

MiniReview

Dolichol biosynthesis in the yeast *Saccharomyces cerevisiae*: an insight into the regulatory role of farnesyl diphosphate synthase

Kariona Grabińska, Grażyna Palamarczyk *

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Laboratory of Fungal Glycobiology, Pawińskiego 5a, 02 106 Warsaw, Poland

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Abstract

Dolichol, an isoprenoid lipid, known mainly for its function in protein glycosylation, is synthesised in the mevalonate pathway. The pathway is highly regulated, on multiple levels, by sterol and non-sterol derivatives of mevalonic acid. Farnesyl diphosphate (FPP) and/or FPP-derived molecules have been identified as the main non-sterol compounds regulating degradation of 3-hydroxy-3-methylglutaryl-CoA reductase, one of the regulatory enzymes in the mevalonate pathway. In the present review we concentrate on the effect of overexpression of farnesyl diphosphate synthase on dolichol biosynthesis in yeast. In this context the role of the Yta7 protein, belonging to the AAA ATPase family, in the regulation of FPP flux to the dolichol branch of the mevalonate pathway is discussed, and the effect of FPP and/or derived molecules on the transcription of genes encoding the first enzyme committed to dolichol biosynthesis, i.e. *cis*-prenyl transferase. © 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: *Saccharomyces cerevisiae*; Dolichol biosynthesis; Farnesyl diphosphate synthase

1. Dolichol structure and biosynthesis

Dolichol, an isoprenoid lipid, in addition to being indispensable for protein *N*-glycosylation and *O*-mannosylation (for reviews see [1–3]), is an important cellular membrane component. Yeast strains defective in dolichol biosynthesis show defects in *N*- and *O*-glycosylation as well as abnormal accumulation of the endoplasmic reticulum (ER) and Golgi membranes [4].

The early steps in dolichol biosynthesis are identical to those leading to sterol and ubiquinone [5,6] and yeast mutants deficient in the first reactions of the ergosterol biosynthetic pathway have been shown to be impaired in the synthesis of lipid-linked saccharides in the absence of exogenous dolichyl phosphate [7]. The pathways diverge

after the synthesis of farnesyl diphosphate (FPP) (Fig. 1). *cis*-Prenyl transferase is considered to be the first enzyme committed to dolichol biosynthesis [6]. De novo synthesis involves 1'–4 condensation of FPP with 11–15 isopentenyl pyrophosphate units in *cis*-configuration to form polyprenyl diphosphate (Fig. 2) (for reviews see [8–10]). It should be underlined that bacterial *cis*-PTases synthesise undecaprenyl (C₅₅ polyprenyl) diphosphate, whereas their eukaryotic counterparts form dolichyl diphosphate (α -dihydropolyprenyl diphosphate) of the various chain lengths characteristic for the given organisms. The first *cis*-prenyl elongation enzyme, undecaprenyl diphosphate synthase (undecaPTase), was cloned from *Micrococcus luteus* [11]. Shortly after, genes encoding undecaPTase from *Escherichia coli* [12,13] and two *cis*-PTs from *Saccharomyces cerevisiae* [4] and from *Arabidopsis thaliana* [14,15] were also cloned. Moreover, putative genes for *cis*-PTs from *A. thaliana* (GenBank 8843798), *Caenorhabditis elegans* (GenBank 3877579), *Drosophila melanogaster* (GenBank 7290854), *Hevea brasiliensis* (GenBank 16751461), *Schizosaccharomyces pombe* (GenBank 4038613), *Candida albicans* (orf 6.7706 and orf 6.7441) and human (GenBank 17438514) were also identified. It is worth noting that among the microorganisms whose genomes have been completely sequenced, *Mycoplasma* sp., a microbe lacking

* Corresponding author. Tel.: +48 (22) 658 7402;
Fax: +48 (39) 12 1623.

E-mail address: gp@ibb.waw.pl (G. Palamarczyk).

