

Short-chain polyisoprenoids in the yeast *Saccharomyces cerevisiae* - new companions of the old guys

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Abstract

Dolichols are, among others, obligatory cofactors of protein glycosylation in eukaryotic cells. It is well known that yeast cells accumulate a family of dolichols with Dol-15/16 dominating while upon certain physiological conditions a second family with Dol-21 dominating is noted. In this report we identified the presence of additional short-chain length polyprenols - all-*trans* Pren-7 in three yeast strains (SS328, BY4741 and L5366), Pren-7 was accompanied by traces of putative Pren-6 and -8. Moreover, in two of these strains a single polyprenol mainly-*cis*-Pren-11 was synthesized at the stationary phase of growth. Identity of polyprenols was confirmed by HR-HPLC/MS, NMR and metabolic labeling. Additionally, simvastatin inhibited their biosynthesis.

Short title: Short-chain polyisoprenoids in yeast

Keywords

polyisoprenoid alcohols; dolichol; prenol; *Saccharomyces cerevisiae*, yeast

Abbreviations

HPLC, High Performance Liquid Chromatography; LC/MS, Liquid Chromatography – Mass Spectrometry; NMR, Nuclear magnetic resonance; IPP, isopentenyl diphosphate; i.u., isoprenoid unit; Pren/ Dol-n, prenol/dolichol composed of n i.u.;

Highlights

This is the first report describing the presence of short-chain length polyprenols, besides dolichols, in *S. cerevisiae*.

Tested strains accumulate an all-*trans* Pren-7 accompanied by traces of Pren-6 and -8 at the logarithmic phase of growth.

Some *S. cerevisiae* strains accumulate a single mainly-*cis*-Pren-11 at the stationary phase of growth.

1. Introduction

Polyisoprenoid alcohols, linear five-carbon unit mainly-*cis* polymers, are widespread components of all living organisms. These molecules consist of up to 100 isoprene residues with either a hydrogenated (dolichols) or an unsaturated (polyprenols) double bond in the α -terminal residue. Dolichols are present mainly in animal and yeast cells and in plant roots

whereas polyprenols - in bacteria and plant photosynthetic tissues. Polyisoprenoids are accumulated in the form of free alcohols and/or esters of carboxylic acids along with a small fraction of mono- and diphosphates. The content of polyisoprenoids increases during the life span of organisms, upon pathological conditions and environmental stress [for review see 1 – 5].

Dolichols participate in vital cellular events, such a protein *N*-, *O*- and *C*-glycosylation [2, 6-9], intracellular protein transport [10, 11] and protein prenylation [12]. They are also postulated to play a part in cell adaptation to adverse conditions [13].

Isopentenyl diphosphate (IPP) molecules built into the polyisoprenoid hydrocarbon skeleton can be synthesized *via* two different pathways: the mevalonate (MVA) pathway and the 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway. The MVA pathway is present in the majority of organisms, including yeasts [14], animals [15] and some gram-positive bacteria [16], whereas most gram-negative bacteria (e.g., *Escherichia coli* and *Bacillus subtilis*) [17] and green algae use only the MEP pathway [18]. In plants both pathways, the MVA and MEP are used to synthesize dolichols [19].

Polyisoprenoids are typically accumulated in cells as homologous mixtures (families) with one or more components dominating. Unique in this context are bacteria possessing a single polyprenol (Pren-11). Dolichol families consist of six to eight members and the size of dolichols is variable depending on the species. Under certain circumstances, e.g. during stationary phase or upon carbon starvation *Saccharomyces cerevisiae* cells accumulate a two-family mixture of dolichols: the family of dolichols, from Dol-14 to Dol-18, with Dol-15 or Dol-16 dominating and the family of longer ones, from Dol-19 to Dol-32, with Dol-21 dominating [20 – 22], while upon exponential growth only the former family is detectable. Similarly to yeast, the family of dolichols (Dol-14 to Dol-18) with Dol-17 and Dol-16 dominating is detected in the tissue of the mushroom *Lentinus edodes* [23] and in the human fungal pathogen *Candida albicans* [24], respectively. Dolichols detected in the fish liver are composed of 19 – 22 isoprene units with Dol-20 as the predominant prenologue [25]. Mammalian cells contain one family of dolichols, Dol-19 is dominating in human cells whereas Dol-18 in rat. Interestingly, three families of dolichols are present in the hairy culture of *Arabidopsis* roots: short-chain (Dol-12 to Dol-14), medium-chain (Dol-15 to Dol-18) and long-chain (Dol-19 to Dol-30) with Dol-13, -16 or Dol-21/-23 dominating, respectively [26]. Moreover, a small amount of Dol-7, a representative of very short-chain polyisoprenoids, is also detected [27]. In contrast to dolichols, the diversity of polyprenol families isolated from

photosynthetic tissues is much broader. The multi-family mixtures with the chain length ranging from Pren-6 up to Pren-130 are sometimes noted [summarized in 4].

In this study we describe the occurrence of short chain polyisoprenoid alcohols in three strains of *S. cerevisiae*. Their structure was confirmed by high resolution mass spectrometry analysis. Moreover, incorporation of [³H] mevalonate, a metabolic precursor of isoprenoids, into their molecules further clearly shows that yeast cells synthesize a family of short polyprenols (Pren-6 – Pren-9) and, in some strains also a single polyprenol Pren-11. Additionally, simvastatin inhibited their biosynthesis. To our knowledge this is the first report showing the occurrence of short-chain polyprenols in *S. cerevisiae* cells.

