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Review

Systems biology of lipid metabolism: From yeast to human

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ABSTRACT

Lipid metabolism is highly relevant as it plays a central role in a number of human diseases. Due to the highly interactive structure of lipid metabolism and its regulation, it is necessary to apply a holistic approach, and systems biology is therefore well suited for integrated analysis of lipid metabolism. In this paper it is demonstrated that the yeast *Saccharomyces cerevisiae* serves as an excellent model organism for studying the regulation of lipid metabolism in eukaryotes as most of the regulatory structures in this part of the metabolism are conserved between yeast and mammals. Hereby yeast systems biology can assist to improve our understanding of how lipid metabolism is regulated.
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1. Introduction

Systems biology has evolved as a very broad research field and it has been defined in many different ways [1–7], but the essence of most of these definitions are twofold: (1) to obtain new insight into the molecular mechanisms occurring in living cells or sub-systems of living cells, through the combination of mathematical modeling and experimental biology and (2) to obtain a quantitative description of biological systems in the form of mathematical models that can be used for predictive analysis. According to this definition mathematical modeling plays a central part in systems biology, and the role of mathematical models is particularly relevant in studies of complex biological systems where it is difficult to extract causal information from experimental data. Analysis of large biological networks is often considered to be the core of systems biology, but this is mainly due to the fact that such networks can only be studied through the integration of mathematical models with experimental data. Also, smaller biological systems can gain much from the combination of mathematical models with experimental data as illustrated in a seminal study of the Hog pathway in yeast [8]. The above definition does not say anything about the use of global data, e.g. transcriptome or proteome data, nor the development of technologies for generation of high-throughput data sets. Although omics analysis is often considered to be systems biology, there are many systems biology studies that do not rely on such data, and there are clearly also some studies that use omics data that in their essence are not systems biology. Mathematical models have, however, shown to be particularly useful for analysis of global data, as the complexity and integrative nature of biological systems makes it difficult to extract information on molecular processes from global data without the use of models as either scaffolds for the analysis or for hypothesis driven analysis of the data.

The type of model that one will use in a systems biology study depends completely on the objective of the study, i.e. is the objective to analyze a given data set and obtain new biological insight or is the objective to obtain a mathematical model that can be used to perform predictive simulations of the biological system. Often, there is a distinction between top-down systems biology and bottom-up systems biology [9]. Top-down systems biology is basically a data-driven process, where new biological information is extracted from large data sets. The models used in this kind of study can be soft models like neural networks, graphs or even statistical models. In many cases there is not a specific hypothesis and the analysis may be rather inductive [10], but often the initial analysis leads to some kind of hypothesis that then leads to establishment of a coarse model which can be evaluated against the experimental data. Bottom-up or hypothesis driven systems biology, on the other hand, is based on the availability of very detailed knowledge, which is translated into a mathematical formulation that can be used to simulate the behaviour of the system. Generally there is not enough knowledge available to build detailed mechanistic models, and an important element of bottom-up systems biology is therefore an evaluation of different model structures [11].

It is difficult to classify mathematical models applied in either top-down or bottom-up systems biology as many different types of models may be used, e.g. models based on ordinary differential equations, stochastic models, stoichiometric models and graph

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models. Most bottom-up driven models only describe a sub-set of the complete biological system, as there is simply not enough quantitative information available to include interactions between all the components within the cell. There is, however, one type of bottom-up model that is fairly global in its approach, namely metabolic network models [12]. Metabolic network models are based on collecting the stoichiometry for all metabolic reactions into a stoichiometric matrix and through the use of flux balance analysis it is possible to use these stoichiometric models for simulation of growth and product formation [13]. As metabolic pathways and architecture are well established, it is possible to expand this modeling concept to cover practically all parts of metabolism, and it may even be possible to expand these models to cover regulation [14,15]. Thus, even though these models are bottom-up driven, they actually provide much information about the connectivity between the different enzymes participating in the metabolic network [16], and they can therefore be used as a scaffold for organizing and integrating x-ome data [17].

Here will be given a brief overview of yeast genome-scale metabolic models and how they can be used as scaffolds for analysis of complex regulatory networks in yeast. It will be illustrated that these models are well suited for studies of lipid metabolism, as this represents a complex metabolic network. In this context it will be further discussed how yeast can be used as a model organism for studies of lipid metabolism in humans. It will be shown that there is a very high degree of conservation between lipid metabolism between yeast and human, hence studies in yeast can bring new insights into regulation of lipid metabolism in human cells. Considering the epidemic of obesity related diseases such as diabetes type II, arteriosclerosis, hypertension and fatty liver, such new insights is of significant relevance.

2. Yeast as a model organism

Saccharomyces cerevisiae (baker's yeast) is a widely used model organism for studying eukaryal cell physiology and molecular events relevant for human disease. There are several inherent features that make yeast a good model organism: (1) they are unicellular microorganisms that are easy to cultivate fast in inexpensive media which enables performing controlled experiments at many different conditions, (2) they come both in haploid and diploid forms and with the possibility for either sexual crossing or clonal division (budding) enabling easy genetic manipulations and screenings, (3) yeast can express heterologous genes either from an episomal plasmid or from a chromosomal integration and it is possible to fairly easy insert, delete or mutate any sequence in the genome, (4) a collection of single deletion mutants is available for diploid cells and for non-essential genes also for haploid cells, (5) there is an extensive research infrastructure available with a well-curated Saccharomyces Genome Database (SGD) (www.yeastgenome.org) and a large number of technologies are available for high-throughput analysis (yeast has been used to pioneer transcriptome, proteome and interactome studies), and (6) a large fraction of the yeast genes have human orthologues.

