# Lipid Biosynthesis in Eukaryotic Cells

## Studies on Enzyme Activities Involved in Fatty Acid Activation and Acylation

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# Abstract

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Lipid molecules are fundamental to cellular function and structure. Lipid metabolic events leading to these basic building blocks has been intensely studied, but are still not fully understood. Through five different studies on enzymes involved in activation and acylation of acyl groups done in three different model organisms we have uncovered novel data on lipid biosynthesis and utilization.

Saccharomyces cerevisiae contains two homologous glycerol-3-phosphate acyltransferases, GPT2 and SCT1, which catalyses the first step in the acyl-lipid assembly. Deletion or over expression of the corresponding genes has not only strong effects on the glycerol-3-phsophate activity but also on the total acyl content as well as on lipid and acyl composition. (Paper I)

The main form of storage lipids in seeds is Triacylglycerol. The *TAZ1* from Saccharomyces cerevisiae, *At104.1*, and *At104.2* from Arabidopsis thaliana are acyltransferase homologous genes that when overexpressed increase oil quantity in Yeast and Plants. (Patent I)

Wax esters and ethyl esters are unique constituents that have roles in energy storage and cell structure. In this work we show that microsomal preparations from Arabidopsis thaliana leaves efficiently acylate free fatty acids to long chain alcohols with no activation of the fatty acids to thioesters prior to acylation. We also show that in this enzymatic reaction long chain (C18–C24) unsaturated fatty alcohols and C18–C22 unsaturated fatty acids were preferred. (Paper II)

Long-chain acyl-CoA synthetases activate fatty acids and provide substrates for virtually every metabolic pathway that catabolizes FA or synthesizes complex lipids. In Mus musculus heart tissue we show that ACSL1 mRNA levels increase by 2.5 fold after birth, which correlates with the 14 fold increase in total ACSL activity at this time. In contrast, the levels of ACSL3, 4, and 6 mRNA were high in the embryonic heart, and decreased dramatically after birth suggesting that these isoforms may play a critical role during the development of the fetal heart. (Paper III)

Microsomes of Saccharomyces cerevisiae and developing Elm (Ulmus glabra) seeds contain a novel type of acyltransferase activity, a acyl-CoA dependent acylation of glycerophosphodiesters. Glycerophosphocholine was acylated by yeast microsomes with a Vmax of 8.7 nmol/min/mg protein and a Km of 2.5 mM. The GPCAT activity was sensitive to NEM, phenantroline and $Zn^{2+}$ ions. (Paper IV)

Keywords: glycerol-3-phosphate acyltransferase, tafazzin, long chain acyl-CoA synthetase, wax ester, ethyl ester, triacylglycerol, glycerophosphocholine, glycerophosphoethanol-amine, Mus musculus, Arabidopsis thaliana, Saccharomyces cerevisiae

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**This Thesis is Dedicated to My Parents,**
**Julia and Gordon Neal**

*The human mind is powerful, we can imagine many worlds that don't exist and it's tempting to try and force reality to meet those views. I think you have to keep real things close and follow where nature takes you. If you set up experiments to test things you already know, then you can learn nothing, but if you let nature show you, and follow, then in that sense, serendipity might play a role*.

– **Sir Paul Nurse**

# Contents

# Appendix

## Papers I-IV and patent 1

The present thesis is based on the following papers and patent, which will be referred to by their Roman numerals.

**I.** Neal, A.C., Banas, A., Ronne, H. & Ståhl, U. Changes in Lipid Composition of *Saccharomyces cerevisiae* strains with Altered Glycerol-3-Phosphate Acyltransferase Gene Expression. *Manuscript*

**II.** Neal, A.C., Banas, A., Banas, W., Ståhl, U., Carlsson, A.S. & Stymne, S. 2006. Microsomal preparations from plant and yeast acylate free fatty acids without prior activation to acyl thioesters. *Biochim Biophys Acta* 1761, 757-764

**III.** Neal, A.C., Coleman, R.A. & Lewin, T.M. Ontogeny of mRNA expression and activity of long-chain acyl-CoA synthetase (ACSL) isoforms in *Mus musculus* heart. *Manuscript*

**IV.** Stålberg, K., Neal, A.C. & Ståhl, U. Glycerophosphodiester Acyltransferase; a New Class of Acylation Activity in Lipid Biosynthesis. *Manuscript*

**1.** Cirpus, P., Oswald, O., Ronne, H., Dahlquist, A., Lenman, M., Neal, A.C., Ståhl, U., Liu, T., Banas, A., Wiberg, E. Use of TAZ1 for Increasing Oil Quantity in Plants. International Patent Number: PCT/EP2004/003845 Filed: April 13$^{th}$ 2004

# Abbreviations

| | |
|---|---|
| ACS | Acyl-CoA synthetase |
| ACP | Acyl carrier protein |
| ACSL | Long chain acyl-CoA synthetase |
| AGPAT | 1-acyl-glycerol-3-phosphate acyltransferase |
| AT | Acetyl transferase |
| CPT-1 | Carnitine palmitoyltransferase-1 |
| DAG | Diacylglycerol |
| DH | Dehydrogenase |
| DHAPAT | Dihydroxyacetonephosphate acyltransferase |
| EnR | Enoyl reductase |
| ER | Endoplasmic reticulum |
| FA | Fatty acid |
| FAS | Fatty acid synthase |
| GPAT | Glycerol-3-phosphate acyltransferase |
| GPCAT | Glycerophosphocholine acyltransferase |
| GPEAT | Glycerophosphoethanolamine acyltransferase |
| KS | Ketoacyl synthetase |
| KR | Ketoacyl reductase |
| LCAD | Long chain acyl-CoA dehydrogenase |
| LPA | Lysophosphatic acid |
| LPCAT | Lysophosphocholine acyltransferase |
| LPEAT | Lysophosphoethanolamine acyltransferase |
| MA | Malonyl acetyl |
| MAG | Monoacylglycerol |
| MAM | Mitochondrial associated membrane |
| MCAD | Medium chain acyl-CoA dehydrogenase |
| mMDH | Mitochondrial malate dehydrogenase |
| MPT | Malonyl palmitoyl-transacylase |
| ORF | Open reading frame |
| PA | Phosphatidic acid |
| PC | Phosphatidylcholine |
| PDAT | Phospholipid:diacylglycerol acyltransferase |
| PE | Phosphatidylethanolamine |
| PPAR | Peroxisome proliferator-activated receptor |
| PS | Phosphatidylserine |
| SCAD | Short chain acyl-CoA dehydrogenase |
| TAG | Triacylglycerol |
| TE | Thioesterase |
| WT | Wild type |

# Introduction

The metabolic steps leading to the synthesis of lipids are very complex. Today there are still many unanswered questions about phospholipid biosynthesis. Understanding these processes can have large impacts on the developments of biotechnological advances in industry, medicine, consumer products and nutrition. Today these advances are becoming more important with the ever-growing population of the world and consumption of natural products. As well as increased consumption of natural products, there is an increasing epidemic of obesity. This epidemic is especially clear in children in the United States of America. Healthier oils have become an important component in good nutrition and of major concern to the general public. Enhancement of oil seed quality and quantity is growing in importance every year. In this thesis I address; lipids, model organisms used to study lipid biosynthesis, glycerol-3-phosphate acyltransferase, tafazzin, long chain acyl CoA synthetase, and novel wax ester and ethyl ester synthetases.

## Why do we study lipids?

Lipids are components essential to all organisms. They are utilized in many cellular functions such as membrane formation (fig. 1), defense mechanisms and as an energy source for cellular processes. To date, there have been thousands of studies

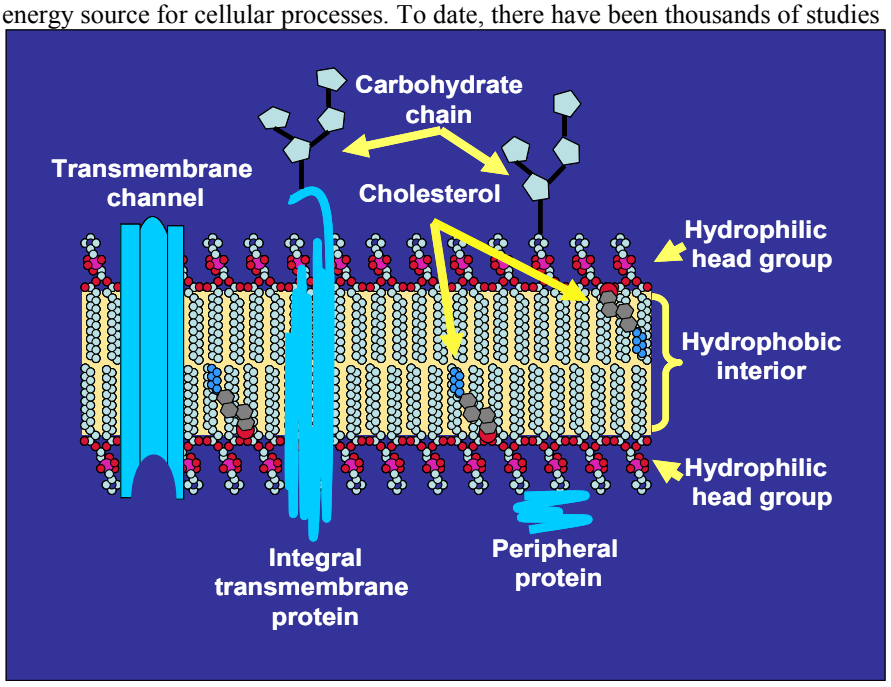



**Figure 1**. The fluid mosaic model: This schematic shows a representation of the fluid mosaic model of the plasma membrane based on theories by Singer and Nicholson in 1972 (Singer & Nicolson, 1972). The plasma membrane is comprised of phospholipids as represented here with their hydrophilic head groups and hydrophobic tails. The large masses here represent integral proteins imbedded or attached to the membrane.

conducted over the last century on the production and metabolic processes of lipids. Understanding lipid formation is an important step towards directed bioengineering. The potential for large breakthroughs in medicine and agriculture are unlimited with the increased understanding of lipid forming processes. While many of the enzymatic steps leading to glycerolipids have been characterized and the genes that influence them have been cloned, there are still many missing pieces in the puzzle. There is vast potential for using knowledge of these cellular functions to improve existing products and techniques.

## The different classes of lipids

In the past, lipids have been described as biological substances that are generally hydrophobic in nature. William Christie has submitted the following definition: lipids are fatty acids and their derivatives, and substances related biosynthetically functionally to these compounds (Christie, 2003). This is close to the traditional definition from Bloor in the 1920's, where he classified lipids into three groups: simple lipoids (fats and waxes), compound lipoids (glycolipids and phospholipids), and derived lipoids (fatty acids, alcohols and sterols) (Bloor, 1920). Christie's definition allows us to include cholesterol, plant sterols and possibly bile acids and tocopherols as lipids. It is accepted now that lipids can be separated into two major classes: simple and complex lipids. Simple lipids as defined by Christie are lipids that yield at most two primary products per mole and complex lipids yield three or more primary products per mole (Christie, 2003). Simple lipids include triacylglycerol, diacylglycerol, monoacylglycerols, fatty acyls, sterols, sterol esters, waxes, and acylglycerols. Complex lipids include glycerophospholipids, glycosphingolipids, and glycoglycerolipids. Fahy et al., 2005 has proposed that these two main classes should be broken further into eight classes: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides (Fahy et al., 2005). In this thesis, I will discuss three of these classes: fatty acyls, glycerolipids, and glycerophospholipids.

## Fatty Acyls

Fatty acyls is a definition which includes diverse simple structures such as fatty acids (FAs), fatty alcohols and simple acyl lipid structures (Table 1; Fig. 2). They are constituents of neutral lipids, polar lipids, as side chains of some co-enzymes, secondary metabolites, as covalent attachments to distinct eukaryotic proteins, as parts of eukaryotic second-messenger molecules, energy storage, in the integrity and dynamic of biological membranes, as well as being a main controlling factor in cellular metabolism. There are five fatty acids that are normally referred to as common that are usually found in membranes and are the main components of vegetable oils. These are palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid (Table 1). The enzyme complex that produce fatty acids is the Fatty acid synthase (FAS). The mechanism of *de novo* FA synthesis is similar in all biological systems. However, there is considerable variation in the organization of the different FAS complexes/polypeptides and subcellular localization. In plants, *de novo* FA synthesis takes place in the plastids, while in other eukaryotes it takes place in the cytosol. The main building blocks of FAS are ac(et)yltransferase,

malonyl/acetyl- or malonyl/palmitoyl-transacylase, ketoacyl synthase (KAS), ketoacyl reductase, dehydratase, enoyl reductase, acyl carrier protein (ACP), and thioesterase (Schweizer & Hofmann, 2004). Saturated acyl chains are formed by the condensation of two carbon units from malonyl-ACP to acetyl/acyl chains with palmitoyl- and stearoyl-ACP as the predominant products (Voelker & Kinney, 2001). In this process the growing FAs are attached through an ester bond to ACP. Each two carbon elongation cycle includes four reactions: condensation of malonyl-ACP and ac(et)yl-ACP, reduction of 3-ketoacyl-ACP, dehydration of 3-hydroxyacyl-ACP, and finaly reduction of *trans*-$\Delta^2$-enoyl-ACP. The final product of these four steps is a fully reduced acyl chain extended by two carbons. The condensing enzymes are very specific for chain length. KAS III condenses C2-C4, KAS I condenses C4-C16 and KASII condenses C16-C18, (Clough *et al.*, 1992; Shimakata & Stumpf, 1984). In plants the elongated FA are then cleaved from ACP by thioesterases exported out of the plastids and activated to acyl-CoA (Schnurr, Shockey & Browse, 2004). The acyl-CoAs are then utilized by various metabolic pathways such as membrane synthesis, seed storage lipid synthesis as well as cuticular lipid synthesis (Aharoni et al., 2004).

**Figure 2.** Three examples of fatty acyl structures utilized in this thesis, Structures were drawn with ACD/ChemSketch.

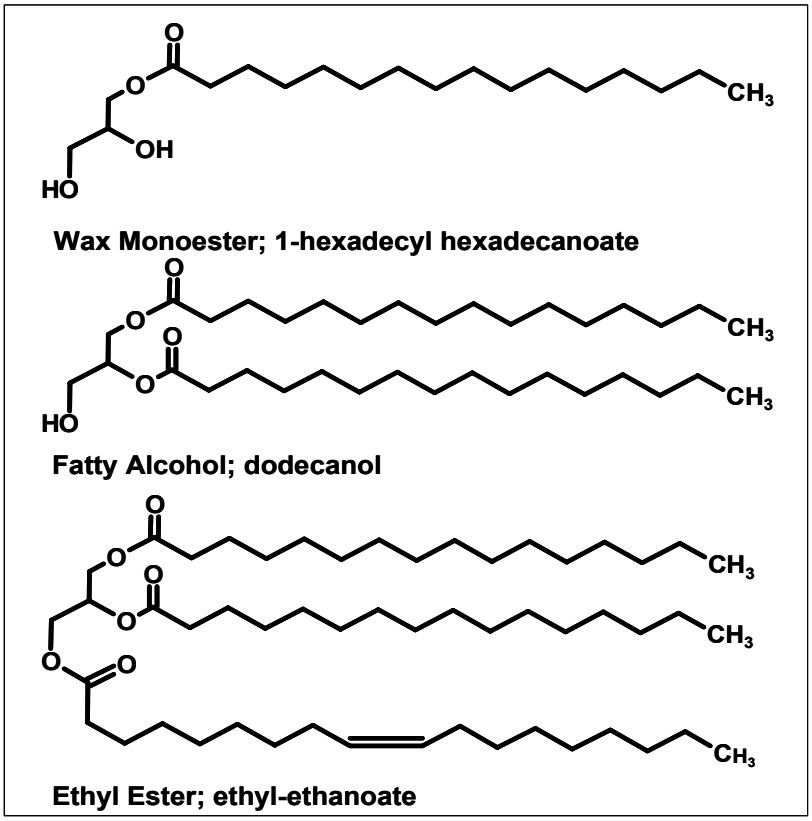


Table 1. **Fatty acids utilized in this thesis. The shorthand designation marked with a star (*) are not naturally produced in plants.**

| **Systematic name** | Trivial name | Shorthand designation |
|---|---|---|
| **Butanoic** | butyric | 4:0 |
| **Hexanoic** | caproic | 6:0 |
| **Octanoic** | caprylic | 8:0 |
| **Decanoic** | capric | 10:0 |
| **Dodecanoic** | lauric | 12:0 |
| **Tetradecanoic** | myristic | 14:0 |
| **Hexadecanoic** | palmitic | 16:0 |
| **Δ 9-hexadecanoic** | palmitoleic | 16:1(ω-7) |
| **Octadecanoic** | stearic | 18:0 |
| **Δ 9-octadecenoic** | oleic | 18:1 |
| **Δ 9,12-octadecadienoic** | linoleic | 18:2(ω-6) |
| **Δ 9,12,15-octadecatrienoic** | α-linolenic | 18:3(ω-3) |
| **Δ 6,9,12-octadecatrienoic** | γ-linolenic | 18:3(ω-6) * |
| **Eicosanoic** | arachidic | 20:0 |
| **Eicosenoic** | gadoleic | 20:1 |
| **Δ 8,11,14-eicosatrienoic** | dihomo- γ-linolenic | 20:3(ω-6) |
| **Δ 5,8,11,14-eicosatetraenoic** | arachidonic | 20:4(ω-6) * |
| **Δ 5,8,11,14,17-eicosapentanoic** | EPA | 20:5 * |
| **docosanoic** | behenic | 22:0 |
| **docosenoic** | erucic | 22:1 |
| **Δ 7,10,13,16,19-docasapentaenoic** | clupanodonic (DPA) | 22:5 |
| **Δ 4,7,10,13,16,19-docosahexaenoic** | cervonic (DHA) | 22:6 * |
| **tetracosanoic** | lignoceric | 24:0 |
| **tetracosenoic** | nervonic | 24:1 |
| **hexacosanoic** | cerotic | 26:0 |
| **hexacosenoic** | | 26:1 |

## Glycerolipids

This class of lipids encompasses simple lipids that contain glycerol. These molecules have important roles as metabolic fuels with the most common molecular structure being the triacylglycerol (TAG, Fig. 3), in membrane composition and as signaling molecules. 2-monoacylglycerols are intermediates or end products of the hydrolysis of TAG or diacylglycerol (DAG, Fig. 3) during digestion (Christie, 2003). While typically a small percentage in total lipid content, some monoacylglycerols (MAGs, Fig. 3) have been found to be integral to certain membranes and to have specific physiological functions. MAGs also serve importance in industry as food surfactants. Derivatives from MAGs are also utilized in many cosmetic products. Some examples of this are 2-arachidonoylglycerol, which is found to be approximately half of the total MAG content in nervous tissue; 2-sciadonoylglycerol, containing the rare unsaturated fatty acid, sciandonic acid, that has cannabimimetic activity and is found in seeds and leaves of all conifers; 1-butyrylglycerol, which appears to be a key regulatory molecule in angiogenesis; and monoacylglycerols, which are a major constituent of

cutin polymers (Dobson *et al.*, 1990; Graca *et al.*, 2002; Kondo *et al.*, 1998; Nakane *et al.*, 2000).

DAGs (Fig. 3) are important intermediates in TAG synthesis. DAGs are also a precursor to phosphatidylethanolamine (PE) and phosphatidylcholine (PC) synthesis. They are important signaling molecules, which can activate cellular mechanisms directly with direct protein interaction or indirectly with fatty acid release. The formation of DAG comes from the lipid biosynthesis pathway trough the dephosphorylation of phosphatidic acid (PA) by phosphatidic phosphatase or from degradation of phospholipids through phospholipases. In the food industry, DAGs are becoming key components of cooking sprays. Oils rich in DAGs have recently become an important tool for fighting obesity (Rudkowska et al., 2005).

Triacylglycerols are important molecules in energy metabolism. They are the main component of all important commercial plant, animal fat and oil products. TAG is formed by the acylation of DAG at the sn-3 position with Acyl-CoA. TAG molecules can also be formed by an acyl-CoA independent formation called phospholipid:diacylglycerol acyltransferase PDAT (Dahlqvist *et al.*, 2000; Stahl *et al.*, 2004). TAG molecules accumulate to form lipid bodies either in the outer membrane of the ER or on the surface of the ER.

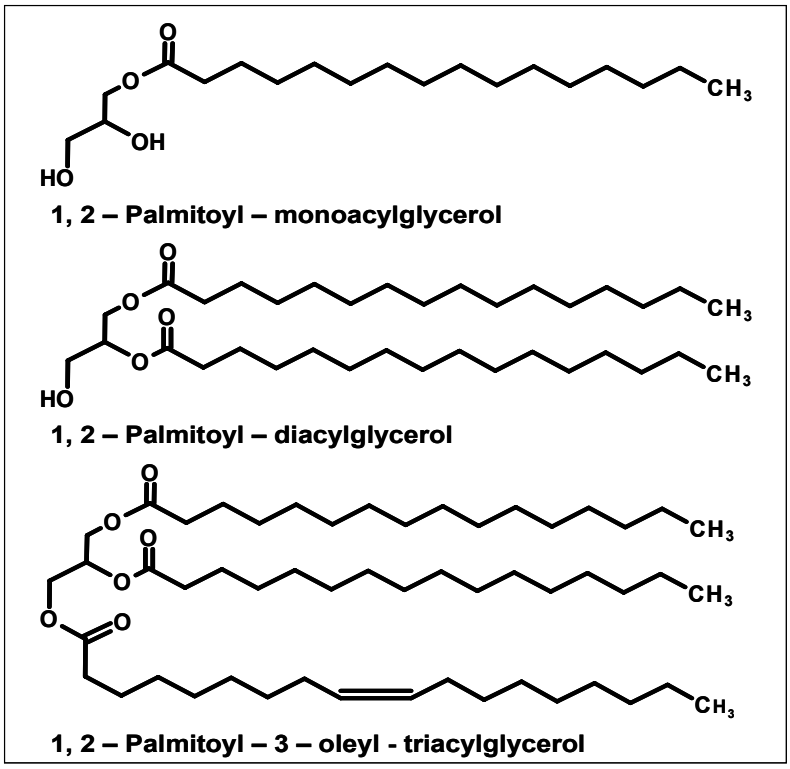



**Figure 3.** Three examples of glycerolipids utilized in this thesis, Structures were drawn with ACD/ChemSketch.

## Glycerophospholipids

The major glycerophospholipid group is the phospholipids, which are amphiphatic molecules that are biologically important for all organisms. They are utilized by the cells in hundreds of cellular functions such as membrane structure, permeability, and specialized defense mechanisms and as a source of energy for cellular processes. Phospholipids have a polar head group at the *sn*-3 position on their glycerol backbone (Fig. 4). Understanding phospholipid biosynthesis can allow us to utilize these cellular functions for designing druges as well as for manipulating storage lipid deposition in seeds. In *S. cerevisiae*, the phospholipi classes that make up the membrane composition are PC, phosphatidylserine (PS), PE, and phosphatidylinositol (Paltauf, 1992). Phospholipid composition in the membranes can vary drastically with environmental changes, and substrate availability.

