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Carnitine translocase in cardiac cell: structural, functional and flux balance analysis in pre-β-oxidation pathway of *Homo sapiens*

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***Abstract* --Fats are stored in the body in the form of fatty acids and reused whenever the body is unable to consume essential fats (at the time of exercise, fasting etc). In the human heart cell, the transport of certain fatty acids into the mitochondria is required to produce energy. This is done via a certain enzyme called Carnitine Translocase. This work deals with the detection and manipulation of carnitine translocase in order to analyze its importance in avoiding certain cardiovascular disorders like hyperglycemia, arrhythmia etc.**

***Keywords*-- carnitine translocase, flux balance analysis, transcription factor**

I. Introduction

The heart demands a high supply to energy in order to function and sustain the human body. Energy in the human heart is stored in the form of ATP. The heart has a comparatively low level of ATP content as compared to the process of ATP hydrolysis. Therefore, in order to sustain enough ATP generation, the heart uses various metabolites present in the cell. Most of these metabolites are present in the form of glucose, free fatty acids, essential fatty acids, co-factors, co-enzymes etc. It is known that maximum number of ATP required for the heart is generated from the β-oxidation of fatty acids, which takes place in the mitochondrial matrix. The fatty acids with chain lengths of 12 or fewer carbons enter mitochondria without the help of membrane transporters. However, those with 14 or more carbons, constituting the majority of free fatty acids obtained in the diet cannot pass through the mitochondrial membrane. They must go through the *carnitine shuttle*. There are various enzymes present in this shuttle. One such enzyme is the Carnitine Translocase (CT) which is responsible for the transportation of the fatty acyl carnitine from the outer mitochondrial membrane to the matrix. In this manuscript we have used Flux Balance Analysis to maximize the objective of finding the CT enzyme in the inter-membrane space of mitochondria to check if the metabolite passes through the enzyme [1].

II. Carnitine Translocase

Carnitine Translocase also known as the Carnitine acylcarnitine translocase is an enzyme present in the inter-membrane space of mitochondria. This enzyme is the coded by an SLC gene (solute carrier gene, family 25, member 20). The main function of this enzyme is to transport the fatty acyl Carnitine from the outer membrane to the inner membrane, where it is free from its bound form and the free carnitine moves back to the outer mitochondrial membrane. Fig. 1 denotes the placement of carnitine translocase in the mitochondrial membrane.

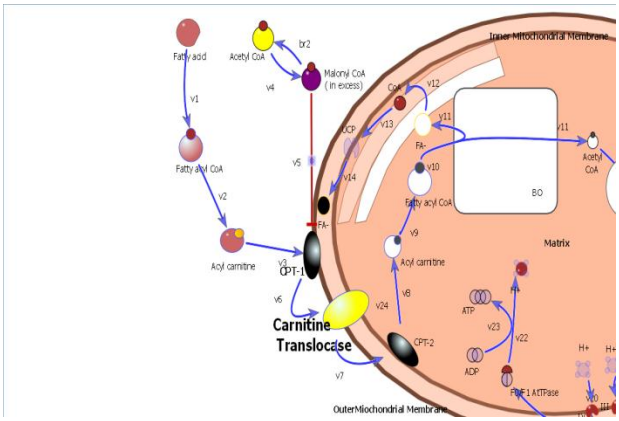

Fig. 1 The transport of fatty acid into the mitochondrial membrane

III. Flux Balance Analysis

Flux balance analysis (FBA) calculates the flow of metabolites through a given metabolic network thereby predicting the growth rate of the concerned metabolite(s) present in that network. Metabolic reactions are represented as a stoichiometric matrix *S* of the order *m*n*, where *m* is the number of metabolites and *n* is the number of reactions present in the given network. The values in each column are the stoichiometric numbers present in the reaction. Negative values are assigned for every flux leaving a metabolite, positive values are assigned to every metabolite that receives a flux and zero values are given to the metabolites that do not participate in the reaction. The flux through the network is represented by the vector 'v'. At quasi- steady state, the mass balance equation is considered at [3-4].

$$S.v = 0 \quad (1)$$

Any *v* that satisfies the equation is said to be the null space of *S*. We are interested in maximizing the possibility of CT in the inter-membrane space of mitochondrial membrane in the heart cell in humans.

