# 私立東海大學資訊工程所

# 碩　士　論　文

指導教授：呂芳懌 教授

## 使用 Work Flow Petri Net 來模擬脂質的能量生成代謝路徑

## Modeling Energy Producing Process in Lipid Metabolism by Using Work Flow Petri Nets

研究生：李樹人

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Thesis Report

Master's Degree

# Modeling Energy Producing Process in Lipid Metabolism by Using Work Flow Petri Nets

by Su-Ren Lee

advisor : Fang-Yie Leu

Computer Science and Information Engineering,

Tunghai University

# Table of Contents

## 中文摘要:

在後基因體時代中，生物資訊領域的研究重心就逐漸的轉移到蛋白質的結構、功能分析及其相關生化代謝路徑的探討上。其中代謝路徑的探討更是格外重要，因為生物體內的每種機能都是由許多的蛋白質(酶)與反應物及代謝產物之間所交織成的網狀連鎖反應。因此探討代謝路徑不僅僅是研究蛋白質功能的必要步驟，同是更是解譯遺傳密碼如何調控生命現象的最佳方式。而代謝路徑資料模型的建立可說是相關研究的基礎。有了資料模型就可以將代謝路徑的生化特性具體的展現在資訊系統之中。將來更可藉此模擬代謝路徑並可應用在教學用途或者是預測可能反應，這對於代謝性疾病的控制及新藥的開發將會有很大的幫助。此外有了大量的資料模型後，亦可將這些模型拿來做比對，藉此找出代謝路徑的相似性，這樣有助於瞭解生化反應發生的原理，並進一步的推導出基因的表現調控機制。

從現有的文獻中，我們雖然可以找到不少使用 Petri Net 的圖型表示法來建立與模擬代謝路徑的研究結果。不過這些研究中雖然可以正確的表現出某些生化反應的結果，可是對於該反應的控制機制卻未加著墨。事實上控制機制乃是生化反應最重要的部份，生物體內的生化反應多到難以計數，若是沒有一個完善的控制機制，生命現象絕對難以維持。

而本論文主要是著重在代謝路徑控制機制，以構造較簡單的脂肪為對象，使用 Work Flow Petri Net 的圖型表示法來建立其資料模型來展現代謝路徑的控制機制。



關鍵字：Petri Nets、Workflow Nets、代謝路徑模型(metabolic pathway model)、代謝路徑模擬(metabolic pathway simulation)

## Abstract

Motivation: To convey the hierarchical metabolic pathways knowledge to software engineers when developing biochemical applications is a frustrating chore for biochemists. Our goal is to provide a simple method to present the behavior of metabolic pathways in a graphical and dynamic ways, and hope that this method is acceptable and helpful to both biochemists and programmers.

Petri Nets, as a formal dynamic model, have been widely used in the fields of computer science and biological processes to model systems. Computer networks and genetic network are two typical examples. Since the reactions in a metabolism are dynamic and variable, it is adequate to simulate these processes with Petri Nets. Therefore, in this study, we propose a Petri-Nets-based discrete event system, called Workflow Petri Nets (WF Nets for short), with which workflow control functions can be used to effectively model and simulate the regulation and cooperation of biochemical reactions in metabolic pathways.

With the model, the biochemical properties of metabolic pathways in an organism can be presented concretely. The model can also be used as a educational system to tell students how metabolic pathways work or as a prediction tool to predict the possible biochemical reactions. Through the simulation researchers can realize the reason of a metabolic disease and even develop new drugs.

Our methodology is mainly based on WF-Net, which is an extended version of typical Petri Nets, particularly suitable for modeling the control behavior of the conversions and interactions of metabolites in biochemical reactions.

# 1. Introduction

In recent years, bioinformatics research has been transferred from genomics to proteomics[1]. The latter is the research on the structure, kinetics, functional analysis of proteins, and, above all, the reconstruction and prediction of metabolic pathways regarding to specific proteins (e.g., enzymes)[2]. All functions of organisms are achieved by biochemical reactions among metabolic pathways. These reactions catalyzed by enzymes are usually performed by reactants and products. Generally, the studies of metabolic pathways mainly emphasize the indispensable procedures to discover how proteins function, and the best ways to explain how genes regulate the phenomena of lives.

From biologist's viewpoint, understanding how enzymes work in metabolisms is especially helpful in deciphering the blueprint of life and in studying how genes work. That is why nowadays data modeling of metabolic pathways has become one of the important topics in computational biology[3].

A chemical reaction is a process that converts chemical substances to another kinds of substances. The substances initially involved in chemical reactions are called reactants, whereas the substances produced after chemical reactions are called products. The materials that catalyze reactions are called catalyzers or more commonly enzymes.

Symbolic description, one of the symbolic execution mechanisms, uses symbols to represent the reactants and products of biochemical reaction[4]. For example, triangles present proteins, and squares are compounds. A symbolic system is very often a friendly system through which biologists can effectively present the detailed steps and phenomena of a biological mechanism. However in such a mechanism, it is hard to describe the quantity changes of each reactants and products in large scale. Currently, there is no proper methods that can effectively describe the conditions needed by a reaction, e.g., in what circumstance a reaction can proceed.

For biologists, the static graph presentation is the most intuitive method to model a metabolic pathway. However, static graphs lack the ability of simulating dynamic bioprocess. These processes widely appear in most main metabolic databases, e.g. KEGG[5], WIT[6], and MetaCyc[7].

A Petri Net, as a strict mathematical model, can be used to model formal

concepts, such as linear algebraic equations and application of the probability theory so as to investigate the behavior of the modeled system. That is why Petri Nets have been widely utilized in many fields as the modeling and simulation tools.

Guy Karlebach,*et al*. 8, have destributed a review that summarized some studies based on Petri Net. Anyway, their approach did not deal with the biological control mechanism. In this study, we proposed a discrete event system, called Workflow-Petri-Nets-based Model (WFPeM for short), which can simplify the representations and data modeling of metabolic pathways, so as to make these tasks easier for those who are not familiar with molecular biology. We hope that our works can help them understand how lives work via graphic presentation. The pathways of fatty acids that generate adenosine triphosphate(ATP) is chosen as the targets. We also build Petri Net models for energy generation process of lipid and describe control mechanisms with workflow presentation.

The rest of this thesis is organized as follows. Chapter 2 briefly review background and related research of this study. Chapter 3 introduces Petri Nets and the tools that we use to model and simulate metabolic pathways. Data modeling examples are presented and discussed in chapter 4. How to simulate the metabolic pathways is explained in chapter 5. Chapter 6 concludes this study and addresses our future work.

# 2. Enzymes and Metabolic pathway

In this chapter, we introduce the roles that enzymes plays in metabolism, and some basic concepts of metabolic pathways. Several existing studies of metabolic pathway modeling approaches are also briefly introduced.

## 2.1. Enzymes and Metabolic Pathway

Proteins, essential parts of all living organisms, participate in every process within cells. Enzymes are a special kind of proteins that catalyze biochemical reactions in metabolism[9]. Different proteins have different structural or mechanical functions, e.g. the proteins in the cytoskeleton forms a system of scaffolding that maintains cell shape. Proteins are also important in cell signaling, immune responses, cell adhesion, and the cell cycle[10].

Protein molecular is also a type of linear polymer made of 20 different kinds of amino acids. Every two amino acids are linked by carboxyl atom of one amino acid and the amine nitrogen of the other. These bonds are called peptide bonds, and the short amino acid sequences are called polypeptides, which join together to form the primary structure of a protein.

Metabolic pathways, as parts of metabolism, are series of biochemical reactions which, catalyzed by enzymes, occur within a cell. A metabolic pathway is normally used to describe the formation of some specific metabolic products, or to initiate another metabolic pathway, called a flux generating step. Many pathways involve a step-by-step modification of the initial substance to shape the substance into the product with the exact chemical structure desired.

## 2.2. Related work

Many studies concerning Metabolic Pathways by means of computers and information technologies have been published[11] [12] [13].

A number of computer programs have been designed to assist biochemists to model and simulate metabolic pathways[14] [15]. Most of them utilized reaction equations, mathematics modules, symbolic description, stoichiometric matrices, and graph presentation, which are briefly introduced below.

Reaction equations as the formal notations of chemical reactions are used to

construct the computational data model for chemical reaction[16]. But this kind of model is merely a qualitative representation. It cannot describe the circumstance that a reaction will be initiated or terminated.

Cornish-Bowden *et al.*[17] first took chemical kinetics into account and developed METAMODEL which is a program to calculate steady-state fluxes and metabolite concentrations of metabolic systems. However, it utilized many mathematics modules, making it difficult to be understood.

Christophe *et al.*[18] used the stoichiometric matrix to reflect the functional capabilities of biochemical reaction networks. The authors presented an algorithm for the synthesis of a set of basis vectors for spanning the null space of the stoichiometric matrix. The basis vectors represented the underlying biochemical pathways fundamental to the corresponding biochemical reaction networks. Hong Q., *et al.*[19] also demonstrated both Kirchhoff's flux law over a biochemical species and potential law over a reaction loop, and developed a theory for nonequilibrium steady-state biochemical systems applicable to analyze large-scale complex isothermal reaction networks.

Hofestädt *et al.*[20] first showed that the theory of Petri Nets is suitable for the quantitative modeling of biochemical networks. This model, due to its mathematic nature, not only provides static state representation, but also supports dynamic simulation of metabolic pathways. In fact, it is a discrete event system models of biochemical pathways. However, their work are mainly focused on kinetics of enzyme and do not deal with the control mechanism of bioreaction.

# 3. Petri Nets and Workflow Nets

## 3.1. Petri Nets

A Petri Net as shown in Figure 3.1 consists of places, transitions, tokens and directed arcs. Places are drawn as circles, transitions as rectangles and arcs as arrows. Arcs are used to connect places and transitions. Input arcs connect transitions to places, whereas output arcs connect places to transitions. Places from (to) which arcs connect to (from) a transition are called the input places (output places) of the transition.

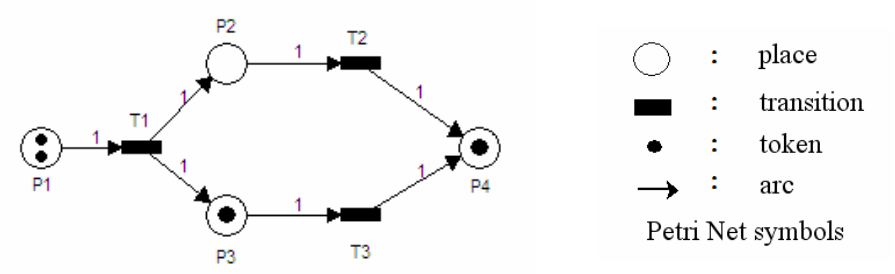
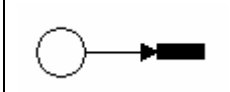
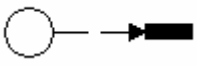
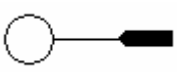



Figure 3.1 An example of a Petri Net

The arc of Petri nets has been expended to three types by David and Alla[21]. They are normal arc, static test arc and inhibitor arc. The corresponding biological meanings in this study are showed as follow:

Table 1 Expanded Arcs for Petri Nets and their biological meaning where M is the number of tokens in a place, and W is the weight of an arc.

| Arc Type | Graph | Enabling Condition | Token Remove from Input Place | biological meaning |
|---|---|---|---|---|
| Normal arc |  | M ≥ W | Yes | A reactant participating a reaction |
| Static test arc |  | M ≥ W | No | An enzyme catalyzing a reaction |
| Inhibitor arc |  | M < W | No | A compound that inhibit a reaction |

Examples of biological meaning of these three arcs are showed in Figure 3.2. The arc that connects place reactant to transition reaction and the arc that connects transition reaction to place product are normal arcs. The arc that connects place inhibitor to transition reaction is an inhibitor arc. The arc that connects place enzyme

to transition reaction is a static test arc. This Petri Net works only if there are tokens in both place reactant and place enzyme, and there is no token in place inhibitor. Therefore, only the (a) can work properly and (b), (c), (d) do not work.

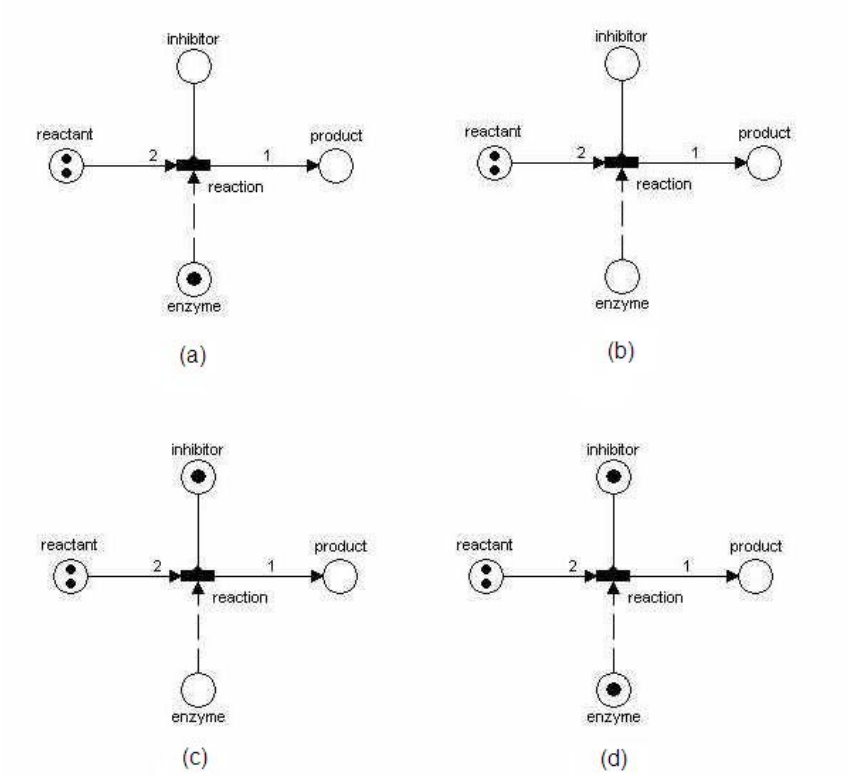



Figure 3.2 biological meaning of arcs.

(a) There is a token in place enzyme. This net works properly.

(b) There is no token in places enzyme and in place inhibitor. This net doesn't works.

(c) There is no token in places enzyme, but there is a token in place inhibitor. This net doesn't works.

(d) There is a token in places enzyme and a token in place inhibitor. This net doesn't works.

## 3.2. Workflow Nets

Nowadays, Petri Nets have been widely utilized to model business processes and manage workflows. For instance, the Event-driven Process Chain (EPC) notation used in the well-known Architecture of integrated Information Systems (ARIS) methods and many other business process modeling languages were mainly inspired by Petri Nets[22]. In 1996, a special class of Petri Nets called Workflow Nets (WF Nets), which extends the original Petri Net notation by including lots of useful features needed in the area of business processes modeling without sacrificing the mathematical nature of the underlying Petri Net formalism, was introduced[23]. However, there are some

characters that WF-Nets do not support, such as the weight of arcs, and extended arc types, e.g., inhibit arc and static test arc mentioned previously.