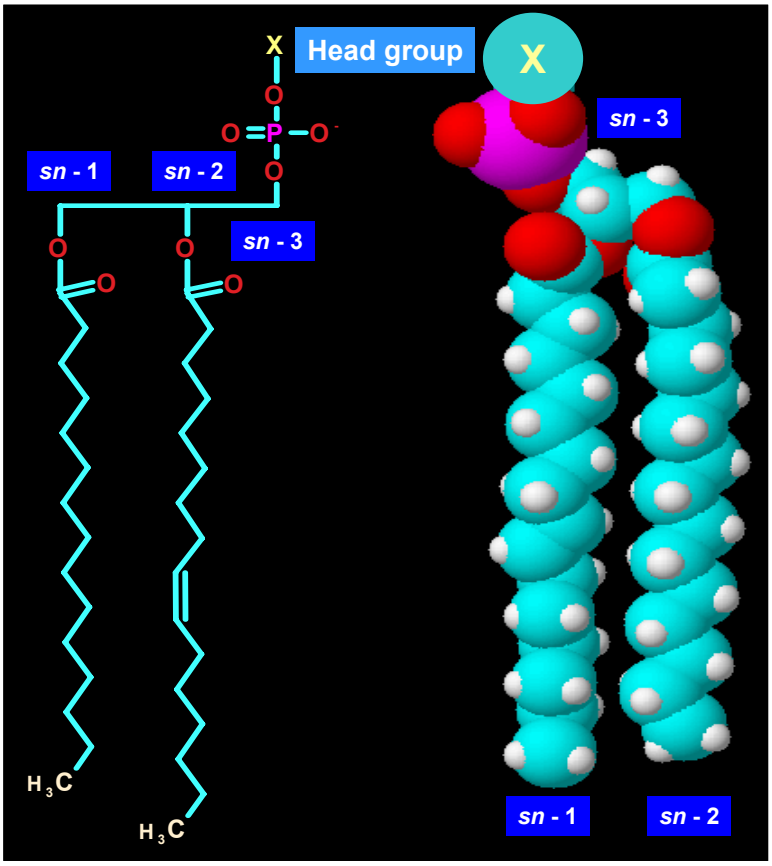



**Figure 4.** Diagram of a phospholipid structure, structures were drawn with ACD/ChemSketch.

Glycerol-3-phosphate is the main precursor in phospholipid biosynthesis in the Kennedy pathway (Kennedy, 1961). Lysophosphatidic acid (LPA) can be produced

by two conjoining sections of the Kennedy pathway. The first and the main source of LPA occur from the acylation of glycerol-3-phosphate at the *sn*-1 position. The second way LPA is produced starts with the acylation of dihydroxyacetone phosphate (DHAP) at the *sn*-1 position to yield 1-acyl-DHAP that is further reduced in a NADPH dependent reaction to form LPA (Athenstaedt & Daum, 2000; Sandager *et al.*, 2002; Schlossman & Bell, 1978). In vertebrates, LPA is found to affect cell proliferation, cell growth, calcium regulation, and apoptosis (Fueller *et al.*, 2003; Ye *et al.*, 2002). In mammals, there are four reported G-protein-coupled receptors that are activated by LPA (Anliker & Chun, 2004). LPA is also an important intermediate in TAG biosynthesis.

PA is an important intermediate on the way to TAG synthesis in the Kennedy pathway. It is also accepted that PA is an important second messenger in both animals and plants and activate G-proteins and protein kinases (McPhail *et al.*, 1999; Munnik, 2001; Testerink *et al.*, 2004). PA is formed by the acylation of LPA at the *sn*-2 position (Fig. 5). The two enzymatic steps glycerol-3-phosphate acyltransferase (GPAT) and lysophosphatic acid acyltransferase (LPAAT or AGPAT) that catalyze the biosynthesis of LPA and PA, respectively, both utilize acyl-CoA as the acyl donor substrate.

There are many reasons for manipulating phospholipid biosynthesis. Diseases caused by problems in producing phospholipids afflict many people. In medical research and the pharmaceutical industry, drugs and medical techniques can be designed based on data from basic research. Increasing oil specificity and quantity in plants can also increase our ability to create sustainable resources for industrial products and personal consumption. Through manipulation of phospholipid biosynthesis, we may also be able to create “healthier” foods that can allow us to have longer, more productive lives. Basic scientific research on the steps that affect TAG accumulation is very important for improving many aspects of life.

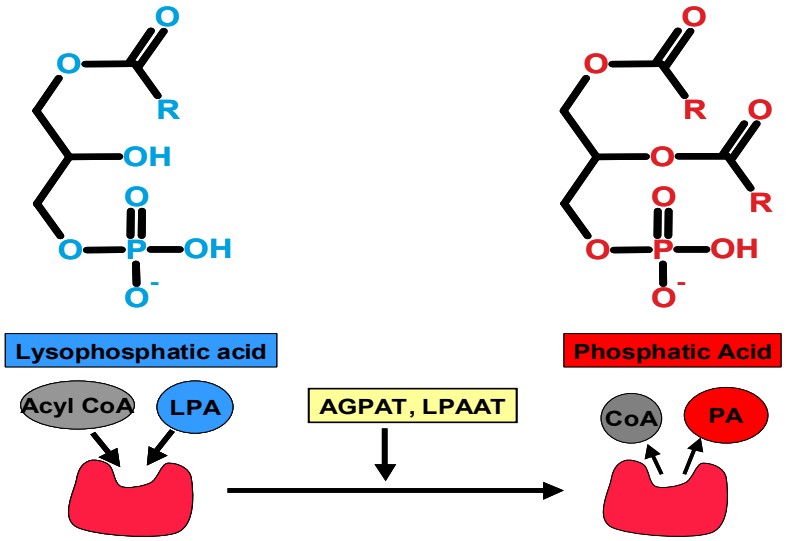



**Figure 5.** Scheme showing the acylation of LPA to PA at the sn-2 position, structures were drawn with ACD/ChemSketch.

# Different Model Systems Used in Studying Lipid Biosynthesis

Better understanding of the natural world not only enhances all of us as human beings, but can also be harnessed for the better good, leading to improved health and quality of life. – Sir Paul Nurse

## Why do we use model organisms to study lipid biosynthesis?

In the last century, model organisms have become a prominent way to examine inheritance and genetics. In recent years, they have been used to look at metabolic events and cellular systems. Model organisms have become a very important tool because of the ease and speed in which new discoveries can be made with them. In recent years, model organisms have become a very prominent tool due to the large increase in gene sequence information and bioinformatics tools. To date, there there is a rapidly growing number of organisms with complete or nearly complete genome sequences. They include the Bakers Yeast (*Saccharomyces cerevisiae)* (Goffeau et al., 1996), *Schizosaccharomyces pombe* (Wood et al., 2002), fruit fly (*Drosophila melanogaster*) (Adams et al., 2000), malaria parasite (*Plasmodium falciparum*) (Gardner *et al.*, 2002; Holt *et al.*, 2002), ciliated protozoan (*Tetrahymena pyriformis*) (Tetrahymena Genome Database), *Candida albicans* (Candida Genome Database*), Escherichia coli*, mustard cress weed (*Arabidopsis thaliana*) (2000), Domestic Rice (*Orza sativa*) (Goff *et al.*, 2002; Yu *et al.*, 2002), puffer fish (*Fugu rebripes*) (Aparicio et al., 2002), mouse (*Mus musculus*) (Waterston et al., 2002), rat (*Rattus norvegicus*), and human (*Homo sapiens*) (Lander *et al.*, 2001; Venter *et al.*, 2001).

All of these model organisms have become important tools in analyzing the function of various genes and gene families. The proteins that are encoded by our genome are often very complex and possess multiple functional domains. Protein modifications such as phosphorylation, glycosylation and subcellular localization often have a large determination on protein function (Barr, 2003). Understanding genetic function is a very complex story. The use of model organisms allows us to look at these complex mechanisms with greater speed. In the past, before Genome Sequencing, model organisms were used to study classic molecular genetics, development and physiology (Barr, 2003). Today, good model organisms must be able to be examined with a genomic approach. This means the ability to see changes in phenotype, metabolism, and gene products due to gene manipulation. An ideal model organism must also be able to be used to determine dominance, complementation, and recombination. The organism's usefulness in these areas is largely dependent on the information and tools available concerning its sequence. As well as being able to meet the criteria above, an ideal model organism must also meet the traditional standards of small size, quick generation time, ease of maintenance and low cost. In this thesis, we explored the use of three model organisms to analyze lipid biosynthesis: *Saccharomyces cerevisiae, Arabidopsis thaliana,* and *Mus musculus*. All these organisms fit both the modern and traditional criteria for being good model organisms.

## *Saccharomyces cerevisiae*

*Saccharomyces cerevisiae* is one of the most powerful model organisms to date. It is the most cited model organism with over 50,000 references listed in PubMed. In *S. cerevisiae*, 6,419 open reading frames have been identified. *S. cerevisiae* has several unique properties that give it advantages over more complex eukaryotic organisms. It is able to grow on defined media. This allows control over its chemical and physical environments. The life cycle of *S. cerevisiae* is well suited for experimental design and allows for rapid accumulation of data. *S. cerevisiae* is not a pathogen, which allows for ease in uses and handling for experiments. Beyond being a great model organism, *S. cerevisiae* has been a first in many high throughput genomic approaches. It was the first eukaryote to be transformed by plasmids, the first to have targeted gene disruption through homologous recombination, and it was the first to be fully sequenced (Beggs, 1978; Goffeau*, et al.*, 1996; Rothstein, 1983). With its unique characteristics, we are able to start identifying several protein functions with several tools such as gene specific knockouts and/or overexpressions, two-hybrid and modified two-hybrid screens, subcellular localization with GFP, co-immuno precipitation, gene and mRNA expression patterns, and enzymatic activity/function analysis.

## *Arabidopsis thaliana*

*Arabidopsis thaliana* is a small weedy flowering plant from the mustard family, *Brassicaceae*, which has been considered the foremost model organism in plants. *A. thaliana* is a weed and has no economical importance in agriculture, but has vast significance in biotechnology. *A. thaliana* has become an established model organism for a variety of reasons. The foremost of these is its relatively small genome size, which contains approximately 27,855 genes that was mostly sequenced and published in the Nature Journal in 2000, and was annotated for the fifth time in July of 2004 by the TIGR database (2000; Haas *et al.*, 2005). Besides having a fully sequenced genome there are a variety of bioinformatics tools available for *A. thaliana.* It has further more a small seed size, fast life cycle of approximately 6 weeks from germination to mature seed, ability to grow in a small space, efficient and quick transformation protocols, and commercial availability of a large number of mutant lines. *A. thaliana* has been used as a model organism to study a number of physiological processes such as disease resistance, pathogen host interactions, plant hormone responses, and environmental responses, thereby increasing our understanding of plant utilization in industry.

## *Mus Musculus*

*Mus musculus* has become a very important model organism, helping us understand human diseases and enzyme function. Mice and humans diverged approximately 75 million years ago (Madsen *et al.*, 2001; Murphy *et al.*, 2001; Waterston*, et al.*, 2002). When comparing the mouse and human genome, 40% of the nucleotides are identical and 80% of the genes have one to one corresponding counterparts (Emes et al., 2003). An almost complete mouse genome was published in 2002 by the mouse genome sequencing consortium, and is comprised of approximately 30,000 protein coding genes (Waterston*, et al.*, 2002). Mice have become an important tool for understanding human physiology. This is not only because of their fairly closely related genomes, but because a large number of human genes do not have homologues in invertebrates. It has also been important to look at tissue specific genes when trying to understand human physiology. This cannot be done using invertebrate eukaryotes. Mice is a great model organism because it allow us to look at important enzymatic and physiological changes in controlled environments through knockouts and mutant strains as well as overexpression of genes through viral replication.

# Background to studies included in the thesis

## Glycerol-3-Phosphate Acyltransferase (GPAT).

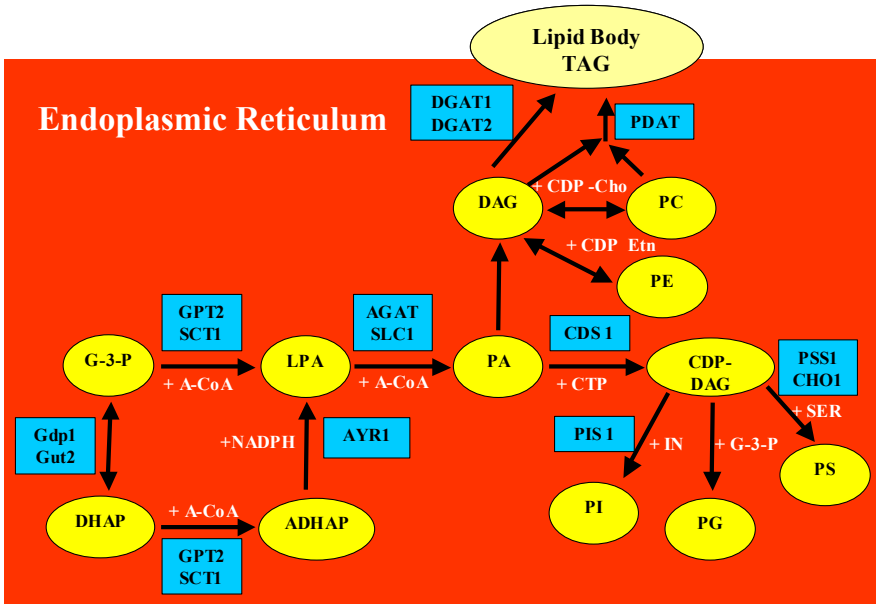



**Figure 6.** Scheme of glycerophospholipid biosynthesis (Kennedy Pathway) in yeast Saccharomyces cerevisciae. Abbreviations not listed previously acyl.Co-A (A-CoA), inositol (IN).

The first step in lipid biosynthesis is the acylation of glycerol- 3-phosphate at the *sn*-1 position to LPA, which is catalyzed by the enzyme glycerol-3-phosphate acyltransferase (GPAT). In eukaryotic cells, multiple isoforms of GPAT are present and they are localized in different intracellular compartments (Dircks & Sul, 1997; Murata & Tasaka, 1997). This initial step in acyl lipid biosynthesis is predicted to be important for the regulation of production of membrane glycerolipids and TAG. Manipulation of TAG synthesis is an important goal for the future of biotechnology. TAG is an important storage compound and is a major reserve source of energy for the plant. The production of TAG can also act as a sink for DAG and acyl-CoA, preventing toxic concentrations to accumulate in the cell (Alvarez *et al.*, 2001; Olukoshi & Packter, 1994). TAG from plants in the form of vegetable oils and fats are major commodities in agriculture. These products have a global annual production of 100 million tons. For these reasons, GPAT is an obvious candidate to manipulate, for use in plant biotechnology, to increase the oil yield and quality in oil crops. Aberrations in GPAT activities are also highly important in many human diseases which manifest due to dysfunctional phospholipid production (Hajra & Bishop, 1982).

The major site of complex lipid assembly in the cell is in the ER. A prokaryotic GPAT gene was cloned two decades ago in *Escherichia coli* and encodes an integral membrane protein with a molecular weight of 83 kDa (Green, Merrill & Bell, 1981). In 2001, two ER localized GPAT genes were cloned in *S. cerevisiae*, GPT2 and SCT1 (Zheng & Zou, 2001). Zheng and Zou showed that GPT2 and SCT1 affect GPAT, and dihydroxyacetone phosphate acyltransferase (DHAPAT) activities in both wild type and overexpression mutants of *S. cerevisiae* (Zheng & Zou, 2001). These yeast GPATs share conserved amino acids of the acyltransferase motif. In 2001, there were no obvious GPAT homologues to these genes in plants and animals. In 2003, a family of ER localized GPATs was identified in *A. thaliana* (Zheng et al., 2003).

While many questions about GPAT in acyl lipid biosynthesis have recently been answered, there is still a need to characterize this part of the phospholipid pathway. GPAT exhibits the lowest specific activity of enzymes in the glycerol lipid pathway which suggests that this step is rate-limiting in acyl lipid biosynthesis (Bell & Coleman, 1980; Coleman *et al.*, 1978). It is a widely accepted view that there are two ER GPAT isoforms in *S. cerevisiae,* SCT1 and GPT2. Studies from other organisms suggest that more than two GPAT isoforms may exist in *S. cerevisiae.* These additional isoforms are predicted to encode GPAT in different organelles other than ER. It is also apparent that there are separate lipid pools in the cell (Rustow & Kunze, 1985). This would suggest there are different GPAT isoforms, which have specific functions for varying conditions, and have different organelle distribution.

In eukaryotic cells, multiple isoforms of the enzyme GPAT are present and localized to different intracellular compartments (Dircks & Sul, 1997; Murata & Tasaka, 1997). A soluble plant plastidial and a murine mitochondrial localized GPAT have been cloned (Ishizaki *et al.*, 1988; Yet *et al.*, 1993). The plant plastidial and the animal mitochondrial GPAT carry out specialized functions. In plants, plastidial GPAT is the part of the prokaryotic pathway responsible for acylating glycrol-3-phosphate utilizing acyl-ACP as the acyl donor for plastidial lipid biosynthesis (Thelen & Ohlrogge, 2002). The animal mitochondrial GPAT has a specific role in TAG biosynthesis and is located in the outer mitochondrial membrane (Gonzalez-Baro, Granger & Coleman, 2001; Igal *et al.*, 2001). The specific function and compartmentalization in plants and animals indicates that this may also be the case in *S. cerevisiae*.

Even though it has been shown that the two yeast GPAT enzymes, GPT2 and SCT1, complement each other, there are some differences between properties and effects of the two enzymes. First, the *SCT1* gene was cloned as a multicopy suppressor of a *ctr1 ise* double mutant yeast strain (Zaremberg & McMaster, 2002). The *ctr1 ise* double mutant is dependent on choline when grown on a high inositol medium due to a mutated phosphatidylethanolamine methyltransferase. It also has the choline transporter (*ctr1*) mutated, leading to an increased Km value for choline uptake (Thai et al., 1997). *GPT2* was not cloned as a suppressor in this screen, indicating a difference in function between the two yeast GPAT enzymes. Furthermore, the reduction in GPAT activity in crude extract of a *GPT2* deleted

yeast strain was about 90%, whereas deletion of *SCT1* led to only a 30% reduction (Zheng & Zou, 2001). In 2001, Zhen and Zou determined that there was a difference in acyl specificity. GPT2 and SCT1 show an ability to efficiently utilize four substrates; 16:0-CoA, 16:1-CoA, 18:0-CoA and 18:1-CoA, although GPT2 has a reduced efficiency in utilizing 18:0-CoA, while SCT1 has distinct preference for 16:0-CoA, and 16:1-CoA (Zheng & Zou, 2001). In 2001, Zheng et al. established that in the knock-out mutant strains *gpt2*Δ and *sct1*Δ, there was a decrease in PA production.

These results showed that *gpt2*Δ increased both PC and PS levels, and decreased in PE, and that the *sct1*Δ strain had a different composition with a little increase in PE levels and a slight increase in PS production compared to the *gpt2*Δ strain (Zheng & Zou, 2001). They also found that *gpt2*Δ did not have an overall effect FA composition except for a decrease in 16:1 content in PE that was compensated by an increase in the 16:0 and 18:1 PE content. The *sct1*Δ deletion impacted fatty acid composition in PC, PI, PS, and PE with a general decrease in 16:0 balanced by a proportional increase in other fatty acids primarily 18:0 (Zheng & Zou, 2001). It has been conclusively shown that a double knockout of *GPT2* and *SCT1* is a synthetically lethal strain (Zaremberg & McMaster, 2002; Zheng & Zou, 2001).

Zaremberg and McMaster (2002) further characterized these genes by feeding yeast cells with $^{14}$C-labeled acetate and showed that the *gpt2*Δ strain had a quite strong increase in incorporation into TAG as compared to the wt strain and that deleting the *SCT1* gene had the opposite effect. They further more showed that when the *gpt2*Δ strain was labeled with [$^{14}$C]choline, there was a strong increase in radiolabled glycerophosphocholine (GPC) incorporation. In the *sct1*Δ strain, there was a decrease in radio-labeled PC and also a decrease in radio-labeled GPC incorporation.

Comprehension of how genes influence FA composition can help us understand how to manipulate these genes to create products with very specific acyl specificities. This information is important for the future of biotechnology and in the creation of genetically modified oils for specific uses. These changes in composition may also be important to understand what is happening in many human genetic disorders when fatty acid compositions are abnormal. Understanding the role of GPATs in these processes gives us important tools to further our understanding of lipid production. The papers cited above give further evidence to the extreme complexities of lipid biosynthesis.

**Tafazzin in *Homo sapiens*, *Saccharomyces cerevisiae*, and *Arabidopsis thaliana*.**

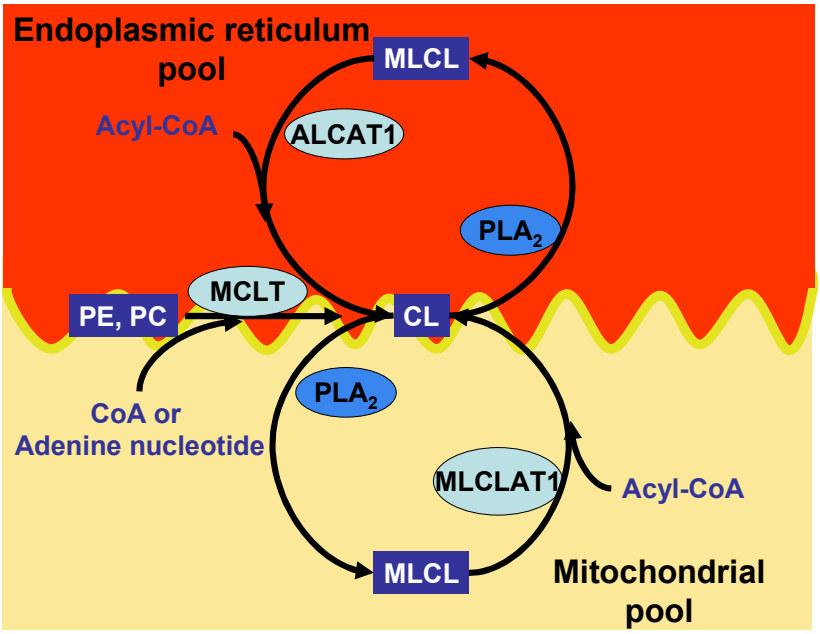



**Figure 7.** Suggested model for cardiolipin remodeling. ALCAT1-Acyl-CoA cardiolipin acyltransferase, MLCLAT1-monolysocardiolipin acyltransferase, MCLT-mitochondrial cardiolipin transacylase, PLA2 - phospholipase A2 (Hauff & Hatch, 2006; Schlame et al., 2002).