2. Materials and methods

2.1. Yeast strains and growth conditions

S. cerevisiae strains used in this study were of wild type: SS328 (*MATalpha ade2-101 his3-delta200 lys2-801 ura3-52*), BY4741 (*MATa his3delta1 leu2delta0 met15delta0 ura3delta0*) and L5366 (*MATa/MATalpha deltawhi2::URA3/deltawhi2::URA3*) were obtained from ATCC – LGC Standards (Lomianki, Poland). The inocula of *S. cerevisiae* were added to liquid YPD medium (1% yeast extract, 1% bacto peptone, 2% dextrose) and incubated with shaking at 28°C for 1 to 4 days to logarithmic (4.6×10^7 cells per ml) or early stationary phase (1.2×10^8 cells per ml), respectively. OD₆₀₀ and polyisoprenoid content were measured after each 24 h.

2.2. Chemicals

All polyprenol and dolichol standards were from the Collection of Polyprenols of the Institute of Biochemistry and Biophysics (Warsaw, Poland). Simvastatin was from Calbiochem-Merck Co. (Darmstadt, Germany).

[³H]mevalonolactone was synthesized using sodium boro[³H]hydride (spec. act. 10 Ci/mmol, Perkin Elmer) as described earlier [28].

Components of growth media were purchased from BioShop (Burlington, Canada).

HPLC solvents were from POCh (Gliwice, Poland).

Chromatographic materials were from Merck (Darmstadt, Germany). All other chemicals were of p.a. quality and were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

2.3. Extraction and alkaline hydrolysis of lipids

Yeast cells were harvested by centrifugation at 3300xg (Allegra; Beckman) for 10 min at RT, washed once with water and incubated at 95°C for 1 h in 10 ml of hydrolytic solution (25 g of KOH, 35 ml of distilled water, brought to 100 ml with 99.8% ethanol) [29]. Nonsaponifiable lipids were then extracted three times with hexane, pooled extracts were evaporated to dryness in a stream of nitrogen, dissolved in 1 ml of hexane and loaded on a silica gel column which was subsequently eluted using isocratic elution with 10% diethyl ether in hexane. Purified polyisoprenoids were analyzed by HPLC/UV using external (dolichol and polyprenol native mixtures isolated from horse gonads and silver birch wood, respectively) and internal (Pren-28) standards.

Lipids for NMR analysis were isolated from 3 L yeast cultures using the method described above. Polyisoprenoid mixtures were purified using column chromatography on silica gel with a linear gradient of 0 to 10 % of diethyl ether in hexane. Fractions containing polyisoprenoids were subjected to further purification by HPLC/UV and eluates corresponding to the retention time of selected polyprenols (time-window 1 min.) were collected, pooled, evaporated and used for NMR analysis.

2.4. Extraction of polyprenyl/dolichyl esters

Lipids from yeast pellets were extracted with chloroform / methanol / water (C/M/W) 1:1:0.3 (by vol.) for 7 days at RT and the extract was adjusted to a final C/M/W ratio of 3:2:1 (by vol.). The lower organic phase was evaporated, remaining lipids were dissolved in hexane and purified on a silica gel 60 column using gradient elution with 2-10% diethyl ether in hexane. An aliquot of each fraction was subjected to alkaline hydrolysis as described earlier [30]. All fractions containing both, native and nonsaponifiable lipids were analyzed by HPLC/UV. Polyprenol content in the fractions containing esters was determined after hydrolysis.

2.5. Statin treatment

To test the effect of statin on polyisoprenoid synthesis the YPD medium was supplemented with simvastatin (dissolved in 70% ethanol, final concentration in the growth medium 0.1 mM) or ethanol as a negative control. Cultures were grown as described above. OD₆₀₀ and polyisoprenoid content and profile were measured after each 24 h.

2.6. Metabolic labeling

S. cerevisiae SS328 cells were cultured to A₆₀₀ 1.2. The cells were pelleted, washed with YPD and resuspended in a fresh YPD medium (OD₆₀₀ 0.2) supplemented with [³H]

mevalonolactone at final concentration 20 $\mu\text{Ci/ml}$ (2.5 mM). Pre-labeling was performed at 28°C for 3 days. The obtained yeast culture was subsequently divided into three aliquots for a pulse-chase experiment. The yeast cells were centrifugated at $3300 \times g$ for 10 min, washed with water and pelleted by re-centrifugation. The first aliquot was immediately used for lipid extraction, whereas the second and third aliquots were resuspended in fresh YPD medium devoid of [^3H] mevalonolactone and the incubation was continued for 1 or 2 days, respectively. Subsequently, the yeast cells were recovered by centrifugation, washed and used for lipid extraction. Lipids were analyzed chromatographically with the aid of an HPLC/UV/radioactivity flow detector and the specific activity (dpm/ng of polyisoprenoid) was calculated. The experiments were performed in duplicate.

2.7. Chromatographic analysis of polyisoprenoids

HPLC/UV and HPLC/MS analysis of lipids was performed as described earlier [27]. High resolution MS spectra were collected with the aid of MALDISynapt G2-S HDMS (Waters Inc) mass spectrometer equipped with an electrospray ion source and q-TOF type mass analyzer.

Tritiated lipids were analyzed with an on-line detector of radioactivity (Radiomatic, Packard, Canberra) coupled to the HPLC. Pren-28 was used as an internal standard.

2.8. NMR analysis

^1H and ^{13}C NMR spectra were obtained with Varian INOVA 400MHz spectrometer at 25 °C in C_6D_6 (D-99.5%, Cambridge Isotope Laboratories Inc., Andover, MA, USA). A total of 32K data points were collected and a spectral width of 6 kHz was used in 1D proton experiments. Two-dimensional { ^1H , ^{13}C } gHSQC experiments [31 – 33] with gradients were performed in proton de-coupled mode with a carbon spectral width of 25 kHz and 256 increments. Spectra were calibrated against the residual chemical shift of benzene in proton spectra (7.16 ppm) and chemical shift of benzene in ^{13}C spectra (128.0 ppm).