A WF-Net has a very regular structure which contains at least one place without any incoming arcs as the input place, and at least one place without any outgoing arcs as the output place. The two kinds of places must be strongly connected, i,e., from each input place there exists one or more directed paths to any output place.

### 3.2.1 Operator Transitions

Four special transition types including AND-split, AND-join, XOR-split and XOR-join are added to express branching situations in a more compact way. Each of them as shown in Figure 3.3 is associated with a graphical symbol.

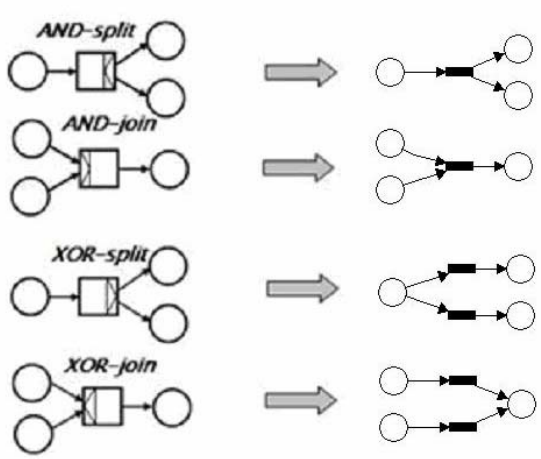



Figure 3.3 Four special Transition types of a Workflow Nets

### 3.2.2 Triggers

Since triggers are unexpendable elements for workflows, four distinct triggers has been added to the transitions of standard Petri net notations. With the four triggers, different kinds of dependency between a task of the workflow process and its operative environment can be clearly presented.

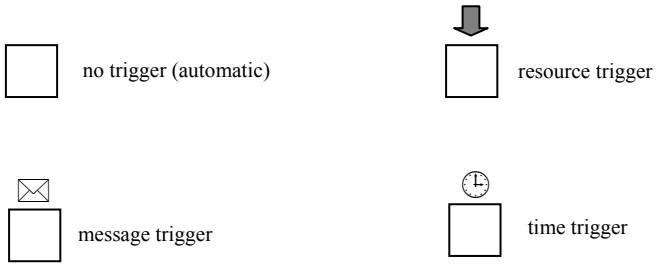



Figure 3.4 Triggers of workflow net

1. No trigger (automatic) : Task execution, done automatically e.g., the combination of oxygen and hemoglobin is independent from its environment.
2. Resource trigger : Task execution depends on the availability of a certain situation or factor instance, e.g., breaking down of glycogen to glucose when the blood sugar concentration is low.
3. Message trigger : Task execution has to wait for arrival of an external signal or event, e.g., initialing a certain segment of DNA to be translated into RNA.
4. Time trigger : Task execution depends on a time-related event, e.g., human estrogen is produced monthly.

Users are allowed to assign exactly one trigger type to a transition of a WF net. A trigger type as shown in Figure 3.4 is graphically represented by a small, self-explaining icon near the associated transition symbol.

### 3.2.3 Basic routing primitives

We can use the transitions defined above as control flow elements to identify four basic routing primitives (see Figure 3.5), including sequential routing in which task A is executed before task B, concurrent routing in which task A and task B are executed in parallel, alternative routing in which either task A or task B is executed, and iterative routing in which task B is repeated.

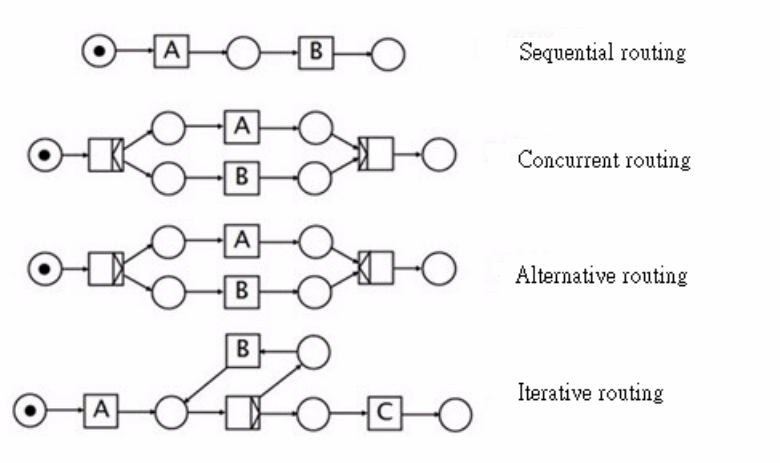



Figure 3.5 four basic routing primitives

There are several available notations which can associate workflow tasks to resources, and with which users can assign one or more resource classes to a resource-triggered transition. These resource classes, which consist of groups and roles, must be defined and maintained in a separate resource model[24]. Elements of a WF Net can be assigned the following quantitative performance parameters, including average number of cases per time unit (arrival rate), average execution duration of a workflow task, and branching probabilities of XOR-Splits.

Soundness is the key property of a WF Net, impling that the model is structurally well-formed (as covered by the definition of a WF-Net), and behaviorally well-formed. If there is only one token in the input place and no tokens exist elsewhere, we call this place the initial marking. If there is only one token in the output place and no tokens exist elsewhere, we call this the final marking. Each execution of the net which starts from the initial marking will eventually lead to the final marking, implying that no deadlocks exist, except that the final marking is a "wanted" deadlock, and the net is bounded.

### 3.2.4 Subprocesses and weights

A subprocess concept is added to handle the graphical and logical complexity of a large WF Net by dividing that net into smaller modules. So that a workflow process can contain another WF Net as one of its parts. Note that a subprocess, a transition symbol surrounded by a rectangle (called a sub net), must be a sound WF Net to support this simple and flexible plug-in concept(see Figure 3.6).

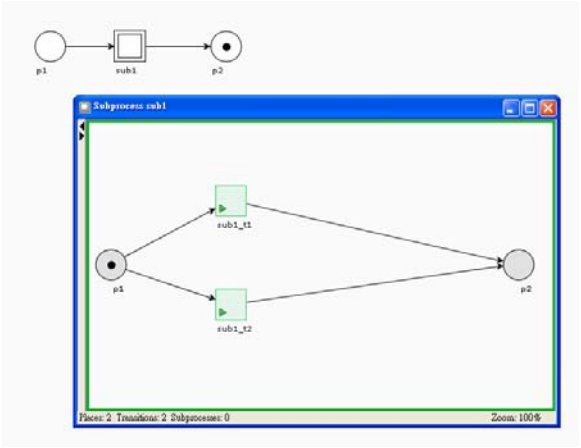



Figure 3.6 A subprocess of WF Net showing how it works.

Though WF-Net is more powerful than original Petri Net in modeling business processes, it is a little bit inconvenient when utilized in biochemical reaction. For example, a WF Net does not support the weight property of an arc. To achieve this kind of task we add an XOR-split transition and an AND-join transition with several places to a WF Net(see Figure 3.7).

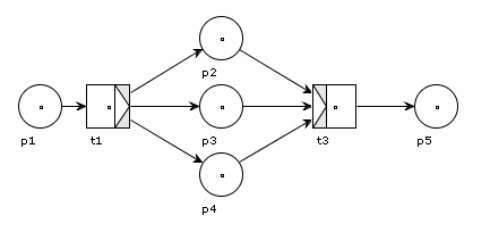



Figure 3.7 Performing the weight property with a WF-Net. The equivalent component in a Petri Net is. an arc with Wight 3

# 4. Modeling Metabolic Pathways

## 4.1. System architecture

The metabolic pathway data in the study is abstracted from the book[25]. The corresponding EC numbers of enzymes are from KEGG LIGAND. We use HPSim 1.1 (http://www.winpesim.de/) and WoPeD 2.3 (http://www.woped.org/) to build Petri Net models and workflow controls, respectively, since currently no tools can fuulfill our needs. Generally, WoPeD that supports the extended "van der Aalst"-like workflow net[26] syntax with special split and join transitions (AND, XOR) and four trigger types (automatic, resource, message, time), can be used to visualize the structures and the dynamics of workflow nets so that users can understand the underlying concepts of workflow more deeply.

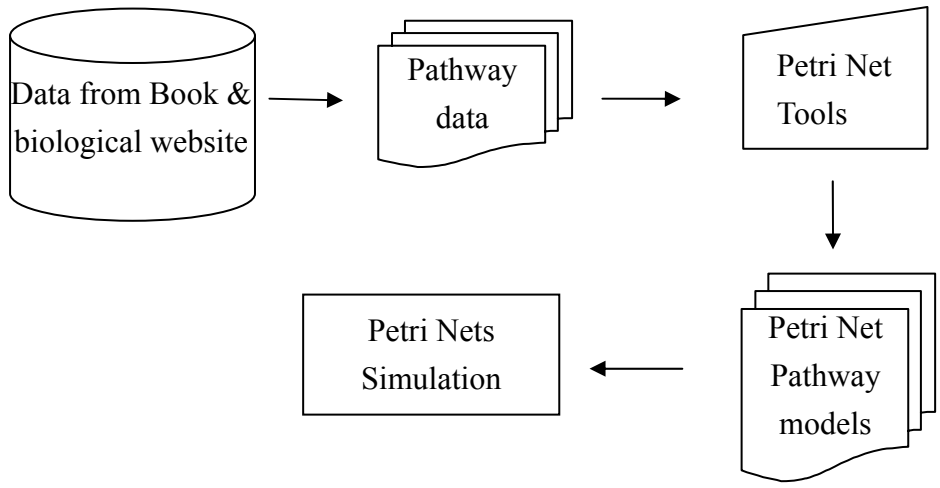



Figure. 4.1 Framework of proposed Modeling and Simulation

The framework of this study as shown in Figure.4.1 has a data source, i.e., biochemistry text books from which pathway data is collected. Petri Net tools are used to generate a set of Petri Net pathway models, which contain compounds that participate in a metabolism, such as reactants, products, enzymes, and co-factors, and through which biochemical equations are presented in computational ways. Hierarchical structure[27] is also utilized to achieve effective visual effects.

## 4.2. Metabolic Pathway

### 4.2.1. Numerical Classification of Enzyme

An enzyme name is often derived from the substrate or the chemical reaction the enzyme is involved[28]. It is generally with the suffix ***–ase.*** For example, lactase, alcohol dehydrogenase and DNA polymerase which are typical enzyme nomenclatures. But this may result in the fact that different enzymes with the same function, which is called an isoenzyme, are given the same basic name. An isoenzyme has a distinct amino acid sequence and might be differentiated by their kinetic properties, such as pH range and immunological attributes. Besides, a biochemical reaction that an enzyme catalyzes may result in different products if the reaction conditions change, consequently causing an enzyme to be delicately identified and then given two different names. A typical example is that glucose isomerase, which is industrially used to convert glucose into the fructose, is also a xylose isomerase.

To reduce such a confusing phenomenon, the International Union of Biochemistry and Molecular Biology (IUBMB)[29] has developed a nomenclature for enzymes called Enzyme Commission number (EC numbers)[30] which is a naming convention that an enzyme is described by a set of four number digits preceded by "EC."

Generally, the four digits are given based on the enzyme's function. The first three approximately define the reactions the enzyme catalyzes and the fourth is merely a serial number called unique identifier.

These four numbers separated by periods indicates a progressively finer classification of an enzyme. For example, EC 3.4.11.4, the tripeptide aminopeptidases, indicates the following groups of enzymes:

- EC 3 denotes hydrolases (enzymes that use water to break up some other molecule).
- EC 3.4 denotes hydrolases that act on peptide bonds.
- EC 3.4.11 denotes those hydrolases that cleave off the amino-terminal amino acid from a polypeptide.
- EC 3.4.11.4 denotes those that cleave off the amino-terminal end from a tripeptide.

As a system of enzyme nomenclature, an EC number is often or always associated with a recommended name for the respective enzyme.

Strictly speaking, an EC number does not specify the enzyme itself, but the

reactions it catalyzes. If two enzymes can catalyze the same reaction, they may have the same EC number, even though they might come from different organisms and have different amino acid sequences.

## 4.3. Energy generation from catabolism of lipids

The energy that an organism can utilize is normally in the form of ATP. Most ATP is produced by the oxidation of carbohydrate, protein and lipid.

### 4.3.1 lipid and carbohydrate

Lipid provides perhaps half of the total energy required by heart and resting skeletal muscles. The fatty acid components of neutral fat (TAG) are the most important for energy production so we use the oxidation of certain fatty acid as our study material. Glucose and fatty acid have the same effect on producing acetyl-CoA (acetyl co-enzyme) at the last process. Glucose, for example, can be oxidized to form acetyl-CoA, which is fed into the citric acid cycle to generate ATP molecules, and so can fatty acid. Two carbon atoms are detached from the glucose or fatty acid molecule at a time to form an acetyl-CoA. Though different in structure, fatty acid oxidation differs from glucose oxidation only in the preliminary formation of acetyl-CoA. Further, in this preliminary processing, NAD+ and FAD are reduced, and the electron they carry is fed into the electron transport system as in glucose oxidation. The relationship between glucose and fatty acids oxidation is shown as Figure 4.2.

Because fatty acid oxidation involves the same citric acid cycle and electron transport system as glucose oxidation, the fatty acid oxidation process is relatively simple for those who are familiar with glucose oxidation. What this study concerns is the catabolism of lipid that chops up fatty acids by removing two carbon atoms at a time as acetyl-CoA to produce bioenergy.

Actually this noteworthy phenomenon of using the same machinery for obtaining energy from all classes of foodstuffs can also be seen in many metabolic pathways. Energy producing processes for different foodstuffs are almost the same except in the preliminary reactions.

Fatty acids are degraded by removing two carbons at a time as acetyl-CoA recurrently. During the conversion process, the fatty acids always in an acyl-CoA form. That is, in the initiation of oxidation, the fatty acids always convert themselves to fatty acyl-CoA compounds. This reaction is called fatty acid activation.

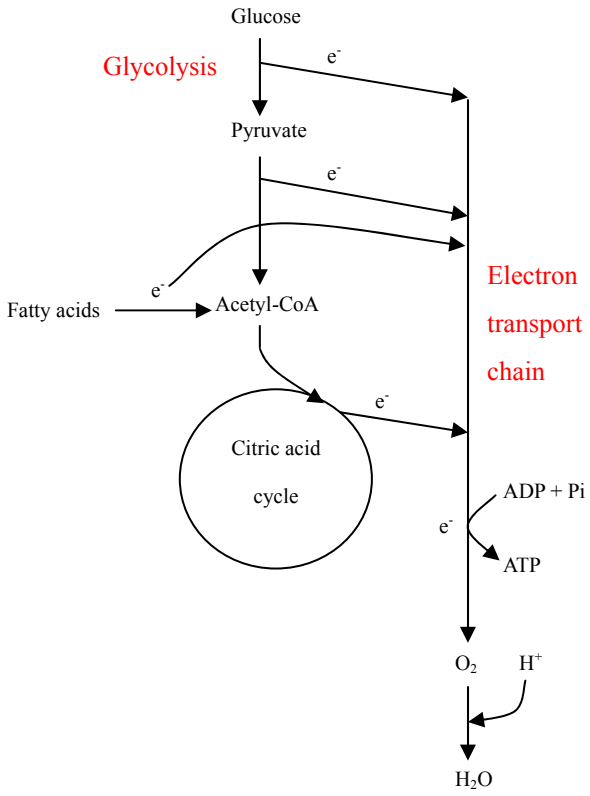



Figure. 4.2 A simplified diagram of the relationship between fatty acids and glucose oxidation. $e^-$ is the electron produced.