Lipids are important storage molecules in seeds of many angiosperms. The main form of lipid storage in seeds is TAG. Plants readily utilize the TAG energy source during germination. In plants, TAG is usually comprised of palmitate, stearate, oleate, linoleate and linolenate (Voelker & Kinney, 2001). Many products in industry and food production come from the processing of lipids from oilseeds. Some of these include cooking oils, biodiesel, industrial lubricants, cosmetics, medicines, plasticizers, surfactants, etc. As well as having multiple uses in industrial applications, seed oils are of major importance to human dietary consumption. With ever-growing concerns about cardiac health, healthier oils have become an important component in good nutrition. Enhancement of oil seed quality and quantity is growing in importance.

TAZ1 is homologous to a region called Xq28 in humans, which is believed to be responsible for Barth Syndrome (Bolhuis et al., 1991). Barth syndrome is an X-linked cardioskeletal myopathy and neutropenia with abnormal mitochondria and increased levels of organic acids in the urine which was first characterized in 1983 (Barth et al., 1983). Barth syndrome is a type of Zellweger syndrome (leukodystrophy), which often causes fatality in childhood due to cardiac failure or sepsis, even in times of apparent good health (Barth, *et al.*, 1983; Wilson *et al.*, 1986). The cardiomyopathy associated with Barth syndrome often leads to

weakness and fatigue (Barth, *et al.*, 1983). In 1996, several mutations in this region were reported and named G4.5, or Tafazzin (Bione *et al.*, 1996). The mutations described in this region are splice-site, missense, nonsense, and frameshift (Bione, *et al.*, 1996; Cantlay *et al.*, 1999; D'Adamo *et al.*, 1997; Johnston *et al.*, 1997). Alterations in the coding region of the gene are linked with Barth Syndrome. There are also lipid abnormalities associated with Barth syndrome including decreased cholesterol, docosahexaenoic acid, arachidonic acid (Barth et al., 2004), and cardiolipin.

The Tafazzin gene has some homology to known acyltransferases that are active in phospholipid biosynthesis (Neuwald, 1997). Recently, it has been suggested that TAZ1 in yeast is involved in the metabolism of cardiolipin, especially in the fatty acid remodeling of this mitochondria specific lipid molecule (Schlame, *et al.*, 2002; Valianpour *et al.*, 2003; Vreken *et al.*, 2000). Cardiolipin is an essential component in mammalian cells. In the mammalian heart, CL makes up 15-20% of the phospholipid phosphorus content (Hatch, 2004). This involvement in cardiolipin remodeling was suggested because cultured skin fibroblasts from patients with Barth syndrome had a decrease in cardiolipin (CL) levels and a reduction in linoleic acid incorporation (Schlame, *et al.*, 2002; Valianpour, *et al.*, 2003; Vreken, *et al.*, 2000). Further studies in 2002 showed that cells from Barth syndrome patients lack tetralinoleoyl-CL (Schlame, *et al.*, 2002). It has been shown that Barth syndrome causes aberrant cardiolipin accumulation (Schlame, *et al.*, 2002; Valianpour, *et al.*, 2003; Vreken, *et al.*, 2000). CL accumulates on the outer membrane of the mitochondrial membrane, mitochondria associated membrane (MAM) fraction (Ma *et al.*, 1999; Rusinol *et al.*, 1994), as well as the inner mitochondrial spaces (Koshkin & Greenberg, 2002; Schlame, Rua & Greenberg, 2000). Earlier studies on Barth syndrome have even shown there is aberrant formation of the mitochondria, where there is a condensation of the inner mitochondrial space (Neustein *et al.*, 1979). Bradner *et al.* (2005), conclusively proved that TAZ1 is localized in the outer mitochondrial membrane in yeast. Tafazzin and TAZ1 have been proposed to be transacylases transferring acyl groups from PC to cardiolipin (Xu et al., 2003). Further evidence of this is that in a *taz1*Δ yeast strain, there was an increase in accumulation of monolysocardiolipin. Also, tafazzin mutations cause changes in cardiolipin acyl composition as well as cardiolipin levels (Gu *et al.*, 2004; Vaz *et al.*, 2003).

Cardiolipin synthesis in *S. cerevisiae* is similar to that in higher eukaryotes. Due to this, yeast TAZ1 has become an important tool for looking at diseases such as Barth syndrome. In eukaryotes, cardiolipin is formed by two glycerol-linked moieties; the four acyl chains are typically mono- and diunsaturated fatty acids (Brandner et al., 2005). There have been two genes identified in de novo cardiolipin biosynthesis in yeast. They are *PGS1*, which encodes phosphatidylglycerol phosphate (PGP) synthase (Chang et al., 1998a). PGP is then dephosphorylated by PGP phosphatase to PG. Then CRD1 catalyzes the phosphatidyl transfer from CDP-DAG to PG to form CL (Chang *et al.*, 1998b; Jiang, Rizavi & Greenberg, 1997; Tuller *et al.*, 1998).

*A..thaliana* At104.1 and At104.2 enzymes have 31.6 and 26.3% identical amino acids, respectively, to *S. cerevisiae*. In plant cells, lyso-PC acyltransferase is located in the plastidial envelope and mitochondrial membranes (Bessoule, Testet & Cassagne, 1995; Testet *et al.*, 1996). These enzymes are supposed to be the key enzymes that import PC from the ER into cellular organelles. In plants, Nebaurer *et al*. (2004) proposed that this import involves the release of lyso-PC from endomembranes in the transfer of these molecules to the mitochondria and chloroplasts for their subsequent acylation into PC. In mammals, newly formed cardiolipin is then rapidly remodeled to generate CL enriched with linoleic acid (Hatch, 2004). It has been proposed that there are two functions of cardiolipin; the first is that it is important for membrane stabilization (Hatch, 2004), secondly, that it is important for protein function by direct protein interactions (Palsdottir & Hunte, 2004).

Our knowledge of Tafazzin has been limited at best. After two decades of intensive research, we still do not understand the true function of this gene. We do understand that Tafazzin is required for proper lipid regulation. There are many unresolved questions associated with Barth syndrome. To date, we do not understand what the true cause of Barth syndrome is or how the functions of TAZ1 influence normal lipid synthesis. Further knowledge in this area is required for us to understand the protein encoded by this gene and to create action plans to maintain healthy lipid production in humans.

## Wax Ester and Ethyl Ester Synthesis

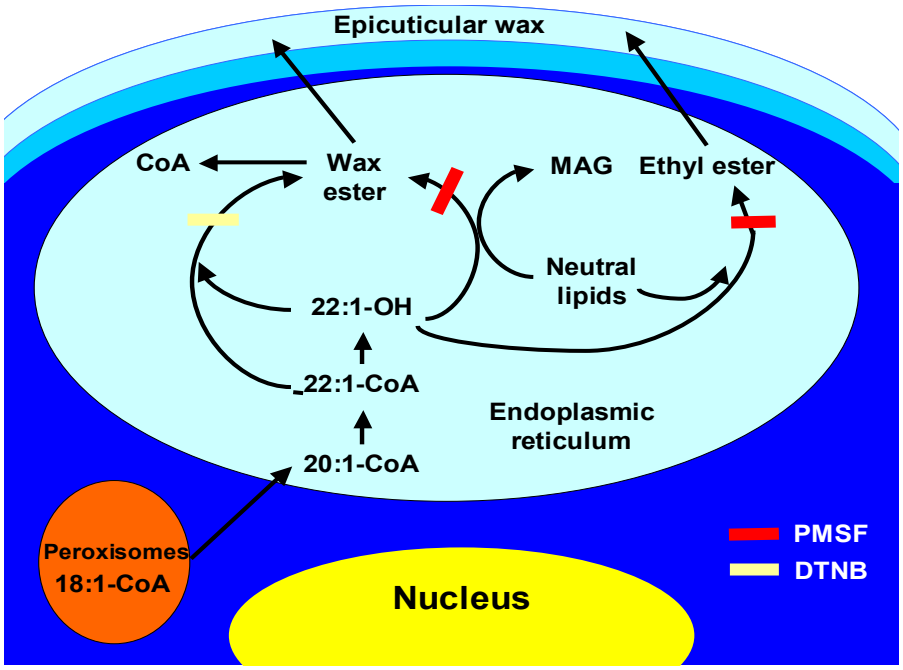



**Figure 8.**. Scheme of wax ester and ethyl ester synthesis in plants.

Epicuticular and intracuticular waxes form hydrophobic barriers that are utilized as a major defense mechanism in plants (Broun et al., 2004). This waxy layer protects plants from environmental changes, insects, and non-stomatal water depletion. Waxes also have a role in protection against bacterial and fungal pathogens (Kolattukudy, 1987).

In addition to being involved in plant protection, processed waxes play an important role in plant developmental metabolism. This role includes the prevention of post-genital organ fusion and pollen-pistil interactions (Aharoni, *et al.*, 2004). Important structural functions of wax esters include regulation of buoyancy and or sound transmission, energy storage, and cuticle permeability, which is hypothesized to influence cell to cell communication by attenuating the passage of signal molecules (Cheng & Russell, 2004; Kalscheuer *et al.*, 2006; Sieber *et al.*, 2000). They are a main constituent of mammalian sebum and meibum, and the epicuticular and intracuticular layer in plants (Broun, *et al.*, 2004; Cheng & Russell, 2004). As well as affecting these processes, Pruitt et al. suggested that cuticle permeability influenced cell to cell communication by attenuating the passage of signal molecules (Sieber, *et al.*, 2000).

Cuticular wax is mainly composed of long chain aliphatic compounds derived from very long chain fatty acids (Kunst & Samuels, 2003). The wax composition in plants varies between species but is also dependent on environmental factors.

In *A. thaliana* leaves, wax esters make up 0.2% of the epicuticular lipid classes (Jenks et al., 1995). In some plants, such as jojoba, wax esters are one of the main components in the cuticular layer as well as the seeds (Benzioni, 1989). Wax esters are produced by the estrification of fatty acids, to fatty alcohols. The only known enzymes involved in this reaction have been fatty acyl CoA: fatty alcohol acyltransferases (wax synthase, WS) (Kolattukudy, 1967; Kolattukudy & Rogers, 1978; Metz *et al.*, 2000).

Little is known about the function of ethyl esters. We know that they play key roles in fermentation in microorganisms and in flavor compounds in food and alcoholic beverages. Ethyl esters are also a component in many pharmaceutical products (Mason & Dufour, 2000; Saerens *et al.*, 2006). In mammals, they may also play a role in organ injury due to acute alcohol intoxication (Diczfalusy et al., 2001). As well as being important constituents in fermentation and food flavors, ethyl esters are beginning to be recognized as important biomarkers in diseases such as chronic alcohol abuse (Kaphalia et al., 2004). Fatty acid ethyl esters (FAEE) are formed by two enzymatic processes; esterification of alcohols and carboxylic acids catalyzed by FAEE synthetases/carboxylases or alcoholysis from alcohols and acylglycerols or alcohols and acyl-CoA's by acyl-CoA:ethanol O-acyltransferases (AEAT) (Laposata, 1998). In yeast, two main medium-chain fatty acid ethyl ester synthetases have been identified, EHT1 and EEB1 (Saerens*, et al.*, 2006). However, the results from Saerens et al. indicate that *S. cerevisiae* may have one or more additional enzymes involved in ethyl ester synthesis (Saerens*, et al.*, 2006). To date, enzymes involved in FAEE synthase in *A. thaliana* have not been characterized.

The precursors for wax ester and ethyl ester production in plants are synthesized by a combination of plastidial or cytosolic *de novo* fatty acid synthesis and cytosolic elongation. In this process, palmitic, stearic, and oleic acid are produced by soluble enzymes in the FAS complex in the plastid (Ohlrogge & Browse, 1995). The growing acyl chain is attached to acyl carrier protein (ACP). These products go through four reactions: a condensation of malonyl-ACP to acetyl-ACP, a reduction of β–ketoacyl-ACP, dehydration of β–ketoacyl-ACP, and lastly, the reduction of *trans*-$\Delta^2$-enoyl-ACP. The final product of these four steps is a fully reduced acyl chain extended by two carbons.

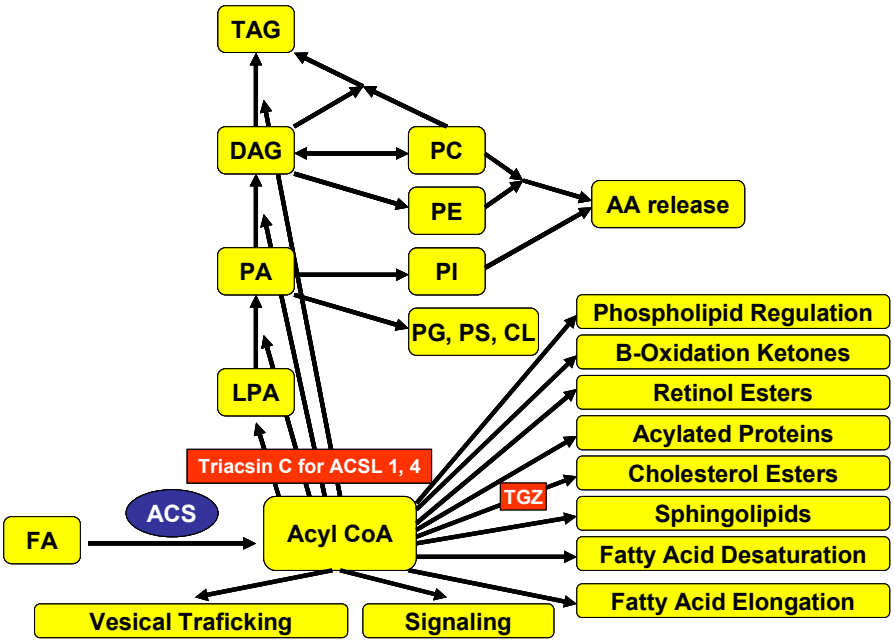
The condensing enzymes in this step are very specific for chain length. KAS III condenses (C2-C24), KAS I condenses (C4-C16) and KASII condenses (C16-C18), (Clough*, et al.*, 1992; Shimakata & Stumpf, 1984). The acyl-acp's are then cleaved from the acyl carrier protein by thioesterases and exported to the endoplasmic reticulum in the form of acyl-CoA's (Schnurr, Shockey & Browse, 2004). The acyl CoA is then utilized in various metabolic pathways such as the synthesis of membrane, seed storage and cuticular lipids (Aharoni*, et al.*, 2004). Plant waxes are mainly composed of very long-chain fatty acids (VLCFA). These

VLCFA are derived by microsomal enzymes that elongate the FA exported from the plastid by two carbons at a time by a similar biochemical reaction as in the *de novo* fatty acid synthesis. Four basic reactions are repeated until the desired chain length is acquired; condensation, reduction, dehydration, and a second reduction (von Wettstein-Knowles, 1986). FA elongation then takes place through the action of the condensing enzyme, Fatty acid elongation enzyme (FAE) (Von Wettstein-Knowles 1982). It has been previously described that after FAE, there are two principle wax biosynthetic pathways, the acyl reduction pathway and the decarbonylation pathway. The decarbonylation pathway starts with the production of aldehydes from VLCFA precursors by fatty acyl-CoA reductase. The next step is a decarbonylation of aldehydes by the aldyhyde decarbonylase to make alkanes. The alkanes can be used to form secondary alcohols and ketones. The step that we are most interested in is the acyl reduction pathway, which in plants is catalyzed by the fatty acid reductase (FAR). In two steps this reaction converts the fatty acid to the aldehyde and then reduces it to produce primary alcohols. Both the reduced aldehydes and the primary alcohols then used to make wax esters by the acylation from acyl-CoA (Kunst & Samuels, 2003). In this thesis, we describe a third branch in the wax biosynthetic pathway. This third division in wax production is a reduction pathway that utilizes reduced aldehydes or primary alcohols to transacylate neutral lipids to form wax esters without involvement of acyl-CoA (This Work).

In *A. thaliana*, there are 22 known Cer mutants that cause defects in wax synthesis. Cer 1-10 mutants have a very glossy phenotype and Cer 11-22 are glossy to a lesser degree (Shimakata & Stumpf, 1984). The identification of these mutants has given us important tools to study the production of wax. By studying these mutations, it has been determined that most of these mutations do not cause an accumulation of metabolic intermediates due to blocks in the pathway. Instead, there is a flux in precursors into alternate branches of the pathway causing complex changes in wax formation (Rhee *et al.*, 1998; von Wettstein-Knowles, 1979). The complexity of this pathway would lead us to conclude that there are many alternatives in the production of waxes. There are many enzymatic steps for which there is no obvious gene yet identified and several mutants for which no function is ascribed (Rashotte et al., 2004). It is most likely these various pathways are activated under different conditions based on needs of the plants, availability, and the accumulation of certain products.

Understanding how waxes are formed in plants can have significant importance for plant biotechnology. By understanding these complex mechanisms we may be able to create plants with enhanced resistance to insect and environmental damage and to modified wax compositions. Modification of wax compositions is highly advantageous for the production of industrial and consumer wax products from plant sources.

## Heart Long-Chain Acyl-CoA Synthetase.




**Firgure 9**. Scheme of products produced and processes influenced by synthesis of acyl CoA. triacsin C and troglitazone (TGZ) are inhibiters.

Long chain acyl-CoA synthetases (ACSLs) are an integral part of nearly all biosynthetic pathways that lead to production of fatty acid derived molecules. ACSLs catalyze the conversion of long chain fatty acids to long chain fatty acyl-CoAs (Kornberg & Pricer, 1953). The formation of acyl-CoAs occurs in a two-step process. The first step converts free fatty acid to an acyl-AMP intermediate (adenylate) through the pyrophosphorolysis of ATP. In the second step, the adenylate is then coupled with the thio group of CoA releasing AMP and acyl-CoA as products (Groot, Scholte & Hulsmann, 1976). There are five characterized isoforms of ACSLs in humans and rodents. ACSL 1, 6, and 5 have over 60% amino acid identity (Oikawa et al., 1998). ACSL 3 and 4 have 68% identity with each other and approximately 30 percent homology with ACSL1 (Kang et al., 1997). These ACSLs differ in cellular and organelle localization, enzyme kinetics, regulation, and substrate preferences (Mashek et al., 2004).

In addition to having varying substrate preferences, the acyl-CoA synthetase products can be utilized in many metabolic processes such as β-oxidation, fatty acid retroconversion, glycerolipid biosynthesis, phospholipid reacylation, desaturation, elongation, cholesterol ester, and wax ester formation. They can also serve as signaling molecules (Coleman, Lewin & Muoio, 2000; Coleman *et al.*, 2002; Faergeman & Knudsen, 1997; Prentki & Corkey, 1996; Schnurr *et al.*, 2002; Stoveken *et al.*, 2005). In addition to these integral differences, there are also diverse quantities of ACSL isoforms in other organisms. In *Arabidopsis thaliana*,

there are nine LACS (ACSLs) (Schnurr, *et al.*, 2002). In *S. cerevisiae*, there are four characterized FAAs (ACSLs) with a possibility of a fifth, FAA5, inferred by the lack of lethality with the quadruple knock: Δ*faa1*, Δ*faa2*, Δ*faa3*, and Δ*faa4* (Knoll et al., 1995). All of the ACS super family share similar composition, with a putative AMP binding and fatty acid binding sites. Previous studies that have characterized the mammalian ACS family have shown integral differences in how acyl-CoAs are channeled. The most compelling evidence is varied response of the ACSL isoforms to different inhibitors.

Triacsin C is a fungal-derived competitive inhibitor. In 1997, Igal *et al*. showed that in human fibroblasts, 5 μM of Triacsin C inhibits *de nov*o synthesis of triacylglycerol by 99% and phospholipids by 83% (Igal, Wang & Coleman, 1997). In the same paper, they also proposed that there are different pools for ACSL products. It was hypothesized that Triacsin sensitive ACSLs can only be used to acylate cholesterol, glycerol-3-phosphate, lysophosphatic acid and diacylglycerol. Triacsin resistant ACSL products can only be used by lysophospholipid acyltransferases, β-oxidation and ceramide N-acyltransferase (Igal & Coleman, 1996; Muoio *et al.*, 2000). In 2001, Kim et al. showed that ACSL1 and ACSL4 in rats were inhibited in a dose dependent manner with an IC 50 of 4-6 μM (Kim, Lewin & Coleman, 2001).

Another important inhibitor family is the thiazolidinediones. Thiazolidinediones exert many of their effects through a mechanism that involves activation of the gamma isoform of the peroxisome proliferator-activated receptor (PPAR gamma), a nuclear receptor. Thiazolidinediones induce activation of PPAR gamma, which alters the transcription of several genes that are considered key regulators of energy homeostasis, insulin sensitivity and inflammation (Berger & Moller, 2002; Chinetti, Fruchart & Staels, 2000; Jiang, Ting & Seed, 1998; Picard & Auwerx, 2002). Troglitazone in particular, blocks incorporation of oleate into triacylglycerol and ketone body production but does not inhibit incorporation of oleate into phospholipids (Fulgencio *et al.*, 1996; Muoio, *et al.*, 2000). 50 μM of Troglitazone inhibits ACSL4 activity by 45% in the mitochondria associated membrane (MAM) fraction (Lewin et al., 2001). Further evidence of specific channeling of ACSL products is their varied substrate specificity between the different ACSL isoforms.

There are many ontological changes that occur between the developmental stages of *Mus musculus* heart. The most pronounced of these changes is the metabolic switch from use of glucose and lactate in the fetal heart to fatty acids and amino acids used by the adult heart (Fisher, Heymann & Rudolph, 1980; Wakefield *et al.*, 2005; Werner *et al.*, 1983).

