Acta Biochimica Polonica

Vol. 52 No. 1/2005

221–232

QUARTERLY

Functional relationships between the *Saccharomyces cerevisiae cis*-prenyltransferases required for dolichol biosynthesis✪

Kariona Grabińska[1,2], Grażyna Sosińska[1], Jacek Orłowski[1], Ewa Swiezewska[1], Thierry Berges[2], Francis Karst[2☼] and Grażyna Palamarczyk[1✉]

[1]*Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warszawa, Poland;*
[2]*Laboratoire de Genetique de la Levure, Université de Poitiers, France*

Received: 22 December, 2004; accepted: 18 February, 2005

Key words: yeast, *cis*-prenyltransferases, farnesyl diphosphate synthase, cross talk

In the yeast *Saccharomyces cerevisiae* the *RER2* and *SRT1* genes encode Rer2 and Srt1 proteins with *cis*-prenyltransferase (*cis*-PT-ase) activity. Both *cis*-PT-ases utilize farnesyl diphosphate (FPP) as a starter for polyprenyl diphosphate (dolichol backbone) formation. The products of the Rer2 and Srt1 proteins consist of 14–17 and 18-23 isoprene units, respectively. In this work we demonstrate that deletion or overexpression of *SRT1* up-regulates the activity of Rer2p and dolichol content. However, upon overexpression of *SRT1,* preferential synthesis of longer-chain dolichols and a decrease in the amount of the shorter species are observed. Furthermore, overexpression of the *ERG20* gene (encoding farnesyl diphosphate synthase, Erg20p) induces transcription of *SRT1* mRNA and increases the levels of mRNA for *RER2* and *DPM1* (dolichyl phosphate mannose synthase, Dpm1p). Subsequently the enzymatic activity of Rer2p and dolichol content are also increased. However, the amount of Dpm1p or its enzymatic activity remain unchanged.

The early steps in dolichol biosynthesis are identical to those leading to sterol and ubiquinone (Jung & Tanner, 1973; Adair & Cafmeyer, 1987). These biosynthetic path-

✪This work was supported by the State Committee for Scientific Research (KBN 6P04A 007)
☼Present address: Université Louis Pasteur, INRA, Colmar, France.
✉Correspondence to: Grażyna Palamarczyk, Institute of Biochemistry and Biophysics, A. Pawińskiego 5a, 02-106 Warszawa, Poland; tel: (48 22) 658 4702; fax: (48 39) 121 623; e-mail: gp@ibb.waw.pl
Abbreviations: *cis*-PT-ase, *cis*-prenyltransferase; DMAPP, dimethylallyl diphosphate; FPP, farnesyl diphosphate; GPI, glycosyl phosphatidyl inositol; GPP, geranyl diphosphate; IPP, isopentenyl diphosphate; OT, disacharyltransferase.

ways diverge after the synthesis of farnesyl diphosphate (FPP) by FPP-synthase encoded by the *ERG20* gene (see Fig. 1). Erg20p catalyses two sequential condensations of the 5-carbon unit dimethylallyl diphosphate (DMAPP) with isopentenyl diphosphate (IPP) to form geranyl diphosphate (GPP) and then GPP with another IPP molecule to form FPP. In wild type *S. cerevisiae* Erg20p produces FPP and GPP in the molar ratio 75:25 (Blanchard & Karst, 1993).

A *cis*-PT-ase is considered to be the first enzyme committed to dolichol biosynthesis (Adair & Cafmeyer, 1987), catalyzing 1′–4 condensation of farnesyl (di-*trans* prenyl) diphosphate (FPP) with 11 to 15 isopentenyl diphosphate (IPP) units to form polyprenyl diphosphate (dehydro- dolichyl diphosphate) of appropriate chain length. This reaction is followed by dephosphorylation and reduction of dehydro-dolichyl diphosphate to dolichol followed by rephosphorylation of the latter by CTP-dependent dolichol kinase (Szkopinska *et al.*, 1988; Sagami *et al.*, 1996; Burda & Aebi, 1999). Mono- and oligosaccharide derivatives of dolichyl phosphate are required in N-glycosylation, O-mannosylation and glycosyl phosphatidyl inositol (GPI) protein anchor formation.

The length of dolichol molecules synthesized in the yeast *S. cerevisiae* by Rer2 protein and most prevalent, at the logarithmic phase of growth (Sato *et al.,* 1999), is 14–17 isoprene units (C_{70}–C_{85}) (Jung & Tanner, 1973). *rer2* mutants of *S. cerevisiae* show characteristic phenotypes, i.e. slow and thermosensitive growth, sensitivity to hygromycin B and resistance to orthovanadate, abnormal accumulation of endoplasmic reticulum (ER) and Golgi membranes as well as defects in N- and O-glycosylation. Deletion of the *RER2* gene results in the slow growth and is concomitant with a decrease of *cis*-PT-ase activity to 2.7% of that in the wild type. However, after several generations some *RER2* deleted cells (Δ*rer2)* exhibit a wild type phenotype due to the compensatory effect of the *SRT1* gene, which is up-regulated in the absence of the *RER2* gene, and they synthesize dolichols that are unusually long for yeast and contain 19–22 isoprene units, similar to mammalian dolichols (Schenk *et al.,* 2001; Sato *et al.*, 2001). Rer2p and Srt1p are localized to different cellular compartments: Rer2p is linked to the outer side of the ER membrane, and Srt1p occurs in lipid bodies (Sato *et al.*, 1999; 2001). Induction of the *SRT1* gene, but not of *RER2,* is observed in the stationary phase of growth (Sato *et al.*, 2001). On the other hand, synthesis of long chain dolichols in early logarithmic phase upon transfer of yeast into starvation medium has been reported (Szkopinska *et al.,* 2002). Moreover, as we have described previously (Szkopinska *et al.,* 1997), overexpression of the *erg20-2* gene encoding mutated Erg20p (Lys197Glu substitution), which is defective in the enzymatic activity (Blanchard & Karst, 1993) results also in the synthesis of long chain polyprenols and dolichols, containing up to 24 isoprene residues in the logarithmic phase of growth. This effect was concomitant with an eight fold increase in the amount of dolichols and induction of the synthesis of poliprenols (dehydrodolichols) as compared to dolichols synthesized in the wild type yeast. Synthesis of long-chain polyprenols and dolichols was also observed upon overexpression of the *SRT1* gene in the Δ*rer2* cells (Sato *et al.,* 2001).

