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Interplay between the *cis*-prenyltransferases and polyprenol reductase in the yeast *Saccharomyces cerevisiae*

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Abstract

Dolichol formation is examined in three *Saccharomyces cerevisiae* strains with mutations in the *ERG20* gene encoding farnesyl diphosphate synthase (mevalonic acid pathway) and/or the *ERG9* gene encoding squalene synthase (sterol synthesis pathway) differing in the amount and chain length of the polyisoprenoids synthesized. Our results suggest that the activities of two yeast *cis*-prenyltransferases Rer2p and Srt1p and polyprenol reductase are not co-regulated and that reductase may be the rate-limiting enzyme in dolichol synthesis if the amount of polyisoprenoids synthesized exceeds a certain level. We demonstrate that reductase preferentially acts on typical polyprenols with 13–18 isoprene residues but can reduce much longer polyprenols with even 32 isoprene residues.

Keywords: Yeast; Dolichols; Polyprenols; *Cis*-prenyltransferases; Polyprenol reductase

1. Introduction

Dolichol was first identified by Pennock et al. [1] in 1960. Behrens and Leloir [2] discovered in 1970 that its phosphorylated form is an obligatory intermediate in the synthesis of some types of glycoproteins. Since then remarkable progress has been made in research on the biosynthetic pathway of dolichol. The initial steps from acetate to farnesyl diphosphate are common with those of the biosynthesis of sterols, ubiquinones and farnesylated proteins. The next steps are the repetitive condensations of isopentenyl diphosphates with farnesyl diphosphate performed by *cis*-prenyltransferase resulting in the formation of polyprenyl diphosphate (PolPP).

The chain length of PolPP is species specific. The genes encoding *cis*-prenyltransferases from yeast (*RER2* and *SRT1*), *Micrococcus luteus*, *Escherichia coli, Arabidopsis thaliana* and human have been cloned [3–9]. The 3D structure determined for undecaprenyl *cis*-prenyltransferase helps to understand the mechanisms restricting the chain length of the *cis*-prenyltransferase products [10]. However, the conversion of PolPP to dolichol, comprising reduction of the α-isoprene residue, still remains unclear. Two suggestions can be considered, one assuming the final condensation of PolPP with free isopentenol with concomitant reduction, and the other one assuming dephosphorylation of PolPP to polyprenol and its reduction to dolichol. At present the second one is thought to be the major pathway even though direct measurement of polyprenol reductase activity in vitro is not available. Sagami et al. [11] investigated the conversion of exogenously added polyprenol to dolichol in a rat microsomal fraction in the presence of nicotinamide adenine dinucleotide phosphate reduced (NADPH) and found it to be extremely low, and in the yeast system it was undetectable.

From the reports on protein glycosylation defects in many different organisms it is clear that some of the perturbations are due to lack or insufficient amount of dolichol. Instead, polyprenol is utilized as the lipid carrier of oligosaccharides involved in N-glycosylation indicating that malfunctioning of the process is related to polyprenol reductase rather than to *cis*-prenyltransferase activity [12].

To gain more insight in the transition from polyprenol to dolichol we asked the following questions. Are the activities of *cis*-prenyltransferases and polyprenol reductase co-

Abbreviations: CHO, Chinese hamster ovary; HPLC, high-performance liquid chromatography; NADPH, nicotinamide adenine dinucleotide phosphate reduced; PolPP, polyprenyl diphosphate.

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regulated? Is reductase a rate-limiting enzyme in dolichol synthesis? Does reductase recognize the chain length of polyprenol? Is its activity expressed mainly in the logarithmic phase and does it diminish in the stationary phase of yeast growth?

2. Materials and methods

2.1. Yeast strains

All strains were *Saccharomyces cerevisiae*. They are listed in Table 1. Plasmid pDD9 is the derivative of a high copy number plasmid pDP51 Not I [13] carrying mutated allele *erg20-2,* under *GAL10/CYC1* promoter with *PGK* terminator. The gene was ligated in EcoR1-BamH1 sites. *erg9::HIS3* strain was constructed as in [14]. Strains DD104, DD94 and *erg9::HIS3* were a gift from F. Karst.

