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Mini-review

Tools for the analysis of metabolic flux through the sphingolipid pathway

Fernando Martínez-Montañés, Roger Schneiter*

University of Fribourg, Department of Biology, 1700 Fribourg, Switzerland

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ABSTRACT

Discerning the complex regulation of the enzymatic steps necessary for sphingolipid biosynthesis is facilitated by the utilization of tracers that allow a time-resolved analysis of the pathway dynamics without affecting the metabolic flux. Different strategies have been used and new tools are continuously being developed to probe the various enzymatic conversions that occur within this complex pathway. Here, we provide a short overview of the divergent fungal and mammalian sphingolipid biosynthetic routes, and of the tracers and methods that are frequently employed to follow the flux of intermediates throughout these pathways.

1. Introduction

Sphingolipids in conjunction with sterols and glycerophospholipids constitute the main lipids of the plasma membrane of eukaryotic cells. They are essential to maintain the permeability barrier of this limiting membrane, but also function as signalling molecules that coordinate surface expansion with cell growth and division. Sphingolipids are amphipathic lipids composed of a hydrophobic part, termed ceramide, which can be substituted by an array of hydrophilic head groups. Ceramide itself is composed of a sphingoid long-chain base (LCB) that is amide-bound to a fatty acid. Despite the fact that sphingolipid constituents such as LCBs and ceramides have emerged as potent signalling molecules, the number of publications on sphingolipids over the last 5–10 years has not increased as dramatically as those containing “lipids” or “fatty acids” as key words (Fig. 1).

Nevertheless, our understanding of sphingolipid synthesis and regulation, particularly in genetic model organisms such as *Saccharomyces cerevisiae*, has been greatly expanded over the last couple of years. This advancement is at least partially due to the development of new analytical tools, most notably mass spectrometry, which opened the door to lipidomics. The steps of sphingolipid biosynthesis and turnover and their homeostatic regulation in both yeast and mammalian cells have recently been reviewed and is not the subject of this more methodologically oriented review [1,2]. Instead, we highlight significant differences in sphingolipid metabolism in less studied models and pathogenic yeast and discuss advantages and shortcomings of different strategies to chemically label different intermediates to follow their flux through the pathway.

2. Diversity of sphingolipid biosynthetic pathways in yeast species

The diversity among different yeast species is large and one of the reasons for their exploration as models to study particular biological processes. While *Schizosaccharomyces pombe* is a preferred subject to study cell division, *Pichia pastoris* is being used as a workhorse for the heterologous expression of eukaryotic proteins. *Saccharomyces cerevisiae*, on the other hand, has yielded many important insights into sphingolipid metabolism, particularly due to the pioneering studies of Lester and coworkers [3,4]. Even

* Corresponding author. University of Fribourg, Department of Biology, Chemin du Musée 10, 1700 Fribourg, Switzerland.
E-mail address: roger.schneiter@unifr.ch (R. Schneiter).

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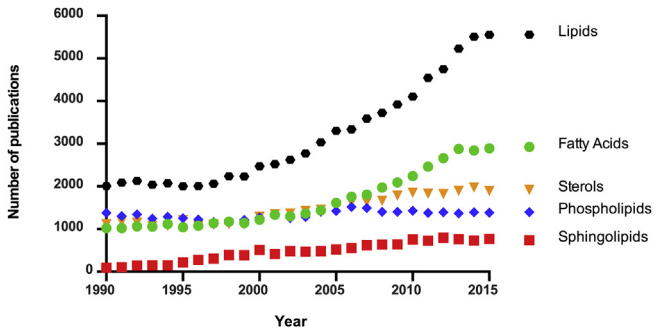

Fig. 1. Lipid-related publications from 1990 to 2015. The number of publications containing the indicated key word in the title were calculated using PubMed Single Citation Matcher (http://www.ncbi.nlm.nih.gov/pubmed/citmatch). Datasets were generated using the following key words: black (lipid/s); green (fatty acid/s); orange (sterol/s, cholesterol/s, ergosterol/s); blue (phospholipid/s, glycerophospholipid/s, phosphoinositide/s, phosphatidylcholine, phosphatidylethanolamine, phostatidylglycerol, phosphatidylinositol, phosphatidic acid, phosphatidylserine, cardiolipin); red (sphingolipid/s, phosphosphingolipid/s, sphingosine/s, ceramide/s, ceramidase/s, sphingomyelin/s, sphingomyelinase/s).

with complete genome sequences of the fission yeast *S. pombe*, the methylotrophic yeast *P. pastoris* and several other pathogenic fungi at hand today, compared to *S. cerevisiae*, only relatively few studies on the sphingolipid pathway in these organisms have been reported [5–9].