A. The Objective Function

The objective function in this study is to maximize the occurrence of Carnitine Translocase (CT) enzyme in mitochondrial membrane. The simulation as well as optimization was carried using MATLAB. A stoichiometric matrix was created on the basis of the network reconstructed (Fig. 1). Initial concentration values for the metabolites were given and considered at 'lower bound' limit, whereas the 'upper bound' limit was kept constant throughout for all metabolites. A metabolite named Malonyl CoA is also involved in this pathway and is produced from Acetyl CoA for fatty acid synthesis. However, when produced in excess (due to the consumption of excess carbohydrates), Malonyl CoA is known to inhibit the activity of Carnitine Palmitoyl transferase-1 (CPT-1) enzyme. Thus, our first strategy consists of the inhibition of Malonyl CoA on CPT-1 enzyme. Stoichiometric matrix was constructed accordingly and the flux was calculated. Here, the initial concentration of Acetyl CoA and Malonyl CoA was kept higher than that of CPT-1. We found that under normal conditions, the fatty acyl CoA is converted to Fatty acyl Carnitine in the presence of the enzyme CPT-1.When Malonyl CoA is not produced in excess, it does not inhibit CPT-1. Hence, in our next step we ruled out the inhibition of Malonyl CoA and included only those reactions that lead to the transport of fatty acyl Carnitine to Carnitine Translocase. Here, the initial concentration of CPT-1 enzyme was kept higher than other metabolites and then the objective function was studied.

IV. Carnitine Translocase-Structural And Functional Analysis

Carnitine Translocase is encoded by a gene SLC25A20. This gene belongs to a solute carrier family and is responsible for translocation purpose. The structure of this particular protein was found out with the help of 'Phyre2', an online protein structure prediction software (Fig. 2). Every gene is needs a transcription factor to signal the RNA polymerase to start the process of transcription on a DNA strand in order to make an mRNA strand that translates to a protein. A major transcription factor of this gene is PPAR-alpha (Peroxisome Proliferater- Activated Receptor alpha. From PIR (Protein Information Resource) database, the protein patterns or motifs were found that are responsible for the structure and the functioning of carnitine translocase. The original sequence was mutated 3 times to confirm the functional aspect of the data.

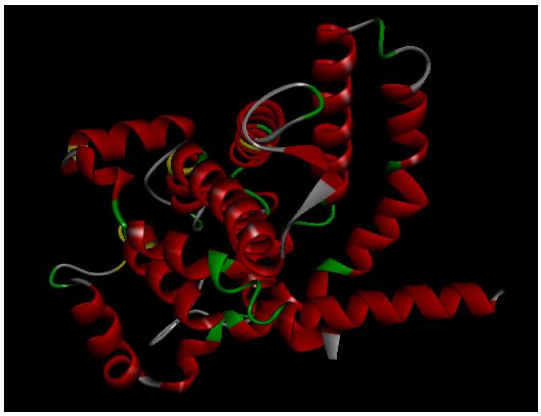

Fig. 2: The structure of Carnitine Translocase viewed under Accelrys Discovery Studio Visualizer

Gene expression values of SLC25A20 gene under normal condition show no signs of over-expression or under-expression of this whereas in diseased state, the gene was observed to be under expressed, that is, 0.003 units lower than its normal value. The study was done using the Array Expression Atlas web server which is collaborated with the ArrayExpress Database

V. Results And Analysis

In our first step, the objective function showed a zero value meaning that due to the inhibition by another metabolite, the fatty acyl CoA was unable to pass through the membrane and hence it got deposited in the cytosol itself (Table I).

TABLE I. Inhibition of CPT1 by Malonyl CoA

Metabolites	Concentration (mM)	Objective Function	Final Concentration (mM)
Acetyl CoA	0.05	0.0	0.05
Malonyl CoA	0.05	0.0	0.05
CPT1	0.005	0.0	0.05
Fatty acid	0.0	0.0	0.05
Fatty acyl CoA	0.0	0.0	0.05
Fatty acyl carnitine	0.0	0.0	0.05
Carnitine translocase	0.0	0.0	0.05
CPT2	0.0	0.0	0.05

TABLE II. Transport of Fatty-acyl CoA to carnitine translocase

Metabolites	Concentration (mM)	Objective Function	Final Concentration (mM)
Acetyl CoA	0.00	0.0	0.05
Malonyl CoA	0.00	0.0	0.05
CPT1	0.05	0.05	0.05
Fatty acid	0.00	0.0	0.05
Fatty acyl CoA	0.00	0.0	0.05
Fatty acyl carnitine	0.00	0.0	0.05
Carnitine translocase	0.00	0.0	0.05
CPT2	0.00	0.0	0.05

In our second step, the objective function showed a positive value which meant that the fatty acyl CoA was easily able to pass through the mitochondrial membrane. In this case there was no inhibition by another metabolite (Table II).

Also, in order to analyze the production of the carnitine translocase, we first found out its functional motifs (Fig. 3). These motifs were found using Protein Information Resource(PIR), an online database. A sequence, `FRDVLREIR`

was used to mutate the protein sequence as it was known to not have any function in particular.