The fetal rat heart primarily consists of slow-twitch oxidative voluntary muscles. After birth, these muscles mature to mainly be composed of fast twitch glycolytic fiber (Close, 1964; Dubowitz, 1963). After birth, there is a significant up-regulation of many genes in the mouse and rat heart. These genes include carnitine palmitoyltransferase-1, mitochondrial malate dehydrogenase, short chain acyl-CoA dehydrogenase, medium chain acyl-CoA dehydrogenase, long chain acyl-CoA

dehydrogenase, and peroxisome proliferator-activated response elements α, and δ, (Brown *et al.*, 1995; Carroll *et al.*, 1989; Disch *et al.*, 1996; Kelly *et al.*, 1989; Nagao, Parimoo & Tanaka, 1993; Steinmetz *et al.*, 2005). Many of these genes are in the acyl-CoA dehydrogenase family, which is involved in fatty acid oxidation (Carroll*, et al.*, 1989).

It is of interest that genes involved in mitochondrial fatty acid oxidation are up-regulated as well. In 1976, Foster and Bailey showed an up-regulation of several mitochondrial fatty oxidation genes after birth which included acyl-CoA synthetase (Foster & Bailey, 1976). The elevated expresion of mitochondrial fatty acid oxidation genes could be due to the increase in mitochondria content in the heart in response to changes in ventricular loading and increased demands in cardiac output (Attardi & Schatz, 1988; Mayor & Cuezva, 1985). There are also changes at postnatal day eighteen as the mouse pups begin to eat more mouse chow and depend less on their mother's milk. Smith et al. published that mouse milk consists primarily of medium chain fatty acids, mainly capric acid, lauric acid, myristic acid, palmitic acid, and oleic acid (Smith, Watts & Dils, 1968). Further evidence of the importance of glucose for the fetal heart is shown by a 10-fold greater sensitivity to insulin compared to the adult (Clark, 1971; Clark, 1973). In rats, during the last four days before birth, both plasma glucogen and insulin concentration rise several fold with the preservation of a ratio of more than ten with respect to the adult. After birth, there is a large fall in insulin and a steep rise in plasma glucogen, creating a drop in the same ratio (Girard et al., 1985). In the fetal rat heart, there is also a sharp increase in the adrenaline stimulation of adenylate cyclase right before birth (Clark et al., 1980). Before birth, in rats, cyclic AMP is immediately involved in the contraction process and in the mobilization of glycogen in the heart. There is also a sharp increase in the ability of adrenalin to stimulate adenylate cyclase in the fetal rat heart (Clark*, et al.*, 1980). In the rat early newborn period, retention of protein is high and agents such as cyclic AMP that normally inhibit protein synthesis do not do so (Klaipongpan, Bloxham & Akhtar, 1977). In rats, these changes may be preparing the heart for perinatal mobilization of cardiac glycogen.

Fetuses also have high oxygen binding capacity with associated leftward shift of the hemoglobin dissociation curve. The higher oxygen binding capacity enhances oxygen affinity on the blood (Bartels, 1964). This allows the fetus and neonates to be less sensitive to hypoxia (Singer, 1999). Some other major physiological differences are that the fetal heart has a reduced number and less active mitochondria, little fatty acid or amino acid oxidation, and poorly developed tubular systems with low $CA^{2+}$, $Na^{+}$, $K^{+}$, -ATPase and relatively high rates of glycolysis and lactate production in the aerobic state (Jones & Rolph, 1981).

Besides the major physiological differences that occur before and just after birth, many other changes occur during developmental stages of *Mus musculus.* There is a switching-off of thermoregulation by the fetus related to a metabolic reduction. This phenomenon can even occur in extremely young neonates. This allows the fetus to create a near-induced hypothermia in case of exposure to cold (Singer, 1999). By postnatal day five in mice, fuzz starts to appear on mouse bodies and by

postnatal day eleven, they are fully furred. There are also changes in diet that include, for example, a major change when teeth erupt at day ten and again after weaning at day twenty-one. There is a postnatal rise in total cholesterol (Roberts, Huff & Carroll, 1979). This may be due to dietary changes, such as a change from mother's milk being the primary food source to a chow diet. Brain immaturity plays an additional role in reducing metabolic needs (Thurston & McDougal, 1969).

It is important for us to understand the functional roles of each ACSL isoform. Understanding this can give us important tools to help us create treatment strategies for many diseases such has diabetes and obesity. With the alarming increase in these disorders in the last few years, it is imperative for us to increase our understanding of the causes and possible treatment plans for them.

## Phosphatidylcholine synthesis in *Saccharomyces cerevisiae*

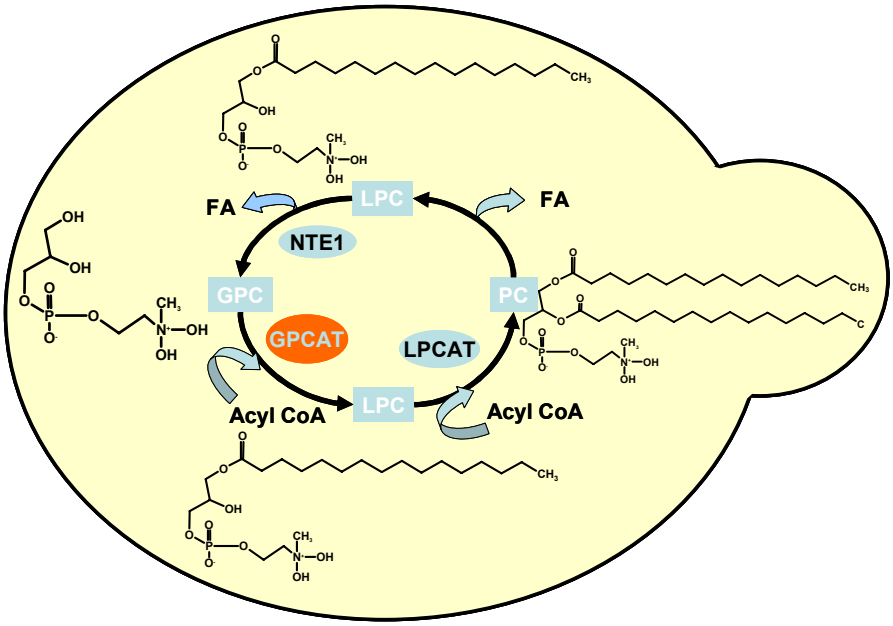



**Figure 10.** Scheme of Novel GPCAT Pathway in S. cerevisiae, structures were drawn with ACD/ChemSketch.

Phosphatidylcholine is the major phospholipid in eukaryotic cellular membranes. PC formation is highly regulated and plays critical roles in cellular biosynthesis, degradation, and distribution in cells (McMaster, 2004). To date, the knowledge on the biosynthesis of PC in yeast involves two pathways; the CDP-choline or Kennedy pathway and the PE methylation pathway. The CDP-choline pathway contains the genes: CKI1, PCT1 and CPT1, and the PE metylation pathway the

gens: CHO2 and OPI3 (Dowd, Bier & Patton-Vogt, 2001; Fernandez-Murray & McMaster, 2005).
Minimal levels of PE are essential for cellular growth (Birner et al., 2001). In WT yeast GPC is somewhat generated extracellularly by the action of three phospholipase B enzymes: PLB1, 2 and 3 (Merkel *et al.*, 2005; Patton, Pessoa-Brandao & Henry, 1995) but mainly intracellularly through deacylation of PC molecules derived from the CDP-choline pathway by the NTE1 hydrolase. (Dowd, Bier & Patton-Vogt, 2001; Fernandez-Murray & McMaster, 2005; Zaccheo *et al.*, 2004).

In yeast outer mitochondrial membranes, PC and PI are equally distributed across both sides of the membrane while PE is localized in the intermembrane space (Sperka-Gottlieb et al., 1988). GPC is generated extracellularly by the action of three phospholipase B enzymes: PLB1, 2 and 3 (Merkel*, et al.*, 2005; Patton, Pessoa-Brandao & Henry, 1995). PC molecules derived from the CDP-choline pathway can be deacylated from NTE1 esterase, which utilizes the product lysophosphatidylcholine (LPC) to form glycerophosphocholine (GPC) (Dowd, Bier & Patton-Vogt, 2001; Fernandez-Murray & McMaster, 2005; Zaccheo*, et al.*, 2004).

The regulation and synthesis of PC is integral to proper function of metabolic function. This is shown by the induction of many diseases and physiological distress when there are problems with the production or regulation of PC. There are several mutant strains with defects in PC biosynthesis and these mutants have the tendency to generate respiratory-deficient petites (Griac, Swede & Henry, 1996). When there are deficiencies in PC regulation, many pathological events such as apoptosis (Anthony, Zhao & Brindle, 1999) and cancer occur (Aboagye & Bhujwalla, 1999). In *Saccharomyces cerevisiae*, GPC accumulation increases in response to high temperatures, choline in the media, and osmotic stress (Dowd, Bier & Patton-Vogt, 2001; Kiewietdejonge *et al.*, 2006). Altered GPC accumulation has been seen in a number of disorders in the human brain such as Alzheimer's disease (Walter *et al*, 2004) and schizophrenia (Shirayama et al., 2004).

In this thesis, we describe novel acyltransferase activities that influence PC regulation. These reactions are catalyzed by glycerophosphocholine acyltransferase (GPCAT) and glycerophosphoethanolamine acyltransferase (GPEAT) activities. The combined reaction of known acyltransferases LPCAT with GPCAT and LPEAT and GPEAT create a novel recycling pathway of glycerophosphodiesters into phospholipids. GPCAT and GPEAT are more efficient and energy saving than previously described mechanisms by Patton-Voigt et al. in 2006 (Patton-Vogt, 2006).

# Aims of this thesis

**"Most things are simple when you understand them." – Ulf Ståhl**

**Overall:** The overall aim of this thesis was to study different fatty acid acylation and CoA-activation activities regarding biochemical activity properties, cellular functions, localization and regulation. Three different model organisms: *S. cereviseae, A. thaliana* and *M. musculus*, were used in these studies on basic lipid metabolism.

**Paper I:** To study the effects on lipid content and composition as well as on fatty acid composition in yeast induced by altering the expression levels of either of the two glycerol-3-phosphate acyltransferase genes, *GPT2* and *SCT1*

**Patent 1:** To characterize the effects on the lipid accumulation in yeast and especially in oil seeds by overexpressing the yeast acyltransferases *TAZ1* and its plant homologues At104.1 and At104.2

**Paper II:** To study the CoA-activation independent acylation of fatty acids into waxesters and ethanolesters in *A. thaliana* and *S. cerevisiae*

**Paper III:** To investigate long chain acyl-CoA synthetase (ACSL) isoforms and their expression, activity and function in *Mus Musculus* heart at different developmental stages

**Paper IV:** Characterization of the novel glycerophosphodiester acyltransferase activity in *S. cereviseae*

# Results and discussions

## Changes in Lipid Composition of *Saccharomyces cerevisiae* strains with Altered Glycerol-3-Phosphate Acyltransferase Gene Expression (paper I)

If we knew what it was we were doing, it would not be called research, would it? - Albert Einstein

In this paper, we studied the effects in yeast of deletion and overexpression of the two yeast GPAT genes on fatty acid content and composition. Previous results, indicating SCT1 is more involved in TAG biosynthesis (Zaremberg & McMaster, 2002). These studies lead to the conclusions that GPT2 and SCT1 are the major ER localized GPATs in *S. cerevisiae*. This data also suggests that these genes have different functions and may be involved in different types of lipid production based on the needs of the organism. In this study, we will give further evidence that while GPT2 and SCT1 are the major ER localized GPATs in *S. cerevisiae*; they may have different functional roles.

**In-vitro, the *GPT2* and *SCT1* deletion and overexpression strains have different GPAT activities-** In the *gpt2*Δ strain, GPAT activity was higher than in the *sct1*Δ strain. There is a clear decrease in GPAT activity for both knockout strains. For *gpt2Δ* and *sct1Δ* strains, there is a 9- and 2-fold reduction, respectively, as compared to WT (Fig. 1A, Paper I). In accordance to this decrease in the deletion strains, there is an increase in GPAT activity in crude extract from both yeast strains overexpressing either *GPT2* or *SCT1* of 3.8- and 3-fold, respectively, compared to the WT yeast strain (Fig. 1B).

**Effcts on acyl lipids are seen in *gpt2Δ* and *sct1Δ* strains when grown on synthetic media containing a non-fermentable carbon source to stationary phase -** Growth conditions strongly affect the total acyl-lipid content (nmol FA/mg dry weight) in both knockout and WT strains, with a strong increased content as an effect of growth to stationary as compared to logarithmic phase, of growth on synthetic as compared to rich media and growth on a nonfermentable as compared fermentable carbon source. There is only a small difference in total acyl-lipid accumulation in GPAT knockout as compared to WT in all growth condition, except at stationary stage on synthetic media supplemented with a non-fermentable carbon source (Fig. 2A). At stationary phase on synthetic media supplemented with a non-fermentable carbon source, the *gpt2Δ* and *sct1Δ* strains have approximately a 20% and 4% decrease in total lipids compared to the wt. The different lipid classes are also affected in the *gpt2Δ* strain when grown on synthetic media supplemented with a non-fermentable carbon source. The *gpt2Δ* strain had a relative decrease in saturated acyl groups and a relative increase in unsaturated acyl groups (Fig. 2B). The stronger decrease in total acyl-lipid content in the *gpt2Δ* as compared to the *sct1Δ* strain correlates with the larger decrease in *in-vitro* GPAT activity in the

crude fraction of the *gpt2Δ*. The total acyl lipid content per dry weight is highest at this growth condition. It is logical that the demand for GPAT activity also is highest and that a reduced GPAT activity would become noticeable. A rate-limiting GPAT reaction would lead to an increase in the half-life of acyl-CoA molecules and an increase in the acyl-CoA pool. The 16:0-CoA and 18:0-CoA are substrates for the yeast desaturase, which would have an increased substrate availability, which is an temting explanaition to the enhanced desaturation seen.

**Deletion of *GPT2* and *SCT1* affect acyl composition –**The acyl composition of total lipids as well as lipid class composition was analyzed on *S. cerevisiae* cells grown to stationary stage on synthetic media supplemented with a non-fermentable carbon source. In the *sct1Δ* strain hardly no effect was seen on acyl composition of the total lipid content when compared to WT cells. The *gpt2Δ* strain on the other hand showed a puite drastic enhancment in desturation level, which correlates with the effect seen on total lipid content (Fig 2B and 2A). In the *gpt2Δ* deletion strain, there was 5% decrease in relative DAG content and a 4% increase in relative TAG content (Fig.3), which indicates that a larger amount of LPA produced by SCT1 is incorporated into TAG molecules than those produced by GPT2. This is in agreement with the findings by (Zaremberg & McMaster, 2002) by *in vivo* feeding of [$^{14}$C]acetate.

**Overexpression of *GPT2* and *SCT1* affects the conten, composition and acyl distribution of lipids –** Acyl lipid composition and content was analyzed in *S. cerevisiae* strains overexpressing either *GPT2* or *SCT1* behind the strong inducible *GAL1*-promoter, grown to stationary stage on synthetic media supplemented with a non-fermentable carbon source. In the *GPT2* and *SCT1* overexpression strains there were a 50% and 70% increase in total acyl content (nmol FA/g dry weight), respectively (Fig. 4). This is interesting as the *SCT1* overexpression induces a higher increase in total acyl lipid content as compared to *GPT2*, while *in-vitro* GPAT activity levels in the crude extract is the oposite. *GPT2* and *SCT1* overexpression also has effects on the acyl composition seen in the analysis of total lipids, with a quite significant reduction in unsaturation level (Fig. 5C). Overexpression of *GPT2* had a corresponding increase in 18 carbon molecules, while the SCT1 strain had an increase in the 16 carbon molecules (Fig. 5B, Paper I). This increase in 16 carbon species due to SCT1 overexpression correlates with SCT1 *in-vitro* acyl preference. GPT2 does not have a fatty acyl preference *in-vitro*, so the increase in the 18 carbon molecules may be due to a change in SCT1 and GPT2 activities in the yeast cells. When we looked at total saturation versus unsaturation, we found that GPT2 and SCT1 both increased saturated acyl groups (Fig. 5C, Paper I). Both GPT2 and SCT1 overexpression increased DAG and free fatty acid (FFA) production. DAG is a lipid intermediate for TAG, the final step in glycerol lipid biosynthesis. Due to this increase in DAG, we can assume that the final steps in lipid biosynthesis now are rate limiting instead of the earlier one. The increase in FFA could be due to an increase in fatty acid turnover or breakdown of acyl-CoAs. The large increase in *in-vitro* total GPAT activity as compared to the WT would definitely cause a sink for acyl-CoA's, which would argue against the hydrolysis of acyl-CoAs. However, there are some differences. Overexpression of GPT2 leads to a 28% increase in the FA content in

phospholipids as compared to wt. SCT1 increased FA content in TAG molecules by 68% as compared to WT (Fig 6A). The higher increase in PL, FA and DAG molecules due to GPT2 overexpression leads us to believe that the increase in FFA content is due to increased phospholipid turnover. When we looked at acyl composition of the lipid classes, we found even more differences. Both overexpression strains show decreases in 16:1 and 18:1 in PL and DAG and a smaller decrease in the FFA molecules. The decreases in unsaturated species of PL are compensated by a general increase in the saturated species. The decrease in incorporation into unsaturated acyl groups is compensated by an increase in 16:0 in both overexpression strains and an increase in 18:0 in the GPT2 overexpression strain. In the GPT2 overexpression strain, there is an increase in 18:0 and SCT1 16:0. Overexpression of SCT1 increases incorporation in 16:0 TAG molecules (Fig.7, Paper I). From these findings, as well as those above, we conclude that in *S. cerevisiae*, strong GPAT gene overexpression will elevate de-novo FA biosynthesis, which will lead to an increase in acyl lipid content and a reduction in total unsaturation level. The large increase in *in-vitro* total GPAT activity as compared to the WT would definitely cause a sink for acyl CoA's. The result indicates to us that acyl-CoA is a possible signal for FA synthesis in *S. cerevisiae*. The elevated FA production is then separately channeled into the two different GPAT paths. Incorporation by SCT1 causes an accumulation into TAG molecules while that by GPT2 does not.

## Use of TAZ1 for Increasing Oil Quantity in Plants (Patent I)

The most exciting phrase to hear in science, the one that heralds new discoveries, is not "Eureka!" (I found it), but "That's funny..."- Isaac Asimov

**Yeast Tafazzin (*TAZ1*) is involved in the incorporation of Glycerol 3-phosphate into Lysophosphatic acid. -** In initial studies on the incorporation of [$^{14}$C]glycerol-3-phosphate in the presence of acyl-CoA (GPAT assay) in crude extraxts of a *TAZ1 S. cerevisiae* knockout strain as compared to WT we found a reduction of approximately 60%. Overexpression of the *TAZ1* gene behind the strong inducible *GAL1* promotor on a multicopy plasmid resulted in a 2.5-fold increase in GPAT activity in crude extracts as compared to the empty plasmid control (Fig.1, 2). There is strong evidence supporting the involvement of TAZ1 in cardiolipin remodeling and our results show a definite role in activation of GPAT, suggesting that there might be a bifunctionality of this enzyme.

**Yeast tafazzin (TAZ1) affects lipid composition in *S. cerevisiae* -** The effects on lipid composition by overexpressing the *TAZ1* gene was studied by growing a WT strain transformed with the pAN3 vector (multicopy plasmid with a *GAL1-TAZ construct1*) on synthetic media lacking uracil and harvested after induction with galactose for 24 and 28 hours. Inducing the *TAZ1*overexpression strain for 24 and 28 hours increased the total lipid content by 16 and 12%, respectively as compared to control (Fig. 3). In the neutral lipid fraction from cells induced for 28 hours, we found an increase of TAG compared to the empty plasmid strain of 62% (Table 3). We saw similar levels of TAG accumulation in

cells grown for 24 hours. Interestingly, in the polar lipid fraction, the total content per dry weight decreased up to 34% with overexpression of *TAZ1* (Table 4). The relative content of phospholipids was different from the empty plasmid strain as well. The relative content of PI and PE was decreased while PC and PS was increased (Table 4). These results lead to the conclusion that *TAZ1* is a good candidate for use to increase oil quantity in oilseeds.

**Overexpression of *A. thaliana* genes *At104.1* and At*104.2* affect lipid composition in *S. cerevisiae*.** - The effects of the *TAZ1* homologues At104.1 and At104.2 from *A. thaliana* has on lipid composition in *S. cerevisiae* was studied by overexpressing the genes behind the *GAL1* promoter and introduced the multicopy vector and an empty control vector into a WT yeast strain. Yeast cells were grown on synthetic media lacking uracil and harvested after induction with galactose for 24 hours. Total lipid content in the At104.1 and At104.2 transformants were increased 42% and 45%, respectively, compared to the empty plasmid control (Table 5). As with *TAZ1*, overexpression of At104.1 and At104.2 increased triacylglycerol content significantly, 79% and 92% respectively. The accumulation of phospholipids was slightly lowered and the FA and DAG content increased slightly (Table 5). In the major polar lipid classes, the most pronounced effect was seen with PI, where there was a decrease with overexpression of AT104.1 and At104.2 of 39% and 33%, respectively (Table 6). For potential use of these genes in lipid manipulation of TAG, we were also interested in the effect these genes had on fatty acid composition. The overexpression of At104.1 and At104.2 increased the over all oleic acid content approximately 1.5-fold and decreased palmitic acid content approximately 2-fold in *S. cerevisiae* compared to the empty plasmid control (Table 7). There was also a small increase in stearic acid of approximately one fold.

**Transgenic plants expressing *A. thaliana genes At104.1* and *At104, 2* and *S. cerevisiae* genes TAZ1 increase relative seed oil content in *A. thaliana*.** - The effect of overexpression of the yeast and *A. thaliana* tafazzin homologues on seed oil content was evaluated in *A. thaliana*. To do this the plant genes were expressed from a constitutive CaMV35S or a seed specific napin promoter. *TAZ1* was introduced into the plant expression vector pSUN300-USP under the control of the seed specific USP promoter. Seed oil content was increased in T2 and T3 generation of *A. thaliana* by all three overexpressed genes. This confirms that *A. thaliana* genes At104.1 and At104.2 and *S. cerevisiae* genes *TAZ1* do influence lipid accumulation. This makes them prominent candidates for increasing oil quality and quantity in plants through use of plant biotechnology.

## Microsomal preparations from plant and yeast acylate free fatty acids without prior activation to acyl thioesters (Paper II)

If the facts don't fit the theory, change the facts -Albert Einstein

In this paper, we show an alternative to both wax ester and ethyl ester production. Acylation of free fatty acids in cells is assumed to require that the acyl group is first activated to a thioester in the form of either CoA or ACP derivatives prior to acylation. It has been reported that enzymes in subcellular fractions or in aqueous environment can catalyze the acylation of free fatty acids to hydroxy or amine groups (Boutur, Dubreucq & Galzy, 1995; Chapman & Moore, 1993; Friedberg & Greene, 1967; Kolattukudy, 1967; Okumura, Iwai & Tsujisaka, 1979). In this paper, we discovered that the membranes catalyzed efficient acylation of free fatty acids to both endogenous and added fatty alcohols (Banas et al., 2005).

