Interactions of the ergosterol biosynthetic pathway with other lipid pathways

M. Veen[1] and C. Lang[2]
Institut für Biotechnologie, Technische Universität Berlin, FG Mikrobiologie und Genetik, Gustav-Meyer-Allee 25, D-13355 Berlin, Germany

Abstract

Micro-organisms have recently received broad attention as sources of novel lipids. An increased understanding of the effects of fats and oils and their composition on the metabolism and on health has shifted the focus towards the use of lipids for disease treatment and prevention and for the promotion of good health. A large range of lipidic products produced by yeast is known today. Ergosterol and its metabolic precursors are major lipidic components of industrial and commercial interest. Having in mind the aim to increase the productivity of ergosterol and its precursor metabolites, both the knowledge of regulatory mechanisms of the biosynthetic pathway and its interactions with other lipid pathways like those of sphingolipids, phospholipids and fatty acids are crucial.

Introduction

Sterols are essential structural and regulatory components of eukaryotic cell membranes [1]. Ergosterol, the end-product of the biosynthetic pathway and the main sterol in yeast, is responsible for structural membrane features, such as fluidity and permeability similar to the way cholesterol is in mammalian cells [2]. Several intermediates of the ergosterol pathway are economically important metabolites [3]. Lanosterol, the first sterol of the pathway, is used as a non-ionic, auxiliary emulsifier to jellify hydrocarbons and is added to cosmetic preparations, particularly lipstick and cosmetic creams. Ergosterol itself is commonly known and used as provitamin D_2. Sterols also function as moisturizers in skin-conditioning cosmetics and serve as the starting material of choice for the synthesis of various tetracyclic triterpenoid derivatives [4]. Furthermore, sterols act as structural components in liposomes, which are used as carriers of drugs and diagnostic substances in pharmaceutical applications [5]. Last but not least, sterols serve as raw materials for biotransformation of steroids and steroid hormones. Additional novel applications for sterols are outlined in recent papers: Subbiah and Abplanalp [6] demonstrated an *in vitro* anticarcinogenic effect of yeast extracts on breast cancer cells. They concluded that oxidation products of ergosterol are responsible for this effect. Xu et al. [7] described the production of meiosis-activating methylated sterol intermediates. For an overview on the biosynthetic pathway of the post-squalene ergosterol biosynthesis, see Scheme 1.

Membranes of eukaryotic cells have several important functions. They act as barriers between the inside of the cell or the lumen of organelles and the corresponding environment. Membranes also carry proteins that selectively transport molecules or act as enzymes in different metabolic activities. Due to this vital function of ergosterol, the biosynthetic pathway is strongly regulated.

Besides sterols, membranes of the yeast *Saccharomyces cerevisiae* are composed of phospholipids, fatty acids and sphingolipids, constituents that are typical for eukaryotic cells [8]. While these components are detectable in all the subcompartments of a cell, their relative composition differs and depends on growth conditions like temperature, oxygen concentration, medium composition and growth rate [9].

Under normal growth conditions, the relative ratio of membrane components is very stable and highly regulated. Variation in the amount of one component will induce radical rearrangement of the membrane. This may lead to a complete change of regulatory mechanisms in the lipid metabolism [10].

Most enzymes involved in the lipid metabolism of yeast have been identified and the corresponding genes have been cloned. Therefore information on the regulation of the individual pathways is abundant. A number of recent reviews focus on these aspects [3,11–13]. Novel data concerning the connection and interaction of the different pathways are emerging. This paper will summarize the current view on interconnections of lipidic pathways involved in the regulation of ergosterol biosynthesis.

Cross-talk between ergosterol biosynthesis and other lipid pathways

In recent years, a number of papers have shown that ergosterol and sphingolipid biosynthetic pathways are closely connected. An overview of the pathway of sphingolipids and ceramides is shown in Figure 1.

Swain et al. [14] have studied an *erg26* mutant that does not synthesize ergosterol, but methylates ergosterol precursors. The authors observed that ceramide biosynthesis and hydroxylation are also strongly deregulated in this mutant. A similar change in sphingolipid biosynthesis has been observed by Storey et al. [15]. These authors used lovastatin to inhibit 3-hydroxy-3-methylglutaryl-CoA reductase, a

Key words: biosynthetic pathway, co-regulation, ergosterol, lipid metabolism, long-chain fatty acid, sphingolipid.