The original sequence is as given below:
MADQPKPISPLKNLLAGGFGGVCLVFVGHPLDTVKVRLQ
TQPPSLPGQPPMYSGTFDCFRKTLFREGITGLYRGMAAPII
GVTPMFAVCFFGFGLGKKLQQKHPEDVLSYPQLFAAGML
SGVFTTGIMTPGERIKCLLQIQASSGESKYTGTLDCAKKL
YQEFGIRGIYKGTVLTLMRDVPASGMYFMTYEWLKNIFT
PEGKRVSELSAPRILVAGGIAGIFNWAVAIPPDVLKSRFQTA
PPGKYPNGFRDVLRELIRDEGVTSLYKGFNAVMIRAFPAN
AACFLGFEVAMKFLNWATPNL

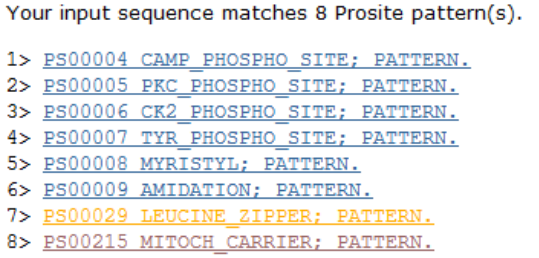

Fig. 3 The original sequence contains 8 patterns.

The 1st mutated sequence was:
MADQPKPISPLKNLLAGGFGGVCLVFVGHFRDVLRELIRT
QPPSLPGQPPMYSGTFDCFRKTLFREGITGLYRGMAAPIIG
VTPMFAVCFFGFGLGKKLQQKHPEDVLSYPQLFAAGMLS
GVFTTGIMTFRDVLRELIRIQASSGESKYTGTLDCAKKLY
QEFGIRGIYKGTVLTLMRDVPASGMYFMTYEWLKNIFTP
EGKRVSELSAPRILVAGGIAGIFNWAVAIFRDVLRELIRTAP
PGKYPNGFRDVLRELIRDEGVTSLYKGFNAVMIRAFPANA
ACFLGFEVAMKFLNWATPNL

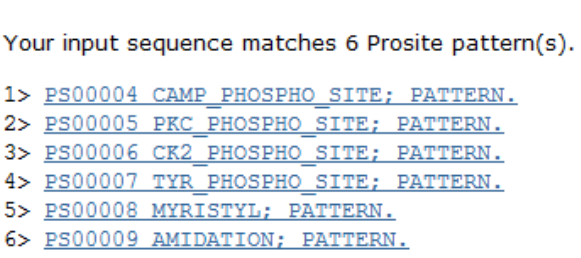

Fig. 4 Mutated sequence obtained after replacing all the motifs of pattern 8 with another sequence.

The sequence in Fig. 4 neither contained a mitochondrial carrier protein motif nor a leucine zipper. The 2nd mutated sequence was:
MADQPKPISPLKNLLAGGFGGVCLVFVGHPLDTVKVRLQ
TQPPSLPGQPPMYSGTFDCFRKTLFREGITGLYRGMAAPII
GVTPMFAVCFFGFGLGKKLQQKHPEDVLSYPQLFAAGML
SGVFTTGIMTFRDVLRELIRIQASSGESKYTGTLDCAKKL
YQEFGIRGIYKGTVLTLMRDVPASGMYFMTYEWLKNIFT
PEGKRVSELSAPRILVAGGIAGIFNWAVAIFRDVLRELIRTA
PPGKYPNGFRDVLRELIRDEGVTSLYKGFNAVMIRAFPAN
AACFLGFEVAMKFLNWATPNL