Abbreviations: *cis*-PTase, Rer2p and Srt1p, *cis*-prenyl transferases encoded by *RER2* or *SRT1* genes; ER, endoplasmic reticulum; FPP, farnesyl diphosphate; FPPS, farnesyl diphosphate synthase; GPP, geranyl diphosphate; HMGR, Hmg1p, Hmg2p, 3-hydroxy-3-methylglutaryl-coenzyme A reductases encoded by *HMG1* and *HMG2* genes; IPP, isopentenyl diphosphate; PC, phosphatidylcholine

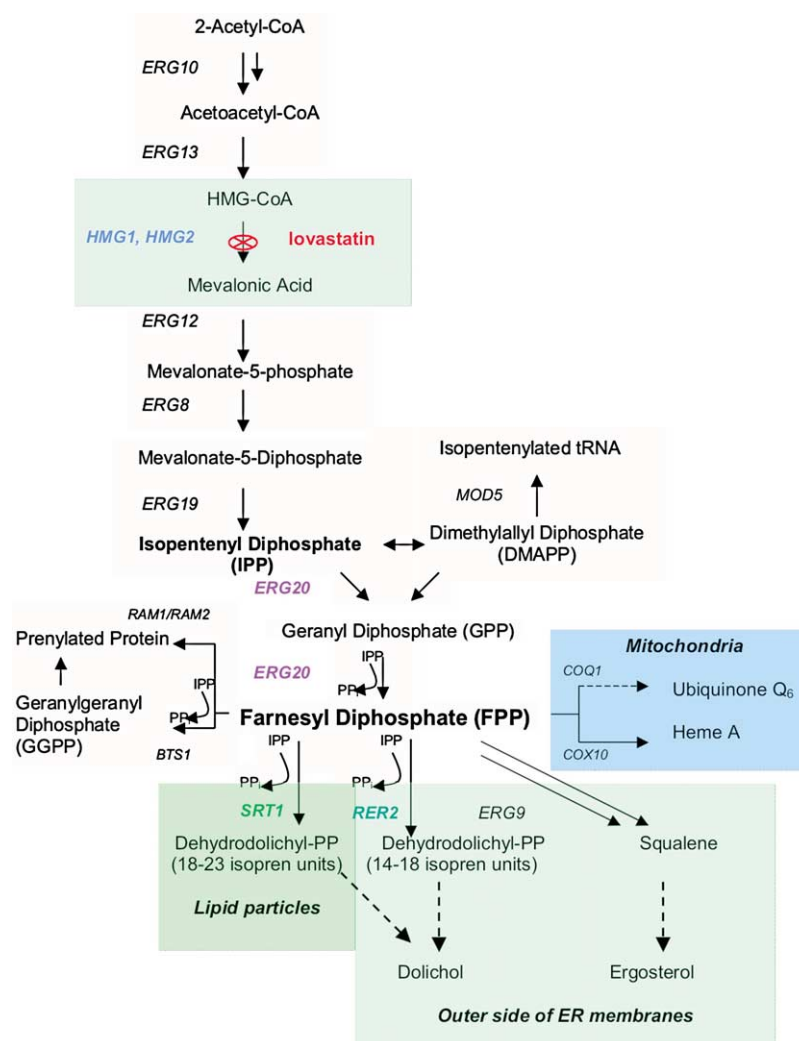


Fig. 1. An outline of the mevalonate pathway in *Saccharomyces cerevisiae*.

a cell wall, is the only one that does not possess a *cis*-PTase homologue.

A comparison of predicted protein sequences and the results of a study on a crystallised undecaPTase from *Micrococcus luteus* indicated that conserved amino acid residues among *cis*-prenyl chain-elongating enzymes are located around a large hydrophobic cleft. The hydrophobicity of this cleft is important for the binding of the hydrophobic chain of the isoprenoid substrate. At the entrance to the cleft it is possible to distinguish a cluster of charged arginine residues proposed to bind the diphosphate group of isopentenyl diphosphate (IPP) and a structural P-loop motif, which frequently appears in various phosphate binding sites and could bind, via a magnesium bridge, the diphosphate group of FPP. The condensation reaction may be started by the release of the diphosphate group of FPP, leading to production of allylic farnesylation. Then a covalent bond may be formed between the C-1 atom of FPP and the C'-4 atom of IPP. By repetition of this stereochemically controlled reaction, the *cis*-prenyl chain is elongated [16].