^1H NMR spectrum of yeast Pren-11 measured in C_6D_6 : 1.5–1.8 ppm (12 CH_3 , including CH_3 *cis*: 3, 1.644 ppm c-c(α); 3, 1.684 ppm c(ω); 3, 1.720 ppm c-c-c(α); 3, 1.743 ppm t-c-c, 15, 1.756 ppm c-c-c. CH_3 *trans*: 3, 1.572ppm t(ω); 3, 1.617 ppm ω -t-t, 1.634; 3, t-t-c), 2.0–2.3 ppm (10 CH_2CH_2), 4.02 ppm (CH_2OH), 5.16–5.40 ppm (11 $\text{C}=\text{CH}$).

¹H NMR spectrum of yeast Pren-7 measured in C₆D₆: 1.5–1.7 ppm (8 CH₃, including CH₃ *cis*: 3, 1.687 ppm $\underline{c}(\omega)$. CH₃ *trans*: 3, 1.477 ppm t- $\underline{t}(\alpha)$; 3, 1.574 ppm $\underline{t}(\omega)$; 15, 1.623 ppm t- \underline{t}), 1.9–2.3 ppm (6 CH₂CH₂), 3.97 ppm (CH₂OH), 5.20–5.40 ppm (7 C=CH).

2.9. Expression of HPS in *E. coli* and polyisoprenoid isolation

Total RNAs were isolated from wild type *S. cerevisiae* cells SS328, BY4741 and L5366 L5366 using the Hot Acid Phenol method [34] and reverse transcribed to cDNAs using a SuperScript® First-Strand Synthesis System (Invitrogen). The cDNAs were used as templates for PCR with gene-specific primers designed according to the cDNA sequences of *S. cerevisiae* S288c trans-hexaprenyltranstransferase (COQ1) (NM_001178351.1) from the GenBank, ScHPS-F (5'- caccatgtttcaaaggtct -3') and ScHPS-R (5'- ttactttcttctgttagtatact -3'). PCR was performed in a final volume of 20 µl containing 20 pmol of amplification primer pair for 50 cycles of 30 s at 98°C, 40 s at 55°C and 1 min at 72°C, with a 3-min preheat at 98°C and a 10-min final extension at 72°C. The PCR product was purified by agarose gel electrophoresis, subcloned into the pGEX-4T-1 vector (GE Healthcare) and transformed into chemically competent TOP10 *E. coli*. pGEX-4T-1 – HPS construct was purified, transformed into *E. coli* BL21 strain and expressed using mM IPTG induction for 24 h at 37°C. The cells were pelleted and polyisoprenoid isolation was performed as described in Section 2.3.

3. Results and discussion

3.1. *S. cerevisiae* cells accumulate short-chain polyprenols

The HPLC/UV analysis of polyisoprenoids extracted from *S. cerevisiae* SS328 strain grown on YPD medium to the early stationary phase revealed the presence of two families of polyisoprenoids. Besides, always detected typical yeast dolichol family composed of 13 to 18 isoprene units (i.u.) (Dol-13 – Dol-18) [22] short-chain polyisoprenoids corresponding to the retention time of polyprenols containing 6 to 9 i.u. (Pren-6 – Pren-9) with the dominating homologue composed of 7 i.u., and a well-pronounced single peak corresponding to a polyprenol consisting of 11 i.u. (Pren-11) were additionally found. Moreover, careful inspection of the HPLC/UV chromatograms revealed a double peak corresponding to prenol and dolichol containing 12 i.u (Pren-12 and Dol-12) (Fig. 1A). The identity of these lipids was conclusively confirmed by the accurate mass measurements (HR-HPLC/MS) summarized in Table 1.

To finally verify the structure of the newly identified yeast polyprenols NMR analysis of isolated Pren-11 and Pren-7 was performed. Polyprenol standards, mainly *cis*-Prenol-11 and

all-*trans*-Prenol-9 were analyzed too as references (Fig.2). It was found that yeast Pren-11 was a mainly-*cis* polyprenol (two internal *trans* and seven *cis* i.u. in the molecule) while Pren-7 appeared to have an all-*trans* structure (Fig. 2 and Materials and methods section 2.8). Both, mainly-*cis* and all-*trans* polyprenols are accumulated in various plant species [4, 35, 36] whereas their accumulation in yeast cells was overlooked during past years.

To verify that these polyisoprenoids are present in other *S. cerevisiae* strains the lipids were extracted from two additional *S. cerevisiae* strains BY4741 and L5366 at the logarithmic and early stationary growth phases. The HPLC/UV and HPLC/MS analyses demonstrated the presence of medium-chain dolichols (Dol-13 – Dol-18) and short-chain polyprenols (Pren-6 – Pren-9) in all studied strains at both the logarithmic and early stationary phases. The single polyprenol Pren-11 was found in the extract of SS328 and BY4147 strains at the early stationary phase only but not for the L5366 strain (Fig. 1B). No peaks corresponding to dolichols in the range from 19 to 30 i.u. were observed either in the logarithmic or early stationary phase. This last observation is in agreement with the previous data reporting the accumulation of these long-chain dolichols exclusively in the stationary phase [22].