## 4.3.2 Mechanism of acetyl-CoA formation from fatty acids

### 4.3.2.1. Activation of fatty acids

The activation reaction of a carboxylic acid is

$$RCOO^- + ATP + CoA\text{-}SH \rightarrow RCO\text{-}S\text{-}CoA + AMP + PPi \quad \Delta G^{0'} = -0.9 \text{ kJ mol}^{-1}$$

Because of the high energy of the thiol ester, the free-energy of this reaction is small. But the hydrolysis of the PPi by the ubiquitous enzyme, i.e,. inorganic pyrophosphatase, makes the overall process strongly exergonic and irreversible ($\Delta G^{0'} = -32.5$ kJ mol$^{-1}$). There are three different kinds of fatty acyl-CoA synthetases (EC 6.2.1.3) that are specific for short, medium, and long chain acids, respectively.

### 4.3.2.2. Transporting fatty acyl-CoA derivatives into mitochondria

Activation of fatty acids occurs on the outer mitochondrial membrane. But the conversion of fatty acids to acetyl-CoA occurs only in the mitochondrial matrix. Because fatty acid molecular is too large to pass through the membrane, the CoA of fatty acyl-CoA is removed. Only the acyl group can be transported into mitochondrion by a special transport mechanism and then react with CoASH to become fatty acyl-CoA again. The high-energy acyl-bond is preserved during the transport, so the reform of fatty acyl-CoA needs no extra energy expenditure. To achieve this, the acyl group is transferred to an odd hydroxylated molecule called carnitine outside the mitochondrion. To be clear, the carnitine can be thought as a vehicle that carries an acyl group to pass through membrane and enter the mitochondrion.

Although a carboxylic ester is usually of the low-energy type, the acyl group has a high group transfer potential, making the fatty acyl-carnitine bond belonging to the high-energy type. This is also why carnitine becomes the carrier molecular in this transport system. When the fatty acyl-carnitine is transported into the mitochondrial matrix, its carnitine will be replaced by a CoA-SH. The carnitine will be sent back to the cytoplasm to transfer another fatty acyl group into a mitochondrion (see Figure. 4.3).

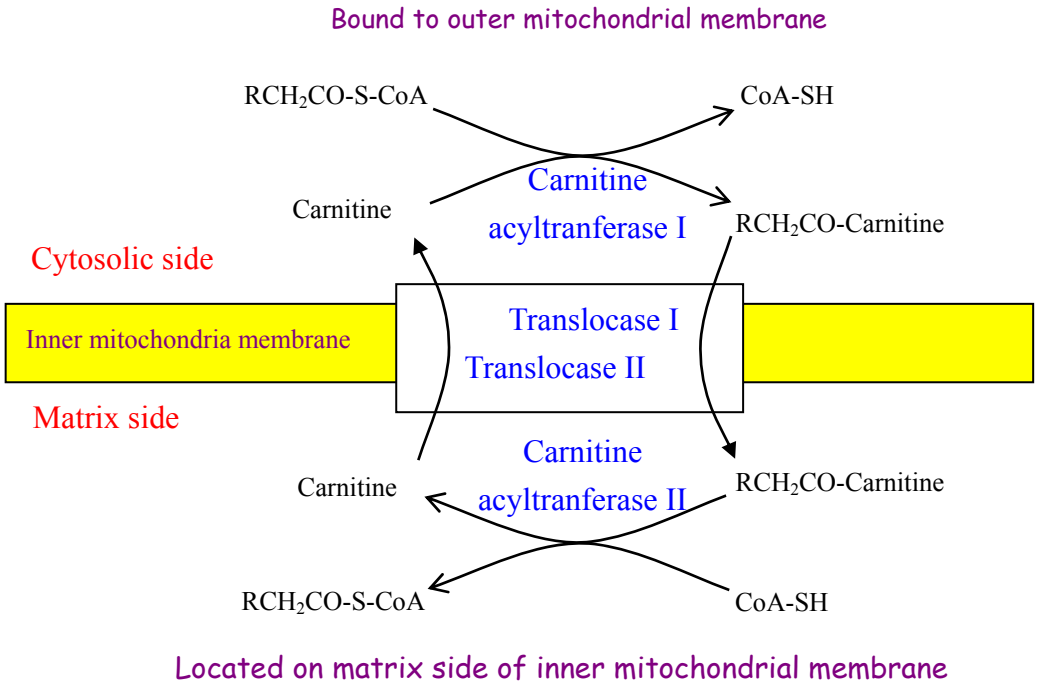



Figure 4.3 Mechanism of transferring $RCH_2CO$-S-CoA into mitochondria by carnitine.

The acyl transfering from fatty acyl-CoA to carnitine is catalyzed by an enzyme called **carnitine acyltransferase I** on the cytoplasmic side of the mitochondrion. And the acyl transfer from fatty acyl-carnitine to CoA-SH is catalyzed by an enzyme called **carnitine acyltransferase II** on the matrix side. According to the nomenclature of IUBMB, these two enzymes share the same EC number, even though they are actually two different kind of enzyme, and so do Translocase I and II.

### 4.3.2.3. Conversion of fatty acyl-CoA to acetyl-CoA molecules inside the mitochondrion

This process of converting a fatty acyl-CoA to acetyl-CoA molecules as shown in Figure 4.4 is composed of four separate reactions. The ketoacyl-CoA is cleaved by CoA-SH, consequently splitting off two carbon atoms as the form of acetyl-CoA and forming a shorter fatty acyl-CoA derivative. Since the molecule is split by the –SH group of CoA-SH, the enzyme is named a thiolase (EC 2.3.1.* ). The free energy is preserved as thiol ester of fatty acyl-CoA by the thiolase reaction.

The fatty acyl group would undergo seven successive rounds of acetyl-CoA producing process until it is shortened to the $C_4$ stage (butyryl-CoA). Then the butyryl-CoA is further transformed into a acetoacetyl-CoA in the last round of reaction, and is finally split by CoA-SH into two molecules of acetyl-CoA. A specific thiolase in mitochondria is responsible for this reaction.

$CH_3COCH_2CO\text{-}S\text{-}CoA + CoA\text{-}SH \rightarrow 2CH_3CO\text{-}S\text{-}CoA$

Acetoacetyl-CoA  Acetyl-CoA

Each round of the repeating reactions involves conversion of saturated fatty acyl-CoAs to $\beta$ –ketoacyl-CoA. Therefore this process is considered as a $\beta$–oxidation of fatty acids. And the NADH and the $FADH_2$ feed electrons into the electron transport to produce energy (ATPs).

### 4.3.3. Biochemical Reaction Model

As chemists use different chemical reactions in combinations of chemical synthesis process to produce a desired products. Metabolic pathways are composed of series of chemical reactions catalyzed by enzymes.

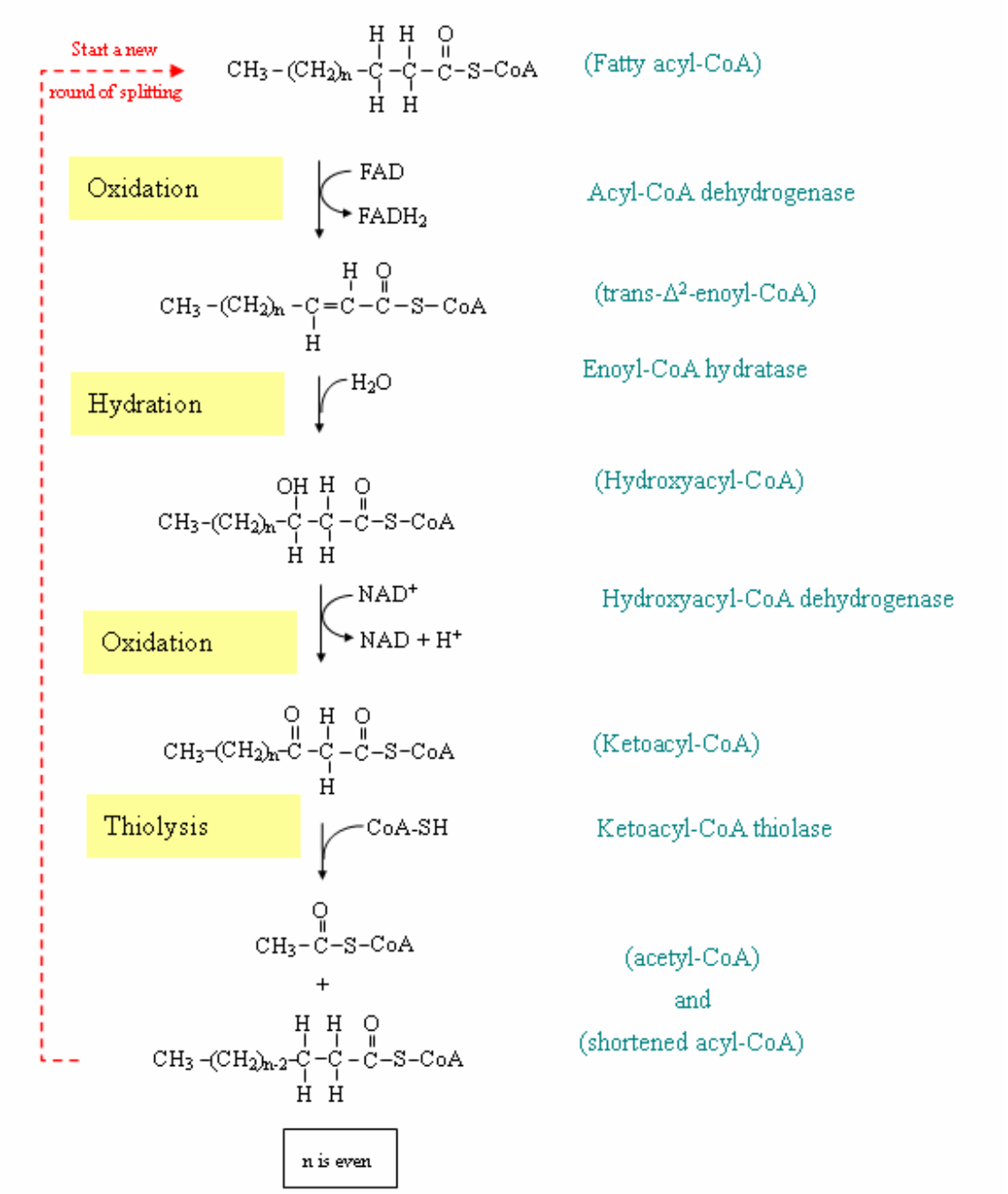



Figure 4.4 A C16 fatty acyl-CoA is shortened by two carbon atoms with the production of a molecule of acetyl-CoA. This process is composed of four reactions.

The protocols in WF-Nets are basically the same as Petri Nets, except those flow control mechanisms, which are presented by operator transitions and triggers. We use transitions without any triggers or operators, which are labeled with E.C. number or a short description, to represent specific biochemical reactions. If the enzyme of a reaction is well known, an extra place is added and connected to the transition with a

two-way arc. Operator transitions and triggers are used to describe the factors that induce or affect reactions to proceed, and they do not refer to any biochemical reactions directly.

### 4.3.4. Dynamic Petri Net Graph

In addition to places and transitions, tokens in a simulation process can also be added to a Petri net to represent status of a place and transition so as to reflect the dynamic behaviors of the simulated system. A function U is also used to denote the number of tokens in a node, i.e., a place. For instance, $U(P_i)$ is the number of tokens in place $P_i$, $U(P_i) \geq 0$, and keeps changing during the simulation process. An arc that connects a place $P_i$ and a transition $T_j$ is given a weight $W(P_i, I(T_j))$ which as an integer and is also the firing threshold used to determine whether the arc could transfer tokens from place $P_i$ to the transition $T_j$ , where $I(T_j)$ is the input transition $T_j$. If $U(P_i) \geqq W(P_i, I(T_j))$ , $T_j$ will be fired, inducing two actions.

(1) Number of tokens in place $P_i$ is subtracted by the weight $W(P_i , I(T_j))$.

(2). Number of tokens in the next place $P_k$ is increased by $W(P_i, I(T_j))$.

### 4.3.5. Simulation of Biochemical Reaction

The dynamic property of Petri Nets is suitable for modeling and simulating biochemical reactions, especially the metabolic pathways. For instance, the Petri Net can be used to simulate the formation of water molecular from two hydrogen and one oxygen atoms (see Figure 4.5).

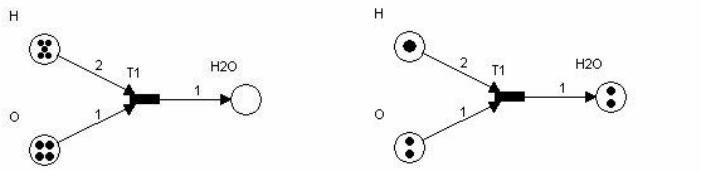



Figure 4.5 The simulation of the formation of water molecular

## 4.4. Petri Nets Models of Metabolic Pathways

The Biochemical processes in organisms can be described at many levels of details, ranging from atomic mechanisms to general processes such as bioenergetics, signal transduction or hormone actions.

Here, we use the Petri Nets to model biochemical reactions. Reactants, products and co-enzymes, e.g., acetyl-CoA and CoA-SH, are represented by places. Reactions which are catalyzed by enzymes are presented by transitions. Since the biochemical

reactions are always associated with specific enzymes, the arcs are used to indicate the direction of the reaction. The weight of an arc is the quantity of reactant molecules that are needed or the quantity of product molecules that are produced in a reaction.

Transitions with an EC number represent the reactions that mainly utilize a group of enzymes to catalyze specific biochemical processes. EC numbers instead of the chemical nomenclature are used here because different enzymes ( including unknown enzymes) may catalyze the same reaction. If the enzyme is already confirmed, an extra place and test arcs are used to connect the corresponding transition. If a reaction does not need any enzyme, it is merely labeled with a short text, for example "spontaneous".

The Petri Nets model of phosphorylation of glucose catalized by the enzyme hexokinase is shown in Figure 4.6.

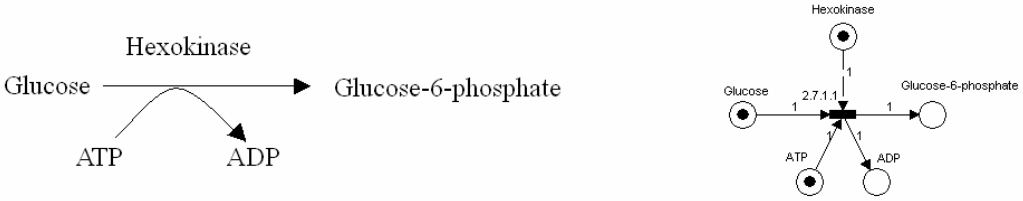



Figure 4.6 Petri Net model of phosphorylation of glucose

### 4.4.1. Examples of Petri Net Model of Lipid Metabolism

Since the transport of fatty acyl-CoA derivatives into mitochondria as shown in Figure 4.3 is the initial point of the metabolism of fatty acids. We use this mechanism as our first Petri Net model (see Figure 4.7).