In the present work we demonstrate that dolichol biosynthesis in *S. cerevisiae* might be affected by a post-transcriptional interaction between *RER2* and *SRT1* encoded proteins or/and their end products. Moreover, overexpression of the *ERG20* gene specifically induces *SRT1* and up-regulates *RER2* and *DPM1* gene expression. In consequence, the activity of Rer2p and dolichol content are increased but the level of Dpm1p and its enzymatic activity remain unchanged.

In conclusion we discuss functional interactions between *cis*-PT-ases and FPPS or/and their products in dolichol biosynthesis.

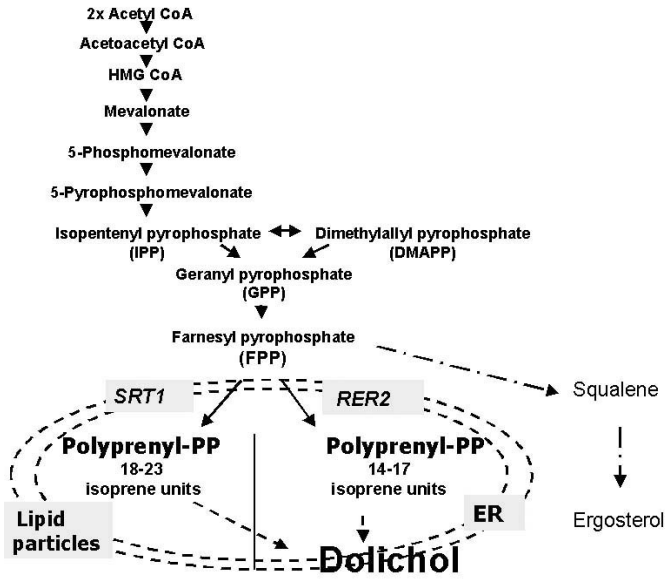

Figure 1. Outline of the dolichol biosynthetic pathway in *S. cerevisiae* according to Grabińska & Palamarczyk, 2002).

MATERIAL AND METHODS

Chemicals. All reagents were of analytical grade. Dehydro-dolichol and dolichol standards were obtained from the Collection of Polyprenols of the Institute of Biochemistry and Biophysics (Warsaw). [^{14}C]Isopentenyl diphosphate (58 mCi/mmol) was from Amersham, U.K.

Yeast strains and plasmids. The strains used in the study are listed in Table1. Plasmid pDD5 was described previously (Szkopinska *et al.,* 1997). p*SRT1* was constructed as follows: the *SRT1* gene coding sequence was amplified by PCR with oligonucleotides: SRT1-ATG: 5′-ATGAAAATGCCCAGTATT-3′ and SRT1-3: 5′ CCCGGG CTTTTACT TATTCATCTCC-3′, cloned into pGEM-T Easy Vector (Promega) and subcloned into the *Not*I restriction site of pNEV-1 replicative vector containing the *PMA1* yeast gene promoter and terminator (Palmgreen *et al.,* 1991).

Media and growth condition. Yeast cells were grown at 28°C in synthetic complete YNB (0.67% yeast nitrogen base and mixture of amino acids and nucleotides to final concentration 20–30 mg/l with nutrition markers omitted) medium with 2% glucose or galactose as a carbon source. Membrane fraction was prepared according to the method described in (Lehle & Tanner, 1974).

Northern blot analysis. Total RNA was prepared from exponentially growing yeast cells (A_{600} = 0.5–0.8) according to the method

Table 1. Yeast strains used in the study

Strain name	Genotype	Source
BY4742	Matα; *his3*Δ*1*; *leu2*Δ*1*; *lys2*Δ*1*; *ura3*Δ*1*;	EUROSCARF
BY4742pDD5	BY4742 2μ $p_{GAL/CYC1}$*ERG20*	This study
BY4742Δ*rer2*	as for BY4742, *rer2::kanMX4*	EUROSCARF
BY4742Δ*srt1,* ACC no Y16541	as for BY4742, *srt1::kanMX4*	EUROSCARF
BY4742p*SRT1*	As for BY4742 2μ p_{PMA1}*SRT1*	This study

described earlier (Cordier *et al.,* 1999). Twenty micrograms of total RNA were separated in a 1.2% denaturing agarose gel containing 12.32 M formaldehyde, and blotted onto a nylon membrane (Hybond N Amersham). Hybridization and washes were performed as described previously (Cordier *et al.,* 1999). Transcripts for the *ACT1, SRT1, RER2, ERG20, SEC59* and *DPM1* genes were visualized using specific [^{32}P]dATP-labeled DNA probes. Gene expression was quantified using the Gene Tools v. 3.00 (Syngene) computer software and presented as the ratio of mRNA of a given gene to the mRNA level of *ACT1*.