The total content of polyisoprenoids extracted from yeast cells collected during the logarithmic growth phase for SS328 strain was 251 µg of polyisoprenoids per gram of yeast wet weight, while for BY4741 and L5366 strains the total content was 268 and 216 µg/g, respectively. The yield of total polyisoprenoids was increased at the early stationary phase to 370, 275 and 270 µg/g, respectively. The most prominent increase was observed for the single polyprenol Pren-11 (from 3- to more than 100-fold) of the content observed at the logarithmic phase while the amount of short-chain polyprenols and dolichols was enhanced by 1.2- up to 1.8-fold of the initial value (Table 2).

The results described above are the first data describing the occurrence of polyisoprenoid alcohols of short chain-length with an unsaturated bond in the α -residue in yeast. *S. cerevisiae* cells are known to produce ubiquinone-6 containing all-*trans*-Pren-6 as a side-chain [37], despite this fact accumulation of Pren-6 has never been noted in these cells. On the other hand careful inspection of the HPLC/UV spectra of the Erg20/Erg9 yeast strains [38] suggests the presence of the signals which in fact might correspond to Pren-11 in these cells. The authors of this report have not, however, raised this possibility. It has either never been verified experimentally.

The biological role of polyprenols in yeast is not known and requires further studies. The enzymes responsible for the synthesis of these lipids are also not known. So far, two active yeast *cis*-prenyltransferases (Rer2 and Srt1) with different properties to terminate the

IPP condensation reaction, different localization and physiological roles have been described. Srt1 is involved in the synthesis of a family of dolichols with Dol-21 dominating whereas Rer2 – with Dol-16 dominating [10, 20]. *RER2* is mainly expressed in the early logarithmic phase, while the expression of *SRT1* is induced in the stationary phase. Rer2p is localized to ER, whereas Srt1 – to lipid bodies [10, 20].

3.2. Short-chain polyprenols are accumulated in yeast cells as free alcohols

In cells polyisoprenoids are accumulated in the form of free alcohols and/or esters of carboxylic acids along with a small fraction of mono- and diphosphates. To analyze the composition of polyisoprenoids vs. polyisoprenoid carboxylic esters lipids extracted from yeast cells were fractionated and fractions corresponding to putative esters ('ester fraction' eluted from silica gel column by 3% diethyl ether in hexane) and free alcohols ('alcohol fraction' eluted later by 5% diethyl ether in hexane) were compared. HPLC/UV analysis of the crude ester fraction revealed the presence of signals overlapping with the retention time of dolichols (Dol-15 – Dol-17) however after alkaline hydrolysis these signal disappeared and only traces of dolichols were visible. Simultaneously, a signal corresponding to ergosterol was increased considerably suggesting that ergosteryl esters might be present in the crude ester fraction (Fig. 3). Thus analysis performed in this report revealed that free alcohols are the predominant forms of all polyisoprenoids accumulated in *S. cerevisiae* cells. Polyprenyl and/or dolichyl esters constituted less than 8% of the total polyisoprenoid pool (Table 3).

3.3. Effect of statin treatment on polyisoprenoid accumulation in S. cerevisiae SS328

Dolichols in yeast are synthesized by the mevalonic acid pathway (MVA) [14]. Statins are well-known inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR), the regulatory enzyme of the MVA pathway.

To investigate the effects of statin on short polyisoprenoids accumulation in yeast, cells of the SS328 strain were treated with simvastatin in a non-toxic dose (0.1 mM), lipids were isolated and the levels of polyprenols and dolichols were estimated by HPLC/UV. In simvastatin-treated cells the content of both short-chain polyprenols and longer dolichols was lower than that in control untreated cells (Fig. 4). The level of dolichols (Dol-13 – Dol-18) was diminished to 56% at the logarithmic phase and 63% at the early stationary phase, respectively, of its content in the control cells. Interestingly, simvastatin exerted a stronger inhibitory effect on the synthesis of short-chain polyprenols than dolichols in yeast cells. The content of polyprenols (Pren-6 – Pren-9) was 61% at the logarithmic phase and 27% at the

stationary phase compared to the control. The most prominent decrease was noted for the single polyprenol Pren-11, the content of which was 20% and 12% of the control at the logarithmic and stationary phases, respectively.

These results correlate with reduction of expression of *RER2* and *SEC59* genes in yeast cells treated with statins [39]. Moreover, oral administration of mevinolin (lovastatin) to rats decreased dolichol and dolichyl-P levels in the heart and skeletal muscle, however the amounts of dolichol increased in the liver and of dolichyl-P in the brain [40].

3.4. Labeling of polyisoprenoids with [^3H] mevalonolactone

To further confirm that the newly discovered short polyprenols in the yeast are isoprenoids synthesized via the MVA pathway, incorporation of [^3H] mevalonolactone into these products was measured. During the initial 3 days of incubation of SS328 strain cells with [^3H] mevalonolactone (pulse-labeling), a highly efficient labeling of polyprenols Pren-7 and Pren-11 was observed whereas the labeling of dolichol Dol-15 was low (Fig. 5). Interestingly, during the chase - subsequent 2 days of yeast growth in the medium devoid of [^3H] mevalonolactone, the labeling of dolichols within the range of Dol-13 – 18 was significantly increased (e.g. 7-fold for Dol-15) while the labeling of Pren-7 and Pren-11 was decreased (1.7 and 3.4-fold, respectively) in comparison to the initial level. The extensive [^3H] mevalonolactone incorporation into short-chain polyprenols (Pren-7 and Pren-11) on the one hand again confirms their polyisoprenoid structure and biosynthetic origin from the MVA pathway. On the other hand, the results of the pulse-chase experiment indicate the substrate-product relationship between short-chain polyprenols and dolichols synthesized in the yeast. Consequently, it might be expected that short-chain polyprenols are utilized as substrates by CPT and elongated until the final chain-length (Pren-13 – Pren-18) is achieved. Such polyprenols are finally reduced to dolichols by polyprenol reductase DFG10 which has recently been briefly described [41].