**Microsomal membranes from Arabidopsis leaves and roots efficiently acylate free fatty acids to long chain alcohols with no activation of the fatty acids to thioesters prior to acylation. -** It has been previously thought that a series of enzymatic steps led to two main wax biosynthetic pathways: the acyl reduction pathway and the decarbonylation pathway (Kunst & Samuels, 2003). In this paper, we have characterized a novel biochemical reaction of wax production. This is a reduction pathway that utilizes reduced aldehydes or primary alcohols to transacylate neutral lipids to form wax esters without involvement of acyl-CoA. In this study, we have characterized this novel enzymatic step and proved that it was an enzymatically catalyzed. Microsomal membranes from Arabidopsis leaves and roots efficiently acylated free fatty acids to long chain alcohols with no activation of the fatty acids to thioesters prior to acylation. We went about proving this using several methods. We researched this by optimization of substrates, heat treatment, DTNB addition and PMSF addition. After discovery of this unusual wax ester production, we went about optimizing the assay with respect to time and substrate preferences. Wax ester synthase activity with final optimized conditions in a time course is shown in (Fig. 1 of Paper 2). We wanted to confirm that the reaction was enzymatic. The first step we took towards proving this was by heat treatment of microsomal membranes. We did this by boiling the microsomes at 90ºC for 10 minutes and then running the optimized enzymatic assay on these boiled microsomes. There was no wax ester production with boiled microsomes under our optimized conditions. Addition of phenylmethylsulphonylfluoride (PMSF), an inhibitor of serine proteases, somewhat inhibited the activity in leaf microsomes at low microsomal concentrations (Fig.1B). Root microsomal membrane wax ester synthesizing activity was 5.5-fold less than that of leaf microsomes. In order to investigate whether formation of wax esters required access to free SH groups, the microsomal preparations of Arabidopsis leaves were incubated with [$^{14}$C]18:1 and 22:1-OH in the presence of high amount of dithionitrobenzoic acid (DTNB), which covalently reacts with free SH groups of CoA or ACP. The formation of wax esters was not affected by DTNB (Fig.2, lane A and B of Paper 2). No detectable amounts of other formed radioactive lipids were seen in the presence of DTNB. Addition of CoA and ATP to incubations of microsomes with [$^{14}$C]18:1 in presence or absence of docosa-13-

enol resulted in extensive incorporation of fatty acids into polar lipids (Fig.2, lane B and D of paper 2), demonstrating that the microsomal membranes contained active acyl-CoA dependent acyltransferases. Incubation with CoA and ATP in the presence of surplus molar concentrations of DTNB in relation to the CoA totally inhibited the transfer to polar lipids but did not affect the formation of wax esters (Fig. 2, lane C and E of paper 1 of paper 2). These results clearly showed that the formation of wax esters did not require activation of the free fatty acid to thioesters.

**In Arabidopsis leaves, fatty acids specificity of the reaction showed that long chain (C18-C24) unsaturated fatty alcohols were preferred. -** Major monomers of cutins in Arabidopsis leaf are omega-hydroxylated fatty acids and long chain dicarboxylic acids (Bonaventure et al., 2004). We tested omega-hydroxylated fatty acids and long chain diols as a substrate for the acylation of free fatty acids in leaf microsomal membranes as well as primary fatty alcohols and [$^{14}$C]oleic acid. We found that saturated alcohols were acylated with decreasing rates with increasing chain length (Fig. 3B of paper 2). Unsaturated alcohols were preferred substrates and were acylated with about the same rate, regardless of unsaturation and chain lengths ranging from 18 to 24 carbons (Fig. 3B of paper 2). We also looked at docosa-13-enol as an acyl acceptor in the presence of several fatty acyls. What we found there was a preference for the unsaturated fatty acyls 18:1, 18:2, and 18:3, but ricinoleic acid (12-hydroxy.octadec-9-enoic acid) and saturate fatty acyls were poorly utilized (Fig. 3A of Paper 2). Omega C18 diols were rapidly acylated by free fatty acids in Arabidopsis leaf membranes and the enzyme(s) appeared to prefer diols with one esterified fatty acid.

**Microsomes from yeast and Arabidopsis roots and leaves efficiently synthesized ethyl esters from ethanol and free fatty acids without prior activation of the fatty acid to a thioester. -** While optimizing the assay, we tried an alternate method of addition of substrates. Instead of adding them in benzene to freeze dried microsomes we used ethanol to add substrates to the microsomal membranes. At low membrane concentration, the formation of wax esters was approximately half that of the benzene method and high concentrations of the membrane strongly inhibited the reaction (Fig. 1B, Fig. 4A of paper 2). What was a surprising result from this experiment was a significant formation of radio labeled ethyl esters that increase with an increase in membrane concentrations. This production of radiolabeled ethyl esters was shown in both root and leaf microsomes. However, formation of the radiolabeled ethyl ester in A. thaliana roots was about half of that produced in the leaves. We further characterized this reaction as described above and found that boiling the microsomes at 90ºC for 10 minutes completely abolished this activity. Also, the addition of DTNB had no effect on this activity. Deletion of fatty alcohols from the assay led to only slightly less ethyl ester formation (Fig.4A). The differences in relative activity of the ethyl ester and wax ester formation in leaf and root membranes, the non-competitive nature and the different kinetics of the two acylation reactions strongly suggest that they are carried out by separate enzymes. We also looked at ethyl ester and wax ester production in Saccharomyces cerevisiae. We found no wax ester production in S. cerevisiae with either the

ethanol or the benzene method. However, we found that with the addition of substrates with ethanol there was efficient synthesis of ethyl esters in the membranes. We also tested this in the addition of DTNB and heat treatment. As with the A. thaliana microsomes, there was little effect from the addition of DTNB and heat treatment completely abolished the activity.

**Herbicide treatment with alloxydim in membrane preparations of root tips from wheat had greatly increased phospholipase activity as well as increasing wax ester and ethyl ester formation from free fatty acids. -** Root extracts from wheat treated with alloxydim, a grass herbicide, were shown to have increased phospholipase activity as well as increased capacity to synthesize ethyl esters (Banas, A. and Banas, W., unpublished observation). Addition of substrates in ethanol in microsomal fractions of root tips from alloxydim treated seedlings had over 20 times higher specific phospholipase A2 activity as well as 7-fold higher ethyl ester and 5-fold higher wax ester synthesis from free fatty acids than membranes from untreated seedlings (Fig 5). This demonstrates that enzymes both producing and acylating free fatty acids are strongly activated or induced under certain physiological conditions. Treatment with alloxydim stops root tip growth and the root tip goes through physiological changes that roughen its texture. The increases in enzyme activities with alloxydim could possibly have a connection to induced suberin synthesis in the root tips. These results combined with the results above suggest a possible involvement of these enzymes in cutin and suberin synthesis. It was found that the lipase inhibitor dehydrolipstatin was an very efficient inhibitor of both wax ester and ethyl ester formation in microsomal membranes from Arabidopsis but had less effect on the formation of ethyl esters in membranes from yeast.

## Ontogeny of mRNA expression and activity of long-chain acyl-CoA synthetase (ACSL) isoforms in *Mus musculus* heart.

## (Paper III)

No, no, you're not thinking; you're just being logical. - Niels Bohr

In this study, we investigated the ACSL isoforms, their expression and their activity in *Mus Musculus* heart at different developmental stages. The story of the ACSL family in developmental stages is a very complicated one. What is clear is that previous data and ours clearly suggest that there are different metabolic functions for each of these isoforms. We know that understanding these mechanisms is not only important for understanding fetal and neonate development but for understanding many adult cardiac diseases as well. It is well understood that in many cases of hypertrophy and heart failure, the heart switches back to what is referred to as “fetal energy metabolism” (Calvani, Reda & Arrigoni-Martelli, 2000; van Bilsen *et al.*, 2004). Understanding how the genes are regulated may give us important markers as indicators for heart disease. Truly understanding these heart metabolic functions will require much more research.

**ACSL1 is the major ACSL isoform involved in fatty acid oxidation** - Due to past data that shows an increase in fatty acid oxidation along with an up-regulation of genes involved in this process, we expected to see at least one ACSL isoform to be up-regulated as well. We looked at total ACSL activity in heart at different developmental stages. We found very low ACSL activity at embryonic day 16 (1.3 nmol/min/mg). In contrast, on postnatal day 1, ACSL activity increased 14-fold to 18.9 nmol/min/mg. ACSL activity remained unchanged through postnatal day 25. In the adult mouse heart, ACSL activity is 6.6-fold higher (124.7 nmol/min/mg) than that of the neonate (Fig. 1, Paper III). The only isoform whose mRNA transcript level increased after birth was ACSL1 (Fig. 2A, Paper III). ACSL1 message level was 2.5-fold higher at postnatal day 1, compared to embryonic day 16. The amount of ACSL1 mRNA remained stable throughout the neonatal period, but was 1.6-fold higher in the adult as compared to day 25. This data suggest that ACSL1 is the isoform contributing activated long-chain fatty acids for oxidation in the heart.

**ACSL3 and ACSL6 may not play a large role in postnatal ACSL activity -** We wanted to look at which isoforms were responsible for the majority of heart ACSL activity at different developmental stages. To separate out the isoforms, we used known inhibitors triacsin C and troglitazone. Incubation of heart total membrane with 10 μM triacsin C inhibited ACSL activity 87 - 97% at all developmental time points (Fig. 3, Paper III). Because triacsin C only inhibits ACSL 1, 3 and 4, this data suggests that ACSL5 and ACSL6 do not contribute significantly to heart ACSL activity. ACSL4 is the only isoform inhibited by troglitazone.

Treatment of heart membranes with 50μM troglitazone did not inhibit ACSL activity. These data strongly suggest that ACSL4 does not contribute to the total ACSL activity measured in the heart. We wanted to look at this further. The abundance of ACSL3, ACSL6 mRNAs was inverse to that observed for ACSL1. The ACSL3:β-actin ratios were highest at embryonic day 16, and were 1.8-fold lower after birth (Fig. 2 B, Paper III). ACSL3 mRNA levels at postnatal days 1 and 7 are 75- and 13-fold higher, respectively, than in the adult. Due to the significant amounts of ACSL3 transcripts present at postnatal day 1, we wanted to assess the contribution of the ACSL3 isoform to total ACSL activity. ACSL3 activity assayed with [$^{14}$C]palmitate is inhibited by high concentrations of arachidonate (Van Horn et al., 2005). In heart total membrane, we found that ACSL activity at embryonic day 16 was inhibited up to 32 % by arachidonate when compared to hearts collected on day 1 or in the adult (Fig. 5, Paper III). Although arachidonic acid can also compete ACSL4 activity measured with palmitate (Van Horn*, et al.*, 2005), our data using troglitazone (Fig. 4, Paper III) strongly suggests that ACSL4 activity is not significant in the embryonic heart. This data combined with previous reports allows us to conclude that ACSL3 and ACL6 are not major contributors to postnatal ACSL activity.

**ACSL3 and ACSL4 need post-transcriptional modification in order for activation to occur -** During this study, we saw some unusual changes. We noticed an in concordance of mRNA levels and total ACSL activity in the heart. At

embryonic day sixteen, ACSL 3 and ACSL4 have a 137- and 5-fold increase in mRNA compared to the adult stage. There is a 75-fold increase in mRNA level at postnatal day one for ACSL3 as compared to the adult stage, and comparable levels to the adult stage for ACSL4 (Fig.2, B, C, Paper III). The largest decreases in total activity were at embryonic day sixteen, postnatal day eighteen and the adult stages. There was a 96.8%, 93.2% and 92.3% decrease, respectively, in total activity in the presence of DMSO. However, in embryonic day 16 through postnatal day 7, there was a 16% to 18% decrease in total ACSL activity as compared to the adult (Fig.3, Paper III). This data indicates that a small amount of total ACSL activity in the embryonic and early postnatal stages may be due to ACSL3 and ACSL4. There is a large discrepancy between total ACSL activity and the transcript levels of ACSL3 and ACSL4. Our data suggests that the ACSL3 and ACSL4 need post transcriptional modification in order for activation to occur. We understand that there are many ontological changes occurring between the developmental stages of *Mus musculus* heart, especially between the late embryonic and early postnatal stages. This activation of ACSL enzymes may be due to changes in substrate availability after birth and the high level of transcript that occurs before birth may actually be in preparation for events that occur in the postnatal stages rather than the embryonic ones.

## Glycerophosphodiester Acyltransferase; A New Class of Acylation Enzymnes in Lipid Biosynthesis (Paper IV)

Things should be made as simple as possible, but not any simpler.
–Albert Einstein

Previously it was thought that the synthesis of Phosphatidylcholine occurred through two mechanism; through the reduction of CDP-choline (CDP-Cho) or by methylation of phosphatidylethanolamine (PE) (Dowd, Bier & Patton-Vogt, 2001; Fernandez-Murray & McMaster, 2005). In this study, we have identified two novel acyltransferase activities in eukaryotic cell membranes that carry out acylation of the two glycerophosphodiesters GPC and GPE. These reactions are called glycerophosphocholine acyltransferase (GPCAT) and glycerophosphoethanolamine acyltransferase (GPEAT). These enzymes utilize Glycerophosphocholine (GPC) and Glycerophosphoethanolamine (GPE) as acyl-accepters and acyl-CoA as acyl donors to synthesize lysophospholipids. These new reactions combined with the known LPCAT and LPEAT activities create a novel pathway for recycling glycerophosphodiesters into phospholipids. This set of reactions is extremely energy efficient compared to the previously described glycerophosphodiester pathway by Patton-Vogt et al 2006 (Patton-Vogt, 2006).

***Saccharomyces cerevisiae* membranes catalyzes the acylation of glycerophosphocholine and glycerophosphoethanolamine-** In the early 1950's, Dawson *et al*. and others identified GPC and GPE as metabolites in several mammalian tissues and considered them as being metabolites in the main pathway for lipid biosynthesis. Radioactive phosphate feeding studies, however, strongly indicated them as rather being catabolic products of phospholipid deacylation, which has been proven more lately (Fernandez-Murray & McMaster, 2005;

Zaccheo*, et al.*, 2004). This theory is strongly supported by the fact that deletion of *NTE1* gene in *Saccharomyces cerevisiae* leds to abrogation of GPC accumulation. It is well understood that *S. cerevisiae*, can degrade GPI and GPC take up in media sources (Almaguer *et al.*, 2003; Fisher *et al.*, 2005; Patton, Pessoa-Brandao & Henry, 1995) as well as GPC generated intracellularly (Fernandez-Murray & McMaster, 2005). To test if yeast utilizes GPC in addition to G3P and DHAP as an acceptor for acylation, an in vitro assay for GPAT activity according to Tillman and Bell (1986) with minor modifications was used for assaying GPAT activity and with an exchange of the [$^{14}$C]G3P for [$^{14}$C]GPC to assay for GPC acylation. The TLC image of radioactive lipids extracted from the incubated yeast extracts show apart from the starting point with minor contamination of water soluble $^{14}$C-labelled substrate, a minor background incorporation of radioactivity into lipids in the G3P and GPC minus acyl-CoA lane (Figure 1A). However, in the presence of 16:0-CoA or 18:1-CoA there was significant synthesis of both $^{14}$C-labelled LPA and PA when [$^{14}$C]G3P was added as acyl-acceptor and of $^{14}$C-labelled LPC as well as of PC when [$^{14}$C] GPC was added (Figure 1A). These data clearly show that the crude yeast extract has an enzymatic activity capable of acylating the GPC molecule and that LPC is readily available for a second acylation by the well-known LPCAT enzyme. We further tested all glycerophosphodiesters for acylation activity in yeast membranes and found apart from GPC that also GPE was utilized for acylation. The new acyltransferase activities, the glycerophosphocholine acyltransferase (GPCAT) with a Vmax in microsomes of 8.7 nmol/min/mg (Fig. 4) and glycerophosphoethanolamine acyltransferase (GPEAT) whose activity is only a small percent of the GPCAT activity (Fig 1A and 1C). Comparing the GPCAT activity to other acylation activities in *S. cerevisiae,* we determined GPCAT to be approximately one fourth of that of the relativley active GPAT activity indicating that GPCAT is not a minor activity in this organism. The GPCAT activities showed some week acyl prefences but the further acylation of both LPC and LPE by the LPCAT and LPEAT activities in the yeast extracts were however more strongly favoured by unsaturated acyl-CoAs. (Fig. 1A and C).

**In *Saccharomyces cerevisiae* extracts, GPC acylation is not catalyzed by *GPT2* or *SCT1*-** To test wether any of the two yeast GPATs, GPT2 or SCT1, is responsible for the acylation of GPC we compared GPCAT and GPAT activities in both crude extracts from WT yeast with knockout and overexpression strains. The deletion strains *gpt2*Δ and *sct1*Δ had the same GPCAT activity as WT but strongly reduced GPAT activies (Fig. 3A). Similar results were obtained with the overexpression strains of *GPT2* and *SCT1* with no effects on GPCAT activity as compared to WT but strong effects on GPAT activity (Fig. 3B). We further characterized the GPCAT activity in microsomal preparations of WT yeast cells and compared it to the well-known GPAT activity. The GPCAT activity was linear in respect to added microsomal proteins up to 25 mg at 10 minutes incubations while the GPAT was fairly linear linear to 20 mg at 5 minutes incubations. The GPCAT enzyme can utilize a broad spectrum of pH optimums from 6.5 to 8.5, but activity drops significantly if the pH drops below 6.5 (Fig. 4C). When testing the basic assay conditions, we found that 2 mg/ml of BSA increased the GPCAT activity by approximately 20% while addition of 2 mM of $MgCl_2$ inhibited activity

by 50%. The effect of divalent ions was further tested with the addition of 2 mM of $Ca^{2+}$, $Mn^{2+}$ and $Zn^{2+}$. $Ca^{2+}$ showed similar inhibition to $Mg^{2+}$ while $Zn^{2+}$ and $Mn^{2+}$ completely inhibited the GPCAT activity. Addition of EDTA showed no significant difference whereas the strong divalent ion chelator phenantroline completely inhibited the activity. The sulfhydryl reagent N-ethylmalemid (NEM) also strongly inhibits the GPCAT activity (Table II). We looked at the effect of heat shock on the GPAT and GPCAT activities. *Saccharomyces cerevisiae* membranes were incubated at 50C for 30minutes. This completely inhibited the GPAT activity while it enhanced GPCAT activity by 30% (Table III). This, combined with old data that shows an increase of GPC production with increased growth temperatures (Dowd, Bier & Patton-Vogt, 2001), leads us to conclude GPCAT recycling activity in *Saccharomyces cerevisiae* membranes may be activated by stress elements such as heat.

**GPCAT activity in higher eukaryotes -** We further tested if the GPCAT activity is present in tissue extracts of higher eukaryotes. We tested three different mammalian extracts: the *Homo sapien* renal epithelial cell line 293T, *Mus musculus* liver and *Rat Ratus* developing brains. We found no evidence of GPCAT activity in these tissues (data not shown). This does not rule out the possible existence of GPCAT in mammalian species but rather may point that this enzyme has a very specific function and that these activities might be difficult to measure *in vitro*. A microsomal preparation from developing *Ulmas glabra* (Elm) seed was tested. Plants with high oil content seeds are extremely active in lipid synthesizing enzymes. In this tissue, we found GPCAT activity, which was about 50% of that of the GPAT activity and thus of similar rate as in yeast extracts (Fig. 2). Further more, incorporation of [$^3$H-choline]GPC added to the growth media under low phosphate conditions to a *pct1Δ* and a *pct1Δgde1Δ* knockout strain suggests that the GPCAT pathway for PC biosynthesis is functional in vivo.

# End

**I have no special talent. I am only passionately curious.**
**-Albert Einstein**

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**"Life is, and has always been a struggle. The fishing pole bends heavier for some than others, and nobody has yet to figure out why – just as you never know, when you make a cast, if what attacks your fly is a finger-size baby snapper or a tiger shark that can turn you into bait. Still we struggle with the rod just the same. Life to me is like a fish on the line. When it is there, you feel it. You fight it. You gain the line. You lose the line. But if that line suddenly snaps, or the pole breaks, or a thousand other problems occur that fisherman use as excuses when the tension is gone, you feel it even more."**
**- Jimmy Buffet (Buffet, 2004).**

## Works Cited

2000. Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. *Nature 408*, 796-815.

Aboagye, E.O. & Bhujwalla, Z.M. 1999. Malignant transformation alters membrane choline phospholipid metabolism of human mammary epithelial cells. *Cancer Res 59*, 80-4.

Adams, M.D., Celniker, S.E., Holt, R.A., Evans, C.A., Gocayne, J.D., Amanatides, P.G., Scherer, S.E., Li, P.W., Hoskins, R.A., Galle, R.F., George, R.A., Lewis, S.E., Richards, S., Ashburner, M., Henderson, S.N., Sutton, G.G., Wortman, J.R., Yandell, M.D., Zhang, Q., Chen, L.X., Brandon, R.C., Rogers, Y.H., Blazej, R.G., Champe, M., Pfeiffer, B.D., Wan, K.H., Doyle, C., Baxter, E.G., Helt, G., Nelson, C.R., Gabor, G.L., Abril, J.F., Agbayani, A., An, H.J., Andrews-Pfannkoch, C., Baldwin, D., Ballew, R.M., Basu, A., Baxendale, J., Bayraktaroglu, L., Beasley, E.M., Beeson, K.Y., Benos, P.V., Berman, B.P., Bhandari, D., Bolshakov, S., Borkova, D., Botchan, M.R., Bouck, J., Brokstein, P., Brottier, P., Burtis, K.C., Busam, D.A., Butler, H., Cadieu, E., Center, A., Chandra, I., Cherry, J.M., Cawley, S., Dahlke, C., Davenport, L.B., Davies, P., de Pablos, B., Delcher, A., Deng, Z., Mays, A.D., Dew, I., Dietz, S.M., Dodson, K., Doup, L.E., Downes, M., Dugan-Rocha, S., Dunkov, B.C., Dunn, P., Durbin, K.J., Evangelista, C.C., Ferraz, C., Ferriera, S., Fleischmann, W., Fosler, C., Gabrielian, A.E., Garg, N.S., Gelbart, W.M., Glasser, K., Glodek, A., Gong, F., Gorrell, J.H., Gu, Z., Guan, P., Harris, M., Harris, N.L., Harvey, D., Heiman, T.J., Hernandez, J.R., Houck, J., Hostin, D., Houston, K.A., Howland, T.J., Wei, M.H., Ibegwam, C., Jalali, M., Kalush, F., Karpen, G.H., Ke, Z., Kennison, J.A., Ketchum, K.A., Kimmel, B.E., Kodira, C.D., Kraft, C., Kravitz, S., Kulp, D., Lai, Z., Lasko, P., Lei, Y., Levitsky, A.A., Li, J., Li, Z., Liang, Y., Lin, X., Liu, X., Mattei, B., McIntosh, T.C., McLeod, M.P., McPherson, D., Merkulov, G., Milshina, N.V., Mobarry, C., Morris, J., Moshrefi, A., Mount, S.M., Moy, M., Murphy, B., Murphy, L., Muzny, D.M., Nelson, D.L., Nelson, D.R., Nelson, K.A., Nixon, K., Nusskern, D.R., Pacleb, J.M., Palazzolo, M., Pittman, G.S., Pan, S., Pollard, J., Puri, V., Reese, M.G., Reinert, K., Remington, K., Saunders, R.D., Scheeler, F., Shen, H., Shue, B.C., Siden-Kiamos, I., Simpson, M., Skupski, M.P., Smith, T., Spier, E., Spradling, A.C., Stapleton, M., Strong, R., Sun, E., Svirskas, R., Tector, C., Turner, R., Venter, E., Wang, A.H., Wang, X., Wang, Z.Y., Wassarman, D.A., Weinstock, G.M., Weissenbach, J., Williams, S.M., WoodageT, Worley, K.C., Wu, D., Yang, S., Yao, Q.A., Ye, J., Yeh, R.F., Zaveri, J.S., Zhan, M., Zhang, G., Zhao, Q., Zheng, L., Zheng, X.H., Zhong, F.N., Zhong, W., Zhou, X., Zhu, S., Zhu, X., Smith, H.O., Gibbs, R.A., Myers, E.W., Rubin, G.M. & Venter, J.C. 2000. The genome sequence of Drosophila melanogaster. *Science 287*, 2185-95.