Western blot analysis. Membrane proteins from the strains tested were separated by SDS/PAGE electrophoresis at 20 μg per lane. Separated proteins were transferred to Immobilon^{TM} (Millipore) and probed with anti-*S. cerevisiae* Dpm1p monoclonal antibody (Molecular Probes) and anti-mouse IgG alkaline phosphatase conjugated (Sigma). Alkaline phosphatase activity was detected with the BCIP/NBT detection system (Promega) according to the manufacturer's protocol.

Cis-prenyltransferase activity. The incubation mixture contained, in a final volume of 250 μl, 50 mM sodium phosphate buffer, 0.5 mM $MgCl_2$, 20 mM 2-mercaptoethanol, 10 mM KF, 3×10^5 c.p.m. radiolabeled [^{14}C]IPP (58 mCi/mmol) and 500 μg of membrane protein. After 90 min of incubation at 30°C, the reaction was terminated by addition of 4 ml of chloroform/methanol (3:2, v/v). The mixture was washed three times with 1/5 volume of 10 mM EDTA in 0.9% NaCl. The organic phase was concentrated under a stream of nitrogen and subjected to thin-layer chromatography on HPTLC RP-18 plates. The gel from the zone containing radiolabeled polyprenols was scraped and subjected to liquid scintillation counting.

Farnesyl diphosphate synthase activity. Cells were grown in minimal medium. The cell-free extracts were prepared in 50 mM phosphate buffer, pH 7.5 (Cordier *et al.,* 1999). The reaction mixture (100 ml) containing 50 mM phosphate buffer, pH 7.5, 200 μM allylic substrate (DMAPP or GPP), 5 μM [^{14}C]IPP (58 mCi/mmol), 55 μM IPP, 1 mM $MgCl_2$, 5 mM iodoacetamide and 105 000 $\times$ ***g*** supernatant (10–60 μg of protein) was incubated at 37°C for 5 min and rapidly ice-chilled. Then 0.5 ml of water was added, followed by 1 ml of hexane and 0.2 ml of 1M HCl (for dephosphorylation), and the samples were shaken for 15 min at 37°C. The mixture was ice-chilled and vigorously mixed. The separated upper phase was washed three times with water and subjected to TLC on HPTLC RP-18 plates in acetone/water (5:2, v/v). Radioactive compounds, visualised by autoradiography, were identified by cochromatography with unlabeled standards, scraped from the plates and quantified by liquid scintillation counting.

Measurements of geraniol:farnesol ratio. The assay conditions were as described previously (Chambon *et al.*, 1990) with the following modifications: 200 μM DMAPP was used as allylic substrate and 11 μM [^{14}C]IPP as the homoallylic one; reaction time was 15 min. Reaction products after dephosphorylation and extraction into the hexane phase were analysed on HPTLC RP-18 plates (as above). Geraniol and farnesol spots were identified by internal standards, scraped from the plates and quantified by liquid scintillation counting.

Characterization of dolichols synthesized in vivo. The membrane fraction mixed with an internal standard (dolichol containing 11 isoprene units, C_{55}) was extracted with chloroform/methanol (3:2, v/v) and centrifuged. The organic supernatant was washed three times with 1/5 volume of 10 mM EDTA in 0.9% NaCl and evaporated to dryness. Subsequently lipids were subjected to alkaline hydrolysis. They were dissolved in a methanol/H_2O (10:1, v/v) mixture containing 15% KOH (w/v) and hydrolyzed at 95°C for 2 h. Lipophylic products were extracted with diethyl ether and evaporated to dryness, dis-

solved in hexane and applied on a silica gel column equilibrated with hexane. The column was washed with 6% diethyl ether in hexane. Subsequently the polyisoprenoid fraction was eluted with 20% diethyl ether in hexane and subjected to HPLC analysis.

Chromatography. Thin-layer chromatography was performed on silica gel plates in toluene/ethyl acetate (95:5, v/v) or on HPTLC RP-18 coated plates with a concentrating zone (Merck, Germany) in acetone with 50 mM H_3PO_4.

Quantification of dolichol was accomplished by HPLC equipped with a 4.6 × 60 mm ODS-Hypersil (3 mm) reversed-phase column (Knauer, Germany) using a Waters dual-pump apparatus, a Waters gradient programmer, and a UV detector set at 210 nm; flow rate 1.5 ml/min in gradient A: methanol/isopropanol/water (12:8:1, v/v), and solvent B: hexane/isopropanol (7:3, v/v); Dol-11 (C_{55}) solution was added to the samples as the internal standard.

Synthesis of glycosyl derivatives of dolichyl phosphate. Membrane fraction and the assay conditions for DolPMan and DolPP-$GlcNAc_{1-2}$ synthesis were as described before (Palamarczyk *et al.,* 1980). Synthesis of Dol-PGlc was measured according to (Palamarczyk *et al.,* 1990). Oligosaccharyl transferase (OT) activity was determined in the presence of DolPP[^{14}C]$GlcNAc_2$ as glycosyl donor and Tyr-Asn-Leu-Thr-Ser-Val as acceptor peptide, as described in (Sharma *et al.,* 1981).