3.5. Search for yCPT responsible for the synthesis of short-chain polyprenols in yeast

In order to verify whether the well-known yCPTs, Rer2 or Srt1 might be responsible for formation of short-chain polyisoprenoids lipid profiles of both mutants, *rer2Δ* and *srt1Δ* were analyzed (Fig. 6). In both cases short-chain polyisoprenoid lipids were accumulated clearly confirming that none of the so far described yCPT is involved in this process. Interestingly, the content of short-chain polyprenols was two-fold higher in *rer2Δ* mutant cells than in wild type and *srt1Δ* yeast mutant (Table 4).

To explore further the role of Rer2 and Srt1 in the synthesis of Pren-11 sequencing of cDNA encoding of Rer2 and Srt1 obtained from three stains of *S. cerevisiae* SS328, BY4741 and L5366 was performed. Analysis of deduced amino acid sequences did not reveal any mutation in Rer2 or Srt1 proteins in yeast strains producing short lipids in comparison to Rer2p and Srt1p sequences of SS288c strain deposited in Gene Bank.

Additionally, this possibility seems unlikely since CPTases are considered as processive enzymes and genetic manipulation – shortening of the loop in the product-binding pocket is required to modify its product specificity as shown for Srt1 [42].

Simultaneously, *in silico* search for the putative new ScCPT responsible for the formation of the short-chain polyprenols did not reveal any candidates homologous either to the canonical Rer2 or Srt1. However, this does not preclude the possibility of the presence of still unknown yCPT in yeast cells. Additionally, the product specificity of the CPT might be determined / fine tuned at the level of CPT/accessory protein interactions. Nus1 protein, a homologue of human NogoB receptor, very recently described in *S. cerevisiae* [43, 44] might be a putative candidate for such a regulator although this possibility remains unexplored in any organism.

3.6. Short-chain all-trans Pren-7 is synthesized by trans-hexaprenyltranstransferase (HPS)

Since Pren-7 appeared to possess all-*trans* structure *in silico* search for yeast *trans*-prenyltransferase was performed. Saccharomyces protein data base analysis revealed one putative enzyme responsible for the formation of *trans* polyprenols. This enzyme – *trans*-hexaprenyltranstransferase (HPS) – was described by Ashby and Edwards [45] as COQ1 – the first enzyme involved in the coenzyme Q (UQ-6) biosynthesis in *S. cerevisiae*. Yeast mutant deficient in HPS activity was unable to elongate geranyl pyrophosphate [45].

Here, we tested the ability of HPS to synthesize polyprenols *in vitro*. To this end *E. coli* BL21 strain was transformed by HPS, polyisoprenoids were isolated and analyzed as described in the Materials and methods section. HPLC/UV analysis of polyisoprenoids synthesized by bacteria expressing HPS revealed a single product corresponding to the retention time of polyprenol composed of 7 i.u. (Fig. 7). Accumulation of this lipid was obviously dependent on the presence of HPS since in *E. coli* transformed with the empty vector no such product was observed (Fig. 7). Altogether, HPS might be responsible for the synthesis of short-chain all-*trans* polyprenols in *S. cerevisiae*.

4. Conclusions

Taken together, the experiments described in this report clearly show that *S. cerevisiae* cells synthesize short-chain polyprenols additionally well-known yeast dolichols. Surprisingly, up to now their presence in yeast had escaped discovery. It is clear that like all yeast isoprenoids these newly discovered polyprenols originate from the MVA pathway. While all-*trans* Prenol is most probably synthesized by *trans*-prenyltransferase the mechanism of formation of mainly-*cis* Prenol-11 remains elusive and requires clarification. Similarly, their biological role awaits explanation.

Acknowledgements

The authors wish to thank the anonymous Reviewers for their stimulating comments, and Drs Agata Lipko and Marcin Grynberg, IBB PAS, Warsaw, Poland and Hiroshi Sagami, Tohoku University, Sendai, Japan for their helpful suggestions and discussions.

This investigation was supported by a grant from the National Science Centre [2011/03/B/NZ1/00568] (LS).

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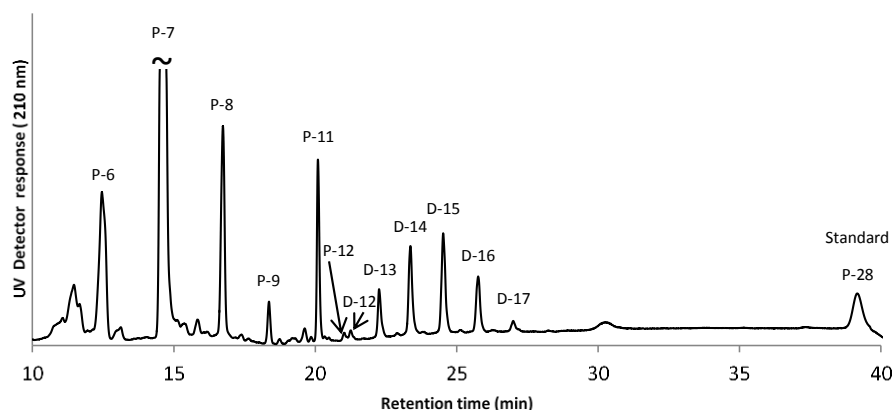
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A)



B)