In terms of glucose metabolism yeast cells do, however, deviate from many other eukaryal cells, including many human cells, as yeast performs aerobic fermentation at high glucose concentrations. This means that despite the presence of oxygen the flux through the tricarboxylic acid (TCA) cycle and the respiratory system is low and most of the carbon is shunted towards ethanol [18]. This phenomenon is generally referred to as the Crabtree effect, and it is explained by extensive glucose repression of the genes encoding enzymes in the TCA cycle and components of the respiratory system [19]. The Crabtree effect is named after a seminal study by Herbert Grace Crabtree in 1929, but it is interesting to note that Crabtree was in fact studying carbohydrate metabolism of tumors [20]. His studies were inspired by studies of Otto Warburg a few years earlier [21], who also looked at the fermentative metabolism of tumors, and that let him to identify iron as an essential component of the respiratory system, which awarded him the Nobel Prize in Physiology and Medicine in 1931. The fermentative metabolism of cancer cells is today known as the Warburg effect and it is interesting to note the many similarities in fermentative yeast metabolism and the metabolism of cancer cells [22] (cancer cells produce lactate instead of ethanol, but both metabolic products are derived from pyruvate). Thus, studies on fermentative yeast metabolism may well represent a very good model for the metabolism of cancer cells, even though the regulatory mechanisms underlying the Crabtree effect and the Warburg effect are likely to be quite different.

3. Genome-scale metabolic modeling of yeast

Genome-scale metabolic models are comprehensive mathematical representations of all biochemical conversion processes in a given cell. These models are reconstructed through a bottom-up approach where any kind of information about the metabolism is collected and used to define the stoichiometry of all possible metabolic reactions that can operate in the studied cell. The stoichiometry of all the different reactions can be specified in a matrix form where the columns represent reactions and the rows represent the different metabolites (see Fig. 1). By assuming that the fluxes into and leaving different metabolite pools are balanced (i.e. steady state), it is possible to set up a material balance for each of the metabolites in the model. These balances can easily be set up using the matrix representation of the metabolic model, and they can constrain the fluxes through the different reactions in the network such that these have to be confined within a feasible space as illustrated by the cone in Fig. 1A. However, despite the large number of constraints provided by the material balances for each of the metabolites in the metabolic network there is still a very large degree of freedoms in terms of how the metabolic network can operate, i.e. what values through the different reactions will the fluxes attain at different conditions. It has, however, been shown that metabolic networks tend to strive towards a given objective, which for microorganisms in most cases involve maximization of fluxes leading to cell mass production [23]. Other objectives, such as maximizing overall ATP production, have also been found to give good predictive strength for some conditions [24]. Thus, using a simple objective function, it is possible to use these genome-scale metabolic models for simulation of metabolism. Even though the models provide a solution for how the fluxes operate in the metabolic network, it is important to be aware that due to the large degrees of freedom in genome-scale metabolic networks the objective based simulation does not necessarily result in a unique solution in terms of the fluxes through the different metabolic reactions in the network, as diversion through different metabolic pathways can result in equivalent contributions to cell mass production. Such detailed flux information can only be obtained through the use of additional data, e.g. measurement of ^{13}C-labeling of different metabolites [24,25].

Besides being useful for simulations, genome-scale metabolic networks represent a valuable resource in terms of providing highly annotated metabolites, reactions, enzymes, genes and even references (Fig. 1B). Thus, these reconstructed metabolic networks represent extensive databases on metabolism, as well as, they enable setting of a graph that represents the metabolic network of the studied organism (Fig. 1C). It is interesting that the analysis of graphs from such metabolic networks shows that the many different metabolic pathways are highly connected [25]. This is due to