RESULTS

Deletion of *SRT1* gene increases activity of *RER2*-encoded *cis*-PT-ase and dolichol content

Deletion of the *SRT1* gene (Δ*srt1*) resulted in a three-fold higher dolichol content (Fig. 2A). There was also an increase of the *RER2* encoded *cis*-PT-ase activity assayed *in vitro* in the presence of exogenous FPP (Fig. 2B). The effect of the *SRT1* gene deletion on dolichol content is post-transcriptional since we observe no changes in the level of *RER2* mRNA in Δ*srt1* as compared to wild type cells (Fig. 2C). As expected, all dolichols, synthesized in the absence of the gene, had a chain length typical for *RER2* en-

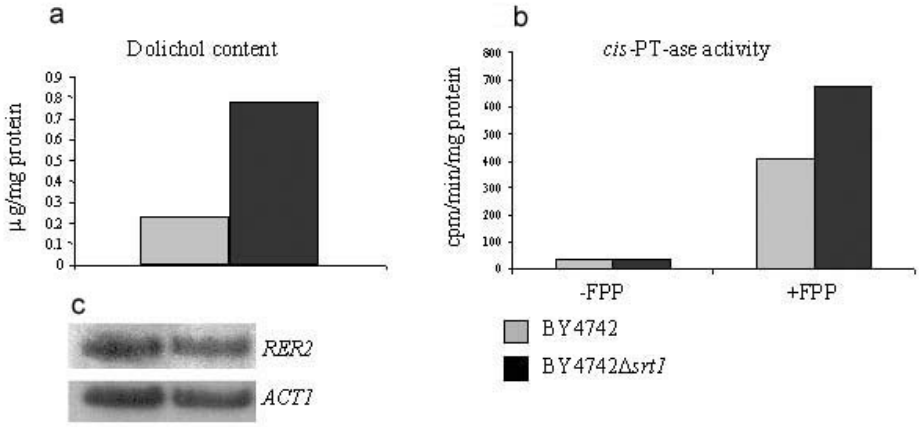

Figure 2. Deletion of the *SRT1* gene increases the activity of *RER2*-encoded *cis*-PT-ase and dolichol content.

Dolichol content (μg/mg of protein) (a); *cis*-PT-ase activity (b); assayed without (left panel) or with (right panel) exogenous FPP. The results represent means ±S.D. from four experiments. Northern blot analysis of *RER2* mRNA (c). RNA was extracted from yeast cells in logarithmic growth phase (A_{600}=0.5–0.8). The same blot was probed with ^{32}P-labelled PCR fragments representing the *RER2* and, as a control, the *ACT1* gene. Yeast strains: BY4742-wild type, BY4742Δ*srt1*-strain with *SRT1* gene deletion.

coded *cis*-PT-ase products i.e., 14–17 isoprene units (Fig. 3).

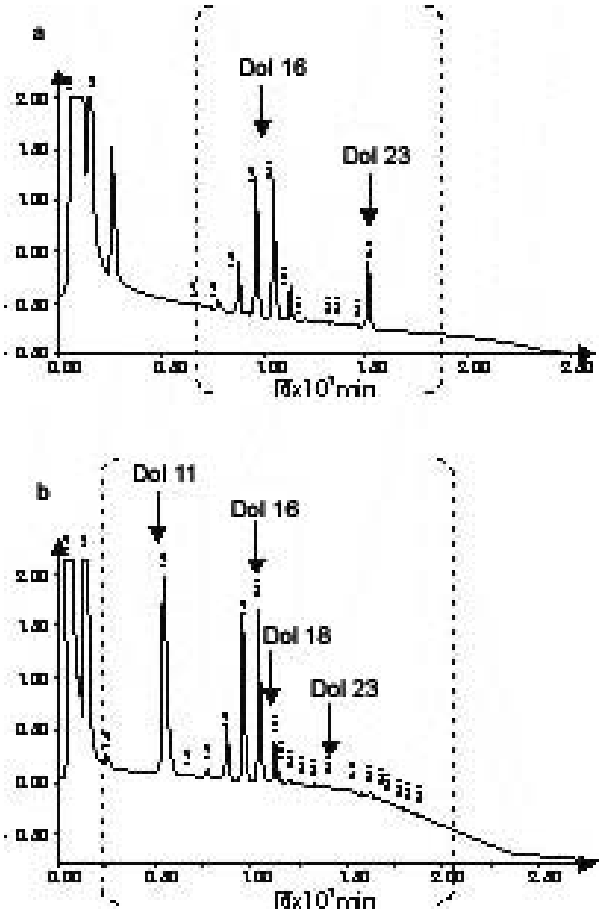

Figure 3. Inactivation of the yeast *SRT1* gene results in the synthesis of shorter dolichols, products of Rer2p.

(a) HPLC analyses of dolichols isolated from the BY4742Δ*srt1* revealed presence of the molecules made up from 14–17 isoprene units. Dolichol containing 23 isoprene units (RT 15,1) was added as internal standard. (b) HPLC analyses of dolichols from wild type strain. In addition to the *RER2* gene products (as in a) the molecules containing 18–23 isoprene, products of Srt1p, are visible. Dolichol containing 11 isoprene residues was added as internal standard.

Overexpression of *SRT1* gene changes the pattern of yeast dolichols

The *SRT1* gene was overexpressed in the wild type strain from a multicopy plasmid under the *PMA1* (**P**lasma **M**embrane H^+ **A**TP-ase) promoter. The dolichol pattern shifted towards longer, Srt1-synthesized species, containing 18–23 isoprene residues (Fig. 4). Moreover, quantification of the products indicated an over a three-fold increase, as compared to the wild type strain, of the total dolichol content and up to 24% reduction in

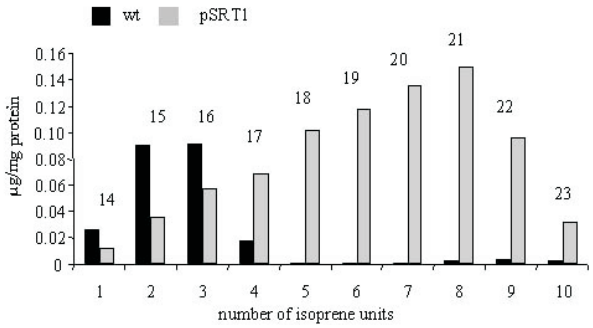

Figure 4. Overexpression of the *SRT1* gene changes the yeast dolichol pattern.