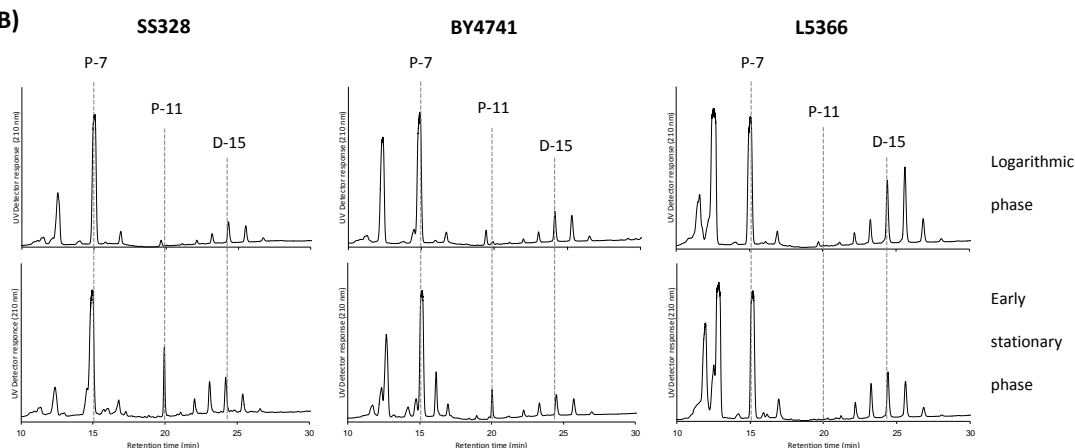


Figure 1.

HPLC/UV analysis of polyisoprenoids extracted from yeast. A) Polyisoprenoids synthesized by *S. cerevisiae* SS328 strain in early stationary growth phase (4-day old yeast culture). B) Polyisoprenoids accumulated in *S. cerevisiae* SS328, BY4741 and L5366 strains harvested at logarithmic (upper panel) and early stationary (lower panel) growth phases. Numbers above the signals indicate the retention time of the respective polyprenol / dolichol standard composed of the indicated numbers of isoprene units. P and D stand for polyprenol and dolichol, respectively.

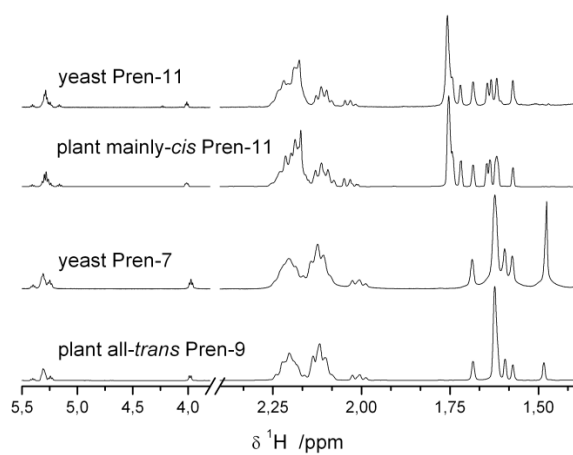


Figure 2.

^1H NMR spectra of yeast Pren-7 and Pren-11, and plant mainly-*cis* Pren-11 and all-*trans* Pren-9 used as standards.

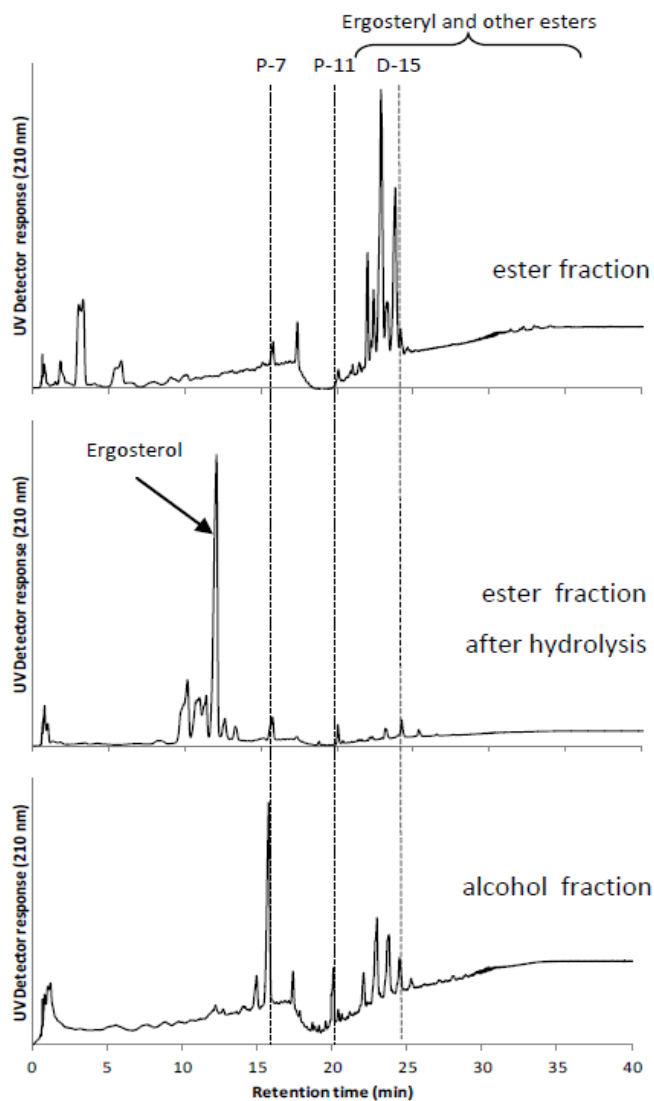


Figure 3.