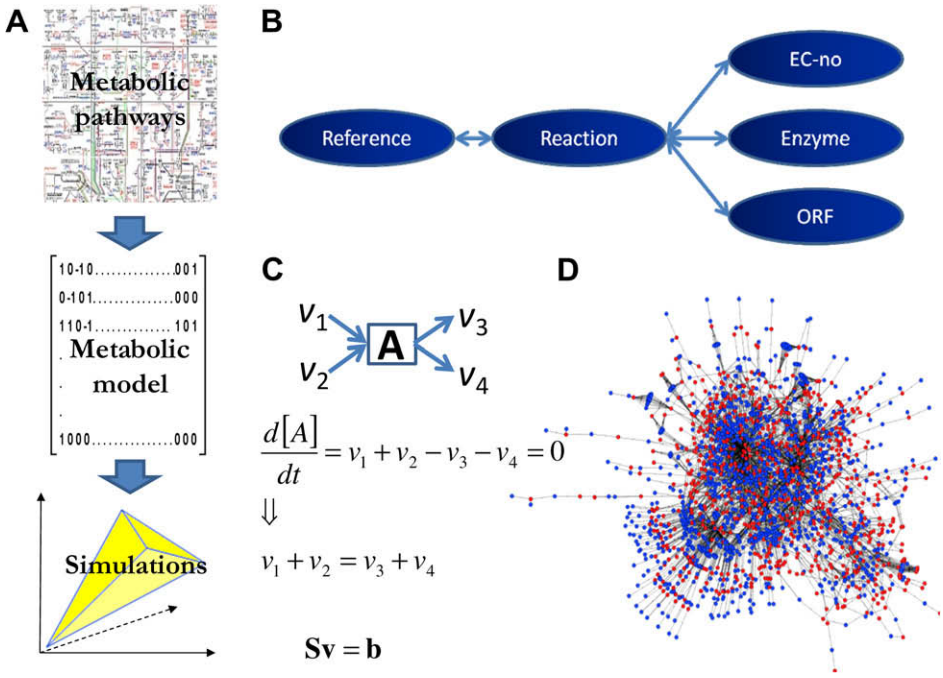

Fig. 1. Illustration of the reconstruction of metabolic network models and how they link ORFs to reactions and metabolites. (A) Metabolic pathway information is translated into defined stoichiometry of all the individual reactions that can then be used for model simulations. (B) Illustration of how each reaction can be linked to a specific reference (paper, database or the like) and also to enzymes and ORFs. (C) Illustration of how balancing of fluxes, represented as v_i, into and out of metabolite pools results in an algebraic equation that constrain the fluxes. For the complete network this can be represented by a matrix equation where **S** is the total stoichiometric matrix, **v** is a vector containing all the fluxes in the network, and **b** is a vector containing the net production rate of the different metabolites. For metabolites that are not taken up or secreted by the cells there is no net production and hence the corresponding element in the **b** vector is zero. (D) Graphical illustration of metabolic networks. It is seen that the network is extremely condensed as a few metabolites participate in a large number of different reactions.

the fact that typically more than 10% of the metabolites in these networks participate in more than 10 metabolic reactions whereas typically around 5% participate in more than 20 metabolic reactions. Among the metabolites being these key "hubs" that are involved in a large number of metabolic reactions are obviously the co-factors ATP, ADP, NADH and NADPH, but also metabolites like pyruvate, glutamate, acetyl-CoA and 2-oxoglutarate [9]. Due to the high degree of connectivity in these metabolic networks we hypothesized that cells will have a coordinated transcriptional response to different types of perturbations, e.g. changes in environmental conditions or introduction of directed mutations [26]. Through the use of the metabolic graphs that result from the connectivity of the different metabolites and their associated enzymes we showed that this hypothesis is likely to hold, as different perturbations result in coordinated gene expression changes in a large number of genes that encode for enzymes sharing the same metabolic neighbors [26]. This actually allows for integrated analysis of different types of omics data where the metabolic networks are used as scaffolds for the analysis [26,27]. This concept can even be used wider for any type of biological network, i.e. protein–protein interaction networks [28] and protein–DNA networks [29].

The first yeast metabolic network was reconstructed in a collaborative effort of the research groups of Palsson and Nielsen [30,31]. This network contains 1175 reactions linked to 708 ORFs and this network has been extensively used for both data analysis and for identification of metabolic engineering targets in connection with the development and/or improvement of biotech processes based on yeast [32]. This network only considered cytosol, mitochondria and extracellular space, and Palsson and co-workers therefore expanded the network to cover more reactions and more compartments, and this resulted in a model with 1498 reactions linked to 750 ORFs [33]. These original models contain several reactions that are not linked to the network, and even though these models do give a fairly good prediction of the phenotype of single gene deletions [31,34] Sauer and co-workers showed that the predictive strength could be improved by removing these non-connected reactions [35]. This resulted in a network with 1038 reactions linked to 672 ORFs.

All of the above-mentioned models do not describe the lipid metabolism in great detail and Nookaew and co-workers derived a new model from the original Förster model that contains many more details in the lipid metabolism [36]. This network contains 1446 reactions linked to 800 ORFs, and the resulting model *iNN800* was shown to have very good simulation performance, and probably represents the best experimentally validated model for simulations. The *iNN800* model has 65 reactions (30 in the mitochondria and 35 in the cytosol) involved in fatty acid biosynthesis, 44 reactions involved in β-oxidation, 26 reactions in lipid degradation, 9 reactions in sphingolipid biosynthesis and 33 reactions in fatty acid elongation and therefore has a very detailed description of the lipid metabolism. This allows for precise calculations of the fluxes through the many different reactions of lipid metabolism based on measurement of the lipid composition in the biomass (Fig. 2).

A major problem with the different yeast models published is that they all use slightly different nomenclatures for metabolites and reactions, and it is therefore difficult to compare these networks directly. In order to solve this issue several research groups organized a jamboree for reaching a consensus in terms of nomenclature for metabolites in yeast metabolic networks and also derive a consensus metabolic network. This resulted in a network containing 1857 reactions linked to 832 ORFs and 1168 metabolites [37]. In this network all metabolites are defined by international chemical identifier (InChi) representations or KEGG identifiers. This allows for a very consistent representation of the metabolic network in the systems biology markup language (SBML). Further-