The *SRT1* gene, in multicopy pNEV-plasmid under *PMA1* (Plasma Membrane H^+ ATPase) promoter, was over-expressed in the wild type BY4742 strain. Dolichols were extracted, hydrolyzed and subjected to HPLC analysis (Material and Methods). Quantities of dolichols, composed of the given number of isoprene residues, are expressed in μg/mg protein in the membrane fraction. The results presented are mean values from two experiments.

the amount of dolichols synthesized by Rer2p, i.e. those containing 14–17 isoprenyl residues. *Cis*-prenyltransferase activity *in vitro* was consistenlly, although moderately increased, but only in the presence of exogenous FPP in the assay (Fig. 5A). HPTLC analysis of the products synthesized *in vitro* (Fig. 5B) is in agreement with the HPLC analysis of dolichols presented in Fig. 3 showing that overexpression of the *SRT1* gene in wild type yeast leads to the synthesis of longer dolichols containing 18–23 isoprene units. On the other hand, it has been reported (Schenk *et al.,* 2001) that deletion of the *RER2* locus is a prerequsite for *SRT1* gene expression, whereas in our hands (Fig. 4) overexpression of *SRT1* gene in wild type

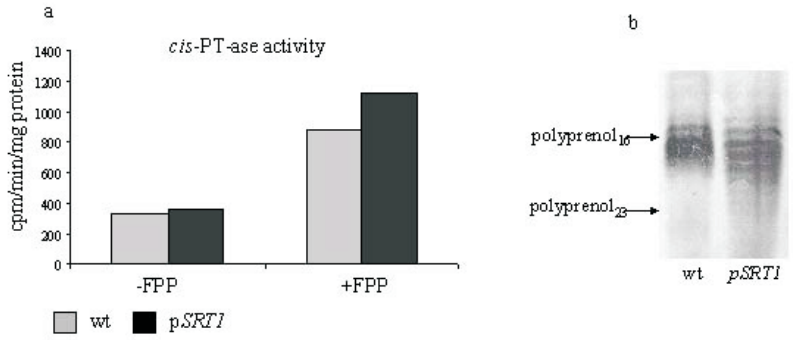

Figure 5. Overexpression of *SRT1* affects *cis*-PT-ase activity *in vitro*.

cis-PT-ase activity assayed without (left) or with (right) exogenous FPP (a); the results represent means ±S.D. from four experiments. HPTLC analysis of polyprenols synthesized in wild type cells and in cells with the *SRT1* gene overexpressed (b).

yeast also leads to the synthesis of the longer polyprenol species synthesized by the *SRT1*-encoded *cis*-prenyltransferase.

Elevated dosage of Erg20p induces *SRT1* and up-regulates *RER2* and *DPM1* transcription

Overexpression of the *ERG20* gene under the inducible *GAL4/CYC1* promoter in wild type *S. cerevisiae*, concomitant with a 16-fold increase in FPP and GPP content (Table 2), resulted in the induction of *SRT1* mRNA, similar to the one observed upon inactivation of the *RER2* gene (Fig. 6A). It is noteworthy that the *SRT1* gene induction was observed in the logarithmic phase of growth. Elevated dosage of *ERG20* resulted also in an increase of *RER2* and *DPM1* mRNA by 20–40% and 50–80%, respectively (Fig. 6B, C). At the same time no changes were observed in *SEC59* mRNA (encoding dolichol kinase).

In the wild type strain Erg20p produces FPP and GPP in the molar ratio 3 : 1 (Blanchard &

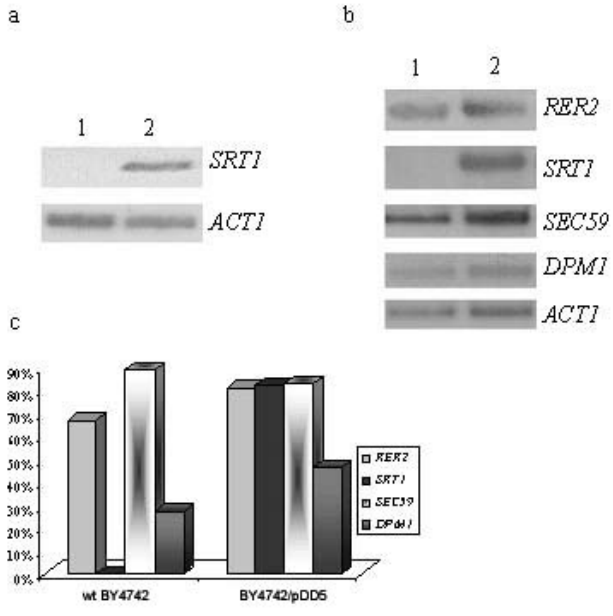

Figure 6. Overexpression of the *ERG20* gene induces *SRT1* and up-regulates *RER2* and *DPM1* transcription in wild type *S. cerevisiae*.

