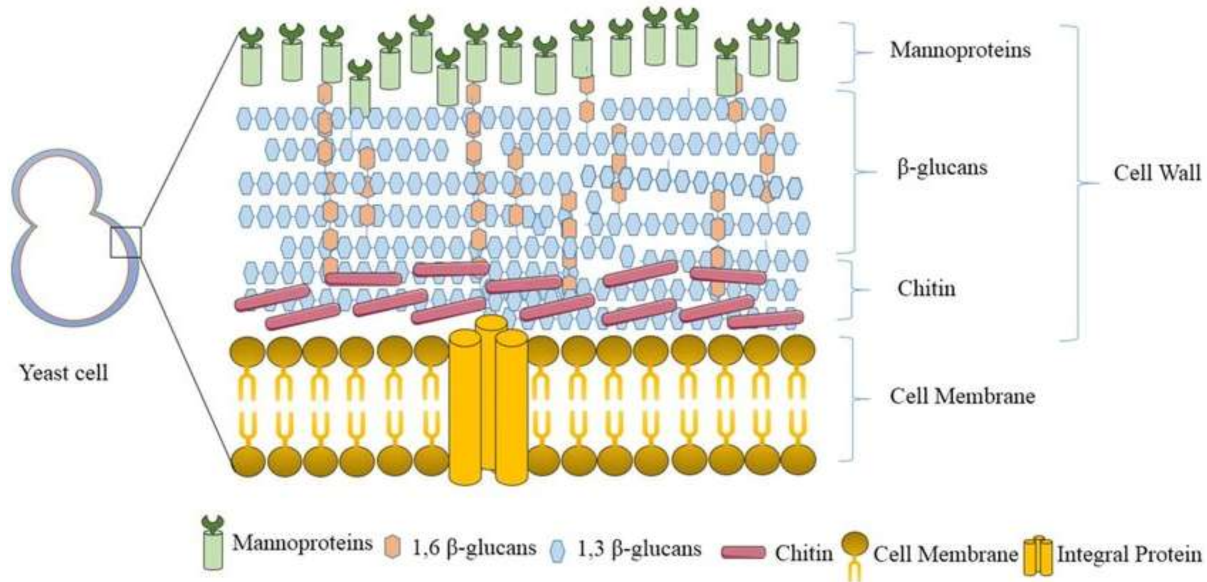

Fig. 1. Cell wall structure of *S. cerevisiae*; according to (8)

Glucans (β -1,3- and β -1,6-) and chitin play mainly structural role and contribute to the cell wall rigidness, however β -1,3-glucan can also stimulate a specific response of immunological system after recognition of cell wall structure by dectin-1 receptors. The spectrum of cell wall proteins is broad as well as their extremely important functions. They are responsible for various properties of the *C. albicans* cell, among them: cell wall maintenance and protection against stress, adhesion and cell–cell interactions, antigenicity, pathogenicity, cell surface hydrophobicity and porosity, virulence and biofilm formation.

3, Biosynthesis and functions of dolichols and its sugar derivatives

Dolichol is a polyisoprenoid alcohol belonging to the lipid compounds found in membranes of Eukarya, Bacteria and Archaea. Dolichols were identified in 1960 (9) and ten years later it was shown that dolichol in phosphorylated form is indispensable for protein glycosylation (10). Subsequent studies revealed that it serves as a carrier lipid of the oligosaccharide core (LLO) in N-glycosylation and delivers glucose (Glc) and mannose (Man) moiety for all glycosylation processes taking place on the lumenal side of endoplasmic reticulum. While dolichols are characteristic for yeast and mammalian cells, their unsaturated counterparts, polyprenols, are usually attributed to bacteria and plants where they play a similar role. However, in addition to its significance for glycosylation, polyisoprenoid alcohols are considered to be responsible for the modulation of cellular membranes properties (i.e. fluidity and permeability), involved in the transport of ER and vacuolar proteins and in vesicle trafficking (11-13). The protective role of dolichol against reactive oxygen species is also suggested (14), as well as its function in the organization and stabilization of some glycosyltransferases (15). It is also known that polyisoprenoids are involved in the formation of bacterial peptidoglycan (16). Few decades of research provided detailed

Copyright 2019 KEI Journals. All Rights Reserved

mechanisms and identified enzymes involved in the biosynthesis of polyisoprenoid alcohols and their contribution to protein modification. Moreover, several human diseases resulting from defects in dolichol biosynthesis have been described [reviewed in (17)] .

