# Differences between Yeast v6.0 and iTO977

# Fatty Acid Metabolism

## Fatty Acid Synthesis

In iTO977, cytoplasmic fatty acid synthesis (FAS) is represented as capable of generating C10:0, C12:0, C14:0, C16:0, and C18:0 free fatty acids and acyl-CoAs. This representation is misleading since the reaction intermediates are shuttled within the FAS enzyme complex and only the terminal acyl-CoA products of C16:0 and C18:0 (with minor amounts of C14:0) are released into the cytosol *in vivo*. In addition, the products of cytoplasmic FAS are acyl-CoAs, not free fatty acids. These two issues were present in the original Yeast v6.0 model as well and were addressed in the changes to the Yeast v6.0 model.

iTO977 depicts mitochondrial FAS in the same manner as cytoplasmic FAS (i.e. individual steps for extension of acetyl-[acp] all the way to stearoyl-[acp]). Mitochondrial FAS and cytoplasmic FAS are connected in iTO977 through transport of C4:0 to C18:0 acyl-ACP between the cytoplasm and mitochondria. This representation in iTO977 is similar to that found in the original Yeast v6.0 model. In contrast, the modified Yeast v6.0 model removes the production of mitochondrial FAS products longer than C8:0 acyl-ACP due to lack of experimental evidence and also removes the transport of acyl-ACP between the cytoplasm and mitochondria based on the observation that mitochondrial FAS is unable to compensate for cytoplasmic FAS in *fas1* or *fas2* mutants.

## Fatty Acid Elongation

iTO977’s representation of fatty acid elongation is in accord with the modified Yeast v6.0 model.

## Fatty Acid Desaturation

Like the original Yeast v6.0 model, iTO977 is missing NADH/NAD+ in the reaction equations for the fatty acid desaturation. The modified Yeast v6.0 model includes these cofactors in the equations.

## ϐ-Oxidation

iTO977 did not have the issue of directionality of the *MDH3* reaction which regenerates NAD+ needed for continued ϐ-oxidation. Nor did it have the issue of directionality of the mitochondrial carnitine acetyl-CoA transferase which is used for transfer of acetyl units into the mitochondria.

However, iTO977 has its own unique issues for ϐ-oxidation. iTO9077’s representation of ϐ-oxidation has multiple blocked reactions since it had reactions for acyl-CoA oxidase in the cytoplasm while the reactions for the other enzymes of ϐ-oxidation are in the peroxisome; this led to discontinuity between reactions. Also, the reactions for 2-enoyl-CoA hydratase should be associated with the gene *FOX2*, not *POX1*. iTO977’s representation of ϐ-oxidation of unsaturated fatty acids is not in accord with the mechanism presented in literature (DOI:10.1016/S0168-6445(03)00017-2) in terms of how the reactions for *ECI1*, *DCI1*, and *SPS19* enter in ϐ-oxidation. In addition, iTO977 has reactions for ϐ-oxidation of even chain lengths from 4 to 18 carbons, but not for very long chains. In contrast, the edited Yeast 6.0 model includes ϐ-oxidation up to 26 carbons.

# Glycerolipid and Glycerophospholipid Metabolism

## New Genes Added

Of the 15 additional genes added to the original Yeast v6.0 model, iTO977 had 4 of these genes (*AYR1*, *GCY1*, *IDP2*, *LRO1*) and was missing 11 of these genes.

## Expansion of Species

iTO977 uses an approach more like that of iFF708, iLL672, iMM904, and iND750 in the sense that defined composites of specific species are used. iTO977 has model-specific reactions that pool various species together as shown below:

Pool\_Acyl1 0.03 decanoyl-CoA [c] + 0.03 lauroyl-CoA [c] + 0.05 myristoyl-CoA [c] + 0.02 tetradecanoyl-9-ene-CoA [c] + 0.19 palmitoyl-CoA [c] + 0.48 hexadecanoyl-9-ene-CoA [c] + 0.08 octadecanoyl-CoA [c] + 0.12 octadecanoyl-9-ene-CoA [c] -> acyl acids [c] + coenzyme A [c]

Pool\_Acyl2 0.01 decanoyl-CoA [c] + 0.01 lauroyl-CoA [c] + 0.02 myristoyl-CoA [c] + 0.01 tetradecanoyl-9-ene-CoA [c] + 0.15 palmitoyl-CoA [c] + 0.60 hexadecanoyl-9-ene-CoA [c] + 0.04 octadecanoyl-CoA [c] + 0.16 octadecanoyl-9-ene-CoA [c] -> acyl CoAs [c]

The successive acylation of glycerol-3-phosphate is then described in iTO977 as:

acyl CoAs [c] + sn-glycerol 3-phosphate [c] -> acyl-sn-glycerol 3-phosphates [c] + coenzyme A [c]

acyl CoAs [c] + acyl-sn-glycerol 3-phosphates [c] -> coenzyme A [c] + phosphatidate [c]

As a side-note, iTO977 also has this reaction utilizing acyl CoAs which does not make sense on a mass-balance basis.

acyl CoAs [c] + acyl-sn-glycerol 3-phosphates [c] -> coenzyme A [c] + sn-glycerol 3-phosphate [c]

The hydrolysis of lipids such as triglycerides or steryl esters yields ‘acyl acids’. However, as is, iTO977 does not have reactions for the activation of these ‘acyl acids’ back to ‘acyl CoAs’ so that they may be re-utilized for acylation reactions or broken down in ϐ-oxidation.

## Compartmentalization

From the iTO977 paper:

“The compartments in iTO977 are Cytoplasm, Mitochondria, Peroxisome and Extracellular… Some of the reactions in the consensus network that takes place in other compartments, such as ER or nucleus, were included in the iTO977 model but localized to the cytoplasm, while some reactions in other compartments were out of scope of the model and discarded.”