Random Weighting through Linear Programming into Intracellular Transporters of Rice Metabolic Network

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Abstract. An eukaryotic cell has different compartments which are specific to different biological activities. The total cellular metabolism is also compartmentalized. The intracellular transporters within a cell are responsible to transport some of the metabolites of one compartment to other. We formulate a model to understand the utility of different transporters. Here, we have taken a partially compartmentalized genome scale metabolic model of rice (*Oryza sativa*). Depending on the gene-expression, the transporters available to transport the metabolites from one compartment to other would change. We study the effect of transporter's capacity on the overall metabolism. We find that depending on the effectiveness of transporters, the photon demand for a rice leaf's biochemical machinery to synthesize the necessary biomass from inorganic nutrients, changes upto three fold. We also observe, interactions of mitochondrial and chloroplastid reactions are associated with this change.

Keywords: Random Weighting, Linear Programming, Metabolic Network, Genome Scale Model, Intracellular Transporter.

1 Introduction

Understanding the plant's cellular metabolism using Flux Balance Analysis (FBA) [1] is one of the active topic in systems biology. FBA is a mathematical approach, mainly based on linear programing, to analyze the flow of metabolites in a metabolic network. The genome scale metabolic model of plants are available [2, 3] and it has been demonstrated that FBA can be used to understand different cellular behaviors like the pathways involved in biomass production and also the change of pathways under varying conditions. In the FBA, one wants to optimize an objective function. In metabolic model, several optimization criterion including maximizing biomass composition or minimizing the total reaction flux have been used.

The human mitochondrial metabolic model has been analyzed for three different objectives using the Pareto optimality criteria and this also uses different

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weights [4]. Here, we have utilized the variation of weight in objective function to mimic the different cellular behavior under perturbed conditions.

Random weighting is used to develop genetic algorithm for bi-level mixed linear integer programming [5]. Multiobjective optimization using NISE method for conflicting objectives like maximizing succinic acid production versus maximizing biomass production for an in silico model of E Coli also uses different weights in each iteration to maximize each objective [6].

Unlike prokaryotes, eukaryotic cells have more complex structures with different compartments for specific tasks. Membrane bounded compartments form a diffusion barrier and prevent uncontrolled exchange of intermediates [7]. While weighting to non-transporters is related to mimicking the gene expression of enzymes, weighting to transporters is used to capture the capacity of transporting the metabolites from one compartment to other. Gene expression of a cell vary under different conditions and these conditions demands different transport mechanism in intracellular level. Thus, we are mimicking the possible active metabolic states of the cell under different conditions, in specific, here we determine the effect of intracellular transporter's transport capacity on the overall metabolism.

2 Materials and Methods

2.1 A Genome Scale Model of Rice

A Genome Scale Model (GSM) of rice (*Oryza sativa*), developed in our group [3] is used to reconstruct the network with different weight among Intracellular Transports (ICT). The constraints are same as used by (Poolman, 2013) [3]. This model has 9 mitochondrial and 14 chloroplastidial intracellular transporters. Here, all intracellular transporters are randomly weighted from 0 (no cost) to 1000 (extreme costly) except photon transporter in chloroplast which is free to use any value. Here, the flux through ATPase is not fixed, however it has a lower cut-off of 0.1. The biomass is fixed in the proportions as experimentally observed and other details are same as described in [3].

2.2 Linear Programming

Linear programming is a well known mathematical method used to achieve best possible solution among several options [8, 9]. Either we can minimize or maximize our objective such as lower flux (low cost) or higher biomass production (high gain) in plant system with some given constraints. Here, metabolic network model of rice is used to form a stoichiometric matrix

$$\mathbf{S}_{m\times n} \tag{1}$$

where m is number of metabolites and n is number of reactions. All the reactions in model is mass balanced; if $\mathbf{v}$ is the flux vector of reactions, then at steady state

$$\mathbf{S}.\mathbf{v} = 0 \tag{2}$$

$$l \leq \mathbf{v} \leq u \tag{3}$$

where l and u correspond to lower and upper bounds of the fluxes, respectively. Objective function in FBA is

$$Z = \mathbf{w}.\mathbf{v} \tag{4}$$

where $\mathbf{w}$ is the weight vector of reactions. We vary $\mathbf{w}$ to mimic the different transport capacity of the transporters. Here, we choose minimization of Z which corresponds to minimization of total reaction fluxes, *i.e.*, optimizing the cellular economy.