Aharoni, A., Dixit, S., Jetter, R., Thoenes, E., Van Arkel, G. & Pereira, A. 2004. The SHINE Clade of AP2 Domain Transcription Factors Activates Wax Biosynthesis, Alters Cuticle Properties, and Confers Drought Tolerance when Overexpressed in Arabidopsis. *Plant Cell*

Almaguer, C., Mantella, D., Perez, E. & Patton-Vogt, J. 2003. Inositol and phosphate regulate GIT1 transcription and glycerophosphoinositol incorporation in Saccharomyces cerevisiae. *Eukaryot Cell 2*, 729-36.

Alvarez, H.M., Souto, M.F., Viale, A. & Pucci, O.H. 2001. Biosynthesis of fatty acids and triacylglycerols by 2,6,10,14-tetramethyl pentadecane-grown cells of Nocardia globerula 432. *FEMS Microbiol Lett 200*, 195-200.

Anliker, B. & Chun, J. 2004. Lysophospholipid G protein-coupled receptors. *J Biol Chem 279*, 20555-8.

Anthony, M.L., Zhao, M. & Brindle, K.M. 1999. Inhibition of phosphatidylcholine biosynthesis following induction of apoptosis in HL-60 cells. *J Biol Chem 274*, 19686-92.

Aparicio, S., Chapman, J., Stupka, E., Putnam, N., Chia, J.M., Dehal, P., Christoffels, A., Rash, S., Hoon, S., Smit, A., Gelpke, M.D., Roach, J., Oh, T., Ho, I.Y., Wong, M., Detter, C., Verhoef, F., Predki, P., Tay, A., Lucas, S., Richardson, P., Smith, S.F., Clark, M.S., Edwards, Y.J., Doggett, N., Zharkikh, A., Tavtigian, S.V., Pruss, D., Barnstead, M., Evans, C., Baden, H., Powell, J., Glusman, G., Rowen, L., Hood, L., Tan, Y.H., Elgar, G., Hawkins, T., Venkatesh, B., Rokhsar, D. & Brenner, S. 2002. Whole-genome shotgun assembly and analysis of the genome of Fugu rubripes. *Science 297*, 1301-10.

Athenstaedt, K. & Daum, G. 2000. 1-Acyldihydroxyacetone-phosphate reductase (Ayr1p) of the yeast Saccharomyces cerevisiae encoded by the open reading frame YIL124w is a major component of lipid particles. *J Biol Chem 275*, 235-40.

Attardi, G. & Schatz, G. 1988. Biogenesis of mitochondria. *Annu Rev Cell Biol 4*, 289-333.

Banas, A., Carlsson, A.S., Huang, B., Lenman, M., Banas, W., Lee, M., Noiriel, A., Benveniste, P., Schaller, H., Bouvier-Nave, P. & Stymne, S. 2005. Cellular sterol ester synthesis in plants is performed by an enzyme (phospholipid:sterol acyltransferase) different from the yeast and mammalian acyl-CoA:sterol acyltransferases. *J Biol Chem 280*, 34626-34.

Barr, M.M. 2003. Super models. *Physiol Genomics 13*, 15-24.

Bartels, H. 1964. Comparative Physiology Of Oxygen Transport In Mammals. *Lancet 18*, 601-4.

Barth, P.G., Majoie, C.B., Gootjes, J., Wanders, R.J., Waterham, H.R., van der Knaap, M.S., de Klerk, J.B., Smeitink, J. & Poll-The, B.T. 2004. Neuroimaging of peroxisome biogenesis disorders (Zellweger spectrum) with prolonged survival. *Neurology 62*, 439-44.

Barth, P.G., Scholte, H.R., Berden, J.A., Van der Klei-Van Moorsel, J.M., Luyt-Houwen, I.E., Van 't Veer-Korthof, E.T., Van der Harten, J.J. & Sobotka-Plojhar, M.A. 1983. An X-linked mitochondrial disease affecting cardiac muscle, skeletal muscle and neutrophil leucocytes. *J Neurol Sci 62*, 327-55.

Beggs, J.D. 1978. Transformation of yeast by a replicating hybrid plasmid. *Nature 275*, 104-9.

Bell, R.M. & Coleman, R.A. 1980. Enzymes of glycerolipid synthesis in eukaryotes. *Annu Rev Biochem 49*, 459-87.

Benzioni, A., Forti, M. 1989. *Oilcrops of the World:Their Breeding and Utilization*. McGraw-Hill. New York. 448-461 pp.

Berger, J. & Moller, D.E. 2002. The mechanisms of action of PPARs. *Annu Rev Med 53*, 409-35.
Bessoule, J.J., Testet, E. & Cassagne, C. 1995. Synthesis of phosphatidylcholine in the chloroplast envelope after import of lysophosphatidylcholine from endoplasmic reticulum membranes. *Eur J Biochem 228*, 490-7.
Bione, S., D'Adamo, P., Maestrini, E., Gedeon, A.K., Bolhuis, P.A. & Toniolo, D. 1996. A novel X-linked gene, G4.5. is responsible for Barth syndrome. *Nat Genet 12*, 385-9.
Birner, R., Burgermeister, M., Schneiter, R. & Daum, G. 2001. Roles of phosphatidylethanolamine and of its several biosynthetic pathways in Saccharomyces cerevisiae. *Mol Biol Cell 12*, 997-1007.
Bloor, W.R. 1920. *Proc. Soc. Exp. Biol. Med. 17*, 138-140.
Bolhuis, P.A., Hensels, G.W., Hulsebos, T.J., Baas, F. & Barth, P.G. 1991. Mapping of the locus for X-linked cardioskeletal myopathy with neutropenia and abnormal mitochondria (Barth syndrome) to Xq28. *Am J Hum Genet 48*, 481-5.
Bonaventure, G., Bao, X., Ohlrogge, J. & Pollard, M. 2004. Metabolic responses to the reduction in palmitate caused by disruption of the FATB gene in Arabidopsis. *Plant Physiol 135*, 1269-79.
Boutur, O., Dubreucq, E. & Galzy, P. 1995. Factors influencing ester synthesis catalysed in aqueous media by the lipase from Candida deformans (Zach) Langeron and Guerra. *J Biotechnol 42*, 23-33.
Brandner, K., Mick, D.U., Frazier, A.E., Taylor, R.D., Meisinger, C. & Rehling, P. 2005. Taz1, an outer mitochondrial membrane protein, affects stability and assembly of inner membrane protein complexes: implications for Barth Syndrome. *Mol Biol Cell 16*, 5202-14.
Broun, P., Poindexter, P., Osborne, E., Jiang, C.Z. & Riechmann, J.L. 2004. WIN1, a transcriptional activator of epidermal wax accumulation in Arabidopsis. *Proc Natl Acad Sci U S A 101*, 4706-11.
Brown, N.F., Weis, B.C., Husti, J.E., Foster, D.W. & McGarry, J.D. 1995. Mitochondrial carnitine palmitoyltransferase I isoform switching in the developing rat heart. *J Biol Chem 270*, 8952-7.
Buffet, J. 2004. *A Salty Piece of Land*. Back Bay Books. Boston. 462 pp.
Calvani, M., Reda, E. & Arrigoni-Martelli, E. 2000. Regulation by carnitine of myocardial fatty acid and carbohydrate metabolism under normal and pathological conditions. *Basic Res Cardiol 95*, 75-83.
Cantlay, A.M., Shokrollahi, K., Allen, J.T., Lunt, P.W., Newbury-Ecob, R.A. & Steward, C.G. 1999. Genetic analysis of the G4.5 gene in families with suspected Barth syndrome. *J Pediatr 135*, 311-5.
Carroll, J.E., McGuire, B.S., Chancey, V.F. & Harrison, K.B. 1989. Acyl-CoA dehydrogenase enzymes during early postnatal development in the rat. *Biol Neonate 55*, 185-90.
Chang, S.C., Heacock, P.N., Clancey, C.J. & Dowhan, W. 1998a. The PEL1 gene (renamed PGS1) encodes the phosphatidylglycero-phosphate synthase of Saccharomyces cerevisiae. *J Biol Chem 273*, 9829-36.
Chang, S.C., Heacock, P.N., Mileykovskaya, E., Voelker, D.R. & Dowhan, W. 1998b. Isolation and characterization of the gene (CLS1) encoding cardiolipin synthase in Saccharomyces cerevisiae. *J Biol Chem 273*, 14933-41.

Chapman, K.D. & Moore, T.S., Jr. 1993. Catalytic Properties of a Newly Discovered Acyltransferase That Synthesizes N-Acylphosphatidylethanolamine in Cottonseed (Gossypium hirsutum L.) Microsomes. *Plant Physiol 102*, 761-769.

Cheng, J.B. & Russell, D.W. 2004. Mammalian wax biosynthesis. II. Expression cloning of wax synthase cDNAs encoding a member of the acyltransferase enzyme family. *J Biol Chem 279*, 37798-807.

Chinetti, G., Fruchart, J.C. & Staels, B. 2000. Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation. *Inflamm Res 49*, 497-505.

Christie, W.W. 2003. *Lipid Anaylsis Isolation, Separation, Identification, and Structual Analysis of Lipids*. 3 3. The Oily Press. Middlesex, UK. 416 pp.

Clark, C.M., Jr. 1971. Carbohydrate metabolism in the isolated fetal rat heart. *Am J Physiol 220*, 583-8.

Clark, C.M., Jr. 1973. Characterization of glucose metabolism in the isolated rat heart during fetal and early neonatal development. *Diabetes 22*, 41-9.

Clark, J.B., Vinicor, F., Carr, L. & Clark, C.M., Jr. 1980. Adenylyl cyclase responsiveness to guanyl nucleotides in the developing rat heart. *Pediatr Res 14*, 291-5.

Close, R. 1964. Dynamic Properties Of Fast And Slow Skeletal Muscles Of The Rat During Development. *J Physiol 173*, 74-95.

Clough, R.C., Matthis, A.L., Barnum, S.R. & Jaworski, J.G. 1992. Purification and characterization of 3-ketoacyl-acyl carrier protein synthase III from spinach. A condensing enzyme utilizing acetyl-coenzyme A to initiate fatty acid synthesis. *J Biol Chem 267*, 20992-8.

Coleman, R.A., Lewin, T.M. & Muoio, D.M. 2000. Physiological and nutritional regulation of enzymes of triacylglycerol synthesis. *Annu Rev Nutr 20*, 77-103.

Coleman, R.A., Lewin, T.M., Van Horn, C.G. & Gonzalez-Baro, M.R. 2002. Do long-chain acyl-CoA synthetases regulate fatty acid entry into synthetic versus degradative pathways? *J Nutr 132*, 2123-6.

Coleman, R.A., Reed, B.C., Mackall, J.C., Student, A.K., Lane, M.D. & Bell, R.M. 1978. Selective changes in microsomal enzymes of triacylglycerol phosphatidylcholine, and phosphatidylethanolamine biosynthesis during differentiation of 3T3-L1 preadipocytes. *J Biol Chem 253*, 7256-61.

D'Adamo, P., Fassone, L., Gedeon, A., Janssen, E.A., Bione, S., Bolhuis, P.A., Barth, P.G., Wilson, M., Haan, E., Orstavik, K.H., Patton, M.A., Green, A.J., Zammarchi, E., Donati, M.A. & Toniolo, D. 1997. The X-linked gene G4.5 is responsible for different infantile dilated cardiomyopathies. *Am J Hum Genet 61*, 862-7.

Dahlqvist, A., Stahl, U., Lenman, M., Banas, A., Lee, M., Sandager, L., Ronne, H. & Stymne, S. 2000. Phospholipid:diacylglycerol acyltransferase: an enzyme that catalyzes the acyl-CoA-independent formation of triacylglycerol in yeast and plants. *Proc Natl Acad Sci U S A 97*, 6487-92.

Diczfalusy, M.A., Bjorkhem, I., Einarsson, C., Hillebrant, C.G. & Alexson, S.E. 2001. Characterization of enzymes involved in formation of ethyl esters of long-chain fatty acids in humans. *J Lipid Res 42*, 1025-32.

Dircks, L.K. & Sul, H.S. 1997. Mammalian mitochondrial glycerol-3-phosphate acyltransferase. *Biochim Biophys Acta 1348*, 17-26.
Disch, D.L., Rader, T.A., Cresci, S., Leone, T.C., Barger, P.M., Vega, R., Wood, P.A. & Kelly, D.P. 1996. Transcriptional control of a nuclear gene encoding a mitochondrial fatty acid oxidation enzyme in transgenic mice: role for nuclear receptors in cardiac and brown adipose expression. *Mol Cell Biol 16*, 4043-51.
Dobson, D.E., Kambe, A., Block, E., Dion, T., Lu, H., Castellot, J.J., Jr. & Spiegelman, B.M. 1990. 1-Butyryl-glycerol: a novel angiogenesis factor secreted by differentiating adipocytes. *Cell 61*, 223-30.
Dowd, S.R., Bier, M.E. & Patton-Vogt, J.L. 2001. Turnover of phosphatidylcholine in Saccharomyces cerevisiae. The role of the CDP-choline pathway. *J Biol Chem 276*, 3756-63.
Dubowitz, V. 1963. Myopathic Changes In A Muscular Dystrophy Carrier. *J Neurol Neurosurg Psychiatry 26*, 322-5.
Emes, R.D., Goodstadt, L., Winter, E.E. & Ponting, C.P. 2003. Comparison of the genomes of human and mouse lays the foundation of genome zoology. *Hum Mol Genet 12*, 701-9.
Faergeman, N.J. & Knudsen, J. 1997. Role of long-chain fatty acyl-CoA esters in the regulation of metabolism and in cell signalling. *Biochem J 323 ( Pt 1)*, 1-12.
Fahy, E., Subramaniam, S., Brown, H.A., Glass, C.K., Merrill, A.H., Jr., Murphy, R.C., Raetz, C.R., Russell, D.W., Seyama, Y., Shaw, W., Shimizu, T., Spener, F., van Meer, G., VanNieuwenhze, M.S., White, S.H., Witztum, J.L. & Dennis, E.A. 2005. A comprehensive classification system for lipids. *J Lipid Res 46*, 839-61.
Fernandez-Murray, J.P. & McMaster, C.R. 2005. Glycerophosphocholine catabolism as a new route for choline formation for phosphatidylcholine synthesis by the Kennedy pathway. *J Biol Chem 280*, 38290-6.
Fisher, D.J., Heymann, M.A. & Rudolph, A.M. 1980. Myocardial oxygen and carbohydrate consumption in fetal lambs in utero and in adult sheep. *Am J Physiol 238*, H399-405.
Fisher, E., Almaguer, C., Holic, R., Griac, P. & Patton-Vogt, J. 2005. Glycerophosphocholine-dependent growth requires Gde1p (YPL110c) and Git1p in Saccharomyces cerevisiae. *J Biol Chem 280*, 36110-7.
Foster, P.C. & Bailey, E. 1976. Changes in the activities of the enzymes of hepatic fatty acid oxidation during development of the rat. *Biochem J 154*, 49-56.
Friedberg, S.J. & Greene, R.C. 1967. The enzymatic synthesis of wax in liver. *J Biol Chem 242*, 234-7.
Fueller, M., Wang de, A., Tigyi, G. & Siess, W. 2003. Activation of human monocytic cells by lysophosphatidic acid and sphingosine-1-phosphate. *Cell Signal 15*, 367-75.
Fulgencio, J.P., Kohl, C., Girard, J. & Pegorier, J.P. 1996. Troglitazone inhibits fatty acid oxidation and esterification, and gluconeogenesis in isolated hepatocytes from starved rats. *Diabetes 45*, 1556-62.
Gardner, M.J., Hall, N., Fung, E., White, O., Berriman, M., Hyman, R.W., Carlton, J.M., Pain, A., Nelson, K.E., Bowman, S., Paulsen, I.T., James, K., Eisen, J.A., Rutherford, K., Salzberg, S.L., Craig, A., Kyes, S., Chan, M.S., Nene, V., Shallom, S.J., Suh, B., Peterson, J., Angiuoli, S., Pertea, M.,

Allen, J., Selengut, J., Haft, D., Mather, M.W., Vaidya, A.B., Martin, D.M., Fairlamb, A.H., Fraunholz, M.J., Roos, D.S., Ralph, S.A., McFadden, G.I., Cummings, L.M., Subramanian, G.M., Mungall, C., Venter, J.C., Carucci, D.J., Hoffman, S.L., Newbold, C., Davis, R.W., Fraser, C.M. & Barrell, B. 2002. Genome sequence of the human malaria parasite Plasmodium falciparum. *Nature 419*, 498-511.

Girard, J., Duee, P.H., Ferre, P., Pegorier, J.P., Escriva, F. & Decaux, J.F. 1985. Fatty acid oxidation and ketogenesis during development. *Reprod Nutr Dev 25*, 303-19.

Goff, S.A., Ricke, D., Lan, T.H., Presting, G., Wang, R., Dunn, M., Glazebrook, J., Sessions, A., Oeller, P., Varma, H., Hadley, D., Hutchison, D., Martin, C., Katagiri, F., Lange, B.M., Moughamer, T., Xia, Y., Budworth, P., Zhong, J., Miguel, T., Paszkowski, U., Zhang, S., Colbert, M., Sun, W.L., Chen, L., Cooper, B., Park, S., Wood, T.C., Mao, L., Quail, P., Wing, R., Dean, R., Yu, Y., Zharkikh, A., Shen, R., Sahasrabudhe, S., Thomas, A., Cannings, R., Gutin, A., Pruss, D., Reid, J., Tavtigian, S., Mitchell, J., Eldredge, G., Scholl, T., Miller, R.M., Bhatnagar, S., Adey, N., Rubano, T., Tusneem, N., Robinson, R., Feldhaus, J., Macalma, T., Oliphant, A. & Briggs, S. 2002. A draft sequence of the rice genome (Oryza sativa L. ssp. japonica). *Science 296*, 92-100.

Goffeau, A., Barrell, B.G., Bussey, H., Davis, R.W., Dujon, B., Feldmann, H., Galibert, F., Hoheisel, J.D., Jacq, C., Johnston, M., Louis, E.J., Mewes, H.W., Murakami, Y., Philippsen, P., Tettelin, H. & Oliver, S.G. 1996. Life with 6000 genes. *Science 274*, 546, 563-7.

Gonzalez-Baro, M.R., Granger, D.A. & Coleman, R.A. 2001. Mitochondrial glycerol phosphate acyltransferase contains two transmembrane domains with the active site in the N-terminal domain facing the cytosol. *J Biol Chem 276*, 43182-8.

Graca, J., Schreiber, L., Rodrigues, J. & Pereira, H. 2002. Glycerol and glyceryl esters of omega-hydroxyacids in cutins. *Phytochemistry 61*, 205-15.

Green, P.R., Merrill, A.H., Jr. & Bell, R.M. 1981. Membrane phospholipid synthesis in Escherichia coli. Purification, reconstitution, and characterization of sn-glycerol-3-phosphate acyltransferase. *J Biol Chem 256*, 11151-9.

Griac, P., Swede, M.J. & Henry, S.A. 1996. The role of phosphatidylcholine biosynthesis in the regulation of the INO1 gene of yeast. *J Biol Chem 271*, 25692-8.

Groot, P.H., Scholte, H.R. & Hulsmann, W.C. 1976. Fatty acid activation: specificity, localization, and function. *Adv Lipid Res 14*, 75-126.

Gu, Z., Valianpour, F., Chen, S., Vaz, F.M., Hakkaart, G.A., Wanders, R.J. & Greenberg, M.L. 2004. Aberrant cardiolipin metabolism in the yeast taz1 mutant: a model for Barth syndrome. *Mol Microbiol 51*, 149-58.

Haas, B.J., Wortman, J.R., Ronning, C.M., Hannick, L.I., Smith, R.K., Jr., Maiti, R., Chan, A.P., Yu, C., Farzad, M., Wu, D., White, O. & Town, C.D. 2005. Complete reannotation of the Arabidopsis genome: methods, tools, protocols and the final release. *BMC Biol 3*, 7.

Hajra, A.K. & Bishop, J.E. 1982. Glycerolipid biosynthesis in peroxisomes via the acyl dihydroxyacetone phosphate pathway. *Ann N Y Acad Sci 386*, 170-82.

Hatch, G.M. 2004. Cell biology of cardiac mitochondrial phospholipids. *Biochem Cell Biol 82*, 99-112.

Hauff, K.D. & Hatch, G.M. 2006. Cardiolipin metabolism and Barth Syndrome. *Prog Lipid Res 45*, 91-101.

