Progress in Lipid Research 52 (2013) 277–293

Contents lists available at SciVerse ScienceDirect

Progress in Lipid Research

journal homepage: www.elsevier.com/locate/plipres

Review

Yeast metabolic engineering – Targeting sterol metabolism and terpenoid formation

Tamara Wriessnegger [a], Harald Pichler [a,b,*]

[a] ACIB – Austrian Centre of Industrial Biotechnology, Graz, Austria
[b] Institute of Molecular Biotechnology, Graz University of Technology, Austria

ARTICLE INFO

Article history:
Received 3 November 2012
Received in revised form 26 March 2013
Accepted 27 March 2013
Available online 6 April 2013

Keywords:
Yeast
Sterol
Mevalonate pathway
Metabolic engineering
Synthetic biology
Isoprene

ABSTRACT

Terpenoids comprise various structures conferring versatile functions to eukaryotes, for example in the form of prenyl-anchors they attach proteins to membranes. The physiology of eukaryotic membranes is fine-tuned by another terpenoid class, namely sterols. Evidence is accumulating that numerous membrane proteins require specific sterol structural features for function. Moreover, sterols are intermediates in the synthesis of steroids serving as hormones in higher eukaryotes. Like steroids many compounds of the terpenoid family do not contribute to membrane architecture, but serve as signalling, protective or attractant/repellent molecules. Particularly plants have developed a plenitude of terpenoid biosynthetic routes branching off early in the sterol biosynthesis pathway and, thereby, forming one of the largest groups of naturally occurring organic compounds. Many of these aromatic and volatile molecules are interesting for industrial application ranging from foods to pharmaceuticals. Combining the fortunate situation that sterol biosynthesis is highly conserved in eukaryotes with the amenability of yeasts to genetic and metabolic engineering, basically all naturally occurring terpenoids might be produced involving yeasts. Such engineered yeasts are useful for the study of biological functions and molecular interactions of terpenoids as well as for the large-scale production of high-value compounds, which are unavailable in sufficient amounts from natural sources due to their low abundance.

Contents

Abbreviations: ABC, ATP-binding cassette; ADH, alcohol dehydrogenase; ADR, adrenodoxin reductase; ADX, adrenodoxin; ARE2, sterol acyltransferase; ARV1, mediator of sterol homeostasis; BTS1, GGPP synthase; CPR, cytochrome P450 reductase; CRT, carotenogenic (genes); CYP, cytochrome P450 enzymes; DCW, dry cell weight; DGPP, diacylglycerol diphosphate; DHCR7, dehydrocholesterol 7-reductase; DHCR24, dehydrocholesterol 24-reductase; DMAPP, dimethylallyl diphosphate; DPP1, DGPP phosphatase; ER, endoplasmic reticulum; ERG1, squalene epoxidase; ERG2, C-8 sterol isomerase; ERG3, C-5 sterol desaturase; ERG4, C-24(28) sterol reductase; ERG5, C-22 sterol desaturase; ERG6, C-24 sterol methyl transferase; ERG7, lanosterol synthase; ERG8, phosphomevalonate kinase; ERG9, squalene synthase; ERG10, acetoacetyl-CoA thiolase; ERG11, C-14 sterol demethylase; ERG12, mevalonate kinase; ERG13, hydroxymethylglutaryl-CoA synthase; ERG19, mevalonate diphosphate decarboxylase; ERG20, geranyl/farnesyl diphosphate synthase; ERG24, C-14 sterol reductase; ERG25, C-4 methyl sterol oxidase; ERG26, C-3 sterol dehydrogenase; ERG27, 3-keto sterol reductase; FF-MAS, follicular fluid meiosis-activating sterol; FPP, farnesyl diphosphate; FPPS, farnesyl diphosphate synthase (ERG20); GGPP, geranylgeranyl diphosphate; GPCR, G-protein coupled receptor; GPP, geranyl diphosphate; HEM1, 5-aminolevulinate; HFA, hydroxylated fatty acids; HMG1/HMG2, hydroxymethylglutaryl-CoA reductase 1/2; HOG1, high osmolarity glycerol response; HPO, *Hyoscyamus muticus* premnaspirodiene oxygenase; HXT1, hexose transporter 1; HXT2, hexose transporter 2; IDI1, isopentenyl diphosphate isomerase; IPC, inositol phosphorylceramide; IPP, isopentenyl diphosphate; LPP1, lipid phosphate phosphatase 1; MAP, mitogen activated protein; MAS, meiosis-activating sterol; MK, mevalonate kinase; NADPH, reduced nicotinamide adenine dinucleotide phosphate; PHA, polyhydroxyalkanoates; PLC1, phospholipase C; PMK, phosphomevalonate kinase, see ERG8; PTC1, protein phosphatase type 2C; PUFA, polyunsaturated fatty acids; SMT1, sterol methyltransferase 1; SMT2, sterol methyltransferase 2; SUE, selection for aerobic uptake of exogenous ergosterol; TAT2, tryptophan permease; tHMG1, truncated 3-hydroxy-3-methylglutaryl coenzyme A reductase; T-MAS, testicular meiosis-activating sterol.

* Corresponding author. Address: Institute of Molecular Biotechnology, Graz University of Technology, Petersgasse 14/2, A-8010 Graz, Austria. Tel.: +43 316 873 4089; fax: +43 316 873 4071.
E-mail addresses: harald.pichler@tugraz.at, harald.pichler@acib.at (H. Pichler).