[1]**Present address:** Organobalance GmbH, Gustav-Meyer-Allee 25, D-13355 Berlin, Germany.
[2]**To whom correspondence should be addressed (email c.lang@lb.tu-berlin.de).**

Scheme 1 | Post-squalene ergosterol biosynthetic pathway

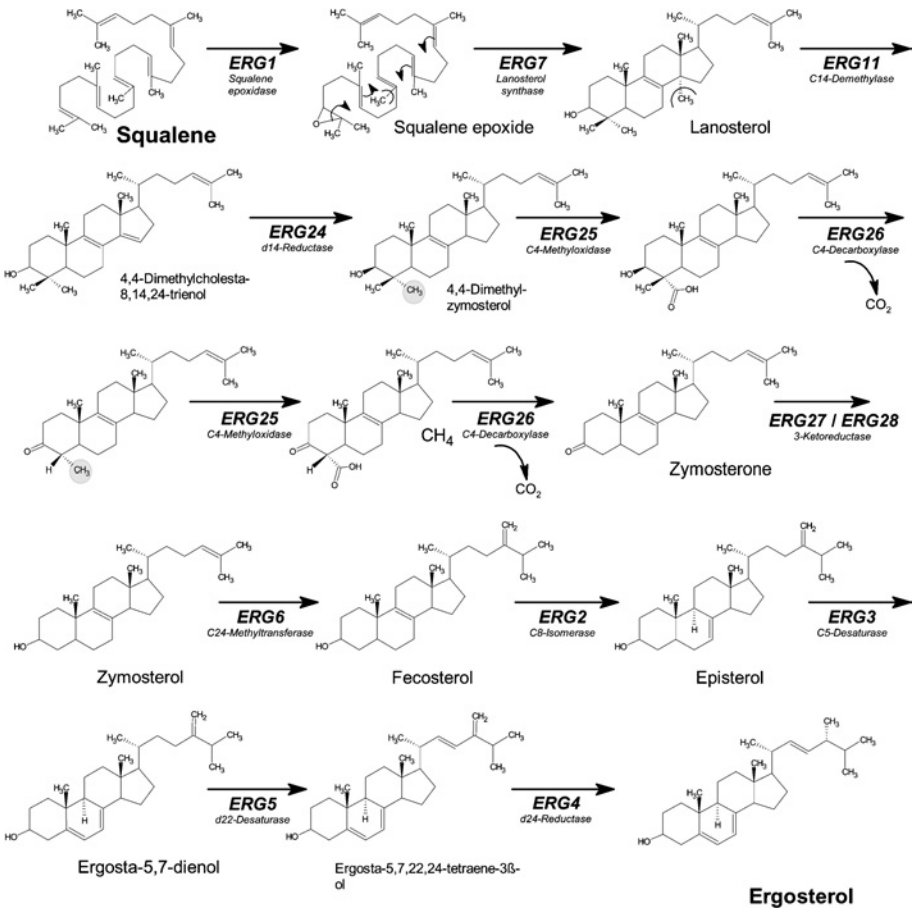
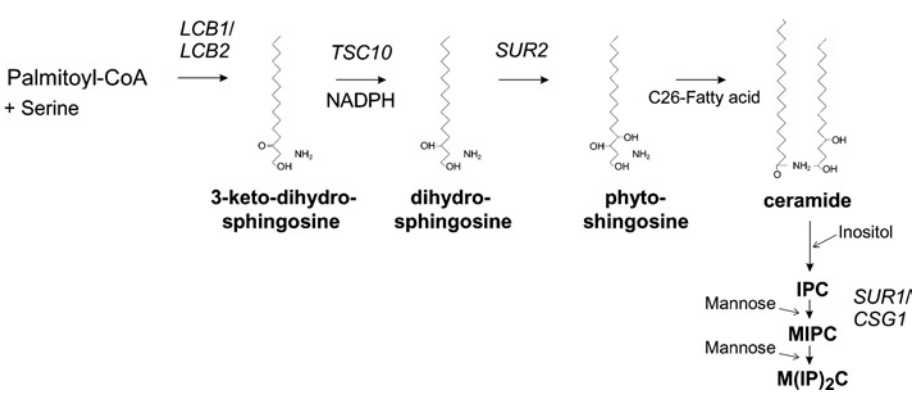


Figure 1 | Sphingolipid biosynthesis in the yeast *S. cerevisiae* (according to [22])