RNA was extracted from yeast cells in logarithmic growth phase (A_{600} 0.5–0.8) cultivated on YNB medium, without nutritional markers, containing 2% galactose as a carbon source. Induction of *SRT1* mRNA in Δ*rer2* cells (a). *RER2, SRT1, SEC59* and *DPM1* genes' mRNA in wild type cells and those transformed with the *ERG20* gene (b). The same blot was probed successively with ^{32}P-labelled PCR fragments representing the *RER2, SRT1, SEC59, DPM1* and, as a control, *ACT1* genes. Quantification of the results from Fig. 4b, (c). Gene expression was measured using the Gene Tools v. 3.00 (Syngene) computer software and presented as the ratio of mRNA of a given gene/*ACT1* mRNA level. Expression in the wild type yeast strain BY4742 was taken to be 100%, with the exception of the *SRT1* mRNA level, where 100% corresponds to expression in BY4242 transformed with the pDD5 (*ERG20*) plasmid.

Table 2. Effect of *ERG20* overexpression on FPPS activity and *SRT1* mRNA level

Yeast strain	FPPS activity (c.p.m./min per mg protein)	GPPS activity of FPPS (c.p.m./min per mg protein)	FPP/GPP ratio	*SRT1* mRNA level
BY4742	100 960 (±24 956)	43 268	2.3	not detectable
BY4742pDD5 (*ERG20)*	1617 900 (±413 706)	726 883	2.2	100%

FPPS activity was measured using [^{14}C]IPP and GPP as substrates. Reaction products were extracted and analysed as described in Materials and Methods. Data represent means ±S.D. from four experiments. The yeast cells were grown on the synthetic complete (YNB) medium containing galactose as a carbon source.

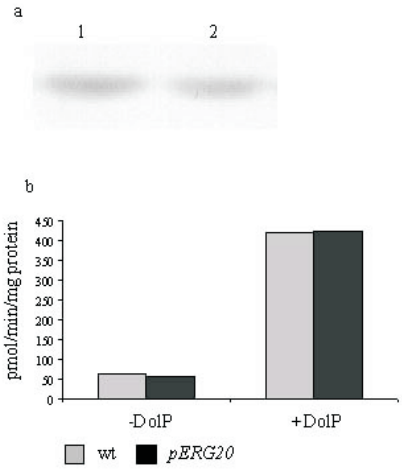

Figure 7. Increase in *DPM1* mRNA does not correlate with Dpm1 protein level and its enzymatic activity.

Western blot analysis of Dpm1p expression level (a) and enzymatic activity (b) 1, wild type; 2, transformed with the *ERG20* gene under *GAL4/CYC1* inducible promoter. Results of the activity assay represent means ±S.D. from four experiments.

Karst, 1993). We assumed, however, that FPPS activities for FPP and GPP formation might change upon Erg20p over-expression. Thus, we compared the enzyme activity in a wild type strain and one with an elevated dosage of the *ERG20* gene. As indicated in Table 2, the strain BY4742pDD5 (*ERG20* overexpressed under the *GAL4/CYC1* promoter) showed a sixteen-fold higher FPP concentration, but at the same time the ratio of FPP to GPP remained almost identical as in the wild type control (BY4742).

We also analyzed by Western blotting the Dpm1 protein level and its enzymatic activity by incubation of the 50 000 × ***g*** membrane fraction from cells overexpressing the *ERG20* gene with GDP[^{14}C]Man. Despite increased *DPM1* mRNA, Dpm1 protein level and its enzymatic activity remained unchanged as compared to wild type cells (Fig. 7). The ability to synthesize dolichyl phosphate glucose, dolichyl diphosphate *N*-acetylglucosamine and the transfer of disaccharide from DolPPGlcNAc$_2$ to the acceptor peptide Tyr-Asn-Leu-Thr-Ser-Val was also assayed. An up to 10% decrease of the activity of the glycosyl transfer catalyzed by the membranes from *ERG20*-transformed strain, was observed, compared to the wild type.

DISCUSSION

In the present work we provide evidence for a functional interaction between the two *S. cerevisiae* proteins with a *cis*-PT-ase activity, i.e. Rer2p and Srt1p. Deletion of the *SRT1* gene increases dolichol content up to three-fold. The *cis*-PT-ase activity measured *in vitro* in the presence of exogenous FPP is also increased. Since Rer2p and Srt1p are localized to different cellular compartments (Sato

et al., 1999; 2001), Rer2p is unlikely to be regulated by a direct interaction with Srt1p. Instead, one can speculate that the enzymatic products of Srt1p, i.e. long-chain dolichols regulate the activity of Rer2p. We have suggested earlier that a specific protein complex is involved in the final step of dolichol biosynthesis (Szkopinska *et al.,* 1997). This is in agreement with the finding (Tateyama & Sagami, 2001) on the specific effect of biotinylated C_{80} polyprenal on the conversion of dehydro-dolichol to dolichol. Tateyama and Sagami's results indicate that the reduction step in the dolichol biosynthetic pathway 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 C_{80} dehydro-dolichol, the product with maximal chain length, preventing its further elongation. Since a massive increase of long-chain polyprenol alcohols in yeast cells is concomitant with their lack of α-saturation (Sato *et al.*, 2001; Szkopinska *et al.,* 1997), it might be that the interaction of Srt1p end products with Rer2p prevents further elongation of dehydro-dolichols and thus ensures saturation of their α-residues.

Deletion of the *SRT1* gene increases dolichol synthesis but does not influence the formation of glycosylated dolichyl phosphate. This result suggests that although the *RER2*-encoded *cis*-PT-ase activity is up-regulated in the absence of the Srt1 protein product, the effect of *SRT1* gene deletion would only be restricted to the one protein, i.e. Rer2p. Thus, we observe unchanged expression of *SEC59* mRNA, encoding dolichol kinase in these cells and an almost unchanged activity of enzymes involved in the synthesis of glycosylated dolichyl phosphate. Overexpression of the *SRT1* gene in wild type strain also increases dolichol formation. The over three-fold increase is due to the synthesis of the longer (18–24 isoprene units) polyprenols which are the products of *SRT1*-encoded *cis*-PT-ase. Simultaneously, an over 20% decrease of Rer2p products (dolichols comprising 14–17 isoprene units) is observed. As mentioned in the Results section these results differ from those described by Shenk *et al.* (2001). Since the behaviour of *RER2* depends on the genetic background (Belgareh-Touze *et al.,* 2003) the discrepancy might be due to the different genetic background of the strains used in the experiments.