2.3 Algorithm

1. Load Model *//From a ScrumPy model file*
2. Build Linear Programming (LP) Object *//Include model in glpk-lp*
3. Set Constraints of LP *//Lower (l) and upper (u) bound of fluxes*

$$\begin{cases} l \leftarrow 0, u \leftarrow +\text{AV for irreversible reaction} \\ l \leftarrow -AV, u \leftarrow +\text{AV for reversible reaction} \end{cases} \quad \textit{//AV is Any Value of fluxes}$$

4. Set Fixed Flux of Biomass *//Flux of all biomass precursors are fixed in desired proportion*
5. Set Objective to Minimize Z *//Minimize total reaction fluxes*
6. T = List of Intracellular Transporters *//Includes 23 intracellular transporters*
   for $i = 1$ to cardinality of T, do
   $\mathbf{w}_{\mathrm{T}_i} \leftarrow$ A random integer in [0,1000] *//uniform integer distribution*
   end for *//Random weight assignment on all ICT is complete*
7. Solve LP Using Simplex Method *//to get flux distributions*
8. Get Solution *//Solution space of reactions with their respective fluxes*
9. End

The algorithm is shown only for a single iteration.

2.4 Package Used for Analysis

ScrumPy - is a Metabolic Modelling Tool implemented by Python programming language [10] and all computations are done using this.

3 Results and Discussion

We repeat the algorithm for 1000 times and accumulate the solution for each weight-set on ICT. These solutions allow us to predict the results described below.

3.1 General Responses

Among 1733 reactions 365 were used and 189 are always active (except biomass precursors which are fixed to active all the time). It indicates that these 189 reactions are essential for cell in any condition. We observe that O_2 and CO_2 transport in chloroplast and pyruvate and AlphaKG transport in mitochondria should remain active irrespective of any cost imposed on them. So they are essential for carrying metabolites to and from compartments which are important for plant survival and biomass production.

3.2 Photon Uses Efficiency

Minimum and maximum photon fluxes are 0.32 and 1.08, respectively. Figure 1 shows that maximum number of combinations of ICT weights use low photon while some of the combinations use high photon. We further observe that the higher amount of photon and thus energy is needed for some combinations of weight factors. Observing the weights we could say which of the intracellular transporters are favorable in different conditions. It is observed that the plant can synthesize the biomass with comparatively lower amount of photon when the chloroplastidial PGA and MalOxAc transporters are highly active.

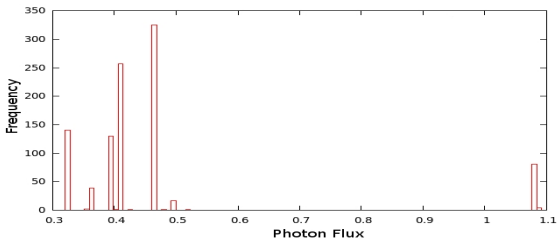

Fig. 1. A histogram of photon use. Photon uses can increase up to three times depending on the usability of ICT. Low photon use occurs for more than 85% of the combinations.

3.3 Observation of Compartmental Activity

There are several modes of TCA cycle throughout the solution, some are conventional cyclic and others are truncated or non-cyclic (Figure 2). Being one of the main energy production mechanism in aerobic organisms, these different modes reflects different energy demand conditions in cell. Cyclic mode establishes when demand for ATP is high or alternative sources are not producing sufficient ATP. When demand for ATP is low or alternative ATP sources are sufficient then TCA cycle becomes truncated. Some of the operative modes of TCA cycle are already reported in [2, 11].

When the photon flux is low, more ATP is generated by the mitochondrial Electron Transport Chain (ETC) and/or by full TCA cycle to achieve sufficient energy requirement. When sufficient ATP is generated by light reactions in the chloroplast, generation of extra ATP is prevented by maintaining low flux through Complex V (shown in the scatter plot in Figure 2) or controlling ETC and using different non cyclic modes. So generation and utilization of energy source is a controlled process and TCA modes are major controller of the same. At the same time, our results indicate that there exists a relationship of chloroplast and mitochondrial metabolism.