3,1. Dolichol biosynthesis and structure

The structure of dolichol and the scheme of its de novo synthesis in yeast are presented in the Fig.2.

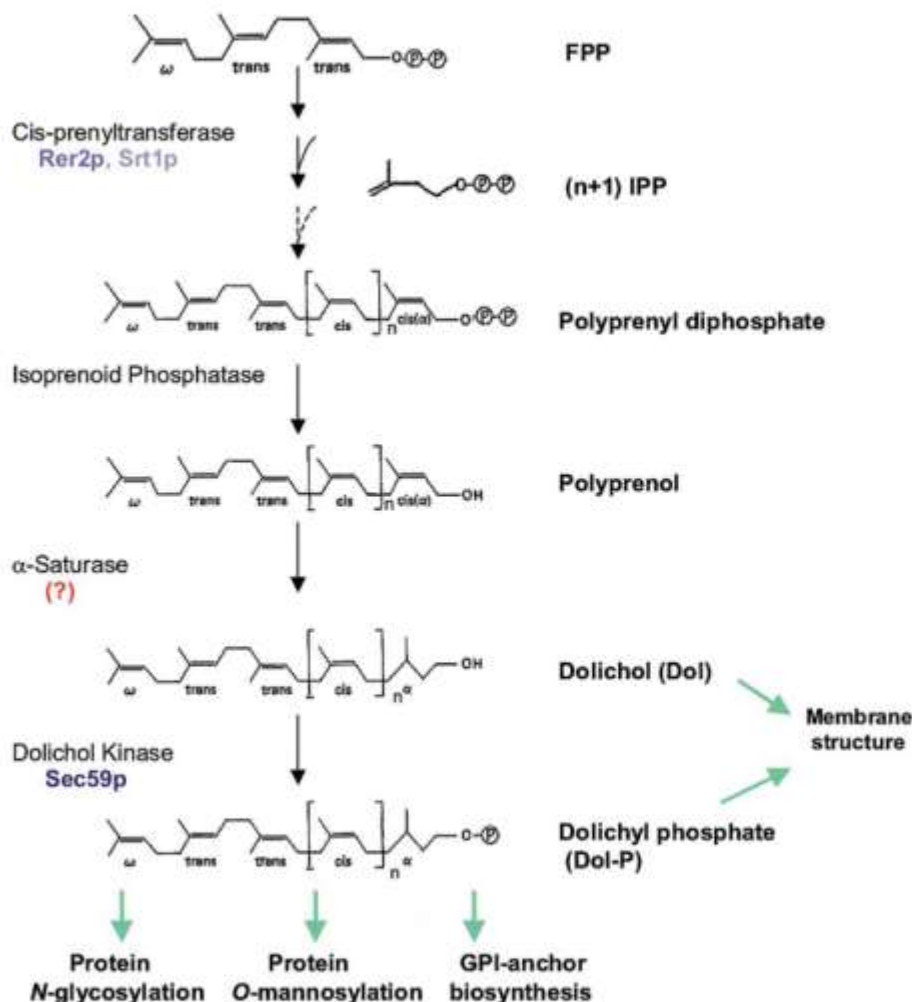

Fig.2. Dolichol and dolichylphosphate; synthesis and structure
A scheme adopted from (18)

Generally, dolichol consists of up to about 25 isoprene residues, linked head-to-tail (19). The alpha hydroxyl group of dolichol may exist free, phosphorylated or esterified with fatty acids (20) The length of dolichol chain depends on the organism, tissue, age and a physiological status of a cell. In general, polyisoprenoid lipids are found as a mixture of four or more (up to app. 100 in plants) different chain lengths, with some

Copyright 2019 KEI Journals. All Rights Reserved

chain lengths predominating. *S. cerevisiae* in the logarithmic growth phase produce molecules comprised of 14 to 18 isoprene units, whereas stationary phase yeast cells additionally accumulate a family of longer dolichols (19–24 isoprene residues) (21). In mammalian cells dolichols with 18–21 units and predominant Dol-19 were found (22) .