In this work we also provide evidence that the Erg20 protein not only synthesizes the substrate for the Rer2 and Srt1 proteins but also plays a regulatory role in the biosynthetic pathway of polyprenyl diphosphate which is the dolichol backbone.

Elevated dosage of the *ERG20* gene, which coincides with 16-fold increase of FPP and GPP level in the cell, induces expression of *SRT1,* encoding an alternative *cis*-PT-ase, in the early logarithmic phase of growth (Table 2). Simultaneously we observe a small induction of *RER2* and *DPM1* expression (Fig. 5) and no effect on the *SEC59* gene. On the other hand, overexpression of *ERG20* has no effect on Dpm1 protein expression or DolPMan formation. This result is in agreement with our recent observation that Dpm1p expression is also regulated post-transcriptionally (Janik *et al.*, manuscript in preparation).

Erg20p is a branch point enzyme in the biosynthetic pathway of isoprenoid lipids i.e. sterols, polyprenols, ubiquinone, heme and prenylated proteins (Song & Poulter, 1994). FPP is also a component of the overall system of control of sterol biogenesis (Brown & Goldstein, 1997). The most highly regulated enzyme of the isoprenoid pathway, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR), has been shown to be regulated by feedback-control of its degradation (Goldstein & Brown, 1990). In mammalian cells the signal for regulation of HMGR stability derives from FPP (Ericsson *et al.,* 1996a; 1996b). In the yeast *S. cerevisiae,* proteasome dependent, regulated degradation of Hmg2p (one of the two proteins contributing to HMGR activ-

ity) is also controlled by an FPP-derived signal (Hampton *et al.,* 1996; Gardner & Hampton, 1999). Moreover, farnesol (FOH), which may be endogenously generated within the cells by enzymatic dephosphorylation of FPP, has been shown to cause apoptotic cell death of human acute leukemia cells (Hang *et al.,* 1994; Melnykovych *et al.,* 1992) and growth inhibition of *S. cerevisiae* due to generation of reactive oxygen species (Machida *et al.,* 1998). All these results confirm the complexity of the regulatory effect of FPP and its derivatives. However, nothing is known about the regulatory effect of FPP on the *cis*-polyprenol (dolichol) branch of the mevalonate pathway. Our results indicate that Erg20p products specifically affect the synthesis of dolichol backbone. Since wild type yeast produce FPP and GPP (Blanchard & Karst, 1993) it is plausible that an intermediate in FPP biosynthesis, i.e. GPP or its derivatives, might also affect the expression of *cis*-PT-ase genes. Although no physiological role for GPP in yeast or mammalian cells has been described so far, it has been demonstrated that GPP together with FPP inhibit human mevalonate kinase activity *in vitro* through a competitive interaction at the ATP-binding site (Hinson *et al.,* 1997).

In conclusion, our results suggest an indirect interaction between the yeast *RER2*- and *SRT1*-encoded proteins at a post-transriptional level. Furthermore, we observe a regulatory effect of elevated dosage of the *ERG20* gene on the *SRT1* and *RER2* gene expression, thus we postulate that the final steps of dolichol biosynthesis are controlled by the specific inter-dependence of Erg20, Rer2 and Srt1 proteins or/and their products.

We thank Dr Ludwig Lehle from the University of Regensburg for the help with oligosaccharyltransferase assay. The help of Dr. Phillip W. Robbins and Dr. Dorothy D. Pless in critical reviewing of the manuscript is gratefully acknowledged.

REFERENCES

Adair WL, Cafmeyer N. (1987) Characterization of the *Saccharomyces cerevisiae* cis-prenyltransferase required for dolichyl phosphate biosynthesis. *Arch Biochem Biophys.*; **259**: 589–96.

Belgareh-Touze N, Corral-Debrinski M, Launhardt H, Galan JM, Munder T, Le Panse S, Haguenauer-Tsapis R. (2003) Yeast functional analysis: identification of two essential genes involved in ER to Golgi trafficking. *Traffic.*; **4**: 607–17.

Blanchard L, Karst F. (1993) Characterization of a lysine-to-glutamic acid mutation in a conservative sequence of farnesyl diphosphate synthase from *Saccharomyces cerevisiae. Gene.*; **125**: 185–89.

Brown MS, Goldstein JL. (1997) The SREBP Pathway: regulation of cholesterol metabolism by proteolysis of membrane bound transcription factor. *Cell.*; **89**: 331–40.

Burda P, Aebi M. (1999) The dolichol pathway of N-linked glycosylation. *Biochim Biophys Acta.*; **1426**: 239–57.

Chambon C, Ladeveze V, Oulmouden A, Servouze M, Karst F. (1990) Isolation and properties of yeast mutants affected in farnesyl diphosphate synthetase. *Curr Genet.*; **18**: 41–6.

Cordier H, Karst F, Berges T. (1999) Heterologous expression in *Saccharomyces cerevisiae* of an *Arabidopsis thaliana* cDNA encoding mevalonate diphosphate decarboxylase. *Plant Mol Biol.*; **39**: 953–67.