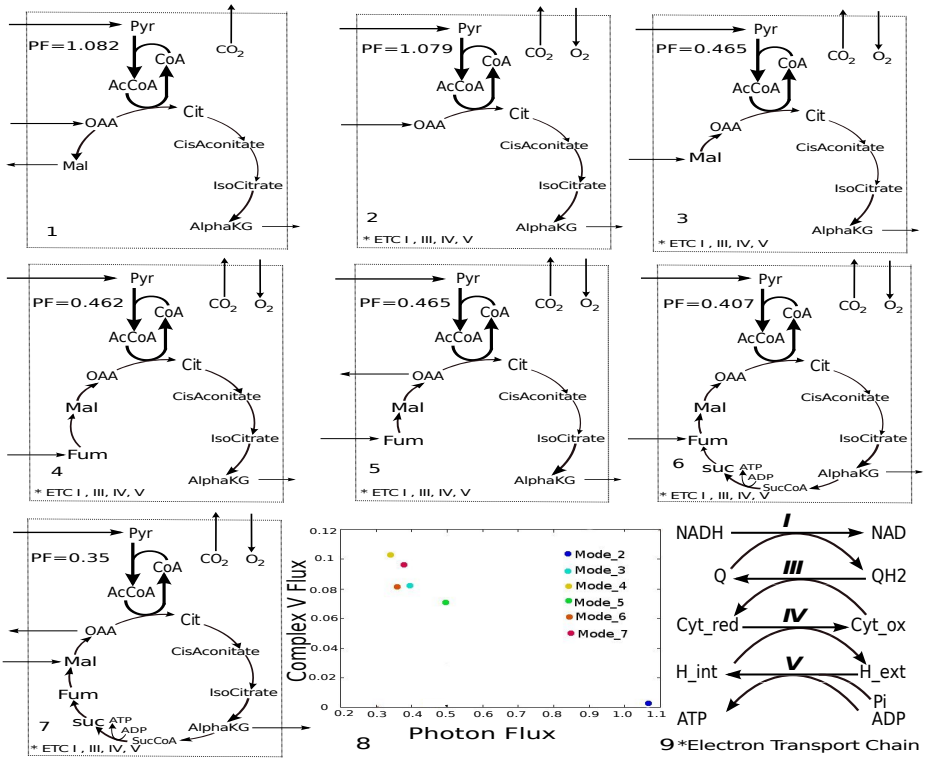

Fig. 2. Modes of TCA Cycle. PF is Photon Flux, ETC is Electron Transport Chain, 1: Non-cyclic mode which doesn't require ETC, 2,3,4,5: Non-cyclic modes using ETC, 6,7: Cyclic Modes, 8: Scatter Plot of Photon Flux Vs Complex V Flux, 9: Electron Transport Chain which is present in modes 2,3,4,5,6,7. Incoming arrow into the dotted box indicates metabolite going into mitochondria and vice versa.

4 Conclusions

Here, we have identified that i) the flux through the chloroplast transporters control the demand of photon to synthesize biomass ii) depending on the transporter's transport capacity, different modes of TCA cycle become active iii) there

exists an interaction of chloroplast and mitochondrial metabolism. Finally, this method can be used to mimic the effect of variations of enzymatic gene expressions in cellular metabolism.

Abbreviations Used in Figures and Elsewhere:
CoA:Coenzyme A; Pyr:Pyruvate; IsoCitrate:isocitrate; AlphaKG:alpha ketoglutarate; SucCoA:succinyl-CoA; Fum:Fumarate; Mal:Malate; OAA:oxaloacetate; AcCoA:Acetyl-CoA; CisAconitate:cis-aconitate; suc:succinate; Cit:citrate; Cyt _red:cytochrome c reductase; Cyt_ox:cytochrome c oxidase; Q:ubiquinone; QH2: ubiquinol; _ext: external; _int: internal; PGA: 3-phosphoglycerate; MalOxAc: malate oxaloacetate; PEP: phosphoenolpyruvate; G6P: glucose 6-phosphate, ATPase: ATP demand.

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