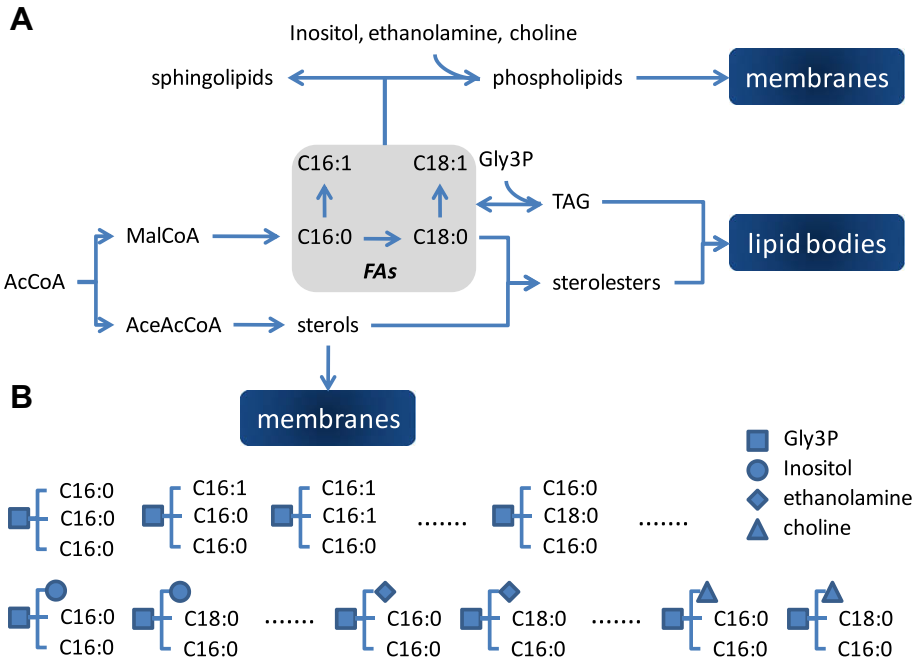

Fig. 2. Overview of lipid metabolism. (A) The main pathways in lipid biosynthesis and illustration of how different lipid species are incorporated into the biomass as either lipid bodies (storage lipids) or membranes. TAG serves, besides as lipid storage, as a buffer of fatty acids that can be used for membrane formation during the cell cycle and it has been shown that there are dynamic changes in the level of the TAG pool in the cell [59]. The formation of TAG from FAs is therefore shown as a reversible reaction. (B) Illustration of the large number of different lipid species that may be present in lipid bodies and membranes. The first row indicates TAGs and the second row indicates three key phospholipids (besides the three shown there are also phosphatidylserine and phosphatidylglycerol species). The complexity even increases with the presence of diacylglycerols and monoacylglycerols (not shown). Sterylesters are not shown, but these can also be present with different fatty acids. AcCoA – acetyl-CoA; MalCoA – malonyl-CoA; AceAcCoA – acetoacetyl-CoA; FAs – fatty acids; Gly3P – glycerol-3 phosphate; TAG – triacylglycerols; C16:0 – palmitic acid; C18:0 – stearic acid; C16:1 – palmitoleic acid; C18:1 – oleic acid.

more, with a stringent definition of the metabolites it is easy to link these metabolic networks with data from mass spectrometry and hereby link metabolomics data directly with metabolic networks. Following the jamboree guidelines we have recently updated the *iNN800* model so all metabolites are presented with InChi strings or KEGG identifiers and the model is presented in SBML format (www.sysbio.se).

4. Comparative analysis of lipid metabolism

Lipid metabolism is quite complex involving a very large number of metabolic reactions spanning different compartments in eukaryotic cells and resulting in the formation of a diverse group of chemical compounds. Lipids can roughly be divided into the following classes: (1) fatty acids (FAs) that mainly serve a role as intermediates in lipid biosynthesis; (2) free sterols that serve as structural components in membranes; (3) sterol esters that are formed from FAs and sterols and that serve as lipid storage compounds, mainly as lipid bodies; (4) triacylglycerols (TAG) that are formed from glycerol and FAs and that serve as lipid storage, mainly stored in lipid bodies; (5) phospholipids that are formed from FAs, glycerol and an alcohol moiety, e.g. inositol, choline or ethanolamine, and that serves as structural compounds in membranes; and (6) sphingolipids that are formed from palmitic acid and are basically very long chain fatty acids that serves as structural components at the cell surface as well as key signaling roles, e.g. regulation of endocytosis, ubiquitin dependent proteolysis and cell cycle control.

Despite the large chemical variety of lipids, they all have the same key carbon precursor, namely acetyl-CoA (see Fig. 2), and all initial steps of lipid biosynthesis occur in the cytosol (see Fig. 3). In mammals, acetyl-CoA in the cytosol is mainly derived from citrate through the reaction catalyzed by ATP:Citrate-lyase. Citrate therefore basically serves as a precursor for lipid biosynthesis in mammals, which explains the important regulatory role of citrate, e.g. on the glycolytic flux through regulation of phosphofructokinase. *S. cerevisisae* does not possess an ATP:Citrate lyase, but it has another very efficient pathway leading to cytosolic acetyl-CoA. This pathway involves decarboxylation of pyruvate to acetaldehyde that is then converted further to acetate and acetyl-CoA (Fig. 2). Acetaldehyde is at fermentative conditions mainly converted to ethanol, but sufficient acetaldehyde is converted to acetyl-CoA to ensure an efficient lipid biosynthesis. Even though this pathway is central for fermentative metabolism in yeast, it is functional also at fully respiratory conditions, where it also can ensure sufficient supply of acetyl-CoA for lipid biosynthesis. *S. cerevisiae* contains two acetyl-CoA synthases, Acs1 and Acs2, and their localization is still under debate, even though Acs2 is believed to be mainly cytosolic and Acs1 is believe to be mainly peroxisomal (both may also have partly nuclear localization to ensure acetyl-CoA synthesis required for acetylation of DNA). Acetyl-CoA can be transported across the mitochondrial and peroxisomal membranes by the carnitine transport system, but even though *S. cerevisiae* contains all the components for this shuttle system it cannot synthesize carnitine and use of this system therefore requires addition of carnitine to the medium.

As illustrated in Fig. 2 lipid biosynthesis basically involves two branches from acetyl-CoA, one leading to sterols and the other leading to fatty acids (FAs) that serve as building blocks for biosynthesis of TAG, phospholipids, sterylesters and sphingolipids. The level of non-esterified fatty acids (NEFAs) is generally low in the cell and "free" fatty acids are mainly present as CoA-esters. Here FA will, however, be used as a general term for NEFAs and CoA-esters of fatty acids.