3,2. *Cis*-prenyltransferases

The early steps in dolichol biosynthesis in yeast and animals are identical to those leading to sterol and ubiqinone and belong to the mevalonate pathway (23). Additionally, in plants and some bacteria, the parallel methylerythritol phosphate (MEP) route is operating (24). To date, several prokaryotic and eukaryotic *cis*-prenyltransferases (*cis*-PTase) have been cloned, including human enzyme (25). In *S. cerevisiae*, at first, two, *cis*-PTase were identified. Rer2 (retention in the endoplasmic reticulum) is a main *cis*-PTase responsible for bulk of the activity in the logarithmic growth phase and synthesizing polyprenols with chains length of 14-18 units, while Srt1 (suppressor of rer-two) is prevalent in the stationary phase or in starving cells and producing polyprenols containing 19-23 isoprene residues (21,26, 27). More recently, occurrence of a third protein presenting *cis*-PTase activity was reported (28) Mammalian *cis*-PTase was described to be a heteromer consisting of NgBR (Nus1 in *S. cerevisiae*) and hCIT (dehydrodolichol diphosphate synthase) subunits, and this composition has been confirmed in plants and fungal *cis*-PTase. Finally, a critical RXG sequence in the C-terminal tail of NgBR that is conserved in other eukaryotes was shown to be essential for enzyme activity (28).

4. An impact of dolichol and dolichyl phosphate dependent glycosylation on *C. albicans* physiology and the cell wall integrity

4.1. Suppression of the *RER2* gene results in the under glycosylation of proteins, compromised cell wall integrity and perturbed morphogenesis (29).

To determine the role of *RER2* in *C. albicans*, one chromosomal copy of the gene was disrupted and the second allele was placed under the control of the regulatable *MET3* promoter. The shevidence was shown that *C. albicans* orf19.4028 encodes protein with *cis*- PTase activity homologous to Rer2p of *S. cerevisiae*, accounting for a bulk of activity as revealed by switching off its expression in conditional *MET3p-RER2* mutant. Moreover, the orf19.5236, encoding protein homologous to Srt1p of *S. cerevisiae* was also found in *C. albicans* genome, Similarly to *S. cerevisiae*, *C. albicans cis*-PTase izoenzymes differ in the product length Rer2 produces polyprenols shorter than Srt1p. In *S. cerevisiae* deletion of *RER2* results in a slow growth, defects in N-linked and O-linked glycosylation and an accumulation of excessive ER and Golgi membrane material, whereas *srt1* null mutant exhibits no apparent phenotype (26,27). What is more, *rer2* cells can undergo a reversion, when the spontaneous activation of *SRT1* leads to a much faster growth rate and concomitant synthesis of longer dolichols. (26). Deletion of both *SRT1* and *RER2* is lethal. In *C.albicans* the defect of glycosylation upon *CaRER2* repression, was demonstrated with the Western blot using polyclonal anti-Gas1p antibodies (29). The *S. cerevisiae* Gas1p is

Copyright 2019 KEI Journals. All Rights Reserved

GPI-anchored N- and O- glycosylated protein located in the plasma membrane, involved in the assembly of the cell wall β-1,3-glucan. This protein is a glycosylation marker commonly used due to clearly visible difference in size between its correctly glycosylated and hypoglycosylated forms. *C. albicans* homologues of Gas1p, Phr1 and Phr2 proteins are in 52% and 56%, respectively, identical to Gas1p of *S. cerevisiae* and their expression depends on pH of the environment. Western blot analysis indicated that glycosylated Phr protein is absent in *MET3pRER2/rer2Δ::hisG* mutant grown in repressive conditions. Instead of, the hypoglycosylated forms of protein are visible. However, a 110 kDa band corresponding to fully glycosylated protein is present in the homozygous *C. albicans* strain (containing only one copy of the *RER2* gene) and the wild-type strain cultivated in the presence of methionine and cysteine (repressive conditions). For all strains grown in non-repressive conditions mostly a native form of Phr protein was observed (29). These data allow to conclude that suppression of the *RER2* gene results in the diminished activity of *cis*-PTase and dolichol level and leads to severe defects in protein glycosylation. Moreover, affects the cell wall composition and integrity and increases susceptibility to some antifungals and chemical agents. In addition, the proper level of cis-PT activity is required for hyphae formation and chlamydospore production in *C. albicans* (29).