The yeast *cis*-PTase gene *RER2* was found by functional complementation of the *rer2* mutant defective in the localisation of ER-resident proteins. Deletion of the gene resulted in a decrease of *cis*-PTase activity to 2.7% of the wild-type but *Δrer2* cells remain viable although they display a severe growth defect. The viability of the cells is most probably due to the existence of the *SRT1* gene, encoding an 'alternative' yeast *cis*-PTase [4,17,18]. Interestingly, we have observed induction of *SRT1* mRNA in *Δrer2* cells (Grabińska and Sosińska, unpublished results). Revertants of *RER2*-deleted cells as well as cells overexpressing *SRT1* in *Δrer2* background synthesise dolichols with a chain length similar to that of mammalian dolichols, containing 19–22 isoprene units, and these long-chain dolichols are utilised for protein *N*-glycosylation [17,18]. The latter is in agreement with the results of in vitro studies on the specificity of yeast glycosyl transferases for polyprenyl phosphates [19]. Subcellular localisation studies of 3HA-tagged Rer2p revealed that the protein is bound to ER membranes [4], although hydropathy analysis of Rer2p indicated absence of a hydrophobic re-

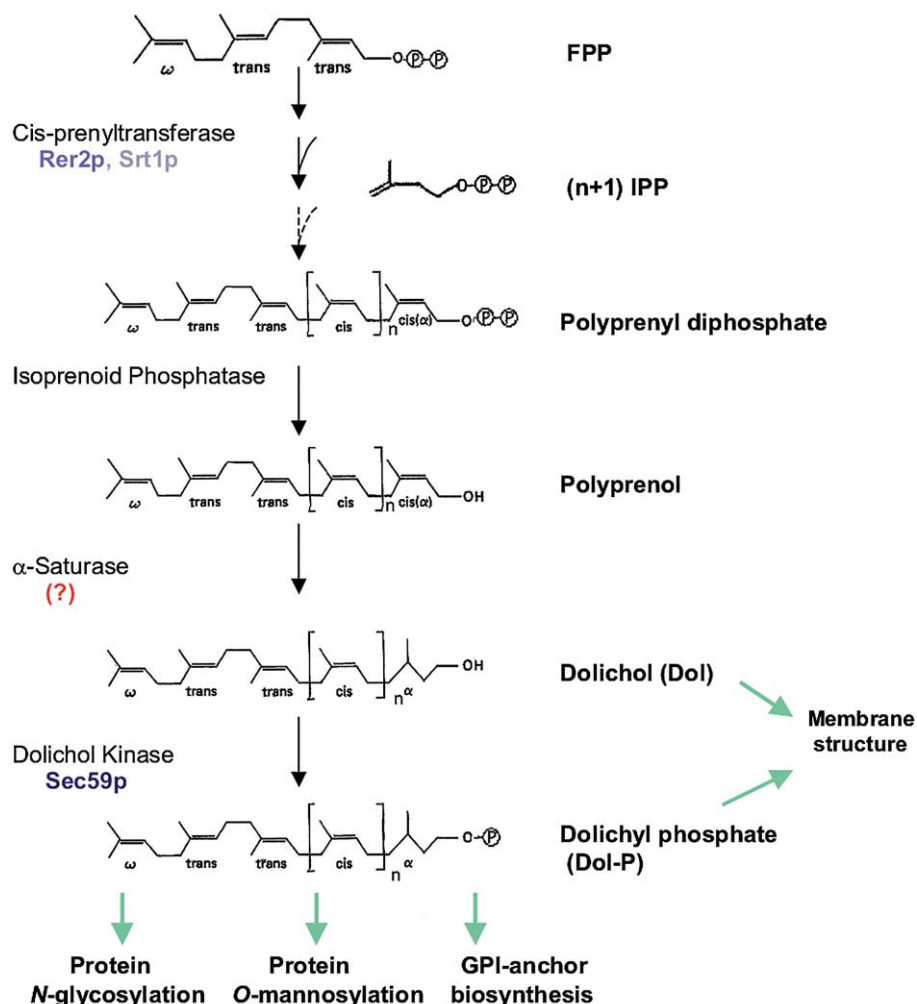


Fig. 2. Dolichyl phosphate biosynthesis de novo in yeast. $n = 10$ –14 isoprene units for Rer2p and 14–23 for Srt1p.