It takes four steps to transfer long chain fatty acyl groups into mitochondria. The Carnitine will be transferred out of mitochondria at the last step and then restarted the mechanism again. The acyl-carnitine bond is a special ester bond that has a high group transfer potential. Because the compound is of the high-energy type so that exchange of carnitine for CoA-SH inside the mitochondria needs no extra energy inputs.

For instance, in the beginning of this metabolic pathway, there are some $RCH_2CO$-S-CoA, carnitines and no CoA-SH in the cytosolic side. No

$RCH_2CO$-S-CoA, no carnitines and some CoA-SH exist in the matrix side. When the process begins, place $RCH_2CO$-S-CoA and place carnitines each pass a token to transition 2.3.1.137 according to the weight assigned to each arc. Transition 2.3.1.137 forwards a token to each place CoA-SH and place $RCH_2CO$ carnitine. The token in place $RCH_2CO$ carnitine is sent to the transition 2.3.1.21 and then to place $RCH_2CO$ carnitine. When the token enters the matrix side, it was forwarded to the transition 2.3.1.137 with another token from place CoA-SH. Transition 2.3.1.137 consequently sends a token to each place $RCH_2CO$-S-CoA and place carnitine. Thus, one $RCH_2CO$-S-CoA molecule is transferred into the mitochondria and the carnitine is sent out to the cytosolic side to help another $RCH_2CO$-S-CoA molecular to be transferred to matrix side. Through this demonstration, we can see that the role which carnitine plays is just like a vehicle. It is utilized again and again to transfer a lot of $RCH_2CO$-S-CoA into mitochondria, which particularly suitable for Petri Net simulation. Therefore, if someone wants to inhibit the reaction that transfer $RCH_2CO$-S-CoA into mitochondria, to interfere the function of carnitine acyltransferase I should be the best method. To be honest, it is difficult for students who are taking their first university course in biochemistry to infer from the chemical equation in Figure 4.3 in which there is nearly no change in the amount of carnitine before and after the chemistry reaction, and not even to say that they could notice the role which a specific reactant may play in designing a new drug. With the use of Petri Net modeling and simulation, even recruits could have a clear concept about what is happening in the metabolic pathway, not merely a collection of tiring static biochemical formulas.

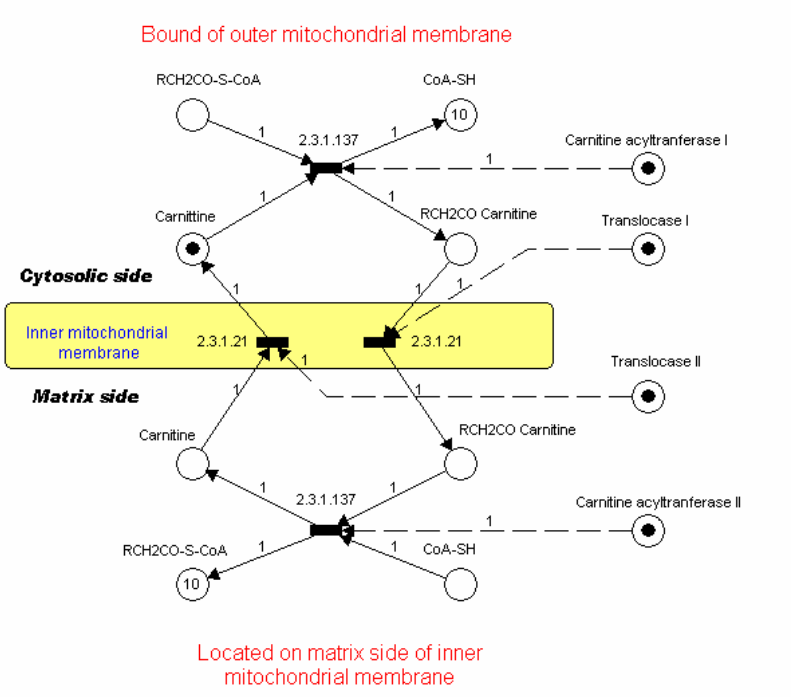



Figure 4.7 Petri Net Model of a transport mechanism that transports long chain fatty acyl groups into mitochondria.

Another Petri Net model as shown in Figure 4.8 is the process that converts fatty acyl-CoA to acetyl-CoA molecule inside the mitochondria (see Figure. 4.4 ). As stated above, this process is a biodegradation that consists of four reactions in each round of splitting off two carbon atoms as an acetyl-CoA. The process is presented as a cycling mechanism to denote each round of biodegradation of fatty acyl-CoA. For example, a molecule of palmitic acid ($C_{16}$) is converted to eight acetyl-CoA molecules, and in this process seven $FADH_2$ molecules and seven NAD molecules are also produced. But there is a problem with the last run of the reaction.

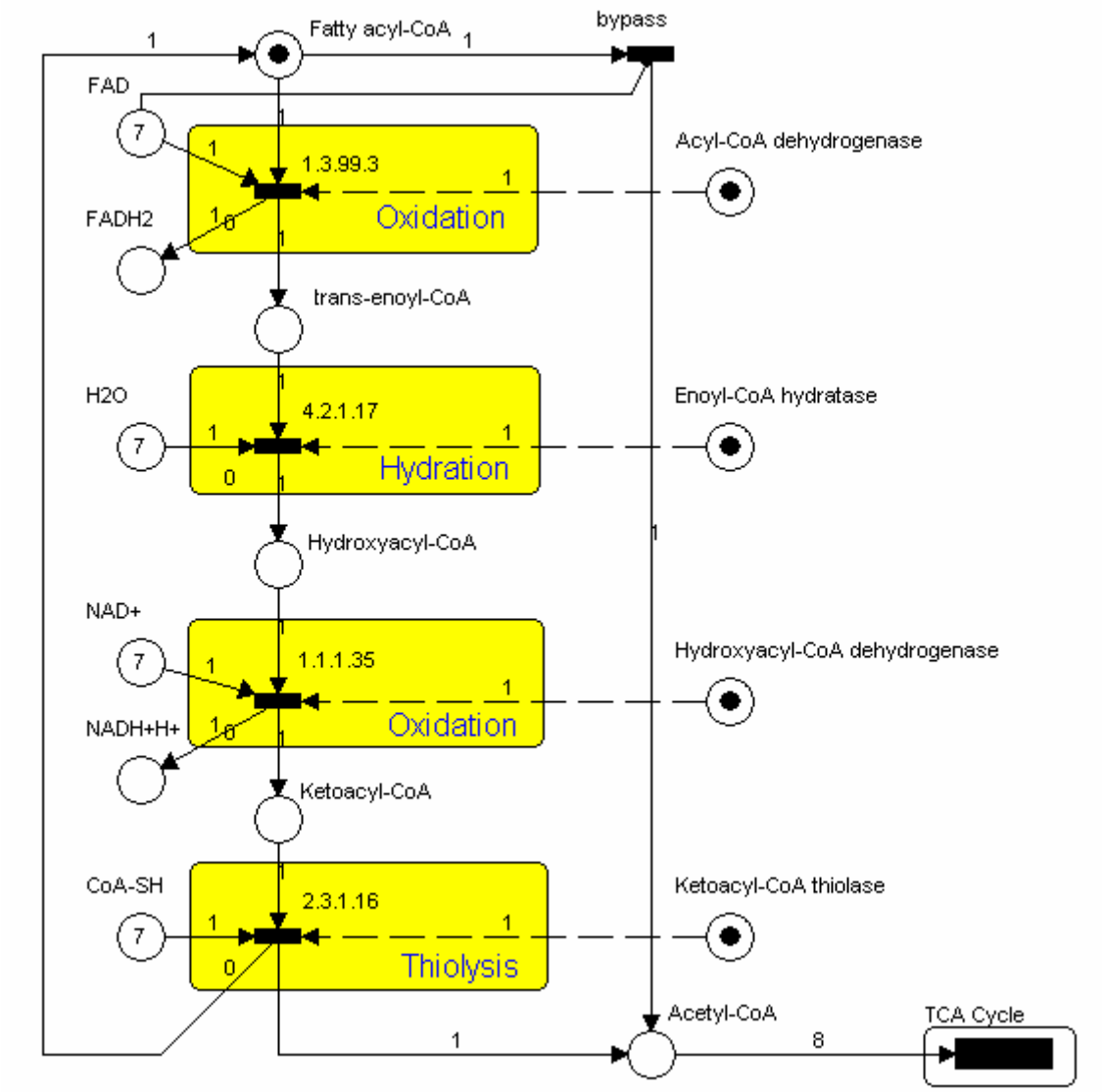



Figure 4.8 A Petri Nets Model of biodegradation of fatty acyl-CoA to acetyl-CoA as shown in Figure

## 4.4.2. Hierarchical Petri Net Model of Metabolic Pathways

A complete metabolic pathway is like a huge network atlas composed of

numerous interconnections of biochemical reactions. A metabolic pathway keeps growing while more biochemical reactions have been confirmed, making the metabolic pathways become too complicated to be read and understood. Therefore, it is a good idea to present a metabolic pathway with a hierarchical method.

A metabolic pathway can be divided into many small parts according to their biochemical functions, e.g., the energy producing process for foodstuffs can be divided into digestion, absorption, transportation, glycolysis, fatty acid $\beta$–oxidation, citric acid cycle and electron transport system. Many compounds and intermediates are shared by different pathways. Products of a pathway may be reactants of another pathway. Thus we can divide the places in hierarchical Petri Nets models into public places and private places.

Public places are used to signify compounds and/or intermediates shared among pathways. So they usually play the role of the bridges between pathways. For example, acetyl-CoA is not only product of oxidation of glucose, but also product of oxidation of fatty acid. And all these acetyl-CoAs get into the TCA cycle, no matter where they are from.

The places, marked as private, are those compounds and/or intermediates that never contact other pathways until the reactions finish. Therefore, in a hierarchical Petri Nets model, private places are usually hidden unless we want to show the details.

We use larger (small) size places to denote public (private) places, and transitions contained in a rectangle to represent a specific sub-pathway. Since the processes in Figures 4.7 and 4.8 are successive (as shown in Figure 4.9), they are represented by a hierarchical method in which the sub-pathway transition labeled “transportation” represents the processes shown in Figure 4.7, and the sub-pathway transition labeled “$\beta$–oxidation” is the processes shown in Figure 4.8.

We implemented a hierarchical Petri Net model, shown in Figure 4.10, to describe the relationship between fatty acid and glucose in energy producing process shown in Figure 4.2. Note that the area labeled by Electron Transport Chain can be further represented by a sub-pathway in which there is another sub-pathway called Respiratory Chain used to denote the complex processes that convert ADP into ATP and release oxygen ion.

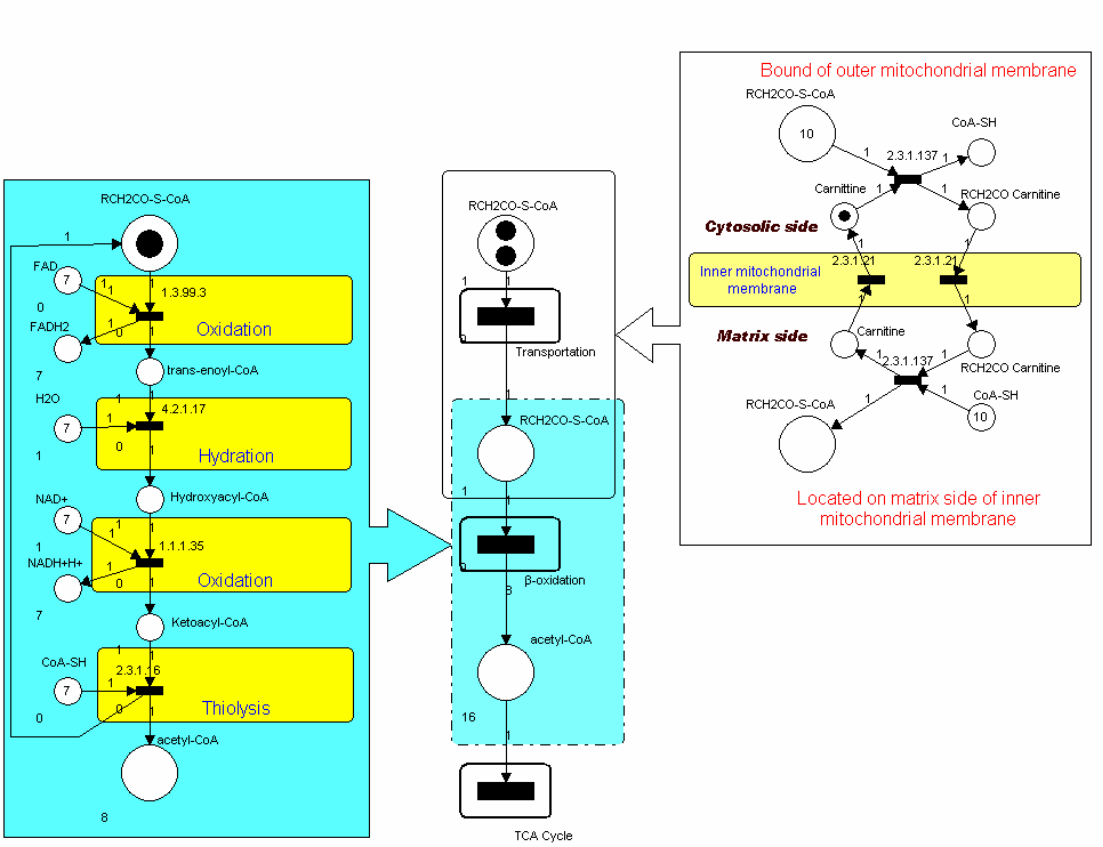



Figure 4.9. An example of hierarchical Petri Nets.
Note that the tokens move along the arcs

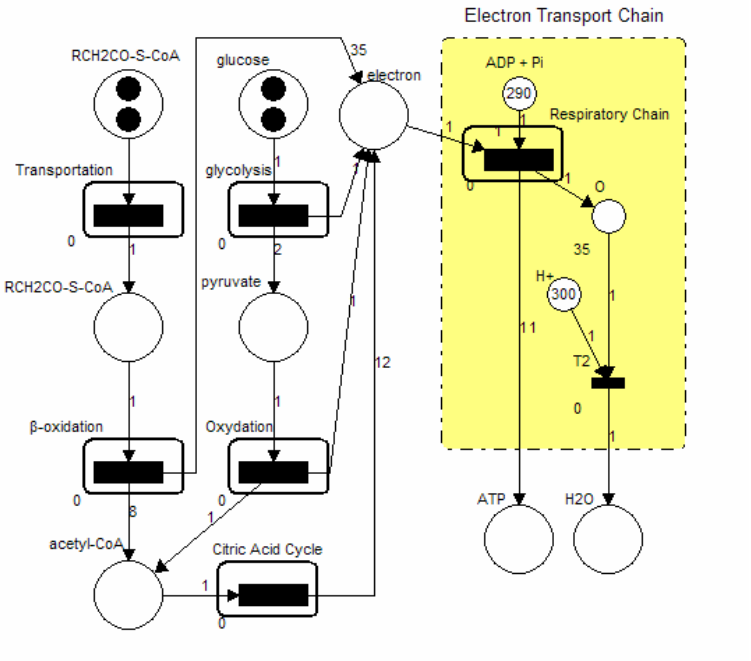



Fig.4.10. Hierarchical Petri Nets
Model of energy production process

## 4.5. Simulation of Metabolic Pathway

The dynamic simulation of Petri Nets shown in Figure 4.8 can be demonstrated by using palmitic acids. This fatty acid would undergo β-oxidation process seven times and produce eight acetyl-CoAs, seven $FADH_2$, seven NADH , and seven hydron cations.

$$C_{15}H_{31}COOH + 7\ FAD + 7\ NAD^+ + 7\ H_2O \rightarrow 8\ Acetyl\text{-}CoA + 7\ FADH_2 + 7\ NADH + 7\ H^+$$

Then, these eight acetyl-CoAs participating in TCA cycle are further oxidized to produce 145 $H_2O$ and 129 ATP. The present of a token in place “Fatty Acyl-CoA” represents that there is a Fatty Acyl-CoA, which is originally a palmitic acid molecule, available for the process of β-oxidation. Each run of the process detaches two carbon atoms from Fatty Acyl-CoA and converts this two carbon-atoms unit to a molecule of acetyl-CoA. When a palmitic acid molecule has undergone β-oxidation process 7 times, the reactions would stop because the residual is naturally an acetyl-CoA molecule already. Because there is no mechanism to determine how many times a loop should take place, we achieve the task by limiting the tokens of place FAD to 7.