Holt, R.A., Subramanian, G.M., Halpern, A., Sutton, G.G., Charlab, R., Nusskern, D.R., Wincker, P., Clark, A.G., Ribeiro, J.M., Wides, R., Salzberg, S.L., Loftus, B., Yandell, M., Majoros, W.H., Rusch, D.B., Lai, Z., Kraft, C.L., Abril, J.F., Anthouard, V., Arensburger, P., Atkinson, P.W., Baden, H., de Berardinis, V., Baldwin, D., Benes, V., Biedler, J., Blass, C., Bolanos, R., Boscus, D., Barnstead, M., Cai, S., Center, A., Chaturverdi, K., Christophides, G.K., Chrystal, M.A., Clamp, M., Cravchik, A., Curwen, V., Dana, A., Delcher, A., Dew, I., Evans, C.A., Flanigan, M., Grundschober-Freimoser, A., Friedli, L., Gu, Z., Guan, P., Guigo, R., Hillenmeyer, M.E., Hladun, S.L., Hogan, J.R., Hong, Y.S., Hoover, J., Jaillon, O., Ke, Z., Kodira, C., Kokoza, E., Koutsos, A., Letunic, I., Levitsky, A., Liang, Y., Lin, J.J., Lobo, N.F., Lopez, J.R., Malek, J.A., McIntosh, T.C., Meister, S., Miller, J., Mobarry, C., Mongin, E., Murphy, S.D., O'Brochta, D.A., Pfannkoch, C., Qi, R., Regier, M.A., Remington, K., Shao, H., Sharakhova, M.V., Sitter, C.D., Shetty, J., Smith, T.J., Strong, R., Sun, J., Thomasova, D., Ton, L.Q., Topalis, P., Tu, Z., Unger, M.F., Walenz, B., Wang, A., Wang, J., Wang, M., Wang, X., Woodford, K.J., Wortman, J.R., Wu, M., Yao, A., Zdobnov, E.M., Zhang, H., Zhao, Q., Zhao, S., Zhu, S.C., Zhimulev, I., Coluzzi, M., della Torre, A., Roth, C.W., Louis, C., Kalush, F., Mural, R.J., Myers, E.W., Adams, M.D., Smith, H.O., Broder, S., Gardner, M.J., Fraser, C.M., Birney, E., Bork, P., Brey, P.T., Venter, J.C., Weissenbach, J., Kafatos, F.C., Collins, F.H. & Hoffman, S.L. 2002. The genome sequence of the malaria mosquito Anopheles gambiae. *Science 298*, 129-49.

Igal, R.A. & Coleman, R.A. 1996. Acylglycerol recycling from triacylglycerol to phospholipid, not lipase activity, is defective in neutral lipid storage disease fibroblasts. *J Biol Chem 271*, 16644-51.

Igal, R.A., Wang, P. & Coleman, R.A. 1997. Triacsin C blocks de novo synthesis of glycerolipids and cholesterol esters but not recycling of fatty acid into phospholipid: evidence for functionally separate pools of acyl-CoA. *Biochem J 324 ( Pt 2)*, 529-34.

Igal, R.A., Wang, S., Gonzalez-Baro, M. & Coleman, R.A. 2001. Mitochondrial glycerol phosphate acyltransferase directs the incorporation of exogenous fatty acids into triacylglycerol. *J Biol Chem 276*, 42205-12.

Ishizaki, O., Nishida, I., Agata, K., Eguchi, G. & Murata, N. 1988. Cloning and nucleotide sequence of cDNA for the plastid glycerol-3-phosphate acyltransferase from squash. *FEBS Lett 238*, 424-30.

Jenks, M.A., Tuttle, H.A., Eigenbrode, S.D. & Feldmann, K.A. 1995. Leaf Epicuticular Waxes of the Eceriferum Mutants in Arabidopsis. *Plant Physiol 108*, 369-377.

Jiang, C., Ting, A.T. & Seed, B. 1998. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature 391*, 82-6.

Jiang, F., Rizavi, H.S. & Greenberg, M.L. 1997. Cardiolipin is not essential for the growth of Saccharomyces cerevisiae on fermentable or non-fermentable carbon sources. *Mol Microbiol 26*, 481-91.

Johnston, J., Kelley, R.I., Feigenbaum, A., Cox, G.F., Iyer, G.S., Funanage, V.L. & Proujansky, R. 1997. Mutation characterization and genotype-phenotype correlation in Barth syndrome. *Am J Hum Genet 61*, 1053-8.

Jones, C.T. & Rolph, T.P. 1981. Metabolic events associated with the preparation of the fetus for independent life. *Ciba Found Symp 86*, 214-33.

Kalscheuer, R., Stoveken, T., Luftmann, H., Malkus, U., Reichelt, R. & Steinbuchel, A. 2006. Neutral Lipid Biosynthesis in Engineered Escherichia coli: Jojoba Oil-Like Wax Esters and Fatty Acid Butyl Esters. *Appl Environ Microbiol 72*, 1373-9.

Kang, M.J., Fujino, T., Sasano, H., Minekura, H., Yabuki, N., Nagura, H., Iijima, H. & Yamamoto, T.T. 1997. A novel arachidonate-preferring acyl-CoA synthetase is present in steroidogenic cells of the rat adrenal, ovary, and testis. *Proc Natl Acad Sci U S A 94*, 2880-4.

Kaphalia, B.S., Cai, P., Khan, M.F., Okorodudu, A.O. & Ansari, G.A. 2004. Fatty acid ethyl esters: markers of alcohol abuse and alcoholism. *Alcohol 34*, 151-8.

Kelly, D.P., Gordon, J.I., Alpers, R. & Strauss, A.W. 1989. The tissue-specific expression and developmental regulation of two nuclear genes encoding rat mitochondrial proteins. Medium chain acyl-CoA dehydrogenase and mitochondrial malate dehydrogenase. *J Biol Chem 264*, 18921-5.

Kennedy, E.P. 1961. Biosynthesis of complex lipids. *Fed Proc 20*, 934-40.

Kiewietdejonge, A., Pitts, M., Cabuhat, L., Sherman, C., Kladwang, W., Miramontes, G., Floresvillar, J., Chan, J. & Ramirez, R.M. 2006. Hypersaline stress induces the turnover of phosphatidylcholine and results in the synthesis of the renal osmoprotectant glycerophosphocholine in Saccharomyces cerevisiae. *FEMS Yeast Res 6*, 205-17.

Kim, J.H., Lewin, T.M. & Coleman, R.A. 2001. Expression and characterization of recombinant rat Acyl-CoA synthetases 1, 4, and 5. Selective inhibition by triacsin C and thiazolidinediones. *J Biol Chem 276*, 24667-73.

Klaipongpan, A., Bloxham, D.P. & Akhtar, M. 1977. Enhancement of the anti-anabolic response to adenosine 3':5'-cyclic monophosphate during development. The inhibition of hepatic protein synthesis. *Biochem J 168*, 271-5.

Knoll, L.J., Schall, O.F., Suzuki, I., Gokel, G.W. & Gordon, J.I. 1995. Comparison of the reactivity of tetradecenoic acids, a triacsin, and unsaturated oximes with four purified Saccharomyces cerevisiae fatty acid activation proteins. *J Biol Chem 270*, 20090-7.

Kolattukudy, P. 1987. *Lipid Derived Defence Polymers and Waxes and Their Role in Plant Microbe Interaction.* Academic. New York. 291-314 pp.

Kolattukudy, P.E. 1967. Mechanisms of synthesis of waxy esters in broccoli (Brassica oleracea). *Biochemistry 6*, 2705-17.

Kolattukudy, P.E. & Rogers, L. 1978. Biosynthesis of fatty alcohols, alkane-1,2-diols and wax esters in particulate preparations from the uropygial glands of white-crowned sparrows (Zonotrichia leucophrys). *Arch Biochem Biophys 191*, 244-58.

Kondo, S., Kondo, H., Nakane, S., Kodaka, T., Tokumura, A., Waku, K. & Sugiura, T. 1998. 2-Arachidonoylglycerol, an endogenous cannabinoid receptor agonist: identification as one of the major species of monoacylglycerols in various rat tissues, and evidence for its generation

through CA2+-dependent and -independent mechanisms. *FEBS Lett 429*, 152-6.

Kornberg, A. & Pricer, W.E., Jr. 1953. Enzymatic synthesis of the coenzyme A derivatives of long chain fatty acids. *J Biol Chem 204*, 329-43.

Koshkin, V. & Greenberg, M.L. 2002. Cardiolipin prevents rate-dependent uncoupling and provides osmotic stability in yeast mitochondria. *Biochem J 364*, 317-22.

Kunst, L. & Samuels, A.L. 2003. Biosynthesis and secretion of plant cuticular wax. *Prog Lipid Res 42*, 51-80.

Lander, E.S., Linton, L.M., Birren, B., Nusbaum, C., Zody, M.C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., FitzHugh, W., Funke, R., Gage, D., Harris, K., Heaford, A., Howland, J., Kann, L., Lehoczky, J., LeVine, R., McEwan, P., McKernan, K., Meldrim, J., Mesirov, J.P., Miranda, C., Morris, W., Naylor, J., Raymond, C., Rosetti, M., Santos, R., Sheridan, A., Sougnez, C., Stange-Thomann, N., Stojanovic, N., Subramanian, A., Wyman, D., Rogers, J., Sulston, J., Ainscough, R., Beck, S., Bentley, D., Burton, J., Clee, C., Carter, N., Coulson, A., Deadman, R., Deloukas, P., Dunham, A., Dunham, I., Durbin, R., French, L., Grafham, D., Gregory, S., Hubbard, T., Humphray, S., Hunt, A., Jones, M., Lloyd, C., McMurray, A., Matthews, L., Mercer, S., Milne, S., Mullikin, J.C., Mungall, A., Plumb, R., Ross, M., Shownkeen, R., Sims, S., Waterston, R.H., Wilson, R.K., Hillier, L.W., McPherson, J.D., Marra, M.A., Mardis, E.R., Fulton, L.A., Chinwalla, A.T., Pepin, K.H., Gish, W.R., Chissoe, S.L., Wendl, M.C., Delehaunty, K.D., Miner, T.L., Delehaunty, A., Kramer, J.B., Cook, L.L., Fulton, R.S., Johnson, D.L., Minx, P.J., Clifton, S.W., Hawkins, T., Branscomb, E., Predki, P., Richardson, P., Wenning, S., Slezak, T., Doggett, N., Cheng, J.F., Olsen, A., Lucas, S., Elkin, C., Uberbacher, E., Frazier, M., Gibbs, R.A., Muzny, D.M., Scherer, S.E., Bouck, J.B., Sodergren, E.J., Worley, K.C., Rives, C.M., Gorrell, J.H., Metzker, M.L., Naylor, S.L., Kucherlapati, R.S., Nelson, D.L., Weinstock, G.M., Sakaki, Y., Fujiyama, A., Hattori, M., Yada, T., Toyoda, A., Itoh, T., Kawagoe, C., Watanabe, H., Totoki, Y., Taylor, T., Weissenbach, J., Heilig, R., Saurin, W., Artiguenave, F., Brottier, P., Bruls, T., Pelletier, E., Robert, C., Wincker, P., Smith, D.R., Doucette-Stamm, L., Rubenfield, M., Weinstock, K., Lee, H.M., Dubois, J., Rosenthal, A., Platzer, M., Nyakatura, G., Taudien, S., Rump, A., Yang, H., Yu, J., Wang, J., Huang, G., Gu, J., Hood, L., Rowen, L., Madan, A., Qin, S., Davis, R.W., Federspiel, N.A., Abola, A.P., Proctor, M.J., Myers, R.M., Schmutz, J., Dickson, M., Grimwood, J., Cox, D.R., Olson, M.V., Kaul, R., Raymond, C., Shimizu, N., Kawasaki, K., Minoshima, S., Evans, G.A., Athanasiou, M., Schultz, R., Roe, B.A., Chen, F., Pan, H., Ramser, J., Lehrach, H., Reinhardt, R., McCombie, W.R., de la Bastide, M., Dedhia, N., Blocker, H., Hornischer, K., Nordsiek, G., Agarwala, R., Aravind, L., Bailey, J.A., Bateman, A., Batzoglou, S., Birney, E., Bork, P., Brown, D.G., Burge, C.B., Cerutti, L., Chen, H.C., Church, D., Clamp, M., Copley, R.R., Doerks, T., Eddy, S.R., Eichler, E.E., Furey, T.S., Galagan, J., Gilbert, J.G., Harmon, C., Hayashizaki, Y., Haussler, D., Hermjakob, H., Hokamp, K., Jang, W., Johnson, L.S., Jones, T.A., Kasif, S., Kaspryzk, A., Kennedy, S., Kent, W.J., Kitts, P., Koonin, E.V., Korf,

I., Kulp, D., Lancet, D., Lowe, T.M., McLysaght, A., Mikkelsen, T., Moran, J.V., Mulder, N., Pollara, V.J., Ponting, C.P., Schuler, G., Schultz, J., Slater, G., Smit, A.F., Stupka, E., Szustakowski, J., Thierry-Mieg, D., Thierry-Mieg, J., Wagner, L., Wallis, J., Wheeler, R., Williams, A., Wolf, Y.I., Wolfe, K.H., Yang, S.P., Yeh, R.F., Collins, F., Guyer, M.S., Peterson, J., Felsenfeld, A., Wetterstrand, K.A., Patrinos, A., Morgan, M.J., de Jong, P., Catanese, J.J., Osoegawa, K., Shizuya, H., Choi, S. & Chen, Y.J. 2001. Initial sequencing and analysis of the human genome. *Nature 409*, 860-921.

Laposata, M. 1998. Fatty acid ethyl esters: ethanol metabolites which mediate ethanol-induced organ damage and serve as markers of ethanol intake. *Prog Lipid Res 37*, 307-16.

Lewin, T.M., Kim, J.H., Granger, D.A., Vance, J.E. & Coleman, R.A. 2001. Acyl-CoA synthetase isoforms 1, 4, and 5 are present in different subcellular membranes in rat liver and can be inhibited independently. *J Biol Chem 276*, 24674-9.

Ma, B.J., Taylor, W.A., Dolinsky, V.W. & Hatch, G.M. 1999. Acylation of monolysocardiolipin in rat heart. *J Lipid Res 40*, 1837-45.

Madsen, O., Scally, M., Douady, C.J., Kao, D.J., DeBry, R.W., Adkins, R., Amrine, H.M., Stanhope, M.J., de Jong, W.W. & Springer, M.S. 2001. Parallel adaptive radiations in two major clades of placental mammals. *Nature 409*, 610-4.

Mashek, D.G., Bornfeldt, K.E., Coleman, R.A., Berger, J., Bernlohr, D.A., Black, P., DiRusso, C.C., Farber, S.A., Guo, W., Hashimoto, N., Khodiyar, V., Kuypers, F.A., Maltais, L.J., Nebert, D.W., Renieri, A., Schaffer, J.E., Stahl, A., Watkins, P.A., Vasiliou, V. & Yamamoto, T.T. 2004. Revised nomenclature for the mammalian long-chain acyl-CoA synthetase gene family. *J Lipid Res 45*, 1958-61.

Mason, A.B. & Dufour, J.P. 2000. Alcohol acetyltransferases and the significance of ester synthesis in yeast. *Yeast 16*, 1287-98.

Mayor, F. & Cuezva, J.M. 1985. Hormonal and metabolic changes in the perinatal period. *Biol Neonate 48*, 185-96.

McMaster, C.R., Jackson, T.R. 2004. *Lipid Metabolism and Membrane Biogenesis*. Springer-Verlag. Berlin. 5-88 pp.

McPhail, L.C., Waite, K.A., Regier, D.S., Nixon, J.B., Qualliotine-Mann, D., Zhang, W.X., Wallin, R. & Sergeant, S. 1999. A novel protein kinase target for the lipid second messenger phosphatidic acid. *Biochim Biophys Acta 1439*, 277-90.

Merkel, O., Oskolkova, O.V., Raab, F., El-Toukhy, R. & Paltauf, F. 2005. Regulation of activity in vitro and in vivo of three phospholipases B from Saccharomyces cerevisiae. *Biochem J 387*, 489-96.

Metz, J.G., Pollard, M.R., Anderson, L., Hayes, T.R. & Lassner, M.W. 2000. Purification of a jojoba embryo fatty acyl-coenzyme A reductase and expression of its cDNA in high erucic acid rapeseed. *Plant Physiol 122*, 635-44.

Munnik, T. 2001. Phosphatidic acid: an emerging plant lipid second messenger. *Trends Plant Sci 6*, 227-33.

Muoio, D.M., Lewin, T.M., Wiedmer, P. & Coleman, R.A. 2000. Acyl-CoAs are functionally channeled in liver: potential role of acyl-CoA synthetase. *Am J Physiol Endocrinol Metab 279*, E1366-73.

Murata, N. & Tasaka, Y. 1997. Glycerol-3-phosphate acyltransferase in plants. *Biochim Biophys Acta 1348*, 10-6.

Murphy, W.J., Eizirik, E., Johnson, W.E., Zhang, Y.P., Ryder, O.A. & O'Brien, S.J. 2001. Molecular phylogenetics and the origins of placental mammals. *Nature 409*, 614-8.

Nagao, M., Parimoo, B. & Tanaka, K. 1993. Developmental, nutritional, and hormonal regulation of tissue-specific expression of the genes encoding various acyl-CoA dehydrogenases and alpha-subunit of electron transfer flavoprotein in rat. *J Biol Chem 268*, 24114-24.

Nakane, S., Tanaka, T., Satouchi, K., Kobayashi, Y., Waku, K. & Sugiura, T. 2000. Occurrence of a novel cannabimimetic molecule 2-sciadonoylglycerol (2-eicosa-5',11',14'-trienoylglycerol) in the umbrella pine Sciadopitys verticillata seeds. *Biol Pharm Bull 23*, 758-61.

Neustein, H.B., Lurie, P.R., Dahms, B. & Takahashi, M. 1979. An X-linked recessive cardiomyopathy with abnormal mitochondria. *Pediatrics 64*, 24-9.

Neuwald, A.F. 1997. Barth syndrome may be due to an acyltransferase deficiency. *Curr Biol 7*, R465-6.

Ohlrogge, J. & Browse, J. 1995. Lipid biosynthesis. *Plant Cell 7*, 957-70.

Oikawa, E., Iijima, H., Suzuki, T., Sasano, H., Sato, H., Kamataki, A., Nagura, H., Kang, M.J., Fujino, T., Suzuki, H. & Yamamoto, T.T. 1998. A novel acyl-CoA synthetase, ACS5, expressed in intestinal epithelial cells and proliferating preadipocytes. *J Biochem (Tokyo) 124*, 679-85.

Okumura, S., Iwai, M. & Tsujisaka, Y. 1979. Synthesis of various kinds of esters by four microbial lipases. *Biochim Biophys Acta 575*, 156-65.

Olukoshi, E.R. & Packter, N.M. 1994. Importance of stored triacylglycerols in Streptomyces: possible carbon source for antibiotics. *Microbiology 140 ( Pt 4)*, 931-43.

Palsdottir, H. & Hunte, C. 2004. Lipids in membrane protein structures. *Biochim Biophys Acta 1666*, 2-18.

Paltauf, F., Kohlwein, SD, Henry, SA 1992. *The Molecular and Cellular Biology of the Yeast Saccharomycese: Gene Expression* Cold Springs Harbor Press. Cold Springs Harbor, NY. 415-500 pp.

Patton-Vogt, J. 2006. Transport and metabolism of glycerophosphodiesters produced through phospholipid deacylation. *Biochim Biophys Acta*

Patton, J.L., Pessoa-Brandao, L. & Henry, S.A. 1995. Production and reutilization of an extracellular phosphatidylinositol catabolite, glycerophosphoinositol, by Saccharomyces cerevisiae. *J Bacteriol 177*, 3379-85.

Picard, F. & Auwerx, J. 2002. PPAR(gamma) and glucose homeostasis. *Annu Rev Nutr 22*, 167-97.

Prentki, M. & Corkey, B.E. 1996. Are the beta-cell signaling molecules malonyl-CoA and cystolic long-chain acyl-CoA implicated in multiple tissue defects of obesity and NIDDM? *Diabetes 45*, 273-83.

Rashotte, A.M., Jenks, M.A., Ross, A.S. & Feldmann, K.A. 2004. Novel eceriferum mutants in Arabidopsis thaliana. *Planta 219*, 5-13.

Rhee, Y., Hlousek-Radojcic, A., Ponsamuel, J., Liu, D. & Post-Beittenmiller, D. 1998. Epicuticular wax accumulation and fatty acid elongation activities are induced during leaf development of leeks. *Plant Physiol 116*, 901-11.

Roberts, D.C., Huff, M.W. & Carroll, K.K. 1979. Influence of diet on plasma cholesterol concentrations in suckling and weanling rabbits. *Nutr Metab 23*, 476-86.

Rothstein, R.J. 1983. One-step gene disruption in yeast. *Methods Enzymol 101*, 202-11.

Rudkowska, I., Roynette, C.E., Demonty, I., Vanstone, C.A., Jew, S. & Jones, P.J. 2005. Diacylglycerol: efficacy and mechanism of action of an anti-obesity agent. *Obes Res 13*, 1864-76.

Rusinol, A.E., Cui, Z., Chen, M.H. & Vance, J.E. 1994. A unique mitochondria-associated membrane fraction from rat liver has a high capacity for lipid synthesis and contains pre-Golgi secretory proteins including nascent lipoproteins. *J Biol Chem 269*, 27494-502.

Rustow, B. & Kunze, D. 1985. Diacylglycerol synthesized in vitro from sn-glycerol 3-phosphate and the endogenous diacylglycerol are different substrate pools for the biosynthesis of phosphatidylcholine in rat lung microsomes. *Biochim Biophys Acta 835*, 273-8.

Saerens, S.M., Verstrepen, K.J., Van Laere, S.D., Voet, A.R., Van Dijck, P., Delvaux, F.R. & Thevelein, J.M. 2006. The Saccharomyces cerevisiae EHT1 and EEB1 Genes Encode Novel Enzymes with Medium-chain Fatty Acid Ethyl Ester Synthesis and Hydrolysis Capacity. *J Biol Chem 281*, 4446-56.

Sandager, L., Gustavsson, M.H., Stahl, U., Dahlqvist, A., Wiberg, E., Banas, A., Lenman, M., Ronne, H. & Stymne, S. 2002. Storage lipid synthesis is non-essential in yeast. *J Biol Chem 277*, 6478-82.

Schlame, M., Rua, D. & Greenberg, M.L. 2000. The biosynthesis and functional role of cardiolipin. *Prog Lipid Res 39*, 257-88.

Schlame, M., Towbin, J.A., Heerdt, P.M., Jehle, R., DiMauro, S. & Blanck, T.J. 2002. Deficiency of tetralinoleoyl-cardiolipin in Barth syndrome. *Ann Neurol 51*, 634-7.