gion capable of acting as a signal sequence or transmembrane domain. Localisation of *cis*-PTase activity in the membrane fraction is in agreement with earlier results obtained in vitro [6,7,20]. Further studies of the green fluorescent protein fusion proteins Rer2p and Srt1p showed different localisation of the two *cis*-PTases. Immunofluorescent double staining with a marker of lipid particles (Erg6p) revealed that Srt1p is mainly localised to this compartment [18]. It was also demonstrated that Srt1p activity is up-regulated in the stationary phase, whereas Rer2p is active in the early stages of growth [18].

The reaction catalysed by *cis*-PTase leads to the synthesis of the dolichol carbon backbone, i.e. polyprenyl diphosphate (dehydro-dolichyl diphosphate) of appropriate chain length, followed by its conversion to dolichol and dolichyl phosphate. The length of dolichol molecules is species-specific and in yeast contains 14–18 isoprene units [5]. Although a great deal of progress has been made in the understanding of the enzymatic steps responsible for polyprenyl chain length termination and conver-

sion of dehydro-dolichol to dolichol, still some open questions remain. In vitro, cell extracts and membrane fractions from *S. cerevisiae* catalyse biosynthesis of dehydro-dolichols (α -unsaturated polyprenols) [20], whereas in vivo yeast synthesises dolichols (α -saturated polyprenols). Thus in *S. cerevisiae* polyprenyl diphosphates synthesised in vitro undergo immediate dephosphorylation [20,21]. A similar conclusion was reached for the rat liver system [22].

Recently a specific effect of biotinylated C₈₀ polyprenol on the reduction pathway of dehydro-dolichol has been described [23]. The results indicate that the reduction step proceeds with the recognition of the chain length of dehydro-dolichol by a 50-kDa protein. The authors suggest that this protein interacts with the hydrophobic part of C₈₀ dehydro-dolichol, of the maximal chain length, preventing further chain elongation. This observation supports our earlier hypothesis concerning the involvement of a specific protein complex in the final step of dolichol biosynthesis [24].

2. Farnesyl diphosphate synthase (FPPS): a regulatory role in the isoprenoid biosynthetic pathway

As was already mentioned, FPPS is the branch point enzyme in the biosynthetic pathway of isoprenoid lipids. As a result, this most widely distributed prenyltransferase, catalysing the sequential condensation of IPP with dimethylallyl diphosphate to form geranyl diphosphate (GPP), further elongated to FPP by addition of the next molecule of IPP, is essential in all organisms [25]. The yeast *ERG20* gene encodes a 40.5-kDa polypeptide of 342 amino acids with a high degree of similarity to FPPS from other organisms, as well as to various enzymes of the *trans*-prenyltransferase protein family, such as geranylgeranyl diphosphate synthases, and hexaprenyl diphosphate synthases. *trans*-Prenyltransferases, homodimers containing two characteristic aspartate-rich motifs: DDXX(X)D, are distinct from already described *cis*-prenyltransferases [8,10,26]. It is interesting to note, however, that a unique protein, forming Z- (*cis*) FPP from *Mycobacterium tuberculosis*, has been recently purified and characterised [27,28].

Determination of the crystal structure of avian FPPS to 2.6 Å resolution has shown that the subunits of the dimer are related by a perfect two-fold axis and in each of them 10 core helices are arranged around a large central cavity, where most of the conserved motifs are located [29]. In the yeast cell the end products of FPPS are FPP and GPP in a 75:25 ratio. A mutated form of FPPS (Lys¹⁹⁷Glu) encoded by the *erg20-2* allele was also described. The mutated FPPS has approximately 10 times lower specific activity and predominantly catalyses GPP formation (70%) [30].