The number of tokens in a place represents the actual molecule number of each kind of reactants or products in the place. Therefore, we can trace the concentration change of each reactant easily.

Table 4.1.lists the number of tokens in each place changes as the process is going.

| Place / Run | fatty acyl-CoA | FAD | $FADH_2$ | trans-enoyl-CoA | $H_2O$ | hydroxyacyl-CoA | $NAD^+$ | $NADH + H^+$ | ketoacyl-CoA | CoA-SH | acetyl-CoA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 7 | 0 | 0 | 7 | 0 | 7 | 0 | 0 | 7 | 0 |
| 1 | 0 | 6 | 1 | 1 | 7 | 0 | 7 | 0 | 0 | 7 | 0 |
| 2 | 0 | 6 | 1 | 0 | 6 | 1 | 7 | 0 | 0 | 7 | 0 |
| 3 | 0 | 6 | 1 | 0 | 6 | 0 | 6 | 1 | 1 | 7 | 0 |
| 4 | 1 | 6 | 1 | 0 | 6 | 0 | 6 | 1 | 0 | 6 | 1 |
| 5 | 0 | 5 | 2 | 1 | 6 | 0 | 6 | 1 | 0 | 6 | 1 |
| 6 | 0 | 5 | 2 | 0 | 5 | 1 | 6 | 1 | 0 | 6 | 1 |
| 7 | 0 | 5 | 2 | 6 | 5 | 0 | 5 | 2 | 1 | 6 | 1 |
| 8 | 1 | 5 | 2 | 0 | 5 | 0 | 5 | 2 | 0 | 5 | 2 |
| 9 | 0 | 4 | 3 | 1 | 5 | 0 | 5 | 2 | 0 | 5 | 2 |
| 10 | 0 | 4 | 3 | 0 | 4 | 1 | 5 | 2 | 0 | 5 | 2 |
| 11 | 0 | 4 | 3 | 0 | 4 | 0 | 4 | 3 | 1 | 5 | 2 |
| 12 | 1 | 4 | 3 | 0 | 4 | 0 | 4 | 3 | 0 | 4 | 3 |
| 13 | 0 | 3 | 4 | 1 | 4 | 0 | 4 | 3 | 0 | 4 | 3 |
| 14 | 0 | 3 | 4 | 0 | 3 | 1 | 4 | 3 | 0 | 4 | 3 |
| 15 | 0 | 3 | 4 | 0 | 3 | 0 | 3 | 4 | 1 | 4 | 3 |
| 16 | 1 | 3 | 4 | 0 | 3 | 0 | 3 | 4 | 0 | 3 | 4 |
| 17 | 0 | 2 | 5 | 1 | 3 | 0 | 3 | 4 | 0 | 3 | 4 |
| 18 | 0 | 2 | 5 | 0 | 2 | 1 | 3 | 4 | 0 | 3 | 4 |
| 19 | 0 | 2 | 5 | 0 | 2 | 0 | 2 | 5 | 1 | 3 | 4 |
| 20 | 1 | 2 | 5 | 0 | 2 | 0 | 2 | 5 | 0 | 2 | 5 |
| 21 | 0 | 1 | 6 | 1 | 2 | 0 | 2 | 5 | 0 | 2 | 5 |
| 22 | 0 | 1 | 6 | 0 | 1 | 1 | 2 | 5 | 0 | 2 | 5 |
| 23 | 0 | 1 | 6 | 0 | 1 | 0 | 1 | 6 | 1 | 2 | 5 |
| 24 | 1 | 1 | 6 | 0 | 1 | 0 | 1 | 6 | 0 | 1 | 6 |
| 25 | 0 | 0 | 7 | 1 | 1 | 0 | 1 | 6 | 0 | 1 | 6 |
| 26 | 0 | 0 | 7 | 0 | 0 | 1 | 1 | 6 | 0 | 1 | 6 |
| 27 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 7 | 1 | 1 | 6 |
| 28 | 1 | 0 | 7 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 7 |
| 29 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 8 |

Table 4.1. The numbers of tokens in different places change during the simulation of palmitic acid β-oxidation

# 5 Workflow Net as a supplement

It is obvious that the major pathways in an organism, such as glycogen synthesis, glycogen breakdown, glycolysis, gluconeogenesis, fat breakdown, fat synthesis, the citric acid cycle, electron transport, etc., cannot all be run at a full speed all the time. If a metabolic pathway is proceeded in one direction, the reactions in the reverse direction must be slow down or even switched off to make sure that the reaction is proceeded in the required direction. The required metabolic rate of a certain pathway, such as glycolysis, will be enormously according to the varying energy expenditure at that time. For example, the basal metabolic rate of a person who is playing basketball is higher than that of the same person when he is sitting on sofa to watch TV.

Metabolic pathways even need to proceed in different directions to respond to physiological needs. For example, if we compare the metabolic pathways after a meal when metabolites are being stored with the metabolic pathways at the time point between meals when stored metabolites (glycogen, fatty acids, etc.) are being utilized, you can find that they are almost in the reverse direction. Further, the metabolic pathways may change in prolonged starvation and in pathological situations, such as diabetes, where carbohydrate metabolism is abnormal. Therefore, the metabolism of the main energy-yielding materials must be regulated to fulfill at least two broad purposes: to respond to energy production needs as energy expenditure varies, and to respond to physiological needs. It does not make sense if we develop a model without dealing the situations mentioned above.

## 5.1. Why are controls necessary?

Current Petri Net functions do not fulfill all the needs of the complex control mechanism in biological processes. We find that the models built on pure Petri Nets has a probability of being trapped with endless loopings or deadlocks. The process of gluconeogenesis provides a good example to illustrate the potential dangers in modeling metabolic systems of futile cycles. Let's see the phosphofructokinase (PFK) step in glycolysis and its reversal in gluconeogenesis by fructose-1:6-bisphosphatase (see Figure 5.1). In one direction, fructose-6-phosphate is phosphorylated by PFK to yield fructose-1:6-bisphosphate, while in the reverse direction the bisphosphatase hydrolyses the fructose-1:6-bisphosphate back to fructose-6-phosphate. A Petri Net based on this cycle would, just like an electrical short circuit (Figure 5.2), achieve nothing but endlessly wasting ATPs and water molecules.

Though this reaction seems like a reaction that merely wasting ATPs and

producing heat, actually, this happened in the nature. Bumblebees utilize this mechanism to warm up their flight muscles before taking off when the weather is cold[31]. This reaction stops when the temperature of their flight muscles is adequate to fly. It is not sufficient to describe such a control mechanism merely using traditional chemical formula or Petri Nets. Therefore, a combined concept of Petri and Workflow Nets seems to be an adequate solution for the issue.

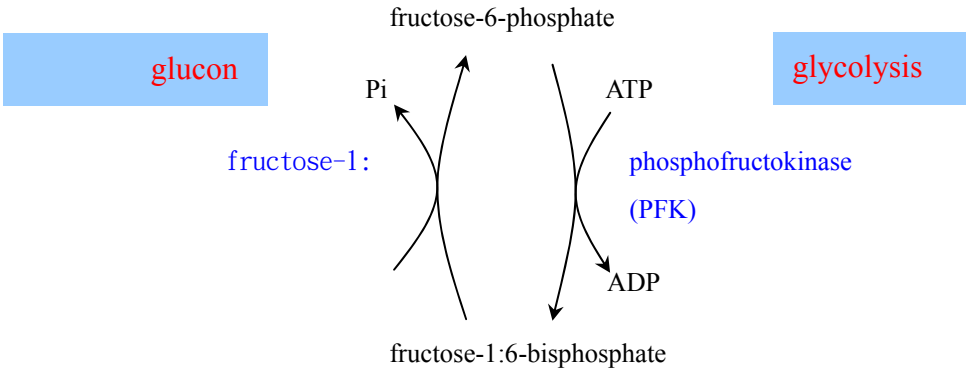



Figure 5.1 A potential futile cycle

With WF Nets, transitions are used to denote biochemical reactions catalized by specific enzymes. But operator and trigger transitions do not refer to any biochemical reactions in our studies. They are purely used to denote controlling factors, such as temperature, pH value, concentration, etc. We add a message trigger transition to both the glycolysis and gluconeogenesis reactions to denote that the organism needs some information to determine whether these reactions should proceed or not. In the example of bumblebees, the message needed is the temperature of the environment. If it is cold enough, the transition allows the WF Net to proceed to the next transition. When it is warm, the transition inhibits the following procedure. The futile cycle therefore needs to be regulated (see Figure 5.3).

This idea can be extended to the glycolysis and gluconeogenesis. These pathways, if not properly controlled, could constitute a giant futile cycle again doing nothing but wasting APT. The same idea may also be applied to glycogen synthesis and breakdown, and to fat synthesis and breakdown, or even to virtually any synthesis and breakdown pathways.

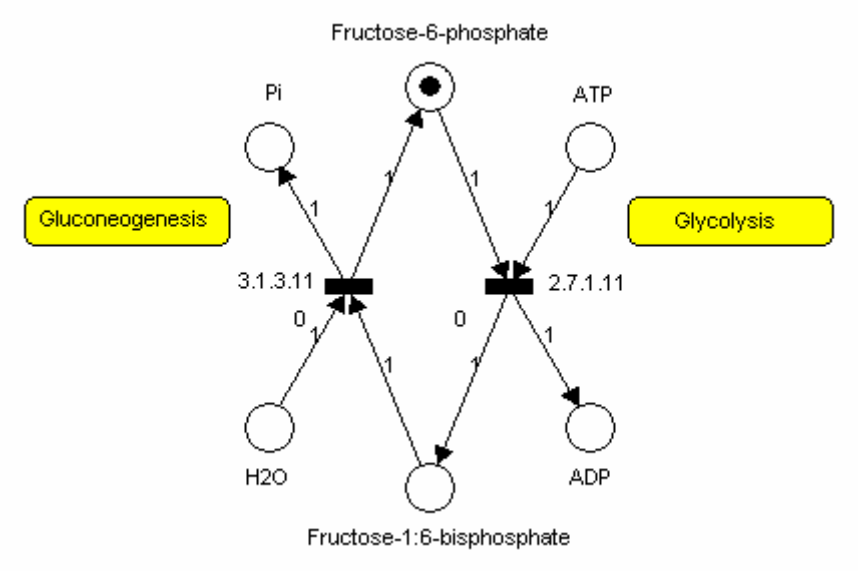



Figure 5.2 A Petri Net of a potential futile cycle

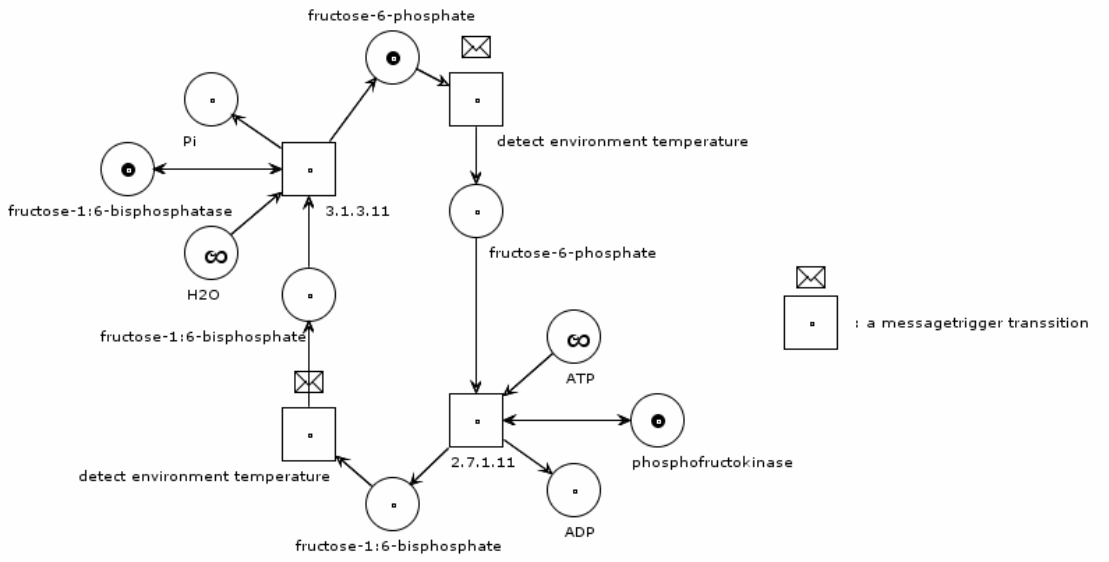



Figure 5.3 A WF-Net of a potential futile cycle

## 5.2 Strategies for metabolic control

Although controls actually exist in all aspects of metabolic pathways, the control and integration of carbohydrate and fat metabolic pathway are of special importance to the energy supply process of an organism due to the flow of chemical change (flux) through these pathways is very large, and the direction of the fluxes changes frequently. For example, the direction of metabolic flows in an animal changes with periodic meals. Control of carbohydrate and fat metabolism illustrates all of the principles of metabolic control. However, it is a rather complex subject to deal with

all carbohydrate and fat metabolism, so we can only choose a simple metabolic pathway as an example to demonstrate the possibility of utilizing WF-Nets to denote the control mechanism.

## 5.3. WF-Nets Modifications to Petri Net Model

During the simulation of our Petri Nets model for β-oxidation of fatty acyl-CoA to acetyl-CoA shown in Figure 4.8, we may see that the model may not be as perfect as we expected because we used FAD number to determine whether a fatty acyl-CoA has undergone seven runs of β-oxidation. We have to give place FAD 7 tokens to insure that the routine runs only 7 runs. There is an inhibit arc placed between place FAD and transition bypass. The inhibit arc would inactive transition bypass if there are tokens in place FAD. Only when the tokens number of place FAD becomes zero can the token in place Fatty acyl-CoA move to place Acetyl-CoA.