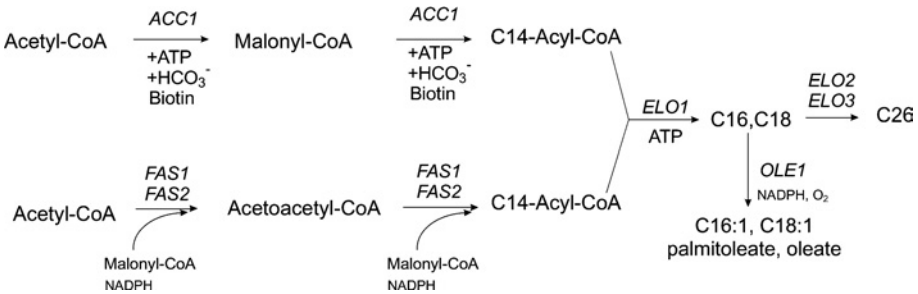
IPC, inositolphosphorylceramide; MIPC, mannosyl-inositolphosphorylceramide; $M(IP)_2C$, mannosyl-diinositolphosphoryl-ceramide.



major bottleneck of the ergosterol biosynthetic pathway. While ergosterol synthesis was down-regulated as expected, ceramide production was also down-regulated. It has been observed in mammalian cells that loss of the sterol regulation element-binding proteins, a major regulatory element of cholesterol biosynthesis, leads to a co-ordinated decrease in both cholesterol and ceramide biosynthesis. Also in mammalian cells, a decreased content of sphingomyelin in membranes results in an increase of sterol esterification by the steryl acyl transferases [16].

Another intriguing connection of sterol and sphingolipid biosynthesis has been observed in an *erg24* ergosterol mutant in *S. cerevisiae*. While this mutation is lethal, the additional deletion of the fatty acid elongase Elo3p renders cells viable.

Scheme 2 | Biosynthesis of fatty acids in yeast



The *elo3* mutant cannot synthesize sphingolipids, because the synthesis of very long-chain fatty acids (C_{26}) is blocked. The viability of the double mutant *erg24elo3* suggests that a lack of sphingolipids compensates for the lack of ergosterol in the membrane. The mechanism for this co-regulation is not yet known, but it might be suggested that, due to steric reasons, the methylated ergosterol precursors that arise in *erg24* mutants are embedded in yeast membranes only if sphingolipids are not synthesized and that the membrane integrity is thus re-established.

Eisenkolb et al. [17] reported that an *erg6* deletion mutant that is normally viable and uses zymosterol as its major membrane sterol intermediate, becomes synthetically lethal when the biosynthesis of very long-chain fatty acids is suppressed by deletion of the gene *ELO3*. In contrast with methylated sterol intermediates that occur in the *erg24* mutant, zymosterol, the major sterol intermediate in an *erg6* mutant, seems to be a membrane constituent that is dependent on the availability of sphingolipids. According to Valachovic et al. [18] a mutation in the otherwise non-essential *ERG2* gene is synthetically lethal when this mutation is combined with mutations in the transcription factors encoded by *UPC2* and *ECM22*. As a suppressor of the triple mutant *erg2upc2ecm22*, they isolated a mutation in the *ELO3* gene. Surprisingly, a deletion of *ELO2*, also required for the synthesis of sphingolipid-containing long-chain fatty acid, did not suppress the *erg2upc2ecm22* triple mutant.

A co-ordinated regulation of sphingolipid and sterol biosynthesis has also been described in a paper by Bammert and Fostel [19]. These authors have performed microarray experiments to analyse the global effects of azole antifungicides (ergosterol biosynthesis inhibitors). Major effects of azole treatment have been observed on genes of the sphingolipid and ergosterol biosynthetic pathways. The genes *SUR2*, encoding the sphingoid base hydroxylase, and *LBC1*, encoding the palmitoyltransferase, are significantly down-regulated.

Baudry et al. [10] observed an accumulation of early ergosterol precursors in an *erg26* mutant and a distinct change in the composition of phospholipids in the membranes. While the content of phosphatidic acid increased by a factor of 1.9, the content of phosphoinositol decreased by 1.7-fold.

In other studies, cross-talk between ergosterol and fatty acid biosynthesis has been observed. The pathway of fatty acid biosynthesis is shown in Scheme 2. Microarray analyses by Bammert and Fostel [19] have shown that blocking the ergosterol biosynthesis leads to a significant change in the expression of genes involved in the *de novo* biosynthesis of long-chain fatty acids. Most significantly, the transcription of the biosynthesis genes *ELO1*, *OLE1* and *FAS1* was decreased. As fatty acids are needed for the storage of steryl ester in yeast lipid particles, this down-regulation might reflect a feedback regulation and a signalling across pathways. This would also point to the cell's ability to adapt its fatty acid biosynthesis to the requirements in metabolic processes, e.g. the storage of sterols as steryl ester.