FPP, in addition to being a precursor for a number of isoprenoid compounds, plays an important regulatory role in their synthesis. It ought to be stressed, however, that the regulation of the pathway exerted by FPP and/or an FPP-derived molecule is a part of an overall system of control and it has been demonstrated that in mammalian cells cholesterol metabolism is controlled by sterol-regulated proteolysis of membrane-bound transcription factors, sterol-regulatory element binding proteins (SREBS) [31]. A general principle for the regulation of this pathway is the existence of multiple levels of feedback inhibition. This feedback inhibition is directed to a variety of intermediates and therefore can act at numerous steps of the pathway, involving changes in transcription, translation and protein stability. In addition, the availability of molecular oxygen also regulates expression of the genes at the key steps of the pathway. Thus the regulatory role of oxysterols in the mevalonate pathway has been also underlined [32]. Experiments using Genome Reporter Matrix [33] confirmed the complexity of the regulation and indicated that multiple points of regulation act to control overall flux through the pathway as well as the relative flux through various branches of the pathway.

2.1. Regulated degradation of Hmg2p in *S. cerevisiae* is controlled by FPP-derived signal

The key enzyme of the isoprenoid pathway, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR), has been shown to be among the most tightly regulated enzymes in nature and determining the rate of cholesterol biosynthesis in mammals [32]. The feedback control of this enzyme occurs mostly by modulation of the amount of HMGR protein and a significant part of it proceeds via the regulation of HMGR degradation. The reductase is an ER membrane protein, and in *S. cerevisiae* two proteins (Hmg1p and Hmg2p) contribute to the overall enzyme activity. Hmg2p, in contrast to Hmg1p, is rapidly degraded with a half-life of 50–60 min. Its degradation, like in mammals, occurs without the exit of the protein from the ER. A genetic analysis aimed at selecting the *S. cerevisiae* mutants deficient in Hmg2p degradation led to the discovery of the *HRD1-3* genes that are involved in the regulated degradation of Hmg2p. Since one of the proteins (Hrd2p) has been predicted to be a component of the mature 26S proteasome, an involvement of proteasomal proteases in the regulated degradation of HMGR was suggested. In agreement with this were subsequent findings that Hmg2p degradation requires neither vacuolar proteases nor passage of the protein through the cell secretory pathway. Moreover, the regulated degradation of Hmg2p depends on its ubiquitination and the signal for this reaction derives from the isoprenoid pathway [34–36]. Lovastatin (a competitive inhibitor of HMGR) drastically increased Hmg2p half-life. A similar effect was observed in the Δ *erg13* mutant impaired in 3-hydroxy-3-methylglutaryl-coenzyme A synthase activity. Thus it was assumed that inhibition of the reaction leading to the synthesis of early intermediates of the pathway decreases the availability of the signal for Hmg2p degradation. On the other hand, presence of zaragozic acid, an inhibitor of squalene synthase, causing accumulation of FPP, increased its degradation. Those results implied that the signal for Hmg2p degradation was a pathway molecule between mevalonic acid and squalene (Fig. 1) [34–36]. It had been demonstrated earlier that in mammalian cells the signal for the regulation of HMGR stability derives from FPP [37,38]. Therefore the hypothesis that FPP or an FPP-derived molecule is the source of the positive signal for Hmg2p degradation in *S. cerevisiae* was tested and confirmed with the use of genetic methods as well as of inhibitors of the mevalonate pathway enzymes. Considering the mechanism by which FPP regulates Hmg2p degradation, several possibilities were offered [34]:

- FPP-derived signal acts as an allosteric regulator, binds to the transmembrane domain of Hmg2p and alters its susceptibility to degradation;
- FPP-derived molecule interacts with the effector protein that alters Hmg2p stability;
- FPP directly or via its derivative alters the structure of

ER membranes and in this way changes the susceptibility of Hmg2p to degradation.