We can exame the model with palmitic acid, a kind of sixteen-carbon fatty acid. When the C16 acyl-CoA molecule has undergone six round of β-oxidation, it becomes a C4 acyl-CoA molecule. If the C4 acyl-CoA proceeds with the seventh rounds of β-oxidation, it would be split into an acetyl-CoA and a C2 acyl-CoA. Because the C2 acyl-CoA is already an acetyl-CoA, the eighth round of β-oxidation is unnecessary. These two acetyl-CoAs as shown in Figure 5.4 are ready to proceed to the following reactions in TCA cycle.

We have to avoid the eighth round occur in the corresponding Petri Nets model in Figure 4.8. Since we used numbers of tokens to denote the molecule numbers of reactants or products, the lack of properties of tokens makes it hard to describe the status of a specific molecule. In other words, we do not have any information to detect whether a token has undergone the seventh round of β-oxidation or not. Though we can limit the Petri Net to run only seven times by setting the number of tokens in place FAD to seven, and use an inhibit arc to inactive the transition bypass. However, it is not the real solution of this issue, because such a model can work only one time.

However, the process of β-oxidation of fatty acyl-CoA in organism happens repeatedly and even simultaneously whenever there is a need of acetyl-CoA. Therefore, the WF Nets is an adequate alternative, even though it is poor in simulation.

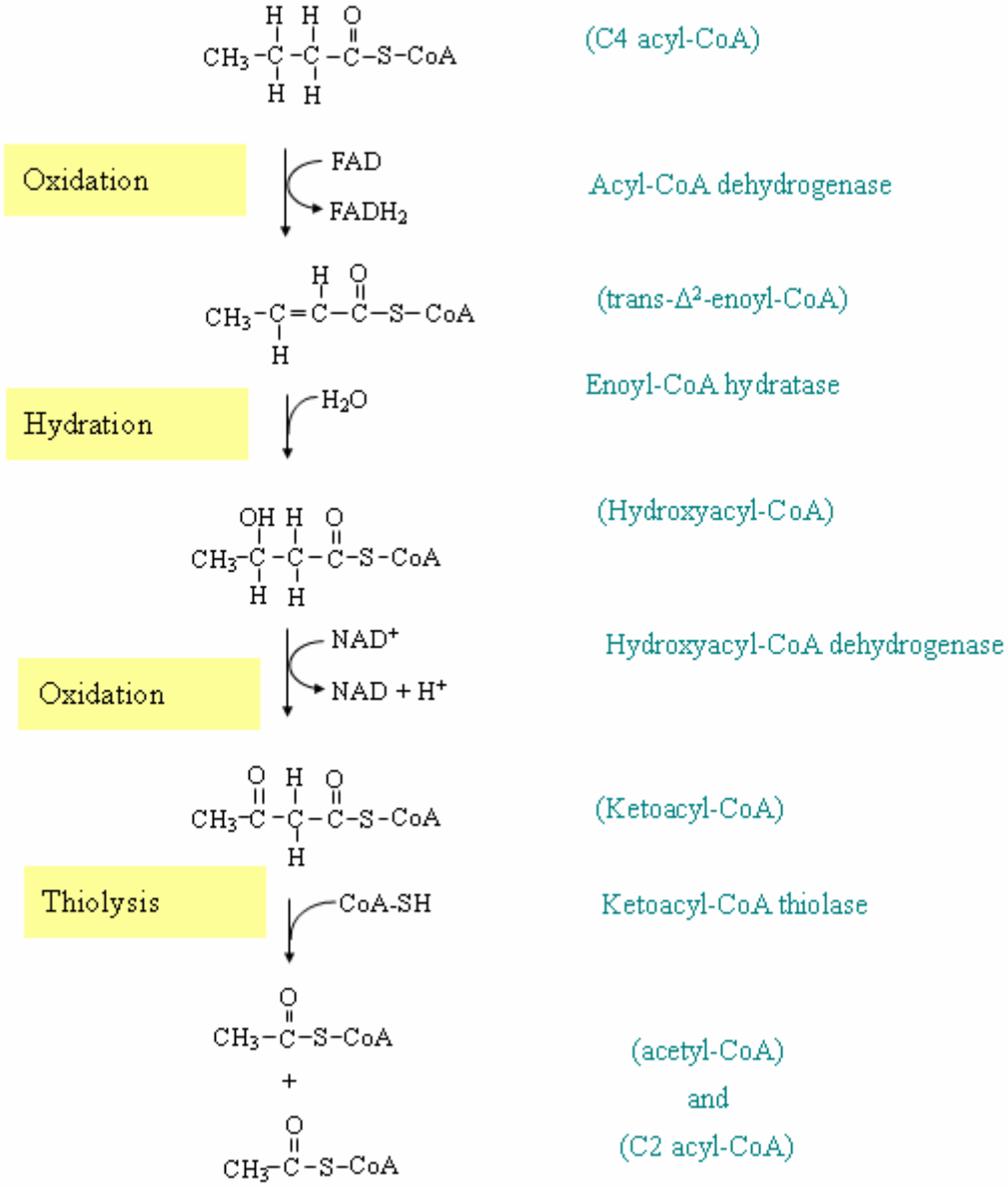



Figure 5.5 shows the modified WF Net of β-oxidation of fatty acyl-CoA. We add an operator transition with resource trigger and an extra place in the net. The operator transition checks to see whether the acyl-CoA has at least four Carbons. If yes, it will undergo the process of β-oxidation. Otherwise it would be sent to the next place acetyl-CoA. We set the operator transition to XOR-Split type and resource trigger to a role (shown in Figure 5.6).

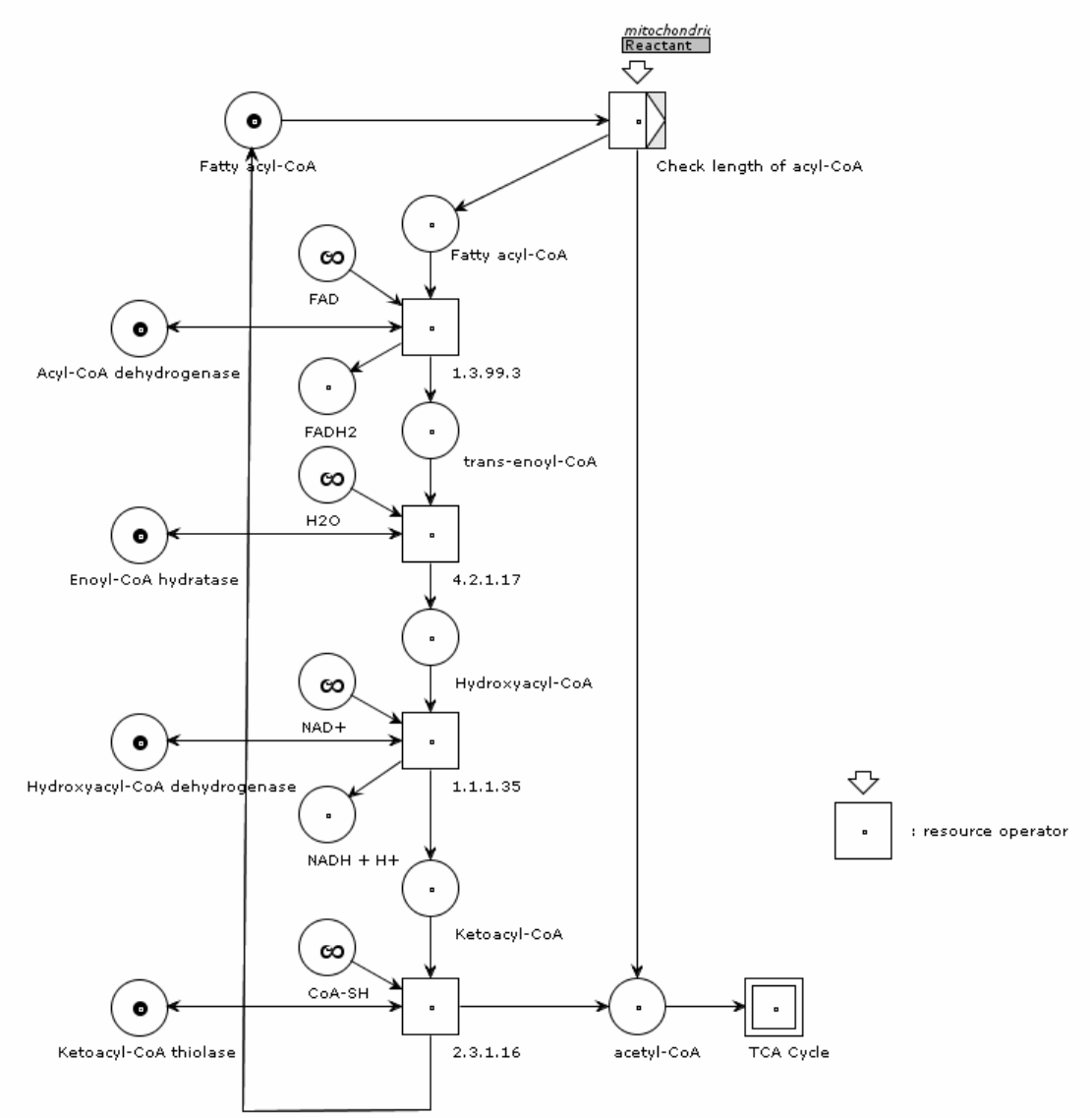



Figure 5.5 A WF Net model for β-oxidation of fatty acyl-CoA

Further, we define a resource object as acetyl-CoA that plays a role as reactant and belongs to group mitochondrion (see Figure 5.7). We use this property to denote that when the reactant of the biochemical reaction in a mitochondrion is already an acetyl-CoA, there is no need to proceed β-oxidation and the reactant will be sent to place acetyl-CoA instead of to place Fatty acyl-CoA.

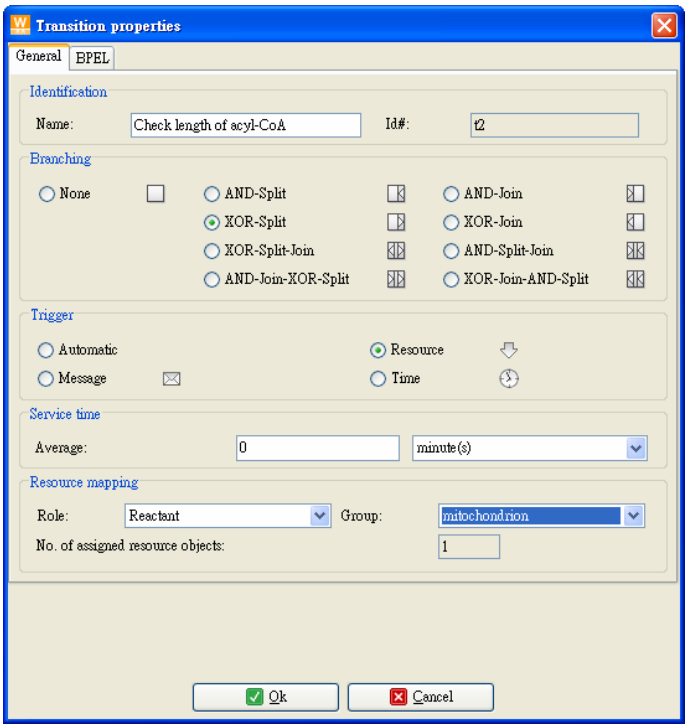



Figure 5.6 Setting the transition properties

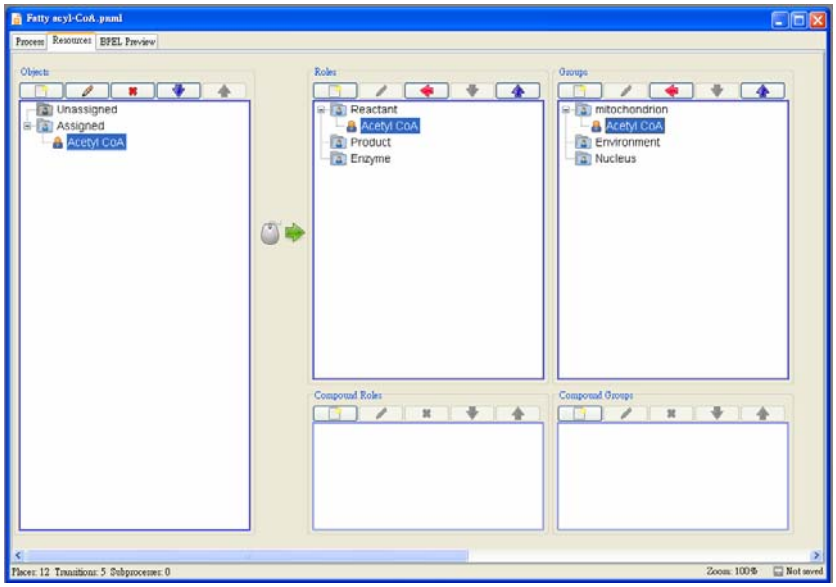



Figure 5.7 Setting Acetyl-CoA as a Resource Object

By doing so, we do not need to limit the number of β-oxidation by setting the number of token in place FAD to 7 to avoid a deadlock. We can even set the number of tokens to place FAD to infinity. This model allows us to deal with more than one fatty acyl-CoA in sequence now.

### 5.3.1 Oxidation of unsaturated fat

So far, the metabolic pathway model concerned is for saturated fat. In our daily food, there exist many kinds of unsaturated fat. In fact, most of the dietary plant oil is unsaturated fat. If unsaturated fat is absorbed by our body, the metabolic pathway cannot be exactly the same asthat of the saturated one. Therefore, the process of β-oxidation would change whenever there is a double bond between two carbon atoms. For example, palmitoleic acid ($C_{16}H_{30}O_2$), which is similar to the palmitic acid in structure, has a double bond between carbon atoms 9 and 10. When a palmitoleic acid molecule is oxidated, it is treated by the mitochondria in the same way as palmitic acid for three rounds of β-oxidation. At this point, the product is cis-$\Delta^3$-enoyl-CoA (see Figure 5.8.).

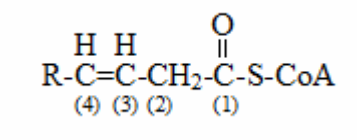



Figure 5.8. cis-$\Delta^3$-enoyl-CoA

The double bond between carbon atoms 3 and 4 prevents the acyl-CoA dehydrogenase from forming a double bond between carbon atoms 2 and 3, as is required in β-oxidation of a saturated acyl-CoA.

An extra isomerase enzyme deals with this by shifting the existing double bond into the required 2-3 position, and generates a trans-isomer in doing so (see Figure 5.9.).