The synthesis of the sphingoid backbone is conserved among eukaryotes and begins in the endoplasmic reticulum with the condensation of L-serine and palmitoyl-CoA, the preferred substrates of serine palmitoyltransferase (SPT), to form 3-ketosphinganine. This sphingoid base is then reduced to dihydrosphingosine (DHS). DHS is hydroxylated to yield phytosphingosine (PHS), and serves as precursor to desaturated ceramides typically produced by *Candida albicans, P. pastoris, S. pombe*, *Kluyveromyces lactis* and *Saccharomyces kluyveri* [5,6,10,11] (Fig. 2, branches on brown/green backgrounds). The diversity of long-chain bases, however, is bewildering and covered in depth in more specialized reviews [12,13].

The long-chain bases, DHS and PHS are *N*-acylated to yield dihydroceramide and phytoceramide, the hydrophobic backbones of the more mature and complex sphingolipids. In *S. cerevisiae,* ceramide is then uniquely converted to phospho-inositol-containing sphingolipids. On the other hand, *P. pastoris*, *C. albicans* and other fungal pathogens, are able to produce two types of mature sphingolipids, the acidic phospho-inositol containing ones and the neutral glucosylceramides, which contain glucose instead of the phospho-inositol, as their polar head group [7,14,15] (Fig. 2). Interestingly, the ceramide synthases of *C. albicans* possess distinct substrates specificities and physiological roles: CaLag1p predominantly synthesizes ceramides with C24:0/C26:0 fatty acid moieties for the generation of phospho-inositol-containing sphingolipids, whereas CaLac1p produces ceramides with C18:0 fatty acid moieties, which serve as precursors for glucosylsphingolipids [8]. It is noteworthy, that the fungal glucosylceramide structurally differs from mammalian and plant glucosylceramides [16]. The double bonds in position Δ4 and Δ8 and the fungal specific C9 methyl group are inserted sequentially into the LCB backbone of ceramide [7,9,17,18] (Fig. 2).

Comparative analyses of the sphingolipid composition of *S. cerevisiae* and other yeast revealed additional differences [19,20]. For example, while mannosyl-diinositolphosphorylceramide ($M(IP)_2C$) is the most abundant mature sphingolipid species in *S. cerevisiae*, the major sphingolipid in *S. pombe* is mannosyl-inositolphosphorylceramide (MIPC) [19–21]. In *S. cerevisiae*, the final step in the biosynthesis of complex sphingolipids requires the

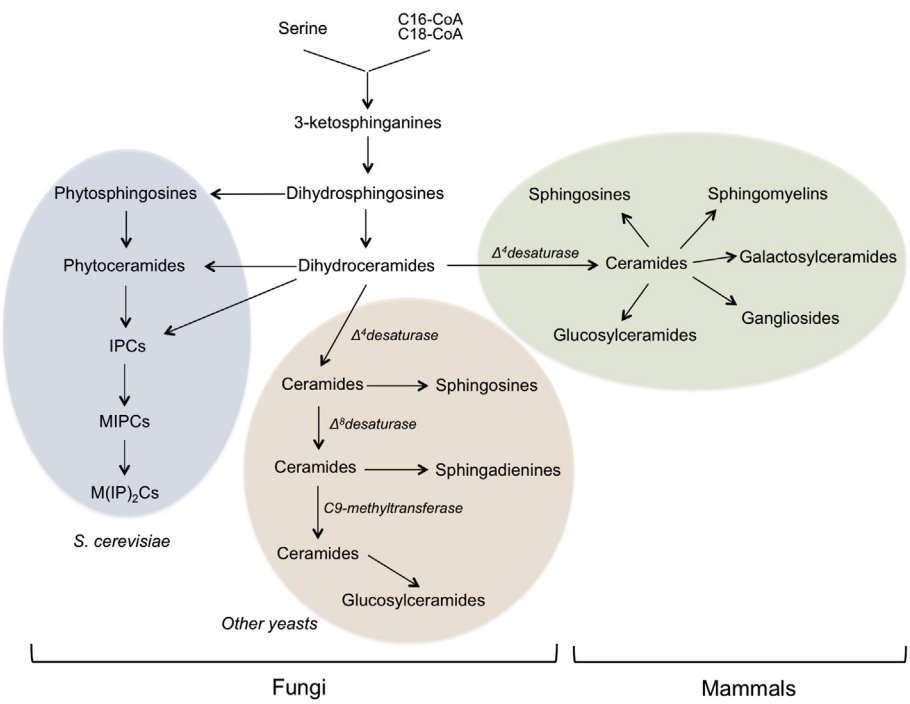

Fig. 2. Overview of the *de novo* sphingolipid biosynthetic pathways in fungi and mammals. For clarity, only anabolic processes are depicted, with the exception of sphingosine and sphingadienine formed by ceramide hydrolysis. Early steps in the pathway until the formation of dihydroceramide are common between fungi and mammals. *S. cerevisiae* produces inositol-containing sphingolipids (left, on blue background), whereas other yeast species can also synthesize glucosylceramides (center, on brown background). On the other hand, mammals (right, on green background) produce glycosphingolipids by addition of one (glucosylceramides, galactosylceramides) or more (gangliosides) sugar residues to the 1-hydroxy group of ceramide.

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