In this context it is worth mentioning that addition of farnesol, which may be endogenously generated within cells by enzymatic dephosphorylation of FPP, to several cell lines in culture resulted in a rapid and dramatic inhibition of the synthesis of phosphatidylcholine (PC), the most abundant membrane lipid in eukaryotic cells [39,40]. Moreover, farnesol could affect membranes independently of the inhibition of PC synthesis, through a diacylglycerol-mediated process, which is downstream of PC synthesis [41]. Thus FPP-derived farnesol might indeed induce changes of Hmg2p stability due to the altered lipid composition of ER membranes. On the other hand, it has been demonstrated that in a phosphatase null mutant regulated degradation of HMG-R still occurs [34].

Moreover, farnesol has been shown to cause apoptotic death of human acute leukaemia cells [39,40] and growth inhibition of *S. cerevisiae* due to generation of reactive oxygen species [42], thus confirming the complexity of the regulatory effect of FPP and its derivatives.

2.2. Dolichol formation in *S. cerevisiae* is affected by FPPS overexpression

Overexpression of the *ERG20* gene or disruption of the *ERG9* gene, leading to the increase of FPP, increases the level of dolichol in the yeast cells [24,43,44]. These results could be explained by simple changes in the substrate level available for polyprenol synthesis.

On the other hand, overexpression of mutated FPPS in the yeast mutant impaired in squalene synthase activity (*erg9*) resulted in a 100-fold increase of dehydro-dolichol content, whereas the amount of dolichol was 10-fold increased [24]. Moreover, in addition to the typical yeast dolichols (C₇₀–C₈₀) the chain length of dehydro-dolichols increased up to C₁₃₅. The simplest explanation of those results is that the dehydro-dolichols (C₇₀–₈₀) of normal chain length were predominantly converted to dolichols but the longer species were not accepted as substrates by dehydro-dolichol reductase, indicating a chain length specificity of the enzyme. On the other hand, the occurrence of dehydro-dolichols instead of dolichols coincides with the massive increase of their synthesis. This might suggest that α -reduction is the rate-limiting step in the conversion of dehydro-dolichols to dolichols.

The molecular modeling of mutated FPPS encoded by the *erg20-2* allele [45] explained previously described changes in biochemical properties of the mutated enzyme [30] but did not give an answer with respect to the biosynthesis of longer species of polyprenol alcohol. Therefore it was proposed that increase of IPP in comparison to FPP could be the crucial factor in inducing biosynthesis of long polyprenols [45]. In rat liver microsomes an increased concentration of IPP over FPP caused in vitro a shift of the end products of *cis*-PTase from prenyl-17 and -18 to

prenyl-20–21 [46]. Biochemical and genetic characterisation of *RER*-deleted yeast strains proved, however, that at least in *S. cerevisiae* long polyprenols are simply the products of an alternative *cis*-PTase encoded by the *SRT1* gene [4,17,18]. Since the long chain α -dehydro-polyprenols are synthesised by Srt1p, which is localised in the lipid particles [18], the impairment in α -saturation might be due to the perturbed transport from the site of their synthesis to the ER where, most probably, the α -saturase is located.