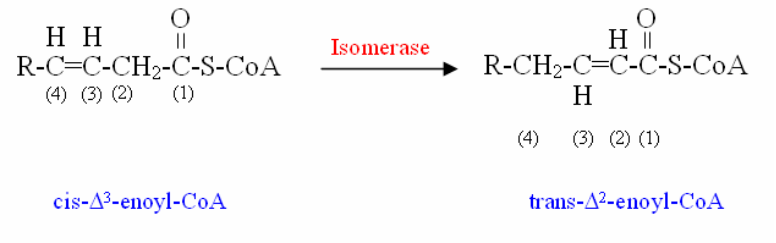



Figure 5.9. Shifting the double bond of a cis-$\Delta^3$-enoyl-CoA to generate a trans-$\Delta^2$-enoyl-CoA

Now, it is adequate for the enoyl-CoA to back to the second step of β-oxidation and thus the problem of monounsaturated fat oxidation is solved.

The corresponding WF-Nets are shown in Figure 5.10 in which an XOR-Split transition with resource trigger is involved to check to see whether there is a double bond between carbon 3 and 4, and the transition's role as a reactant in group mitochondria is set.

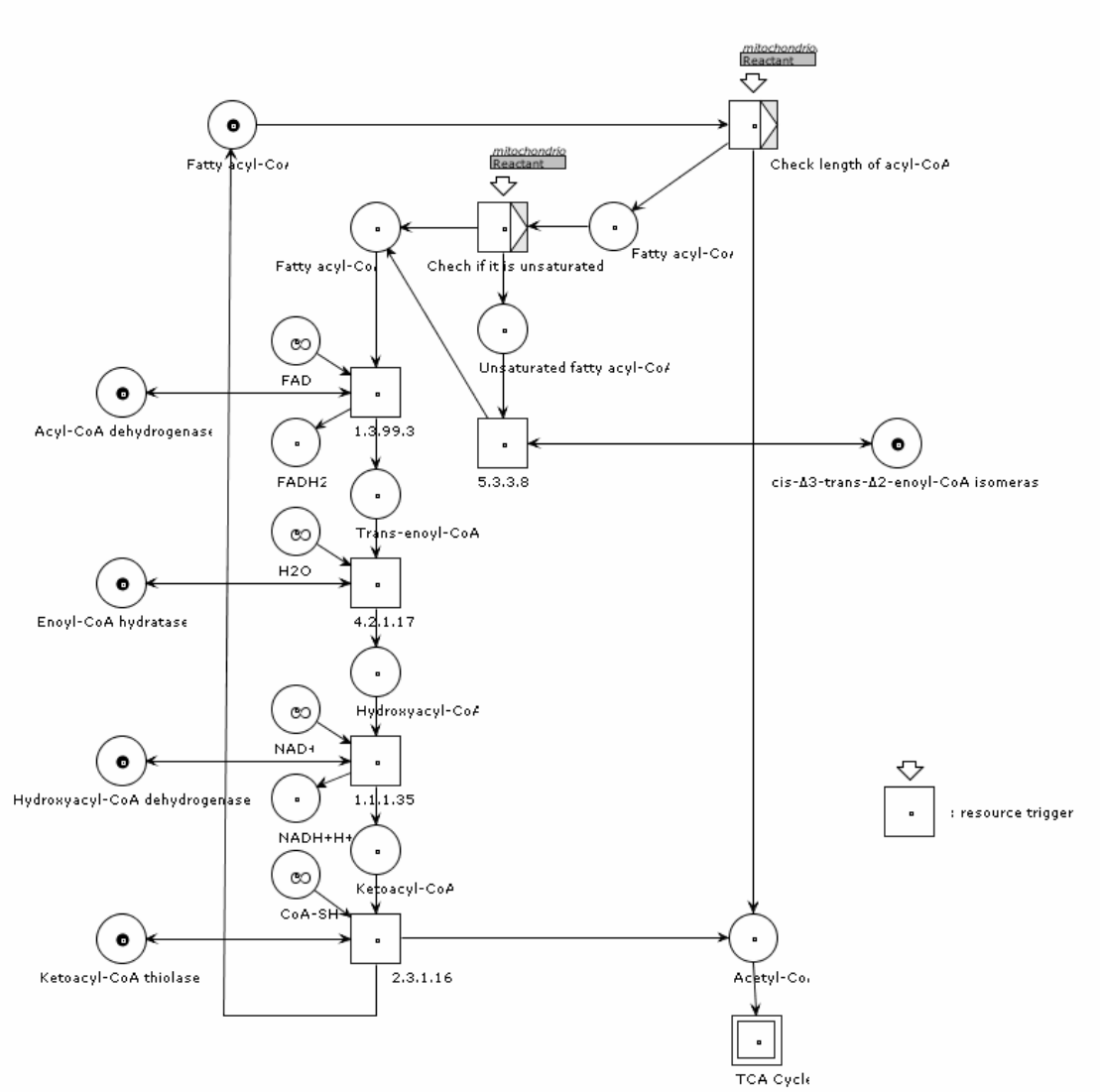



Figure 5.10. A WF-Nets model for $\beta$-oxidation of monounsaturated fatty acyl-CoA

## 5.3.1 Oxidation of odd-numbered carbon chain fatty acids

A small proportion of fatty acids in the diet have odd-numbered carbon chain. For example, fatty acids from plants are not all even-numbered. β-oxidation of these odd-numbered carbon chain fatty acids produces a five-carbon β-ketoacyl-CoA instead of acetoacetyl-CoA as the penultimate product. Cleavage of β-ketoacyl-CoA by thiolase produces-CoA and a three-carbon chain fatty acid is called propionyl-CoA (see Figure 5.11).

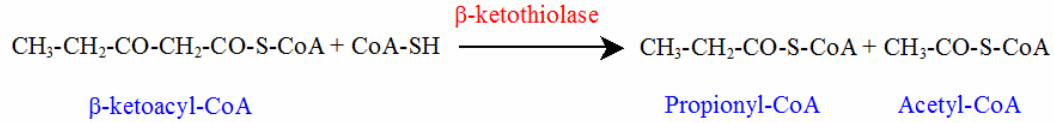



Figure 5.11. Cleavage of β-ketoacyl-CoA

Propionyl-CoA is converted to succinyl-CoA and then joins the citric acid cycle mainstream by the following reactions. First, propionyl-CoA, carboxylized by a propionyl-CoA carboxylase, become propionate called D-methylmalonyl-CoA. Then the methylmalonyl-CoA epimerase catalyses the conversion of D- to L-methylmalonyl-CoA. In the last step, methylmalonyl-CoA mutase, which is a derivative of vitamin B12 called deoxyadenosylcoalamin, converts the L-methylmalonyl-CoA to Succinyl-CoA (see Figure 5.12).

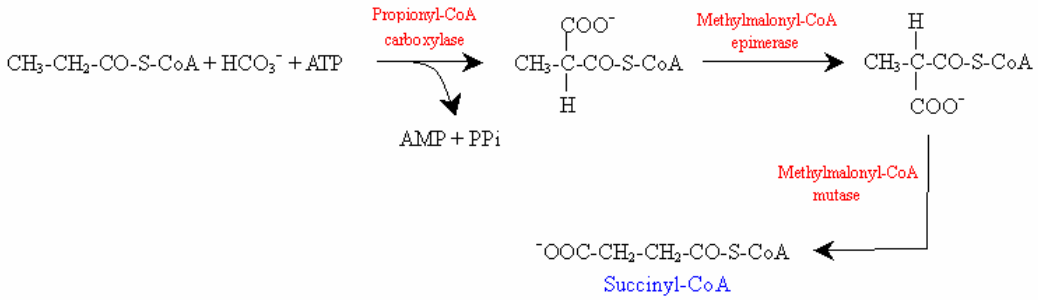



Figure 5.12. Formation of Succinyl-CoA

Our WF Net model (as shown in Figure 5.13) can be further modified to fulfill the scenario. An XOR-Split transition with resource trigger is used to check to see whether the penultimate product is either a five-carbon β-ketoacyl-CoA or an acetoacetyl-CoA. The transition's role is set to be a reactant in group mitochondria. Since the last product, succinyl-CoA, is to join the citric acid cycle, we use a XOR-Split-Join transition with time trigger to check to see whether a reactant is ready to join the TCA cycle or not, and a place named reactant to represent it. This is because more than one reactant will be involved in the TCA cycle at different time. According to the process of TCA cycle, the XOR-Split-Join transition with time trigger will force the appropriate reactant to join the TCA cycle at correct time.

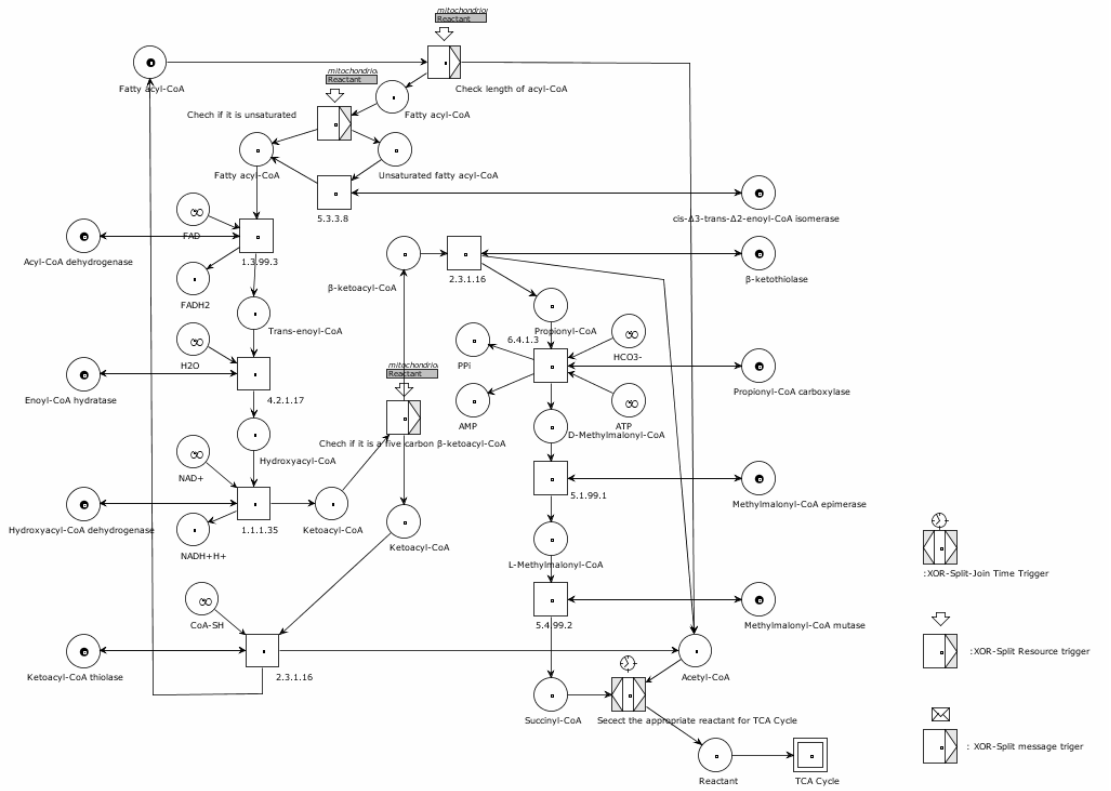



Figure 5.13. A WF-Nets model for $\beta$-oxidation of odd-numbered carbon chain fatty acids

### 5.4.1 Flux control of acetyl-CoA when in abnormal situation

So far we know that the fatty acids are converted to acetyl-CoA, then join the citric acid cycle and at last are oxidized to produce ATP as biological energy. This is correct for all tissues in normal circumstances. But the pathway may change in liver at a particular situation. Basically, the body can be in a physiological situation that fat metabolism is the main source of energy. This occurs in starvation after exhaustion of glycogen storage, and the similar phenomenon can occur in diabetics. It is because the patients are not able to metabolize carbohydrate effectively, thus resultsing in an almost analogous glucose 'starvation,' no matter whether the supply of glucose is sufficient or not. In this situation, the adipose cells are pouring out free fatty acids and the liver may produce excessive amounts of acetyl-CoA. There is definitely too much produced to join the citric acid cycle. The phenomenon may be exacerbated because the malfunction of carbohydrate metabolism leads to a relative shortage of oxaloacetate, which is essential for producing citrate from acetyl-CoA.

What the liver cell does is to join two acetyl groups together to form acetoacetate ($CH_3COCH_2COO^-$), which is partly reduced to β-hydroxybutyrate

($CH_3CHOHCH_2COO^-$) and acetone. These two ketone bodies are released into the blood and are utilized by peripheral tissues to generate energy.

Since acetoacetyl-CoA (as shown in Figure 5.14) is formed from 2 acetyl-CoA by reversal of ketoacyl-CoA thiolase reaction, one would think it is reasonable that free acetoacetate would simply be formed by hydrolysis of the acetoacetyl-CoA.

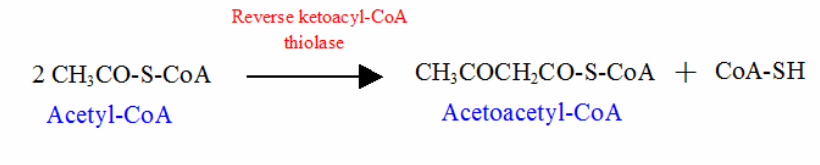



Figure 5.14. Formation of acetoacetyl-CoA

However, it is not such a simple reaction. Liver cells use a third molecule of acetyl-CoA to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). The HMG-CoA (as shown in Figure 5.15) is further decomposed to form acetoacetate and acetyl-CoA. The reason why the synthesis of acetoacetate proceeds in this way is still unknown[32].

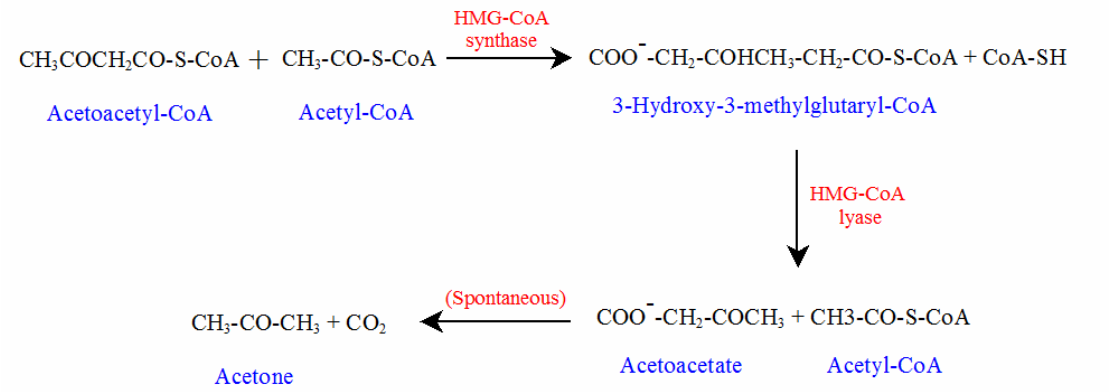



Figure 5.15. Ketone body produced in the liver during excessive oxidation of fat in starvation or diabetes.

If the amount of ketone bodies is overdosed, then some acetoacetate in mitochondria is converted to acetoacetyl-CoA by an acyl transfer reaction in which succinyl-CoA is converted to succinate (see Figure 5.16.).