Ericsson J, Jackson SM, Edwards PA. (1996a) Synergistic binding of sterol regulatory element-binding protein and NF-Y to the farnesyl diphosphate synthase promoter is critical for sterol-regulated expression of the gene. *J Biol Chem.*; **271**: 24359–64.

Ericsson J, Jackson SM, Lee BC, Edwards PA. (1996b) Sterol regulatory element binding protein binds to a cis element in the promoter of the farnesyl diphosphate synthase gene. *Proc Natl Acad Sci USA.*; **93**: 945–50.

Gardner RG, Hampton RY. (1999) A highly conserved signal controls degradation of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase in eukaryotes. *J Biol Chem.*; **274**: 31671–8.

Goldstein JL, Brown MS. (1990) Regulation of mevalonate pathway. *Nature.*; **343**: 425–30.

Grabińska K, Palamarczyk G. (2002) Dolichol biosynthesis in the yeast *Saccharomyces cerevisiae*: an insight into regulatory role of farnesyl diphosphate synthase. *FEMS Yeast Res.*; **2**: 259–65.

Hampton RY, Gardner RG, Rine J. (1996) Role of 26S proteasome and *HRD* genes in the degradation of 3-hydroxy-3-methyl-glutaryl-CoA reductase, an integral endoplasmic reticulum membrane protein. *Mol Biol Cell.*; **7**: 2029–44.

Hang JS, Goldner CM, Yazolvitskaya EM, Voziyan PA, Melnykovych G. (1994) Direct cell killing (apoptosis) in human lymphoblastoid cells incubated in the presence of farnesol; effect of phosphatidyl-choline. *Biochim Biophys Acta.*; **1223**: 133–40.

Hinson DD, Chambliss KL, Toth MJ, Tanaka RD, Gibson KM. (1997) Post-translational regulation of mevalonate kinase by intermediate of the cholesterol and nonsterol isoprene biosynthetic pathway. *J Lipid Res.*; **38**: 2216–23.

Jung P, Tanner W. (1973) Identification of the lipid intermediate in yeast mannan biosynthesis. *Eur J Biochem.*; **37**: 1–6.

Lehle L, Tanner W. (1974) Membrane bound mannosyl transferase in yeast glycoprotein biosynthesis. *Biochim Biophys Acta.*; **350**: 225–35.

Machida K, Tanaka T, Fujita K-I, Taniguchi M. (1998) Farnesol-induced generation of reactive oxygen species via indirect inhibition of the mitochondrial transport. *J Bacteriol.*; **180**: 4460–5.

Melnykovych G, Haug JS, Goldner CM. (1992) Growth inhibition of leukemia cell line CEM-C1 by farnesol: effects of phosphatidylcholine and diacylglycerol. *Biochem Biophys Res Commun.*; **186**: 543–48,

Palamarczyk G, Lehle L, Mankowski T, Chojnacki T, Tanner W. (1980) Specificity of solubilized yeast glycosyl transferases for polyprenyl derivatives. *Eur J Biochem.*; **105**: 517–23.

Palamarczyk G, Drake R, Haley B, Lennarz WJ. (1990) Evidence suggesting that the synthesis of glucosyl phosphoryl dolichol in yeast microsomal membranes involves a 35 kDa protein. *Proc Natl Acad Sci USA.*; **87**: 2666–70.

Palmgreen MG, Sommarin M, Serrano R, Larsson C. (1991) Identification of an autoinhibitory domain in the C-terminal region of the plasma membrane H(+) ATPase. *J Biol Chem.*; **266**: 20470–75.

Sagami HAY, Igarashi S, Tateyama K, Ogura K, Roos J, Lennarz WJ. (1996) Enzymatic formation of dehydrodolichal and dolichal, new products related to yeast dolichol biosynthesis. *J Biol Chem.*; **271**: 9560–6.

Sato M, Sato K, Nishikawa Si, Hirata A, Kato Ji, Nakano A. (1999) The yeast *RER2* gene identified by endoplasmic reticulum protein localization mutations encodes *cis*-prenyl-transferase, a key enzyme in dolichol biosynthesis. *Mol Cell Biol.*; **19**: 471–83.

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

Schenk B, Rush JS, Waechter CJ, Aebi M. (2001) An alternative *cis*-isoprenyltransferase activity in the yeast that produces polyprenols with chain length simmilar to mammalian dolichols. *Glycobiology.*; **11**: 89–98.

Sharma CB, Lehle L, Tanner W. (1981) N-glycosylation of yeast proteins; characterization of the solubilized oligosaccharyl-transferase. *Eur J Biochem.*; **116**: 101–8.

Song L, Poulter CD. (1994) Yeast farnesyl-diphosphate synthase: site directed

mutagenesis of residues in highly conserved prenyltransferase domains I and II. *Proc Natl Acad Sci USA.*; **91**: 3044–8.

Szkopińska A, Nowak L, Swiezewska E, Palamarczyk G. (1988) CTP-dependent lipid kinases of yeast *Saccharomyces cerevisiae*. *Arch Biochem Biophys.*; **266**: 124–31.

Szkopińska A, Grabińska K, Delourme D, Karst F, Rytka J, Palamarczyk G. (1997) Polyprenol formation in the yeast *Saccharomyces cerevisiae*; effect of farnesyl diphosphate synthase overexpression. *J Lipid Res.*; **38**: 962–8.

Szkopińska A, Świeżewska 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 aging. *Acta Biochim Polon.*; **49**: 781–7.

Tateyama S, Sagami H. (2001) Study on the biosynthesis of dolichol in yeast: recognition of the prenyl chain length in polyprenol reduction. *J Biochem.*; **129**: 297–302.