Schlossman, D.M. & Bell, R.M. 1978. Glycerolipid biosynthesis in Saccharomyces cerevisiae: sn-glycerol-3-phosphate and dihydroxyacetone phosphate acyltransferase activities. *J Bacteriol 133*, 1368-76.

Schnurr, J., Shockey, J. & Browse, J. 2004. The acyl-CoA synthetase encoded by LACS2 is essential for normal cuticle development in Arabidopsis. *Plant Cell 16*, 629-42.

Schnurr, J.A., Shockey, J.M., de Boer, G.J. & Browse, J.A. 2002. Fatty acid export from the chloroplast. Molecular characterization of a major plastidial acyl-coenzyme A synthetase from Arabidopsis. *Plant Physiol 129*, 1700-9.

Schweizer, E. & Hofmann, J. 2004. Microbial type I fatty acid synthases (FAS): major players in a network of cellular FAS systems. *Microbiol Mol Biol Rev 68*, 501-17.

Shimakata, T. & Stumpf, P.K. 1984. [Fatty acid synthetase of higher plants]. *Tanpakushitsu Kakusan Koso 29*, 249-65.

Shirayama, Y., Yano, T., Takahashi, K., Takahashi, S. & Ogino, T. 2004. In vivo 31P NMR spectroscopy shows an increase in glycerophosphorylcholine

concentration without alterations in mitochondrial function in the prefrontal cortex of medicated schizophrenic patients at rest. *Eur J Neurosci 20*, 749-56.

Sieber, P., Schorderet, M., Ryser, U., Buchala, A., Kolattukudy, P., Metraux, J.P. & Nawrath, C. 2000. Transgenic Arabidopsis plants expressing a fungal cutinase show alterations in the structure and properties of the cuticle and postgenital organ fusions. *Plant Cell 12*, 721-38.

Singer, D. 1999. Neonatal tolerance to hypoxia: a comparative-physiological approach. *Comp Biochem Physiol A Mol Integr Physiol 123*, 221-34.

Singer, S.J. & Nicolson, G.L. 1972. The fluid mosaic model of the structure of cell membranes. *Science 175*, 720-31.

Smith, S., Watts, R. & Dils, R. 1968. Quantitative gas-liquid chromatographic analysis of rodent milk triglycerides. *J Lipid Res 9*, 52-7.

Sperka-Gottlieb, C.D., Hermetter, A., Paltauf, F. & Daum, G. 1988. Lipid topology and physical properties of the outer mitochondrial membrane of the yeast, Saccharomyces cerevisiae. *Biochim Biophys Acta 946*, 227-34.

Stahl, U., Carlsson, A.S., Lenman, M., Dahlqvist, A., Huang, B., Banas, W., Banas, A. & Stymne, S. 2004. Cloning and functional characterization of a phospholipid:diacylglycerol acyltransferase from Arabidopsis. *Plant Physiol 135*, 1324-35.

Steinmetz, M., Quentin, T., Poppe, A., Paul, T. & Jux, C. 2005. Changes in expression levels of genes involved in fatty acid metabolism: upregulation of all three members of the PPAR family (alpha, gamma, delta) and the newly described adiponectin receptor 2, but not adiponectin receptor 1 during neonatal cardiac development of the rat. *Basic Res Cardiol 100*, 263-9.

Stoveken, T., Kalscheuer, R., Malkus, U., Reichelt, R. & Steinbuchel, A. 2005. The wax ester synthase/acyl coenzyme A:diacylglycerol acyltransferase from Acinetobacter sp. strain ADP1: characterization of a novel type of acyltransferase. *J Bacteriol 187*, 1369-76.

Testerink, C., Dekker, H.L., Lim, Z.Y., Johns, M.K., Holmes, A.B., Koster, C.G., Ktistakis, N.T. & Munnik, T. 2004. Isolation and identification of phosphatidic acid targets from plants. *Plant J 39*, 527-36.

Testet, E., Bessoule, J.J., Mongrand, S., Guillot-Salomon, T., Cantrel, C. & Cassagne, C. 1996. Occurrence of an acyl-CoA:1-acylglycerophosphorylcholine acyltransferase in plant mitochondria. *FEBS Lett 399*, 87-91.

Thai, T.P., Heid, H., Rackwitz, H.R., Hunziker, A., Gorgas, K. & Just, W.W. 1997. Ether lipid biosynthesis: isolation and molecular characterization of human dihydroxyacetonephosphate acyltransferase. *FEBS Lett 420*, 205-11.

Thelen, J.J. & Ohlrogge, J.B. 2002. Metabolic engineering of fatty acid biosynthesis in plants. *Metab Eng 4*, 12-21.

Thurston, J.H. & McDougal, D.B., Jr. 1969. Effect of ischemia on metabolism of the brain of the newborn mouse. *Am J Physiol 216*, 348-52.

Tuller, G., Hrastnik, C., Achleitner, G., Schiefthaler, U., Klein, F. & Daum, G. 1998. YDL142c encodes cardiolipin synthase (Cls1p) and is non-essential for aerobic growth of Saccharomyces cerevisiae. *FEBS Lett 421*, 15-8.

Wakefield, L., Cornish, V., Broackes-Carter, F. & Sim, E. 2005. Arylamine N-acetyltransferase 2 expression in the developing heart. *J Histochem Cytochem 53*, 583-92.

Valianpour, F., Wanders, R.J., Overmars, H., Vaz, F.M., Barth, P.G. & van Gennip, A.H. 2003. Linoleic acid supplementation of Barth syndrome fibroblasts restores cardiolipin levels: implications for treatment. *J Lipid Res 44*, 560-6.

van Bilsen, M., Smeets, P.J., Gilde, A.J. & van der Vusse, G.J. 2004. Metabolic remodelling of the failing heart: the cardiac burn-out syndrome? *Cardiovasc Res 61*, 218-26.

Van Horn, C.G., Caviglia, J.M., Li, L.O., Wang, S., Granger, D.A. & Coleman, R.A. 2005. Characterization of recombinant long-chain rat acyl-CoA synthetase isoforms 3 and 6: identification of a novel variant of isoform 6. *Biochemistry 44*, 1635-42.

Waterston, R.H., Lindblad-Toh, K., Birney, E., Rogers, J., Abril, J.F., Agarwal, P., Agarwala, R., Ainscough, R., Alexandersson, M., An, P., Antonarakis, S.E., Attwood, J., Baertsch, R., Bailey, J., Barlow, K., Beck, S., Berry, E., Birren, B., Bloom, T., Bork, P., Botcherby, M., Bray, N., Brent, M.R., Brown, D.G., Brown, S.D., Bult, C., Burton, J., Butler, J., Campbell, R.D., Carninci, P., Cawley, S., Chiaromonte, F., Chinwalla, A.T., Church, D.M., Clamp, M., Clee, C., Collins, F.S., Cook, L.L., Copley, R.R., Coulson, A., Couronne, O., Cuff, J., Curwen, V., Cutts, T., Daly, M., David, R., Davies, J., Delehaunty, K.D., Deri, J., Dermitzakis, E.T., Dewey, C., Dickens, N.J., Diekhans, M., Dodge, S., Dubchak, I., Dunn, D.M., Eddy, S.R., Elnitski, L., Emes, R.D., Eswara, P., Eyras, E., Felsenfeld, A., Fewell, G.A., Flicek, P., Foley, K., Frankel, W.N., Fulton, L.A., Fulton, R.S., Furey, T.S., Gage, D., Gibbs, R.A., Glusman, G., Gnerre, S., Goldman, N., Goodstadt, L., Grafham, D., Graves, T.A., Green, E.D., Gregory, S., Guigo, R., Guyer, M., Hardison, R.C., Haussler, D., Hayashizaki, Y., Hillier, L.W., Hinrichs, A., Hlavina, W., Holzer, T., Hsu, F., Hua, A., Hubbard, T., Hunt, A., Jackson, I., Jaffe, D.B., Johnson, L.S., Jones, M., Jones, T.A., Joy, A., Kamal, M., Karlsson, E.K., Karolchik, D., Kasprzyk, A., Kawai, J., Keibler, E., Kells, C., Kent, W.J., Kirby, A., Kolbe, D.L., Korf, I., Kucherlapati, R.S., Kulbokas, E.J., Kulp, D., Landers, T., Leger, J.P., Leonard, S., Letunic, I., Levine, R., Li, J., Li, M., Lloyd, C., Lucas, S., Ma, B., Maglott, D.R., Mardis, E.R., Matthews, L., Mauceli, E., Mayer, J.H., McCarthy, M., McCombie, W.R., McLaren, S., McLay, K., McPherson, J.D., Meldrim, J., Meredith, B., Mesirov, J.P., Miller, W., Miner, T.L., Mongin, E., Montgomery, K.T., Morgan, M., Mott, R., Mullikin, J.C., Muzny, D.M., Nash, W.E., Nelson, J.O., Nhan, M.N., Nicol, R., Ning, Z., Nusbaum, C., O'Connor, M.J., Okazaki, Y., Oliver, K., Overton-Larty, E., Pachter, L., Parra, G., Pepin, K.H., Peterson, J., Pevzner, P., Plumb, R., Pohl, C.S., Poliakov, A., Ponce, T.C., Ponting, C.P., Potter, S., Quail, M., Reymond, A., Roe, B.A., Roskin, K.M., Rubin, E.M., Rust, A.G., Santos, R., Sapojnikov, V., Schultz, B., Schultz, J., Schwartz, M.S., Schwartz, S., Scott, C., Seaman, S., Searle, S., Sharpe, T., Sheridan, A., Shownkeen, R., Sims, S., Singer, J.B., Slater, G., Smit, A., Smith, D.R., Spencer, B., Stabenau, A., Stange-Thomann, N., Sugnet, C., Suyama, M., Tesler, G., Thompson, J.,

Torrents, D., Trevaskis, E., Tromp, J., Ucla, C., Ureta-Vidal, A., Vinson, J.P., Von Niederhausern, A.C., Wade, C.M., Wall, M., Weber, R.J., Weiss, R.B., Wendl, M.C., West, A.P., Wetterstrand, K., Wheeler, R., Whelan, S., Wierzbowski, J., Willey, D., Williams, S., Wilson, R.K., Winter, E., Worley, K.C., Wyman, D., Yang, S., Yang, S.P., Zdobnov, E.M., Zody, M.C. & Lander, E.S. 2002. Initial sequencing and comparative analysis of the mouse genome. *Nature 420*, 520-62.

Vaz, F.M., Houtkooper, R.H., Valianpour, F., Barth, P.G. & Wanders, R.J. 2003. Only one splice variant of the human TAZ gene encodes a functional protein with a role in cardiolipin metabolism. *J Biol Chem 278*, 43089-94.

Venter, J.C., Adams, M.D., Myers, E.W., Li, P.W., Mural, R.J., Sutton, G.G., Smith, H.O., Yandell, M., Evans, C.A., Holt, R.A., Gocayne, J.D., Amanatides, P., Ballew, R.M., Huson, D.H., Wortman, J.R., Zhang, Q., Kodira, C.D., Zheng, X.H., Chen, L., Skupski, M., Subramanian, G., Thomas, P.D., Zhang, J., Gabor Miklos, G.L., Nelson, C., Broder, S., Clark, A.G., Nadeau, J., McKusick, V.A., Zinder, N., Levine, A.J., Roberts, R.J., Simon, M., Slayman, C., Hunkapiller, M., Bolanos, R., Delcher, A., Dew, I., Fasulo, D., Flanigan, M., Florea, L., Halpern, A., Hannenhalli, S., Kravitz, S., Levy, S., Mobarry, C., Reinert, K., Remington, K., Abu-Threideh, J., Beasley, E., Biddick, K., Bonazzi, V., Brandon, R., Cargill, M., Chandramouliswaran, I., Charlab, R., Chaturvedi, K., Deng, Z., Di Francesco, V., Dunn, P., Eilbeck, K., Evangelista, C., Gabrielian, A.E., Gan, W., Ge, W., Gong, F., Gu, Z., Guan, P., Heiman, T.J., Higgins, M.E., Ji, R.R., Ke, Z., Ketchum, K.A., Lai, Z., Lei, Y., Li, Z., Li, J., Liang, Y., Lin, X., Lu, F., Merkulov, G.V., Milshina, N., Moore, H.M., Naik, A.K., Narayan, V.A., Neelam, B., Nusskern, D., Rusch, D.B., Salzberg, S., Shao, W., Shue, B., Sun, J., Wang, Z., Wang, A., Wang, X., Wang, J., Wei, M., Wides, R., Xiao, C., Yan, C., Yao, A., Ye, J., Zhan, M., Zhang, W., Zhang, H., Zhao, Q., Zheng, L., Zhong, F., Zhong, W., Zhu, S., Zhao, S., Gilbert, D., Baumhueter, S., Spier, G., Carter, C., Cravchik, A., Woodage, T., Ali, F., An, H., Awe, A., Baldwin, D., Baden, H., Barnstead, M., Barrow, I., Beeson, K., Busam, D., Carver, A., Center, A., Cheng, M.L., Curry, L., Danaher, S., Davenport, L., Desilets, R., Dietz, S., Dodson, K., Doup, L., Ferriera, S., Garg, N., Gluecksmann, A., Hart, B., Haynes, J., Haynes, C., Heiner, C., Hladun, S., Hostin, D., Houck, J., Howland, T., Ibegwam, C., Johnson, J., Kalush, F., Kline, L., Koduru, S., Love, A., Mann, F., May, D., McCawley, S., McIntosh, T., McMullen, I., Moy, M., Moy, L., Murphy, B., Nelson, K., Pfannkoch, C., Pratts, E., Puri, V., Qureshi, H., Reardon, M., Rodriguez, R., Rogers, Y.H., Romblad, D., Ruhfel, B., Scott, R., Sitter, C., Smallwood, M., Stewart, E., Strong, R., Suh, E., Thomas, R., Tint, N.N., Tse, S., Vech, C., Wang, G., Wetter, J., Williams, S., Williams, M., Windsor, S., Winn-Deen, E., Wolfe, K., Zaveri, J., Zaveri, K., Abril, J.F., Guigo, R., Campbell, M.J., Sjolander, K.V., Karlak, B., Kejariwal, A., Mi, H., Lazareva, B., Hatton, T., Narechania, A., Diemer, K., Muruganujan, A., Guo, N., Sato, S., Bafna, V., Istrail, S., Lippert, R., Schwartz, R., Walenz, B., Yooseph, S., Allen, D., Basu, A., Baxendale, J., Blick, L., Caminha, M., Carnes-Stine, J., Caulk, P., Chiang, Y.H., Coyne, M., Dahlke, C., Mays, A., Dombroski, M., Donnelly, M.,

Ely, D., Esparham, S., Fosler, C., Gire, H., Glanowski, S., Glasser, K., Glodek, A., Gorokhov, M., Graham, K., Gropman, B., Harris, M., Heil, J., Henderson, S., Hoover, J., Jennings, D., Jordan, C., Jordan, J., Kasha, J., Kagan, L., Kraft, C., Levitsky, A., Lewis, M., Liu, X., Lopez, J., Ma, D., Majoros, W., McDaniel, J., Murphy, S., Newman, M., Nguyen, T., Nguyen, N., Nodell, M., Pan, S., Peck, J., Peterson, M., Rowe, W., Sanders, R., Scott, J., Simpson, M., Smith, T., Sprague, A., Stockwell, T., Turner, R., Venter, E., Wang, M., Wen, M., Wu, D., Wu, M., Xia, A., Zandieh, A. & Zhu, X. 2001. The sequence of the human genome. *Science 291*, 1304-51.

Werner, J.C., Whitman, V., Fripp, R.R., Schuler, H.G., Musselman, J. & Sham, R.L. 1983. Fatty acid and glucose utilization in isolated, working fetal pig hearts. *Am J Physiol 245*, E19-23.

Wilson, G.N., Holmes, R.G., Custer, J., Lipkowitz, J.L., Stover, J., Datta, N. & Hajra, A. 1986. Zellweger syndrome: diagnostic assays, syndrome delineation, and potential therapy. *Am J Med Genet 24*, 69-82.

Voelker, T. & Kinney, A.J. 2001. Variations In The Biosynthesis Of Seed-Storage Lipids. *Annu Rev Plant Physiol Plant Mol Biol 52*, 335-361.

von Wettstein-Knowles, P. 1979. *Advances in the Biochemistry and Physiology of Plant Lipids*. Elseviver/North Holland Biomedical Press. Amsterdam. 1-26 pp.

von Wettstein-Knowles, P. 1986. Role of cer-cqu in epicuticular wax biosynthesis. *Biochem Soc Trans 14*, 576-9.

Wood, V., Gwilliam, R., Rajandream, M.A., Lyne, M., Lyne, R., Stewart, A., Sgouros, J., Peat, N., Hayles, J., Baker, S., Basham, D., Bowman, S., Brooks, K., Brown, D., Brown, S., Chillingworth, T., Churcher, C., Collins, M., Connor, R., Cronin, A., Davis, P., Feltwell, T., Fraser, A., Gentles, S., Goble, A., Hamlin, N., Harris, D., Hidalgo, J., Hodgson, G., Holroyd, S., Hornsby, T., Howarth, S., Huckle, E.J., Hunt, S., Jagels, K., James, K., Jones, L., Jones, M., Leather, S., McDonald, S., McLean, J., Mooney, P., Moule, S., Mungall, K., Murphy, L., Niblett, D., Odell, C., Oliver, K., O'Neil, S., Pearson, D., Quail, M.A., Rabbinowitsch, E., Rutherford, K., Rutter, S., Saunders, D., Seeger, K., Sharp, S., Skelton, J., Simmonds, M., Squares, R., Squares, S., Stevens, K., Taylor, K., Taylor, R.G., Tivey, A., Walsh, S., Warren, T., Whitehead, S., Woodward, J., Volckaert, G., Aert, R., Robben, J., Grymonprez, B., Weltjens, I., Vanstreels, E., Rieger, M., Schafer, M., Muller-Auer, S., Gabel, C., Fuchs, M., Dusterhoft, A., Fritzc, C., Holzer, E., Moestl, D., Hilbert, H., Borzym, K., Langer, I., Beck, A., Lehrach, H., Reinhardt, R., Pohl, T.M., Eger, P., Zimmermann, W., Wedler, H., Wambutt, R., Purnelle, B., Goffeau, A., Cadieu, E., Dreano, S., Gloux, S., Lelaure, V., Mottier, S., Galibert, F., Aves, S.J., Xiang, Z., Hunt, C., Moore, K., Hurst, S.M., Lucas, M., Rochet, M., Gaillardin, C., Tallada, V.A., Garzon, A., Thode, G., Daga, R.R., Cruzado, L., Jimenez, J., Sanchez, M., del Rey, F., Benito, J., Dominguez, A., Revuelta, J.L., Moreno, S., Armstrong, J., Forsburg, S.L., Cerutti, L., Lowe, T., McCombie, W.R., Paulsen, I., Potashkin, J., Shpakovski, G.V., Ussery, D., Barrell, B.G. & Nurse, P. 2002. The genome sequence of Schizosaccharomyces pombe. *Nature 415*, 871-80.

Vreken, P., Valianpour, F., Nijtmans, L.G., Grivell, L.A., Plecko, B., Wanders, R.J. & Barth, P.G. 2000. Defective remodeling of cardiolipin and phosphatidylglycerol in Barth syndrome. *Biochem Biophys Res Commun 279*, 378-82.

Xu, Y., Kelley, R.I., Blanck, T.J. & Schlame, M. 2003. Remodeling of cardiolipin by phospholipid transacylation. *J Biol Chem 278*, 51380-5.

Ye, X., Ishii, I., Kingsbury, M.A. & Chun, J. 2002. Lysophosphatidic acid as a novel cell survival/apoptotic factor. *Biochim Biophys Acta 1585*, 108-13.

Yet, S.F., Lee, S., Hahm, Y.T. & Sul, H.S. 1993. Expression and identification of p90 as the murine mitochondrial glycerol-3-phosphate acyltransferase. *Biochemistry 32*, 9486-91.

Yu, J., Hu, S., Wang, J., Wong, G.K., Li, S., Liu, B., Deng, Y., Dai, L., Zhou, Y., Zhang, X., Cao, M., Liu, J., Sun, J., Tang, J., Chen, Y., Huang, X., Lin, W., Ye, C., Tong, W., Cong, L., Geng, J., Han, Y., Li, L., Li, W., Hu, G., Huang, X., Li, W., Li, J., Liu, Z., Li, L., Liu, J., Qi, Q., Liu, J., Li, L., Li, T., Wang, X., Lu, H., Wu, T., Zhu, M., Ni, P., Han, H., Dong, W., Ren, X., Feng, X., Cui, P., Li, X., Wang, H., Xu, X., Zhai, W., Xu, Z., Zhang, J., He, S., Zhang, J., Xu, J., Zhang, K., Zheng, X., Dong, J., Zeng, W., Tao, L., Ye, J., Tan, J., Ren, X., Chen, X., He, J., Liu, D., Tian, W., Tian, C., Xia, H., Bao, Q., Li, G., Gao, H., Cao, T., Wang, J., Zhao, W., Li, P., Chen, W., Wang, X., Zhang, Y., Hu, J., Wang, J., Liu, S., Yang, J., Zhang, G., Xiong, Y., Li, Z., Mao, L., Zhou, C., Zhu, Z., Chen, R., Hao, B., Zheng, W., Chen, S., Guo, W., Li, G., Liu, S., Tao, M., Wang, J., Zhu, L., Yuan, L. & Yang, H. 2002. A draft sequence of the rice genome (Oryza sativa L. ssp. indica). *Science 296*, 79-92.

Zaccheo, O., Dinsdale, D., Meacock, P.A. & Glynn, P. 2004. Neuropathy target esterase and its yeast homologue degrade phosphatidylcholine to glycerophosphocholine in living cells. *J Biol Chem 279*, 24024-33.

Zaremberg, V. & McMaster, C.R. 2002. Differential partitioning of lipids metabolized by separate yeast glycerol-3-phosphate acyltransferases reveals that phospholipase D generation of phosphatidic acid mediates sensitivity to choline-containing lysolipids and drugs. *J Biol Chem 277*, 39035-44.

Zheng, Z., Xia, Q., Dauk, M., Shen, W., Selvaraj, G. & Zou, J. 2003. Arabidopsis AtGPAT1, a member of the membrane-bound glycerol-3-phosphate acyltransferase gene family, is essential for tapetum differentiation and male fertility. *Plant Cell 15*, 1872-87.

Zheng, Z. & Zou, J. 2001. The initial step of the glycerolipid pathway: identification of glycerol 3-phosphate/dihydroxyacetone phosphate dual substrate acyltransferases in Saccharomyces cerevisiae. *J Biol Chem 276*, 41710-6.