This suggestion is strongly supported by studies on the regulation of the acyl-CoA-carboxylase (Acc1p) [20]. These authors have shown that the transcription of *ACC1* shows a strong co-regulation with the transcription of *HMG1*, the gene known to be a major regulator of the ergosterol biosynthetic pathway. An increase in the sterol biosynthetic capacity results in a concomitant increase in the availability of fatty acids.

This was also observed in strains accumulating high amounts of sterols. The availability of fatty acids does not seem to be a limiting factor in the esterification and accumulation of sterols in the cells [21]. The amount of triacylglycerides also strongly increases in the cells when sterols accumulate in lipid particles (M. Veen, G. Shin and C. Lang, unpublished work). This might suggest that the total amount of steryl esters and fatty acid esters, the major constituents of lipid particles, rather depends on the equilibrium of both compounds in the lipid particles than on the individual biosynthetic rate.

Conclusion

Several links between the biosynthetic pathway of ergosterol and other pathways involved in the biosynthesis of lipids have been detected in recent studies. This emerging knowledge of regulatory networks promises to be one major key for the systematic deregulation of the sterol biosynthetic pathway and the production of high value sterol metabolites with the yeast *S. cerevisiae*.

References

1 Volkman, J.K. (2003) Appl. Microbiol. Biotechnol. **60**, 495–506
2 Parks, L.W., Smith, S.J. and Crowley, J.H. (1995) Lipids **30**, 227–230
3 Veen, M. and Lang, C. (2004) Appl. Microbiol. Biotechnol. **63**, 635–646
4 Sjostrom, B., Bergenstahl, B. and Kronberg, B. (1993) J. Pharm. Sci. **82**, 584–589
5 Stach, D., Zheng, Y.F., Perez, A.L., Oehlschlager, A.C., Abe, I., Prestwich, G.D. and Hartman, P.G. (1997) J. Med. Chem. **40**, 201–209
6 Subbiah, M.T. and Abplanalp, W. (2003) Int. J. Vitam. Nutr. Res. **73**, 19–23
7 Xu, R., Wilson, W.K. and Matsuda, S.P. (2002) J. Am. Chem. Soc. **124**, 918–919
8 Rest van der, M.E., Kamminga, A.H., Nakano, A., Anraku, Y., Poolman, B. and Konings, W.N. (1995) Microbiol. Rev. **59**, 304–322
9 Tuller, G., Nemec, T., Hrastnik, C. and Daum, G. (1999) Yeast **15**, 1555–1564
10 Baudry, K., Swain, E., Rahier, A., Germann, M., Batta, A., Rondet, S., Mandala, S., Henry, K., Tint, G.S., Edlind, T. et al. (2001) J. Biol. Chem. **276**, 12702–12711
11 Carman, G.M. and Henry, S.A. (1999) Prog. Lipid Res. **38**, 361–399
12 Lees, N.D., Bard, M. and Kirsch, D.R. (1999) Crit. Rev. Biochem. Mol. Biol. **34**, 33–47
13 Dickson, R.C. and Lester, R.L. (2002) Biochim. Biophys. Acta **1583**, 13–25
14 Swain, E., Baudry, K., Stukey, J., McDonough, V., Germann, M. and Nickels, Jr, J.T. (2002) J. Biol. Chem. **277**, 26177–26184
15 Storey, M.K., Byers, D.M., Cook, H.W. and Ridgway, N.D. (1998) Biochem. J. **336**, 247–256
16 Slotte, J.P. and Bierman, E.L. (1988) Biochem. J. **250**, 653–658
17 Eisenkolb, M., Zenzmaier, C., Leitner, E. and Schneiter, R. (2002) Mol. Biol. Cell **13**, 4414–4428
18 Valachovic, M., Wilcox, L.I., Sturley, S.L. and Bard, M. (2004) Lipids **39**, 747–752
19 Bammert, G.F. and Fostel, J.M. (2000) Antimicrob. Agents Chemother. **44**, 1255–1265
20 Hardie, D.G. and Carling, D. (1997) Eur. J. Biochem. **246**, 259–273
21 Veen, M., Stahl, U. and Lang, C. (2003) FEM Yeast Res. **4**, 87–95
22 Daum, G., Lees, N.D., Bard, M. and Dickson, R. (1998) Yeast **14**, 1471–1510

Received 31 May 2005