In the branch towards sterols the first step is the conversion of two molecules of acetyl-CoA to acetoacetyl-CoA (Fig. 3), and even

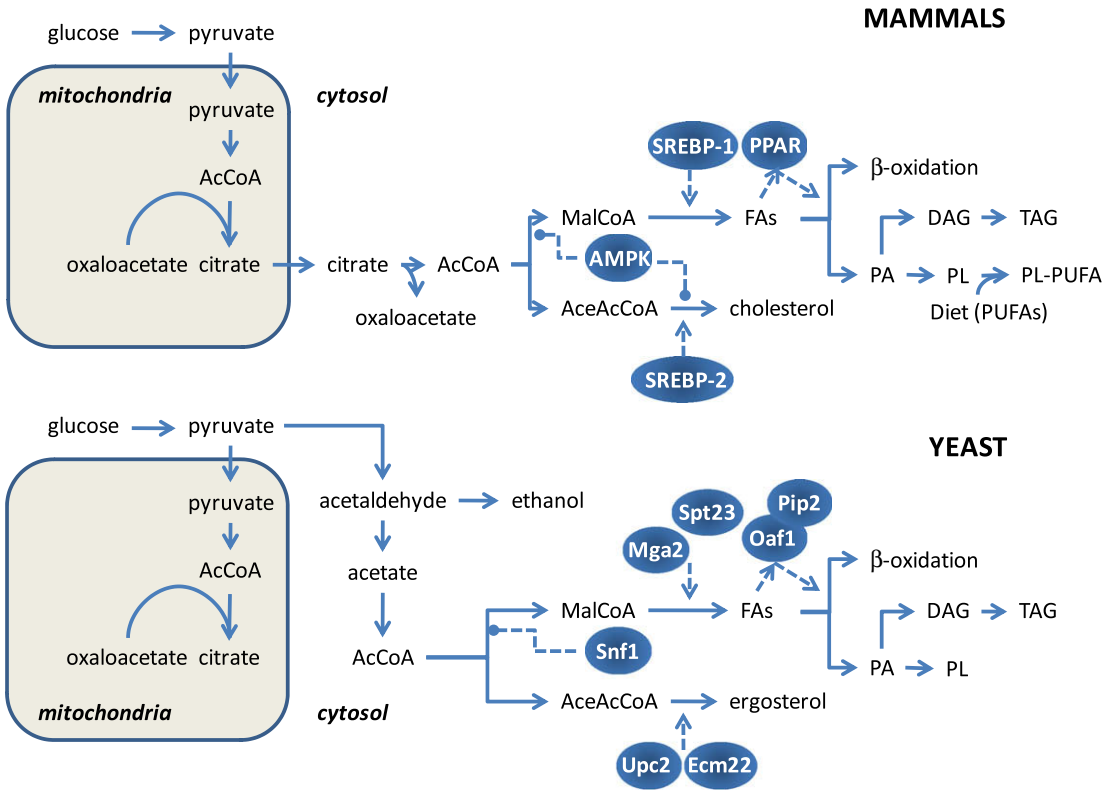

Fig. 3. Comparison of lipid biosynthesis and its regulation in mammals and yeast. For mammals fatty acids are also taken up via the diet and these are incorporated into the different lipid pools (here only illustrated to the PL-pool). AcCoA – acetyl-CoA; MalCoA – malonyl-CoA; AceAcCoA – acetoacetyl-CoA; FAs – fatty acids; PA – phosphatidic acid; DAG – diacylglycerols; TAG – triacylglycerols; PL – phospholipids; PL-PUFA – phospholipids containing poly-unsaturated fatty acids.

though this reaction step is the committed step from the common lipid precursor metabolite acetyl-CoA towards sterols, there is no evidence of stringent regulation of this step. Further downstream of the sterol pathway farnesyl pyrophosphate (FPP) is a key branch point metabolite, as it also serves as a precursor for dolichol that is used for N-glycosylation of proteins. The pathway from acetyl-CoA to FPP is often referred to as the mevalonate pathway due to the intermediate mevalonate. A key reaction step in the mevalonate pathway is the conversion of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) to mevalonate by HMG-CoA reductase (HMGR). HMGR is a highly regulated enzyme in the sterol pathway and it is target of all current cholesterol lowering drugs (often referred to as statins). HMGR is regulated at the transcriptional level, protein level and through inactivation by phosphorylation. In yeast there are two HMGRs, Hmg1 and Hmg2.

Transcriptional regulation of the mevalonate/sterol pathway is managed by transcriptional activators that are members of the sterol regulatory element binding protein (SREBP) family. These transcription factors also up-regulate the expression of several other genes encoding enzymes involved in the sterol pathway [38]. In mammals SREBP-2 is the transcription factor, and even though yeast does not contain any homologues of SREBP-2, the yeast proteins Upc2 and Ecm22 were recently shown to have a similar function as SREBP-2 in mammals [39,40]. SREBPs are bound to the endoplasmic reticulum (ER) membrane, but are activated and released from the ER membrane upon dissociation from SCAP (SREBP-cleavage activating protein), resulting in translocation of SREBPs to the Golgi where the transcription factor part of the protein is proteolytically cleaved of from the rest of the proteins and subsequently migrates to the nucleolus [38]. SCAP contains a multispanning ER membrane anchor that is referred to as a sterol-sensing domain (SSD). In mammals increased cholesterol levels results in a conformational change of the SSD domain of SCAP which results in binding of this protein to the SSD-domain interacting protein INSIG, and hereby SCAP is prevented from activating SREBP resulting in a down-regulation of genes encoding enzymes involved in sterol biosynthesis [38]. Whether there is direct interaction between cholesterol and SCAP or whether cholesterol changes the membrane fluidity and this leads to conformational changes of SCAP is not known [38]. Yeast has two homologues to INSIGs, namely Nsg1 and Nsg2, and these have been shown to have a functional role similar to the INSIG [41]. Thus, it seems like this regulatory system is conserved between yeast and human even though there are no direct sequence homologies between the SREBP and SCAP proteins in yeast and human (SCAP has so far not been identified in yeast).