Our recent data indicate that overexpression, under a strong inducible promoter, of the FPPS-encoding gene *ERG20* and its mutated allele *erg20-2* in wild-type yeast results in the induction of *SRT1* gene transcription and to a small extent (30–50%) increases *RER2* mRNA. On the other hand, *erg20-2*, but not *ERG20*, when overexpressed in Δ *rer2* cells, increased significantly (by 80% as compared with the wild-type) both *SRT1*-encoded *cis*-PTase activity and dolichol content (Grabińska et al., unpublished). To interpret the effect of FPPS overexpression on dolichol biosynthesis several possibilities have to be considered. As has already been mentioned, the wild-type and mutated FPPSs encoded by *ERG20* and *erg20-2*, respectively, differ in their catalytic properties. Thus when an FPPS-defective strain was complemented by overexpression of the *erg20-2* gene, the amount of FPP was not restored to the wild-type level [30]. Moreover, the wild-type FPPS synthesises FPP and GPP in the ratio 70:30 [30] whereas in wild-type yeast transformed with the *erg20-2* gene the ratio of FPP:GPP was almost 1:1 [30], (Grabińska, unpublished). Thus, although no physiological role of GPP in the yeast cell has been described so far, one cannot exclude that GPP rather than FPP induces *SRT1* transcription and affects *cis*-PTase activity of Srt1p as well as dolichol content. On the other hand, the effect of overexpression was measurable only if the *ERG20* or *erg20-2* genes were placed under the inducible *GAL4/CYC1* promoter, ensuring an at least 16-fold increase of the FPPS end products in the case of wild-type gene overexpression. Thus the lack of effect of *ERG20* overexpression on *cis*-PTase activity and dolichol content in Δ *rer2* might also result from the toxic effect of farnesol, accumulating in the cells, as a result of the massive increase of FPP and its subsequent dephosphorylation. Moreover, our data do not exclude the possibility that the synthesis of *SRT1* mRNA is repressed by glucose, since the induction of the gene expression is detectable only when the cells are grown on galactose.

Since FPP, a product of cytosolic FPPS, is utilised as a substrate in different cellular compartments, we speculated that FPPS might require a protein partner, regulating FPP distribution between the various branches of the mevalonate pathway. Putative proteins interacting with FPPS were first identified by the *S. cerevisiae*-based two-hybrid system [47]. In this search we found [48] the ER membrane-located Yta7 protein, a member of the CDC48/PAS1/SEC18 family of ATPases [49]. Deletion of the

YTA7 gene yielded a strain that was hypersensitive to ethanol and lovastatin, thus substantiating further the link of Yta7p with the mevalonate pathway.

Since Yta7p was predicted to be a part of the regulatory subunit of the 26S proteasome we considered briefly the possibility that stability of FPPS depends on the presence of Yta7p. A set of cycloheximide chase experiments with a ProtA-tagged version of FPPS, expressed in the $\Delta yta7$ mutant and in the wild-type yeast strain, ruled out this possibility. Moreover, in these experiments the FPPS, unlike Hmg2p, turned out to be a very stable protein, thus the idea of FPPS regulation via proteasome-dependent proteolysis seemed even more unlikely. A detailed analysis of the intermediates of the sterol pathway in the wild-type and $\Delta yta7$ strains revealed a doubling of the amount of squalene, although the amount of the end product of this branch of the pathway, ergosterol, was not changed. Simultaneously we observed a decrease of dolichol and *cis*-PTase activity in vitro in $\Delta yta7$. This effect was, however, corrected by the addition of exogenous FPP [48]. Our results might suggest that Yta7p governs FPP distribution between different branches of the mevalonate pathway, i.e. those leading to the synthesis of ergosterol and dolichol, respectively, and facilitates substrate availability for *cis*-PTase. Our hypothesis is supported by the fact that overexpression of the *ERG20* gene up-regulates dolichol biosynthesis. A comparison of gene expression revealed that inactivation of the *YTA7* gene leads also to an increase in *cis*-PTase (*RER2*) gene transcription. This could represent an attempt by the cell to compensate for the lower amount of the allylic starter for dolichol production.

The concomitant elevation of squalene and no alteration in the level of its sterol derivatives could be explained by the fact that the regulation of sterol biosynthesis in the terminal portion of the pathway represents an efficient mechanism by which the cell can control the production of sterol without disturbing the production of other essential mevalonate pathway products [50].

In conclusion, our data provide evidence that the ER-located Yta7 protein which belongs to the AAA protein family (ATPases associated with diverse cellular activities), might be involved in the partitioning of FPP between the ergosterol and polyprenol branch of the mevalonate pathway. Moreover, the level of FPPS products, i.e. FPP and GPP, plays an important regulatory role in modifying the polyprenol pattern. This is most probably achieved by the regulation of transcription of the *SRT1* gene (Grabińska et al., unpublished), encoding in yeast an 'alternative' *cis*-PTase, responsible for the synthesis of the mammalian-type long-chain polyprenols.

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