4, 2. Diminished expression of the proteins involved in N- or O- glycosylation results in morphological and physiological changes of the cell, similar to the one observed upon supression of the Rer2 protein.

N-glycosylation starts by the assembly of the oligosaccharide linked to the lipid carrier DolP. (Fig.3)

Copyright 2019 KEI Journals. All Rights Reserved

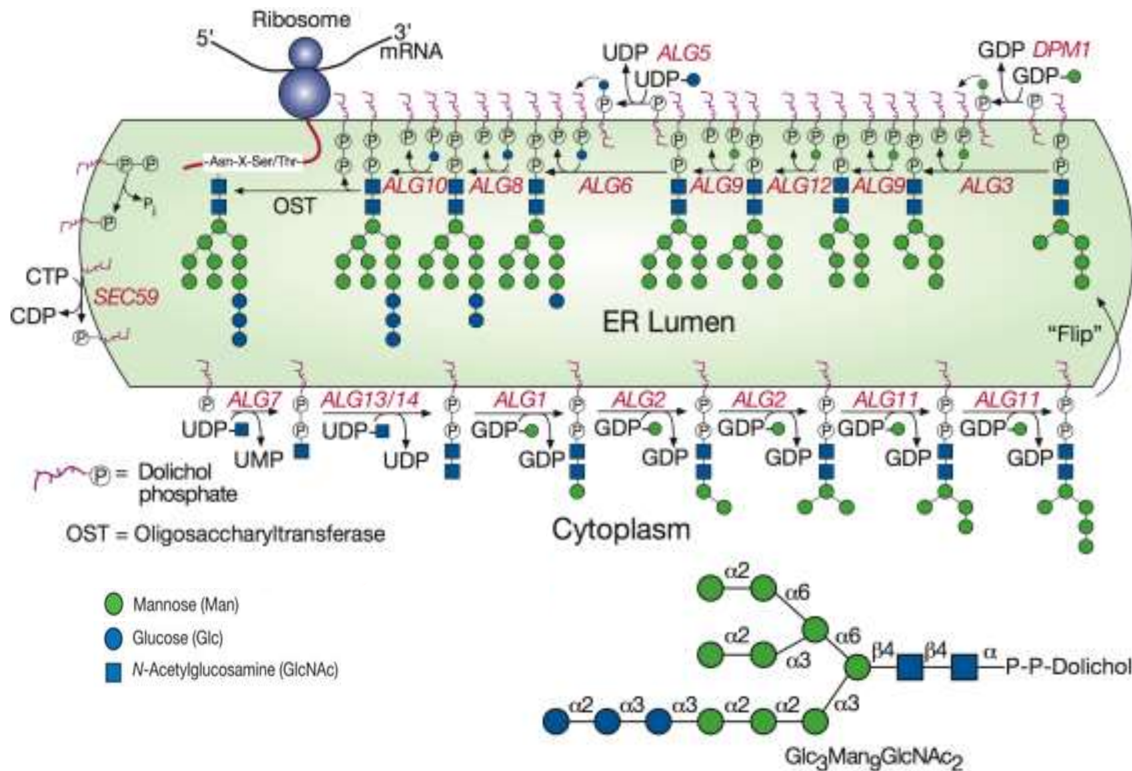

Fig. 3. Synthesis of Lipid Linked Oligosaccharide and its structure.
Adapted from: Essentials of Glycobiology. 2nd edition. Varki A, Cummings RD, Esko JD, et al., editors. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009 (30)