HPLC/UV analysis of polyisoprenoids and polyisoprenoid carboxylic esters extracted from *S. cerevisiae* SS328 strain cells. ‘Ester’ and ‘alcohol fractions’ were eluted from silica gel column by 3% and 5% diethyl ether in hexane. Numbers above the signals indicate the retention time of the respective polyprenol / dolichol standard composed of the indicated number of isoprene units. P and D stand for polyprenol and dolichol, respectively.

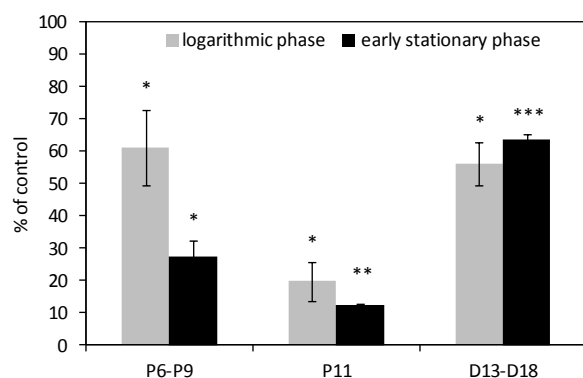


Figure 4.

Effect of simvastatin on accumulation of polyisoprenoid alcohols in *S. cerevisiae* SS328. Content of polyprenols and dolichols was determined in yeast cells treated with 0.1 mM simvastatin and expressed as percentage of the control - yeast cells grown in the medium devoid of simvastatin; lipid quantities were estimated by means of HPLC/UV and normalized per protein content. Cells were collected at logarithmic and early stationary (gray and black bars, respectively) phases. Values are means \pm SD of three independent measurements, the statistical significance is indicated (* $p < 0.05$; ** $p < 0.005$; *** $p < 0.001$).

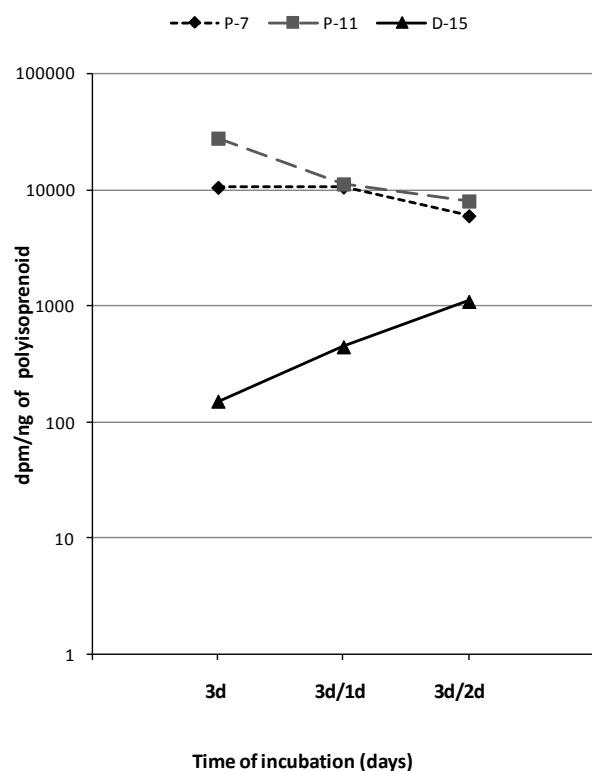


Figure 5.

[³H] polyisoprenoids in *S. cerevisiae* SS328 – cells metabolically labeled with [³H]mevalonolactone for 3 days (pulse) were transferred to the medium without [³H]mevalonolactone and incubation was continued for 2 subsequent days (chase). Lipid content was measured at indicated time points with the aid of HPLC/UV/radioactivity flow detector, shown are data for dominating polyisoprenoids: P-7 (diamonds), P-11 (squares) and D-15 (triangles).

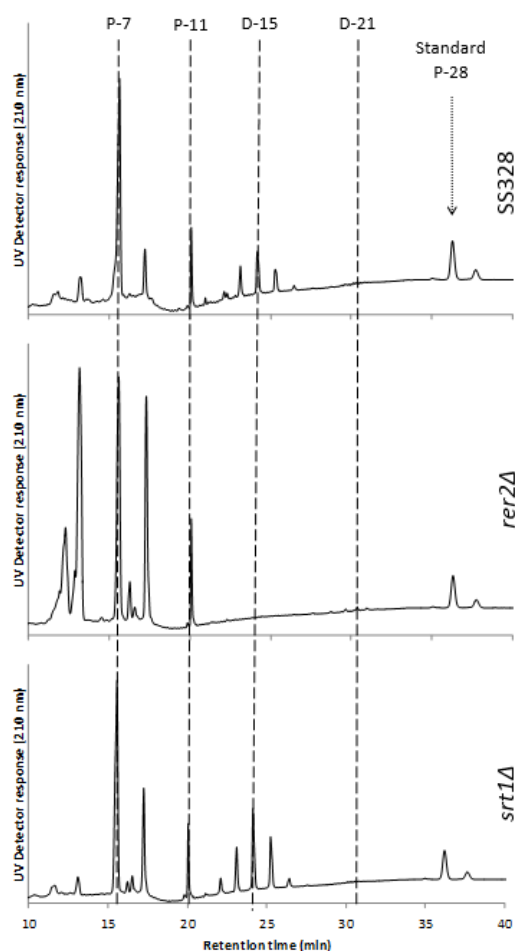


Figure 6.

HPLC/UV analysis of polyisoprenoids accumulated in *S. cerevisiae* SS328, and *rer2Δ*, and *srt1Δ* mutant cells harvested at early stationary growth phase. Numbers above the signals indicate the retention time of the respective polyprenol / dolichol standard composed of the indicated number of isoprene units. P and D stand for polyprenol and dolichol, respectively.