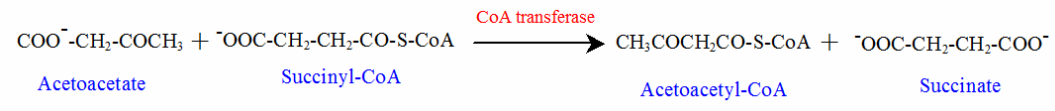



Figure 5.16. The Co-A group of succinyl-CoA is transferred to acetoacetate to form acetoacetyl-CoA and succinate.

Acetoacetyl-CoA is then cleaved by a thiolase by using CoA-SH to form two molecules of acetyl-CoA(see Figure 5.17), making the pathway back to the initial point and becomes a cycle.

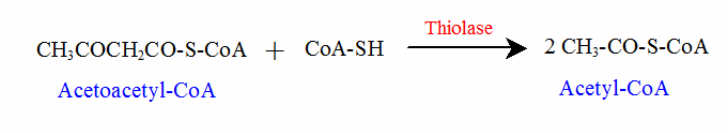



Figure 5.17. Acetoacetyl-CoA is cleaved by a thiolase using CoA-SH to form two molecules of acetyl-CoA

Like a regulator, acetoacetate is partly reduced to β-hydroxybutyrate and the reaction is reversible when there is a need to utilize acetoacetate. This task is achieved by dehydrogenating β-hydroxybutyrate back to acetoacetate (see Figure 5.18.).

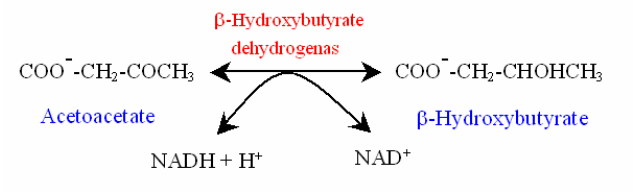



Figure 5.18. $\beta$-hydroxybutyrate is dehydrogenated to acetoacetate. The reaction is reversible.

We now put all of them together to depict the overall flux control of Acetyl-CoA in β-oxidation of fatty acid in abnormal situation (see Figure 5.19). We can see that this metabolic pathway is actually a big futile cycle, and a control mechanism surely exists in the liver cell to handle all of these reactions in a proper way. Figure 5.20 illustrates the corresponding WF Net model that we used to describe this big futile cycle.

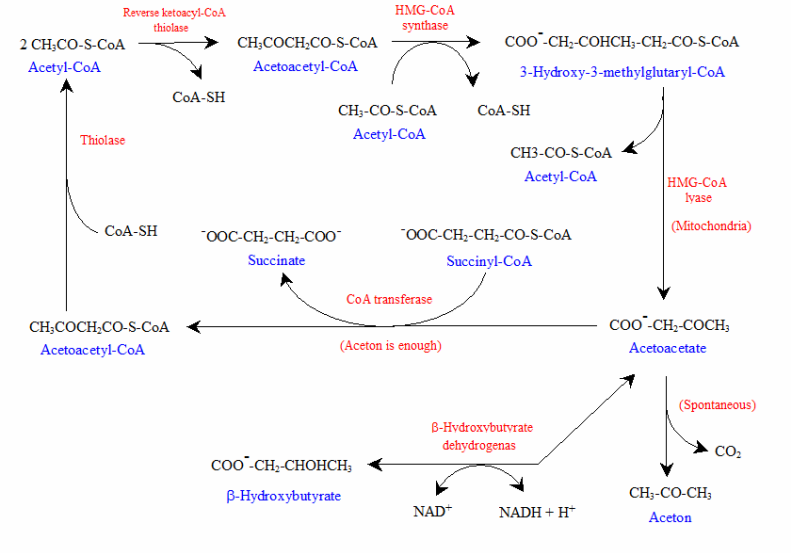



Figure 5.19 The overview of flux control of Acetyl-CoA in β-oxidation of fatty acid in abnormal situation

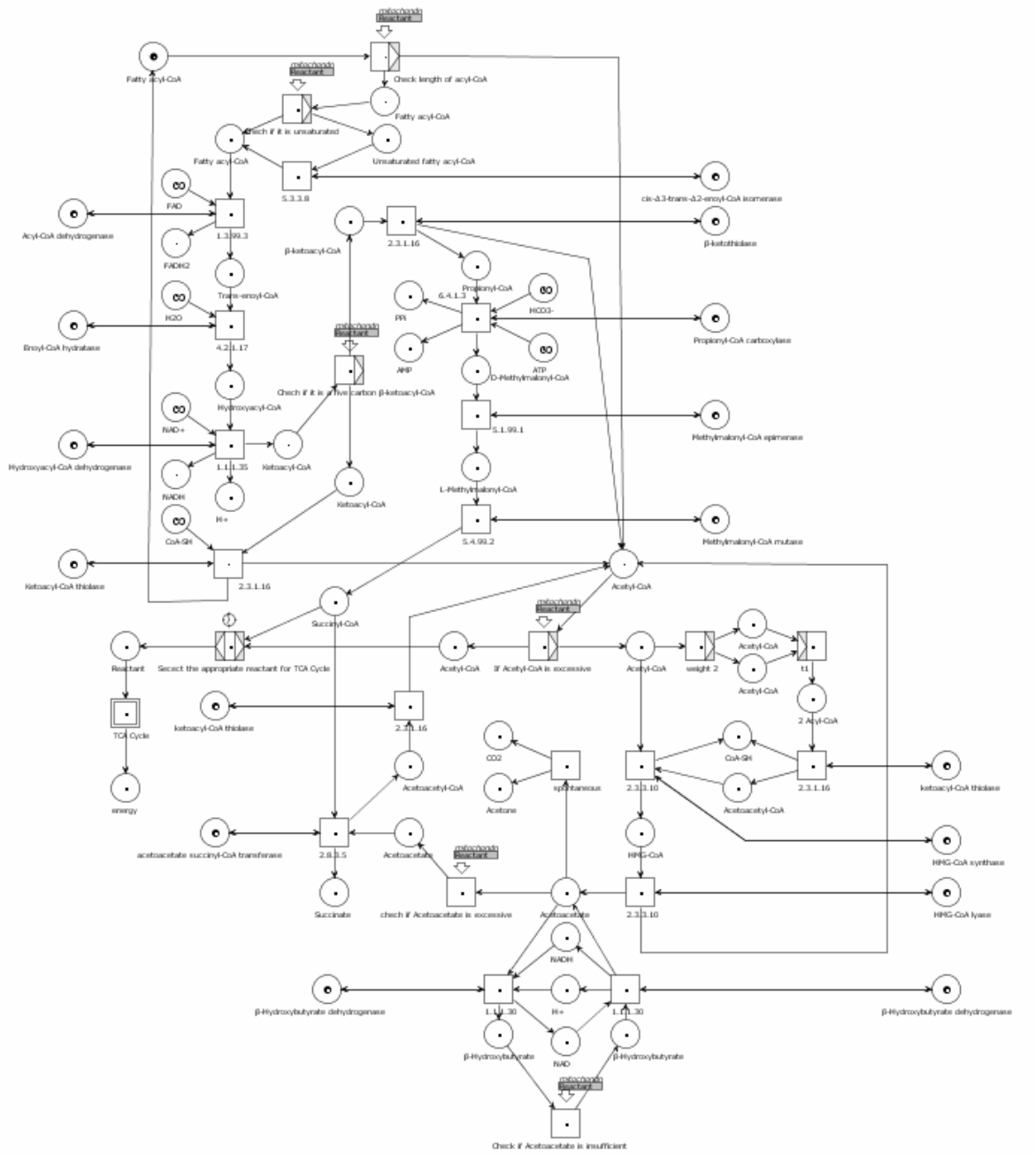



Figure 5.20. The WF-Net model of flux control of Acetyl-CoA in β-oxidation of fatty acid in abnormal situation

## 5.4 Advantage of our work

We have used our model as supplementary material for high school students who are taking their first chemistry course to see if our model can help the novice learn stoichiometry better. There were twelve violenteer students join our project. These students were divided into two groups. The students in test group learned

stoichiometry with our WF Net model simulation, and the students in control group did not use our model as supplementary material. We give them a test with thirty stoichiometry questions. The result showed that the average score of the test group are higher than the control group by four questions. It means that our model really help the novice learn stoichiometry better.

# 6. Conclusion and further discussion

## 6.1 Comparison of our work with other Petri Net works

Since there are already a lot of studies that deal with the modeling method of metabolic pathways with Petri Net, the difference between our method and theirs works is mentioned here. The advantages of our expanded method are also presented.

Samarrai, et al.[33] proposed a Petri Net model for carbohydrate metabolism. They use places to present events, such as “increse on blood glucose”. Thought their model can actually present the reponse for concentration change, it is still not an ideal model because both events and reactans are denoted with places. Such a way may confuse the software engoneer because it is hard to identified whether a token means the change of molecule molecule number or a condition change.

Jasmin Fisher[34], summarized some studies about modeling biological process with Petri Net and propose a model. However, their work deals with enzymes in the same way as reactants. This is not an adequate way to the activities of enzyme because the function of enzyme is to catalyze a bio-reaction not to take part in the process. The amount of enzyme seldom changed after the reaction. In their model, both reactants and enzymes are presented by places with normal arcs. Since tokens may move from enzyme places to transitions, if we give this kind of Petri Net to a programmer and ask him to develop the corresponding simulation software. The programmer may misunderstand what the biochemist try to express. In our model, we use a test arc to connect an enzyme place and a reaction transition. Because a test arc do not change the number of tokens, it does not make any misunderstanding.

The Petri Net models provided by KEGG (as showed in Figure 6.1) are much better than those metioned above. However, their work contain both normal and reverse reactions, this make their model impossible to be simulated because normal and reverse reactions can’t proceed in the same time.

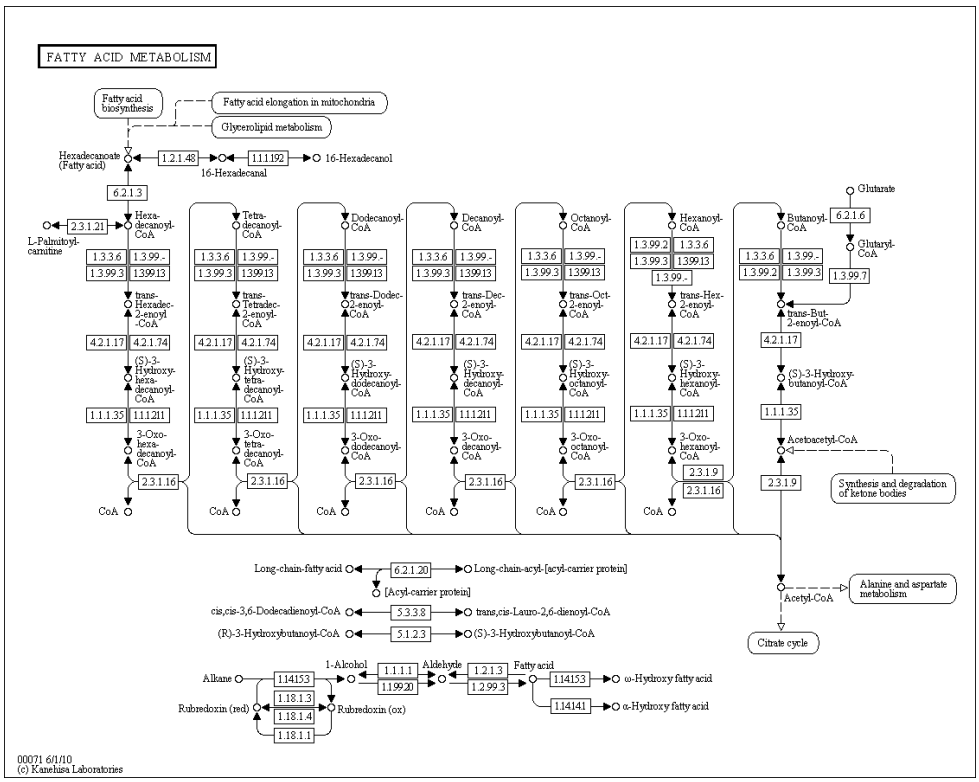



Figure 6.1. A Petri Net model from KEGG

## 6.2 further work

In this thesis, we have introduce the idea of using Work Flow Petri Net as a modeling and simulation tool to depict the metabolic pathway of energy producing process from fats. Petri Net modeling method is a powerful dynamic simulation tool, through which users can understand the dynamic behaviors of reactions and process occurring in life, particularly metabolic pathways. However, the lack of the ability to describe the controlling mechanisms needed in biochemical reactions really limits the accuracy of application simulation. In this study, we integrated Work Flow and a Petri Net to solve the problems.

However, the mechanism of life is more complicated than those we have learned. For example, in a wider sense, the metabolic activities of cells are controlled by circulating hormones and neural inputs that operate in the body as a whole according to current needs. How signals external to the cells, called intrinsic signals, regulate metabolism is also an indispensable part to the simulation of automatic controls of metabolic pathways. How to employ the combined control mechanism of both intrinsic and extrinsic signals by using Work Flow Nets would be another interesting

topic. However, what we have done in this study is limited to the molecular level. If we can extend the proposed approach to cells, or even organism levels, it would be more close to the real world metabolic pathway. These constitute our future research.

# Appendix

## A. List of abbreviations

| | |
|---|---|
| A | Adenine |
| ADP | adenosine diphosphate |
| AMP | adenosine monophosphate |
| ARIS | Architecture of integrated Information Systems |
| CoA | coenzyme A |
| ATP | adenosine triphosphate |
| EPC | Event-driven Process Chain |
| FAD | flavin adenine dinucleotide |
| NAD | nicotinamide adenine dinucleotide |
| PFK | phosphofructokinase |
| PN | Petri Net |
| PNML | Petri Net Markup Language |
| TAG | triacylglycerols |
| VLDL | very low density lipid |
| WF-nets | Workflow Petri Nets |
| WoPeD | Workflow Petri Net Designer |

## B: List of EC number

| | |
|---|---|
| 1.1.1.30 | beta-hydroxybutyrate dehydrogenase |
| 1.1.1.35 | hydroxyacyl-CoA dehydrogenase |
| 1.3.99.3 | acyl-CoA dehydrogenase |
| 2.3.1.137 | carnitine acyltransferase I & II |
| 2.3.1.16 | ketoacyl-CoA thiolase; β-ketothiolase; |
| 2.3.1.21 | translocase I & II;<br>carnitine palmitoyltransferase I & II |
| 2.3.3.10 | 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA)<br>synthase;<br>3-hydroxy-3-methylglutaryl-CoA lyase |
| 2.7.1.11 | phosphofructokinase (PFK) |
| 2.8.3.5 | acetoacetate succinyl-CoA transferase |
| 3.1.3.11 | fructose-1:6-bisphosphatase |
| 4.2.1.17 | enoyl-CoA hydratase |
| 5.1.99.1 | methylmalonyl-CoA epimerase |
| 5.2.1.5 | linoleate isomerase |

| | |
|---|---|
| 5.3.3.8 | cis-Δ3-trans-Δ2-enoyl-CoA isomerase |
| 5.4.99.2 | methylmalonyl-CoA mutase |
| 6.4.1.3 | propionyl-CoA carboxylase |

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