The first two reactions, catalyzed by the complex of Alg (Asparagine linked glycosylation) proteins, Alg7, Alg13, Alg14, involves transfer of GlcNAc-1P, from UDPGlcNAc to DolP, followed by the second GlcNAc to form DolPP-$GlcNAc_2$. Consequently, other proteins from Alg family facilitate the transfer of Man and Glc from the respective sugar nucleotides (at the cytoplasmic side of ER) and from Dol-P linked sugars (in the ER lumen). Eventually formed lipid linked oligosaccharide (LLO; $DolPPGlcNAc_2Man_9Glc_3$) serves as oligosaccharyl donor to the protein bearing N-glycosylation site (Asp-X-Ser/Thr). Glycosylation occurs at the gamma amide group of asparagine.

C. albicans ALG7, ALG13 and *ALG14* genes, encoding members of the Alg7p/Alg13p/Alg14p complex, catalyzing the first two initial reactions in the N-glycosylation process were identified in our Lab.(31). It allowed us to construct the strain *alg13* Δ***::****hisG/TRp-ALG13* with one allele of *ALG13* disrupted and the other under the control of a regulatable tetracycline promoter. Both decreased and elevated expression of *ALG13* changed expression of all members of the complex and resulted in a decreased activity of Alg7p and Alg14p and under-glycosylation of the marker glycoprotein. The *alg13* strain was also defective in hyphae formation and growth of biofilm.(31). These results confer the key

Copyright 2019 KEI Journals. All Rights Reserved

role of the correct N-glycosylation for the cell wall integrity and morphogenesis of *C. albicans.* During the process of O-glycosylation (described elsewhere) DolPMan synthase (DPMS), in *C. albicans* made up from the interacting proteins Dpm1, Dpm2 and Dpm3 (32) catalyse synthesis of DolPMan from GDPMan and DolP. Subsequently, the mannosyl residues from DolPMan are transferred by O-mannosyltransferazes to the hydroxyl group of serine or threonine, in the protein which undergoes the O-glycosylation. Mannosyl oligosaccharide is further elongated by direct transfer of Man from GDPMan (up to 4-5 sugar residues in *S. cerevisiae*). The reduction of DPMS activity, similarly as DolPPGlcNAc$_2$ synthase, causes series of defects in *C. albicans* cells, among which, diminished protein glycosylation is the most evident. Furthermore, the relations between the cell wall composition and integrity, sensitivity to some antifungals and the cell wall disturbing agents is observed (32). Finally, the morphological differentiation of mutants with decreased expression of either *RER2* or *DPM1* or *DPM2* or *DPM3* or *Alg 13* was tested and unraveled relevance of these genes in hyphae formation and chlamydospores production (29,31). At last, the search for the interplay between microbial cell wall protein and the dolichol-dependent steps of glycosylation of the host cells, devoid of the cell wall was followed. To this end we studied the role of the GPI-remnant anchor Cwh8 protein of the *C. albicans* cell wall. According to Fernandez et al. (33) topological studies using different approaches indicate that in *S. cerevisiae* Cwh8p is a transmembrane protein with a luminally oriented active site. The specificity, subcellular location, and topological orientation of this enzyme are consistent with a role in the re-utilization of the glycosyl carrier lipid for additional rounds of lipid intermediate biosynthesis after its release during protein N-glycosylation and it was tentatively assumed that Cwh8p acts as DPP-phosphatase.

In *C. albicans* database the protein homologues to *S. cerevisiae* Cwh8 exist and in the *cwh8* "null mutant" an increase of the endogenous pool of free dolichol as well as the LLO is observed. However, glycosylation of the marker protein Phr1/2 is not restored, compared to the control and the mutant has all characteristic of the strains defective in dolichol dependent glycosylation (33). Our, still preliminary data, suggest that Cwh8p is a regulatory factor affecting expression of the proteins involved in dolichol backbone biosynthetic pathway (in preparation). Since Cwh8p is a microbial cell wall protein it seems to be an attractive candidate target for the antifungal agents.