HMGR has, just like the SCAP, a SSD that ties it to the ER membrane. Just like the INSIG can interact with SCAP, they can interact with HMG and hereby prevent this enzyme from undergoing ubiquitination that leads to proteolytic degradation [41]. Thus, high levels of sterol in the ER membrane results in both down-regulation of gene expression and inactivation of one of the key enzymes in the sterol biosynthetic pathway HMGR. It is interesting to note that this regulation of HMGR at protein level can be removed through removal of the SSD, as illustrated in studies aiming at increasing the flux through the sterol pathway in order to have high level production of biotechnologically relevant compounds derived from FPP, e.g. lycopene and sesquiterpenes [42,43]. The final level of regulation of HMGR is through phosphorylation by AMP-activated kinase (AMPK), resulting in inactivation of the HMGR. Even though this inactivation has been well demonstrated in mammals it has not been confirmed yet to exist in yeast.

In the other "branch" of lipid biosynthesis acetyl-CoA is converted to malonyl-CoA by acetyl-CoA carboxylase (ACC and Acc1

in yeast). This is a committed step towards FAs and compared with the first step of cholesterol biosynthesis this step is highly regulated. Thus, ACC is, like HMGR in mammals, inactivated by AMPK, and this regulation also exist in yeast where it is has been shown that Acc1 is phosphorylated by the yeast AMPK orthologue Snf1 [44]. Malonyl-CoA serves as precursor for synthesis of FAs, that are formed by the large multifunctional fatty acid synthetases (FAS) that in yeast consists of two subunits encoded by *FAS1* and *FAS2*. The main product of the FAS is palmitic acid (C16:0) which can be converted further to stearic acid (C18:0) by an elongase encoded by *ELO1* in yeast. Both palmitic acid and stearic acid can be converted to their corresponding mono-unsaturated fatty acids palmitoleic acid (C16:1) and oleic acid (C18:1) by a Δ9-desaturase encoded by *OLE1* in yeast, see Fig. 4. These steps are the same in mammals, but mammals also take up other fatty acids from the diet and can convert the linoleic acid from the diet into PUFAs containing several double bonds and even further elongated, e.g. arachidonic acid and eicosapentaneoic acid. Yeast basically only contains the above-mentioned four FAs (there is a low content of shorter chain fatty acids), and it lacks the ability to convert oleic acid to PUFAs like linoleic (C18:2) and γ-linolenic acid (C18:3) like many other, even closely associated, yeasts. One may speculate whether the presence of only mono-unsaturated fatty acids gives a certain membrane structure that has evolved in connection with the development of the high ethanol tolerance of *S. cerevisiae*.

In mammals the FAS have been shown to be regulated by SREBP, more specifically by SREBP-1, in a similar fashion as enzymes of the sterol biosynthetic pathway. In yeast the transcription factors Mga2 and Spt23 has been shown to regulate the expression of *OLE1* and these transcription factors have been shown to be bound to the ER membrane and activated in a similar fashion as the SREBPs [45]. Thus, in both yeast and mammals there is a coordinated regulation of biosynthesis of FAs and cholesterol, which may be explained by the requirement for a coordinated biosynthesis of these two different types of lipids that are both needed for proper membrane function and for storage in the form of sterylesters (Fig. 2). In the presence of high glucose levels, mammals do, however, have an additional regulatory system, namely insulin which in the presence of high glucose concentration stimulates FA biosynthesis only, and hence allow for dedicated biosynthesis of storage lipids in the form of TAG [38]. Based on transcriptional analysis of livers from mice that had over-expression of SREBP-1 and SREBP-2 and a knockout of SCAP all genes under regulation of SREBPs have been identified in mammals [46].

The integration of FAs into phospholipids and TAG, and the degradation of FAs through β-oxidation are also conserved. Both in yeast and mammals two FAs are added to glycerol-3 phosphate to form phosphatidic acid (PA), which serves as a precursor for the biosynthesis of phospholipids and TAGs. In the route towards phospholipids PA is first activated to CDP-diacylglycerol through reaction with CTP, and thereafter there is a trans-esterification with an alcohol to form the different types of phospholipids. PA is also the precursor for TAGs as it can be converted to first diacylglycerols (DAGs) to which an additional FA is added to form TAGs. As mentioned above, mammals can incorporate FAs from the diet into phospholipids and TAGs, and further convert these to other PUFAs, resulting in a far more complex and varied lipid composition than in yeast. The insertion of additional double bonds is carried out by specific desaturases that are located in the ER membrane and hence happens when the fatty acids are present as phospholipids. On the contrary, elongation occurs only when the fatty acids are present as CoA-esters, and there is hence a dynamic exchange of fatty acids between the different lipid pools even after they have first been integrated into the phospholipid pool.