2.2. Media and growth conditions

Yeast cells were grown at 28 °C to logarithmic or late stationary phase on YPD medium (1% yeast extract, 1% bacto peptone, 2% dextrose) supplemented with 2 mg/l of ergosterol in 1% Tween 80. One culture of the DD104 strain was on medium with 0.5 mg/l of ergosterol in 1% Tween 80. One culture of the DD94 strain was pregrown on YPD medium supplemented with 2 mg/l of ergosterol in 1% Tween 80 collected at the early logarithmic phase (1.3×10^7 cells per ml), transferred to YPE medium (1% yeast extract, 1% bacto peptone, 2% ethanol) supplemented with 2 mg/l of ergosterol in 1% Tween 80 and grown to the late stationary phase (3×10^8 cells per ml).

2.3. Extraction of lipids

Yeast cells were harvested, washed with water and broken with glass beads 425–600 μm on vortex in 50 mM Tris–HCl buffer, pH 7.4. After the removal of unbroken cells by low speed centrifugation the supernatant was spun at 48,000 × *g* for 90 min. The pellet was extracted with chloroform/methanol 3:2. Denatured protein was discarded by centrifugation. The organic supernatant containing the extracted lipids was washed three times with 1/5 volume of 10 mM EDTA in 0.9% NaCl and evaporated to dryness in a nitrogen stream and suspended in benzene.

2.4. Alkaline hydrolysis of lipids

Lipids extracted from yeast cells suspended in benzene were hydrolyzed at 95 °C for 1.5 h in a mixture of ethanol/H_2O 17:3 containing 15% KOH (w/v). Lipophylic products were extracted with diethyl ether, washed with water, evaporated to dryness in a nitrogen stream, suspended in hexane and applied to a silica gel 60 (230–400 mesh ASTM) column equilibrated with hexane. A step-wise gradient of 3%, 8%, 12% and 15% of diethyl ether in hexane was applied. Fractions containing polyisoprenoids (verified on TLC) were collected and subjected to high-performance liquid chromatography (HPLC) analysis.

Table 1
Strains used in the study

Strain	Genotype
DD104	*MAT α erg9-1 erg20-2 his3-1 leu2-3 ura3-1 ade2-1*
DD 94	*MAT α erg9-1 erg20-2 his3-1 leu2-3 ura3-1 ade2-1* [pDD9]
erg9::HIS3	MAT a ura3-1 his3-1 leu2-1 ade2-1 aux32
W303 1B	*MAT α his3-11 leu2-3,112 trp1-1 ura3-1 ade2-1 can^R 1-100*

2.5. Chromatography

TLC was performed on Silica Gel 60 plates in toluene/ethyl acetate 95:5. HPLC chromatography was performed on an ODS Hypersil 3 μm 60 × 4.5 mm column at a flow rate of 1.5 ml/min in a linear gradient program from A) methanol/isopropanol/water 12:8:1 to B) hexane/isopropanol 7:3 in 30 min. The amount of polyisoprenoids was estimated by comparison with a quantitative standard dolichol 23. The chain length and identity of the polyisoprenoids was confirmed by applying a mixture of natural dolichols from pig liver and polyprenols from *Ginkgo biloba* [15,16]. Polyprenol and dolichol standards were from the Collection of Polyprenols of the Institute of Biochemistry and Biophysics (Warsaw).

3. Results

3.1. The efficiency of polyprenol reductase

We examined the polyisoprenoid profile in two mutated yeast strains *erg9::HIS3* and DD104 to investigate the correlation between the amount of polyprenols synthesized and reductase efficiency in converting them to dolichols. Cells of the *erg9::HIS3* strain collected during the logarithmic growth phase synthesized 3.23 μg of polyisoprenoids per gram of yeast wet weight (ww) (Table 2). All the dolichols recovered belonged to one family consisting of chains with 13–18 isoprene residues (Fig. 1A). The same cells cultivated to the late stationary phase synthesized 42.92 μg of polyisoprenoids per gram of ww. (Table 2). Again the dolichols belonged to only one family with 13–18 isoprene residues. However, the appearance of double peaks indicates that besides dolichols the corresponding polyprenols are present (polyprenols elute

Table 2
Synthesis of polyisoprenoids in different *S. cerevisiae* strains

	Polyisoprenoids (μg/g ww)		
Strain	Logarithmic	Late stationary	
erg9::HIS3	3.23	42.92	
DD104	0.82	30.01	5.48•
DD94	1.48	22.00	21.51*

• Cells grown in the medium with 0.5 mg/l of ergosterol. * Cells transferred from YPD to YPE medium. Cells were grown on YPD or YPE medium at 28 °C with 2 or 0.5 mg/l of ergosterol. Extraction, alkaline hydrolysis and quantification was as described in Section 2.