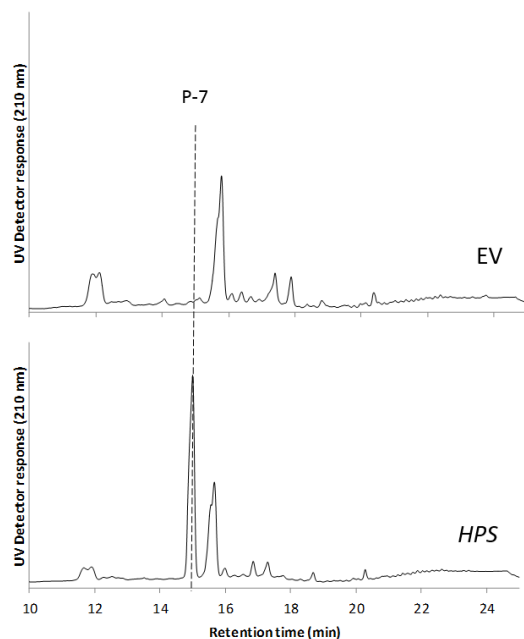


Figure 7.

HPLC/UV analysis of polyisoprenoids isolated from *E. coli* BL21 transformed with pGEX-4T-1 – HPS (HPS) construct or control strain transformed with empty pGEX-4T-1 vector (EV). Polyisoprenoids were isolated and their profile was analyzed as described in Materials and methods. P-7 stands for polyprenol composed of 7 i.u.

Table 1

Polyisoprenoid lipids identified by mass spectrometry (LC/MS) analysis in the extract isolated from *Saccharomyces cerevisiae* SS328 strain collected at the early stationary phase.

Polyisoprenoid alcohol	Molecular formula	m/z	HR error (mDa)
Pren-6	C ₃₀ H ₅₀ ONa	449.3737 [M+Na]	-2,2
Pren-7	C ₃₅ H ₅₈ ONa	517.4388 [M+Na]	0,3
Pren-8	C ₄₀ H ₆₆ ONa	585.5024 [M+Na]	1,3
Pren-9	C ₄₅ H ₇₄ ONa	653.5648 [M+Na]	1,1
Pren-11	C ₅₅ H ₉₀ ONa	789.6932 [M+Na]	4,3
Pren-12	C ₆₀ H ₉₈ ONH ₄	857.7554 [M+NH ₄]	3,9
Dol-12	C ₆₀ H ₁₀₀ ONH ₄	854.8161 [M+NH ₄]	4,3
Dol-13	C ₆₅ H ₁₀₈ ONH ₄	922.8799 [M+NH ₄]	5,5
Dol-14	C ₇₀ H ₁₁₆ ONa	995.8970 [M+Na]	4,6
Dol-15	C ₇₅ H ₁₂₄ ONa	1063.9600 [M+Na]	5
Dol-16	C ₈₀ H ₁₃₂ ONa	1127.0725 [M+NH ₄]	10,3
Dol-17	C ₈₅ H ₁₄₀ ONa	1195.1345[M+NH ₄]	9,7
Dol-18	C ₉₀ H ₁₄₆ ONa	1268.1554 [M+Na]	Weak signal > 10

Table 2

Content of polyprenols and dolichols accumulated in the *S. cerevisiae* SS328, BY4741 and L5366 strains. Cell were harvested at the logarithmic and early stationary growth phases. Lipid quantity was estimated by HPLC/UV, data are means of three independent experiments \pm SD, P and D stand for prenol and dolichol, respectively.

	Polyisoprenoids ($\mu\text{g/g}$ of yeast wet weight)					
Strain	Logarithmic Phase			Early Stationary Phase		
	P-7	P-11	D-12 – D-17	P-7	P-11	D-12 – D 17
SS328	208 (\pm 29)	0.2 (\pm 0.01)	43 (\pm 12)	258 (\pm 25)	33 (\pm 6)	79 (\pm 18)
BY4741	216 (\pm 10)	1.6 (\pm 0.1)	31 (\pm 9)	235 (\pm 19)	12 (\pm 2)	47 (\pm 5)
L5366	158 (\pm 23)	0.1 (\pm 0.01)	58 (\pm 21)	202 (\pm 23)	0.3 (\pm 0.01)	68 (\pm 14)

Table 3

The composition of polyisoprenoids vs. polyisoprenoid carboxylic esters extracted from *S. cerevisiae* SS328, BY4741 and L5366 strains. Cells were harvested at the early stationary growth phase. Lipid quantity was estimated by HPLC/UV. Experiment was performed in duplicate, shown are representative values. P and D stand for prenol and dolichol, respectively. n.d. – Pren-11 was not detected in L5366 strain.

Strain	Fraction of polyisoprenoid esters (% of total polyisoprenoid content)		
	P-7	P-11	Dol mix [D-12 – D-17]
SS328	2	8	5
BY4741	6	8	2
L5366	8	n.d.	1

Table 4

Content of polyprenols and dolichols accumulated in wild type *S. cerevisiae* SS328, and *rer2Δ* and *srt1Δ* mutants. Cells were harvested at the early stationary growth phase. Lipid quantity was estimated by HPLC/UV, data are means of three independent experiments \pm SD, P and D stand for prenol and dolichol, respectively.

Strain	Polyisoprenoids ($\mu\text{g/g}$ of wet weight)		
	P7	P-11	Dol mix [D-12 – D-17]
SS328	228(\pm 27)	26 (\pm 6)	63 (\pm 4)
<i>rer2Δ</i>	567 (\pm 32)	53 (\pm 12)	0
<i>srt1Δ</i>	203 (\pm 17)	34 (\pm 7)	94 (\pm 21)