The reactions of β-oxidation are highly conserved, and also the regulation of β-oxidation is conserved between yeast and mammals. In mammals oleic acid is activating the peroxisome proliferating activating receptor (PPAR), a transcription factor that leads to stimulation of peroxisome formation and hence increased β-oxidation in response to fatty diets. Yeast contains the transcription factor Pip2–Oaf1 that plays a similar role [47], but these proteins share no homology with PPARs of mammals. In yeast β-oxidation is also regulated by the transcription factor Adr1 [48], that is also regulating glycerol metabolism and ethanol oxidation. This transcription factor is probably conserved in the whole eukaryal kingdom as it is present in fungi [49] and in humans [50].

Regulation of phospholipid biosynthesis has been studied in details in yeast and Fig. 4 gives a brief overview of some of the key regulatory processes. As already mentioned, Snf1 the yeast orthologue of human AMPK, is inactivating Acc1, but it is also stimulating β-oxidation through activation of Fox2, a multifunctional

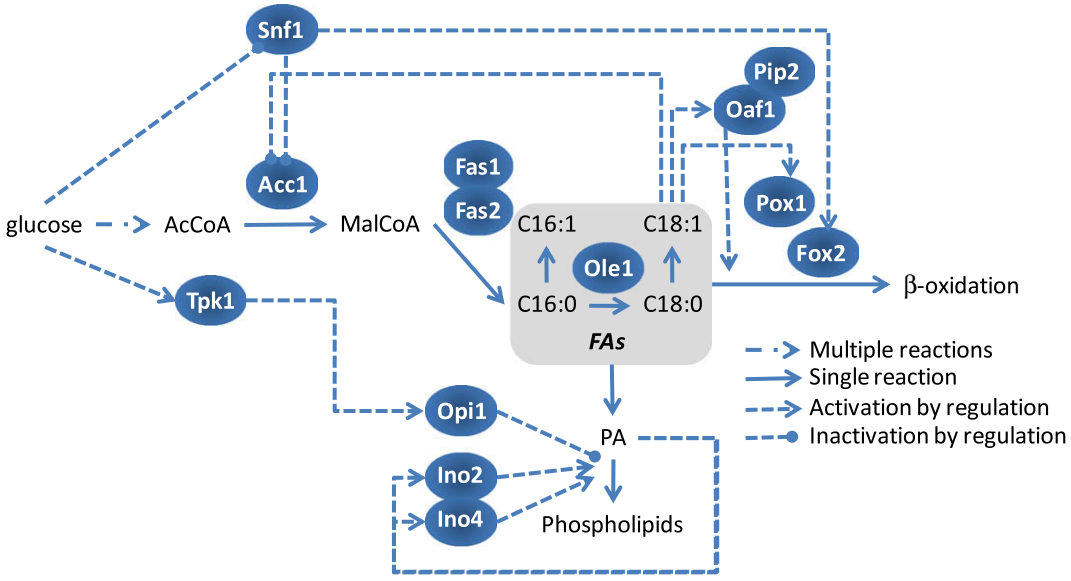

Fig. 4. A simplified scheme of regulation of phospholipid biosynthesis in yeast. AcCoA – acetyl-CoA; MalCoA – malonyl-CoA; C16:0 – palmitic acid; C18:0 – stearic acid; C16:1 – palmitoleic acid; C18:1 – oleic acid; FAs – fatty acids.

enzyme that harbors both 3-hydroxyacyl-CoA dehydrogenase and enoyl-CoA hydratase activities. Both AMPK and Snf1 are activated when the ATP production level is low in the cell, and is hence inactivating ATP consuming processes such as lipid biosynthesis and activating ATP generating processes such as β-oxidation [51]. With oleic acid playing a key role as the entry into phospholipid biosynthesis it is also not surprising that this FA plays a key regulatory role. Thus, it acts as a feed-back inhibitor of Acc1, as an activator of the Pip2–Oaf1 transcription factor and as an activator of Pox1, an enzyme directly involved in β-oxidation. Thus, if there is accumulation of oleic acid due to down-regulation of phospholipid biosynthesis there is feed-back inhibition of its own biosynthesis and feed-forward activation of β-oxidation, a regulatory concept that is used widely in metabolism. Phospholipid biosynthesis is stimulated by the transcription factors Ino2 and Ino4 [52] ensures derepression when phosphatidic acid accumulates, e.g. in response to inositol limitation (another precursor for phospholipids, see Fig. 2). However, there is also negative regulation by the transcription factor Opi1, which is activated by protein kinase A (PKA or Tpk1 in yeast) [53]. PKA is activated at high glucose levels, and the consequence of this regulatory scheme is that in the presence of excess glucose, there is accumulation of FAs that is shunted towards TAG or sterylesters (not shown in Fig. 4).

5. Systems biology of lipid metabolism

From the above it is clear that lipid metabolism involves a large number of enzyme catalyzed reactions with regulation at different levels. It is therefore difficult to dissect the different regulatory modules and map how they interact in response to diet or other environmental conditions. Studies of lipid metabolism are therefore an obvious area where systems biology can contribute, both in terms of assisting with integrated analysis of high-throughput data and in terms of predictive simulations of how different cell types respond to variations, e.g. blood lipid levels. It is also clear that with the high degree of conservation in regulation between yeast and mammals, yeast is an excellent model organism for studying lipid metabolism.

