FEBS Letters 582 (2008) 4032–4038

Reconstruction of a bacterial isoprenoid biosynthetic pathway in *Saccharomyces cerevisiae*

Jérôme Maury[a,*], Mohammad A. Asadollahi[b], Kasper Møller[d], Michel Schalk[e], Anthony Clark[e], Luca R. Formenti[b], Jens Nielsen[c]

[a] *Center for Microbial Biotechnology, DTU-Biosys, Building 223, 2800 Kgs Lyngby, Denmark*
[b] *Center for Microbial Biotechnology, DTU-Biosys, Lyngby, Denmark*
[c] *Department of Chemical and Biological Engineering, Chalmers University of Technology, Sweden*
[d] *CMC Biopharmaceuticals AS, Søborg, Denmark*
[e] *Firmenich SA, Geneva, Switzerland*

Received 9 June 2008; revised 9 October 2008; accepted 28 October 2008

Available online 6 November 2008

Edited by Gianni Cesareni

Abstract **A eukaryotic mevalonate pathway transferred and expressed in *Escherichia coli,* and a mammalian hydrocortisone biosynthetic pathway rebuilt in *Saccharomyces cerevisiae* are examples showing that transferring metabolic pathways from one organism to another can have a powerful impact on cell properties. In this study, we reconstructed the *E. coli* isoprenoid biosynthetic pathway in *S. cerevisiae*. Genes encoding the seven enzymatic steps of the pathway were cloned and expressed in *S. cerevisiae*. mRNA from the seven genes was detected, and the pathway was shown able to sustain growth of yeast in conditions of inhibition of its constitutive isoprenoid biosynthetic pathway.**
© 2008 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

Keywords: Isoprenoid; Yeast; 2-Methyl erythritol 4-phosphate; Mevalonate; *Saccharomyces cerevisiae*

1. Introduction

Bridging between synthetic biology and metabolic engineering, researchers have been attempting to create organisms with new properties of interest by transferring metabolic pathways from one organism to another. Pioneering studies have demonstrated the usefulness of reconstituting and expressing entire heterologous pathways in non-native hosts for production purposes. In a single yeast strain, Szczebara et al. brought to function a complex, artificial and self-sufficient pathway for the biosynthesis of hydrocortisone, which is the major steroid in mammals [1]. After engineering 13 different enzymatic steps, they successfully rerouted the native yeast sterol-biosynthetic pathway, so that hydrocortisone became the major steroid produced [1]. This first report of the total biosynthesis of hydrocortisone by a recombinant microorganism from a simple carbon source constitutes a solid basis for the development of an environmentally friendly and low-cost industrial process for the production of corticoid drugs [1]. In another study, a eukaryotic mevalonate pathway was transferred and engineered in *Escherichia coli* with the aim to produce artemisinin, an antimalarial drug usually isolated from plant material [2]. Associated with the expression of a synthetic amorpha-4,11-diene synthase, it led to significant improvements in amorpha-4,11-diene production, an intermediate in the biosynthesis of artemisinin [2]. Biosynthesis of paclitaxel, a mitotic inhibitor used in cancer chemotherapy, has been extensively studied and partly transferred from *Taxus* species to *E. coli* or *Saccharomyces cerevisiae* [3–4] in order to establish a microbial production process. Ten enzymatic steps from the paclitaxel biosynthesis of *Taxus* species were successfully and independently expressed in yeast [4]. Five sequential steps catalyzing the synthesis of the intermediate taxadien-5α-acetoxy-10β-ol were installed in a single yeast strain [4]. However, the complete pathway from the precursor geranylgeranyl diphosphate to paclitaxel includes nineteen steps in *Taxus* species [4]. These examples showed that transferring entire metabolic pathways into a new host can result in tremendous improvements in the production of desired compounds. Transferring a pathway to another biological host, and making it functional in a non-native environment can also provide substantial knowledge about the mechanisms and regulations of the aforesaid heterologous pathway.