0163-7827/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.plipres.2013.03.001

1. Introduction

Sterols are essential lipid constituents of eukaryotic membranes. The structural features of terminal sterols differ among species. Whereas mammalian and fungal cells generally contain one major sterol, cholesterol and ergosterol, respectively, plants have complex sterol profiles, dominated by sitosterol, stigmasterol and campesterol [1,2]. The structural differences between these sterols are highlighted in the sterol biosynthetic pathways shown in Fig. 1. Common to all sterols is the double bond at C-5 and the 3β-hydroxyl group, enabling proper orientation and anchoring in the membrane [3]. In yeast as in higher eukaryotes, free sterols are predominantly present in the plasma membrane [4]. As "bulk" membrane components, sterols fulfill a major structural function of controlling the physical state of the plasma membrane by modulating its bilayer fluidity and permeability [5]. On the other hand, molecular interaction with specific sterols governs membrane protein stability and function [6,7]. These diverse roles of sterol molecules imply that eukaryotes had to develop elaborate control of sterol structures and sterol abundance. Beside regulating de novo synthesis accordingly, excessive sterols can be acylated and stored in lipid droplets [8,9]. Like higher eukaryotes, yeasts are equipped to acylate elevated amounts of sterols and re-mobilize them by lipases if needed [10–12]. Remarkably high portions of total cellular sterols can thus be sequestered from membranes sustaining their functionality. High metabolic flux rates through the sterol biosynthesis pathway indicate a substantial pool of isoprene units and render yeast an interesting production organism for sterols and other terpenoid compounds. Furthermore, sterol and other lipid biosynthetic pathways in yeasts, especially in *Saccharomyces cerevisiae*, have been intensively studied [13], making yeast an ideal host system for metabolic engineering approaches.

Metabolic engineering of microorganisms is developing into a major field in biotechnology. Metabolic analyses of cells to identify most promising targets for manipulation are followed by genetic engineering of cells [14–17]. The goals of metabolic engineering are the optimization of strains for overproducing recombinant proteins and small molecule chemicals, the extension of substrate range, enhanced productivity and yield, elimination of by-products, improvement of process performance and of cellular properties [18,19]. Regardless of the desired product, the common aim of metabolic engineering is to transfer product-specific enzymes to microorganisms in order to generate an optimized biosynthetic pathway in terms of minimal cost and maximum productivity [20].

Yeast, especially *S. cerevisiae,* has been successfully used as microbial system in metabolic engineering approaches for the

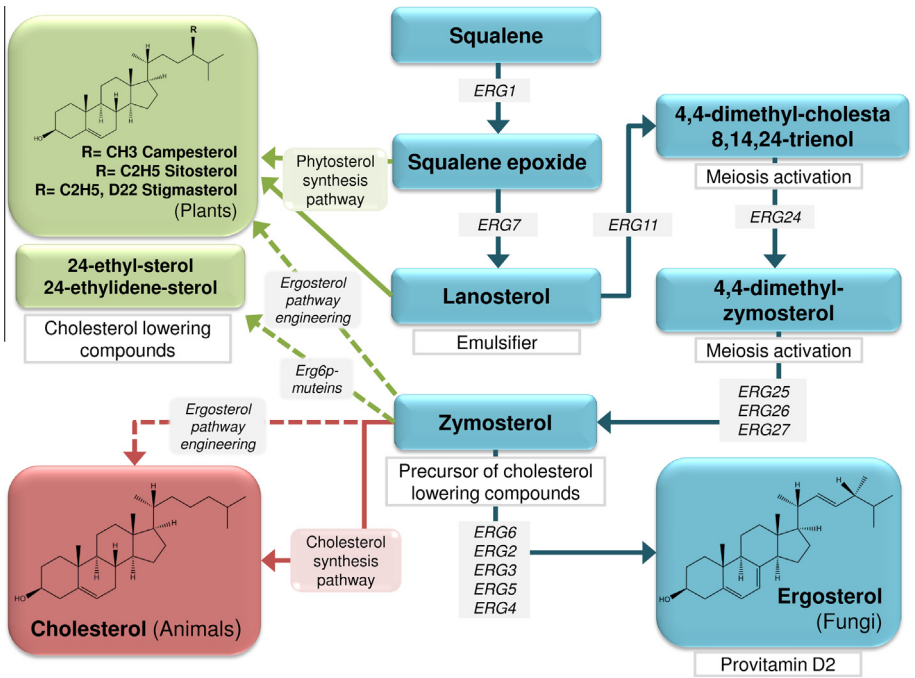

Fig. 1. Sterol biosynthesis pathways starting from squalene with sterol intermediates and end products in fungi (blue), plants (green) and animals (red). Solid arrows indicate naturally occurring reactions while dashed arrows denote engineering approaches described for yeasts. *ERG1*, Squalene epoxidase; *ERG2*, C-8 sterol isomerase; *ERG3*, C-5 sterol desaturase; *ERG4*, C-24(28) sterol reductase; *ERG5*, C-22 sterol desaturase; *ERG6*, C-24 sterol methyl transferase; *ERG7*, Lanosterol synthase; *ERG11*, C-14 sterol demethylase; *ERG24*, C-14 sterol reductase; *ERG25*, C-4 methyl sterol oxidase; *ERG26*, C-3 sterol dehydrogenase; *ERG27*, 3-keto sterol reductase.