Based on the results presented above, it is reasonable to conclude that the level of dolichol and/or N-glycosylation, in *C. albicans,* is crucial not only for the glycosylation but also for the sensitivity of *C. albicans* towards external agents, including antifungal drugs, diminished adherence and the morphogenetic switch enhancing pathogenic feature of this organism.

Copyright 2019 KEI Journals. All Rights Reserved

References

1.Odds F. C. 1988 *Candida and candidosis*. Bailli`ere Tindall: London

2.Ghannoum MA, Jurevic RJ, Mukherjee PK, Cui F, Sikaroodi M, et al. 2010 Characterization of the *Oral Fungal Microbiome (Mycobiome) in Healthy Individuals. PLoS Pathogens* **6**(1)

3. Kam L.W., Lin J.D. 2002 *Management of systemic candidal infections in the intensive care unit*. Am J Health Syst Pharm. **59**, 33–41 K

4. Barnett J. A., 2008 A history of research on yeasts : medical yeasts part 1, Candida albicans. Yeast **25**, 385-417

5. Bennett R.J., Johnson A. D. 2003 Completion of a parasexual cycle in Candida albicans by *induced chromosome loss in tetraploid strains.* EMBO J. **22**, 2505–15

6. Santos M. A., Tuite M. F. 1995 *The CUG codon is decoded in vivo as serine and not leucine in Candida albicans. Nucleic Acids Res.* **23***,* 1481–1486

7. Miller M. G. & Johnson A. D. 2002 *White–opaque switching in Candida albicans is controlled by mating-type locus homeodomain proteins and allows efficient mating.* Cell **110**, 293–302

8. Anwar I., Muhammad F., Awais M. M., Akhtar M. 2017 *A review of β-glucans as a growth promoter and antibiotic alternative against enteric pathogens in poultry;* World's Poultry Science Journal **73**(03):1-11 DOI: 10.1017/S0043933917000241

9. Anderson R. G., Hussey H., Baddiley J. 1972 *The mechanism of wall synthesis in bacteria. The organization of enzymes and isoprenoid phosphates in the membrane.* Biochem. J. **127**, 11–25

10 Pennock J. F., Hemming F. W., Morton R. A. (1960) *Dolichol: a naturally occurring isoprenoid alcohol.* Nature **186**, 470–472

11. Behrens N. H. and Leloir L. F. 1970 *Dolichol monophosphate glucose: an intermediate in glucose transfer in liver.* Proc Natl Acad Sci USA **66**, 153–159

12. Valtersson C., van Duijn G., Verkleij A. J., Chojnacki T., de Kruijff B., Dallner G. (1985) *The influence of dolichol, dolichyl esters, and dolichyl phosphate on phospholipid polymorphism and fluidity in model membranes.* J Biol Chem **260**, 2742–51

13. Sato M., Sato K., Nishikawa S., Hirata A., Kato J., Nakano A. 1999 *The yeast RER2 gene, identified by endoplasmic reticulum protein localization mutations, encodes cis-prenyltransferase, a key enzyme in dolichol synthesis.* Mol Cell Biol **19**, 471–83

14..Belgareh-Touze N., Corral-Debrinski M., Launhardt H., Galan J. M., Munder T., Le Panse S., et al. 2003 *Yeast functional analysis: identification of two essential genes involved in ER to Golgi trafficking.* Traffic **4**, 607–17

15. Albright C. F., Orlean P., Robbins P. W. 1989 *A 13-amino acid peptide in three yeast glycosyltransferases may be involved in*

Copyright 2019 KEI Journals. All Rights Reserved

dolichol recognition. Proc Natl Acad Sci USA **86**, 7366–9.