In this study, we investigated the biosynthesis of the isoprenoid family of compounds in the yeast, *S. cerevisiae*. This is the largest family of natural compounds with more than 40 000 described compounds [5–6]. Isoprenoids are involved in various important cellular biological functions, and many isoprenoids are of commercial interest due to their potent anticancer, antitumor, cytotoxic, antiviral, or antibiotic properties or their characteristic flavour or aroma. Here, the cloning and expression of seven enzymatic steps from a bacterial isoprenoid biosynthetic pathway into *S. cerevisiae* are reported. The seven enzymatic steps of this pathway, the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway have recently been unraveled [7] (Fig. 1). Genes were amplified from *E. coli*, cloned on self-replicating yeast plasmids, and were expressed in *S. cerevisiae*. Their expression and the ability of the bacterial MEP pathway to sustain growth of *S. cerevisiae*, which otherwise depends on another native biosynthetic pathway (the mevalonate pathway) for the generation of isoprenoids, were investigated.

*Corresponding author.
E-mail address: jema@bio.dtu.dk (J. Maury).

Abbreviations: DMAPP, dimethyl allyl diphosphate; G3P, glyceraldehyde 3-phosphate; GC–MS, gas chromatography–mass spectrometry; IPP, isopentenyl diphosphate; MEP, 2-C-methyl-D-erythritol 4-phosphate; MVA, mevalonate; RT-PCR, reverse transcriptase polymerase chain reaction

0014-5793/$34.00 © 2008 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2008.10.045