Integrated analysis of lipid metabolism requires the ability to measure a large number of different lipid species. There are many different methods for analysis of lipid species, and traditionally there was performed a separation of the different lipid classes followed by analysis of the fatty acid composition in each of these classes. However, with the development of advanced mass spectrometry it has become possible to performed detailed lipidomics analysis using a single analysis [54]. Through combination of this kind of measurements and detailed metabolic network models it is possible to quantify the fluxes through different pathways in lipid metabolism. We used this approach to analyze the global regulation of the fluxes in lipid metabolism by analyzing the composition of structural lipids (phospholipids, sterylesters and TAGs) in yeast grown at eight different growth conditions, and using these data to identify global correlations between transcripts, metabolite levels and fluxes through the different branches of lipid metabolism (unpublished data). The detailed metabolic network with a detailed description of the lipid metabolism was essential for this study, and this illustrates how metabolic network models can be used for integrated data analysis and for identification of global regulatory structures.

As mentioned earlier the AMPK protein kinase plays a central role, not only in regulating lipid metabolism, but in overall control of energy homeostasis in the eukaryal cells (Fig. 5A). The yeast orthologue Snf1 has also been extensively studied, but mainly for its role in carbon metabolism. We therefore undertook a study with the objective to identify the global role of Snf1 in the cell. A reference strain and a strain with deletion of *SNF1* were both grown at glucose-limited conditions (where Snf1 is active), and the transcriptome, proteome and metabolome were analyzed [55,56]. The use of a number of different methods for integrated data analysis, including the use of metabolic network models for identification of reporter metabolites and co-regulated modules, resulted in the reconstruction of a global regulatory network of the protein kinase Snf1. A number of targets for regulation by Snf1 not previously identified in yeast were identified by this approach, and overall it was found that Snf1 plays an equally important role for global regulation as AMPK in mammals [56]. Furthermore, the analysis allowed for building a rather detailed model for how Snf1 is regulating lipid metabolism and the results are summarized in Fig. 5B. Consistent with the general model for Snf1 activating energy producing reactions it is seen that genes encoding enzymes of several key steps of β-oxidation are down-regulated. It is interesting to note that *ACC1* is also down-regulated in the Δ*snf1* mutant, and this is despite the fact that the oleic acid concentration is increased and that the transcription of *OAF1* is increased, which according to the model of Fig. 4 lead to increased level activity of β-oxidation. This points to a possible role of Snf1 as a key activator of β-oxidation, and this could well be through activation of Oaf1 and Pip2. In this connection it is worth mentioning that the transcription factor Adr1 is also playing a role in up-regulating β-oxidation, and this is negatively regulated by Tpk1

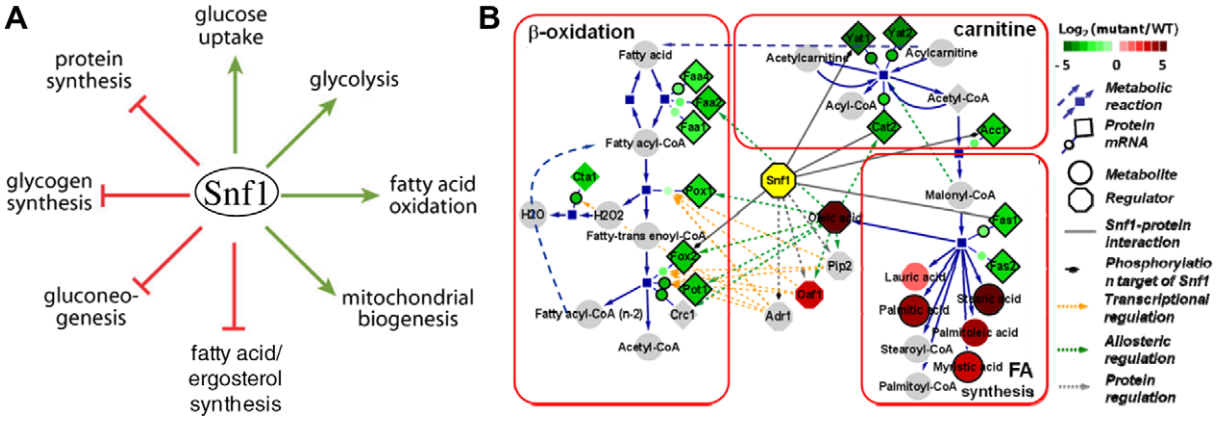

Fig. 5. Overview of the global regulatory role of the protein kinase Snf1 in yeast. (A) Simplified view of how Snf1 is regulating different key metabolic pathways in the cell. The model is based on studies of AMPK in mammals but was found to hold also for yeast in a study of Usaite et al. [55]. (B) Detailed view of how Snf1 regulated different processes in lipid metabolism. The model is adapted from Usaite et al. [56].

(or PKA) and activated by Snf1 [57,58]. Interestingly, the *FAS1* and *FAS2* are also down-regulated as well as Acc1, which could point to a reduced flux towards the fatty acids. This may likely be due to down-regulation of β-oxidation, which leads to an increased level of FAs, in particularly oleic acid, that then also down-regulate fatty acid biosynthesis. Despite the down-regulation of Acc1 and the FAS there may still be sufficient flux towards the FAs to sustain lipid production needed for biomass formation, as the negative regulation of Acc1 by Snf1 obviously is lacking in the Δ*snf1* mutant. The global study on the role of Snf1 clearly resulted in identification of new regulatory structures, and it therefore points to the value of using top-down systems biology for analysis of complex regulatory networks, of which regulation of lipid metabolism is a good example. This kind of study in yeast may therefore lead to building solid regulatory network models that due to the high degree of conservation of regulation between yeast and mammals can then be used as scaffolds for analysis of data from human studies.

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