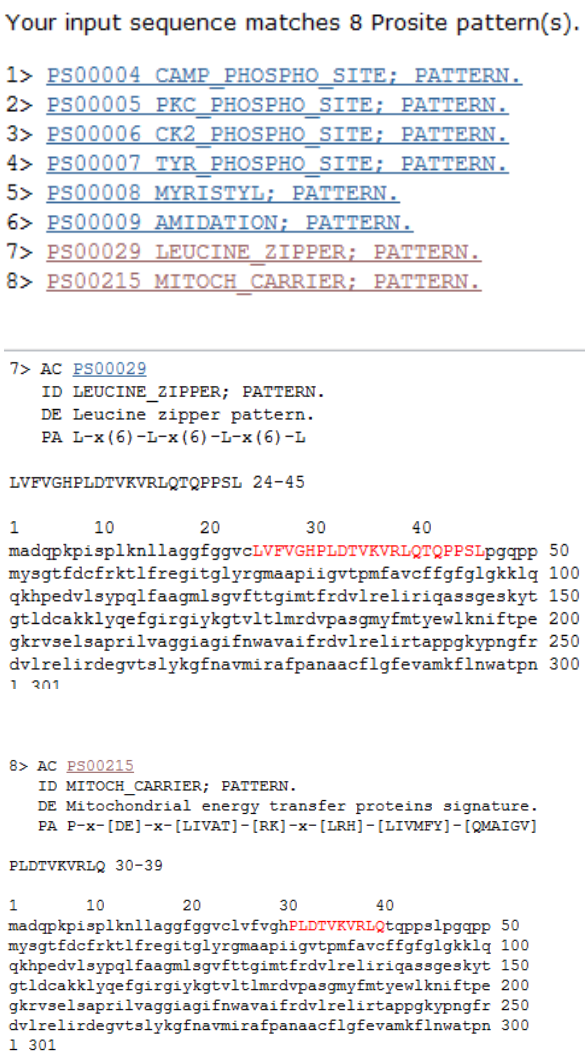

Fig. 5 Mutated sequence obtained after replacing 2nd and 3rd motif of pattern 8 with another sequence.

The sequence in Fig. 5 contained a mitochondrial carrier protein motif and a leucine zipper. The 3rd mutated sequence was:
MADQPKPISPLKNLLAGGFGGVCLVFVGHPLDTVKVRLQ
TQPPSLPGQPPMYSGTFDCFRKTLFREGITGLYRGMAAPII
GVTPMFAVCFFGFGLGKKLQQKHPEDVLSYPQLFAAGML
SGVFTTGIMTFRDVLRELIRIQASSGESKYTGTLDCAKKL
YQEFGIRGIYKGTVLTLMRDVPASGMYFMTYEWLKNIFT
PEGKRVSELSAPRILVAGGIAGIFNWAVAIPPDVLKSRFQTA
PPGKYPNGFRDVLRELIRDEGVTSLYKGFNAVMIRAFPAN
AACFLGFEVAMKFLNWATPNL

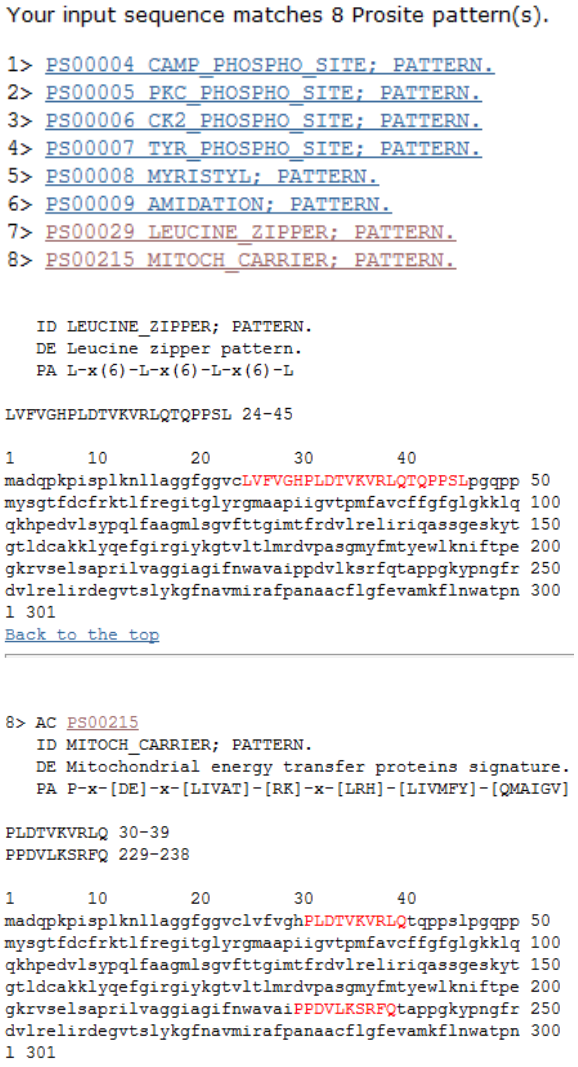

Fig 6 Mutated sequence obtained after replacing 2nd motif of pattern 8 with another sequence.

The sequence in Fig. 6 neither contained a mitochondrial carrier protein motif and a leucine zipper. From the above results we can decipher than when the mutation occurs at all three motifs it is likely that the protein might not function as a port for translocation. Leucine zipper is for dimerization and for the formation of parallel alpha helices. If these two (leucine zipper and mitochondrial carrier protein) are not present, it is possible that there is a carnitine translocase deficiency in the cell

VI. Discussion

Carnitine Translocase is coded by SLC25A20 gene whose major transcription factor is Peroxisome Proliferator- Activated Receptor-alpha (PPAR-α). Any change in the mRNA sequence can bring about a change in the gene product. The alteration in the mRNA sequence could be due to one or more mutations. We are currently studying Nonequilibrium Thermodynamics to incorporate it in the study of translation to maximize the production of Carnitine Translocase, thereby preventing its deficiency in the cell [6][7].

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