16.Bergamini E. 2003 *Dolichol: an essential part in the antioxidant machinery of cell membranes.* Biogerontology **4**, 337–9

17. Cantagrel V. and Lefeber D. J. 2011 *From glycosylation disorders to dolichol biosynthesis defects: a new class of metabolic diseases.* J Inherit Metab Dis **34**, 859–867

18. Grabińska K. and Palamarczyk G. 2002 *Dolichol biosynthesis in the yeast Saccharomyces cerevisiae: an insight into the regulatory role of farnesyl diphosphate synthase* FEMS Yeast Research **2**, 259-265

19. Krag S.S. 1998 *The importance of being dolichol.* Biochem Biophys Res Commun. **243**, 1-5

20. Tollbom O. and Dallner G. 1986 *Dolichol and dolichyl phosphate in human tissues*. Br J Exp Pathol **67**, 757–764

21.Szkopinska A., Swiezewska E., Rytka J. (2002) *Induction of the synthesis of an additional family of long-chain dolichols in the yeast Saccharomyces cerevisiae. Effect of starvation and ageing.* Acta Biochimica Polonica **49**, 781–787

22.. Rip J.W., Rupar C.A., Ravi K., Carroll K.K., (1985) Distribution, metabolism and function of dolichol and polyprenols, Prog Lipid Res 24, 269-309

23..Jung P. and Tanner W. 1973 *Identification of the lipid intermediate in yeast mannan biosynthesis.* Eur. J. Biochem. **37**, 1-6

24 Hunter W.N. 2007 *The non-mevalonate pathway of isoprenoid precursor biosynthesis.* J. Biol. Chem. **282**, 21573–21577

25 Endo S., Zhang Y. W., Takahashi S., Koyama T. 2003 Identification of human dehydrodolichyl diphosphate synthase gene. Biochim Biophys Acta. **1625**, 291-5

26. Schenk B., Fernandez F., Waechter C.J. 2001 *The ins(ide) and out(side) of dolichyl phosphate biosynthesis and recycling in the endoplasmic reticulum*

27. Sato M., Fujisaki S., Sato K., Nishimura Y., Nakano A. 2001 *Yeast Saccharomyces cerevisiae has two cis-prenyltransferases with different properties and localizations. Implication for their distinct physiological roles in dolichol synthesis*. Genes Cells. **6**, 495-506

28. Grabińska K.A., Edani B.H., Park E.J., Kraehling J.R., Sessa W.C. 2017; *A conserved C-terminal RXG motif in the NgBR subunit of cis-prenyltransferase is critical for prenyltransferase activity*. J Biol Chem. 2017 Oct **20**;292 (42):17351-17361. doi: 10.1074/jbc.M117.806034. Epub 2017 Aug 23

29. Juchimiuk M, Orłowski J., Gawarecka K., Świeżewska E., Ernst J.F., Palamarczyk G. (2014 *Candida albicans cis-prenyltransferase Rer2 is required for protein glycosylation, cell wall integrity and hypha formation.* Fungal Genet Biol. 2014 Aug;69:1-12.

Copyright 2019 KEI Journals. All Rights Reserved

doi: 10.1016/j.fgb.2014.05.004. Epub 2014 May 27

30. . Varki A, Cummings RD, Esko JD, et al. 2009 *Essentials of Glycobiology*. 2nd edition., editors.; Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press

31. Niewiadomska M., Janik A., Perlińska-Lenart U., Piłsyk S., Palamarczyk G., Kruszewska J.S. 2017 The role of Alg13 N-acetylglucosaminyl transferase in the expression of pathogenic features of Candida albicans.Biochim Biophys Acta Gen Subj. 2017 Apr;**1861**(4):789-801. doi: 10.1016/j.bbagen.2017.01.019. Epub 2017 Jan 24

32. Juchimiuk M., Kruszewska J., Palamarczyk G. 2015 *Dolichol phosphate mannose synthase from the pathogenic yeast Candida albicans is a multimeric enzyme*. Biochim Biophys Acta. 1850(11):22 doi:10.1016/j.bbagen.2015.08.012. Epub 2015 Aug 2

33.Fernandez F., Rush J. S., Toke D. A., Han G. S., Quinn J. E., Carman G. M., Choi J. Y., Voelker D. R., Aebi M., Waechter C. J. (2001) The CWH8 gene encodes a dolichyl pyrophosphate phosphatase with a luminally oriented active site in the endoplasmic reticulum of Saccharomyces cerevisiae. J Biol Chem 276(44):41455–41464

Copyright 2019 KEI Journals. All Rights Reserved