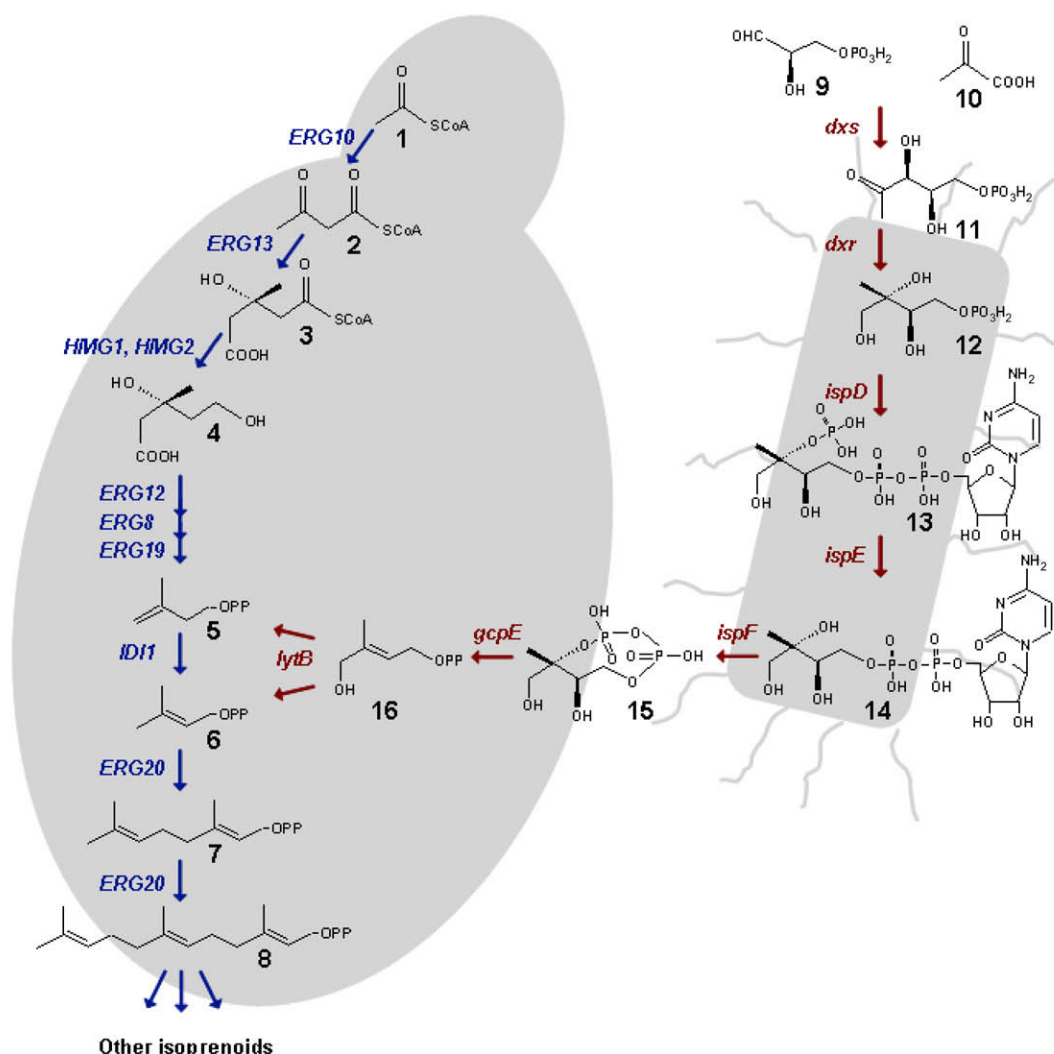

Fig. 1. Isoprenoid biosynthetic pathways. Mevalonate pathway from *Saccharomyces cerevisiae* (blue) and the *Escherichia coli* MEP pathway (dark red) are presented. Intermediates: **1** acetyl-CoA, **2** acetoacetyl-CoA, **3** 3-hydroxy-3-methylglutaryl-CoA, **4** mevalonate, **5** isopentenyl diphosphate, **6** dimethyl allyl diphosphate, **7** geranyl diphosphate, **8** farnesyl diphosphate, **9** D-glyceraldehyde 3-phosphate, **10** pyruvate, **11** 1-deoxy-D-xylulose 5-phosphate, **12** 2-C-methyl-D-erythritol 4-phosphate, **13** 4-diphosphocytidyl-2-C-methyl-D-erythritol, **14** 2-phospho-4-diphosphocytidyl-2-C-methyl-D-erythritol, **15** 2-C-methyl-D-erythritol 2,4-cyclodiphosphate, **16** 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate.

2. Materials and methods

2.1. Strains and plasmids

Table 1 lists all strains and plasmids used in this study.

2.2. Construction of plasmids pIP001 and pIP002

2.2.1. Cloning of the MEP pathway encoding genes. *E. coli* genes encoding Dxs (AAC73523), Dxr (AAC73284), IspD (AAC75789), IspE (AAC74292), IspF (AAC75788), GcpE (AAC75568), and LytB

Table 1
Strains and plasmids.

Strain	Genotype	Plasmid	Plasmid description
Saccharomyces cerevisiae			
YIP-00-02	MATa MAL2-8^c SUC2 ura3-52 trp1-289	None	
YIP-00-03	MATa MAL2-8^c SUC2 ura3-52	None	
YIP-0V-01	MATa MAL2-8^c SUC2 ura3-52	pIP031	pYX212 2µ URA3 P_{TPI1}-GFTpSD
YIP-0V-02	MATa MAL2-8^c SUC2 ura3-52	pIP027	pESC-URA 2µ URA3 P_{GAL1}- GFTpSD
Escherichia coli			
DH5α	F′φ80lacZΔM15 Δ(lacZYA-argF)U169 deoR recA1 endA1 hsdR17($r_K^- m_K^+$) phoA supE44 λ- thi-1 gyrA96 relA1	None	

Plasmid name	Key features
pESC-TRP	2µ *TRP1* P_{GAL1}/T_{CYC1} P_{GAL10}/T_{ADH1} Amp^R (*bla*)
pESC-URA	2µ *URA3* P_{GAL1}/T_{CYC1} P_{GAL10}/T_{ADH1} Amp^R (*bla*)

Download English Version:

https://daneshyari.com/en/article/2050711

Download Persian Version:

https://daneshyari.com/article/2050711

Daneshyari.com