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Hierarchical organization of fluxes in *Escherichia coli* metabolic network: Using flux coupling analysis for understanding the physiological properties of metabolic genes

Zhaleh Hosseini, Sayed-Amir Marashi *

Department of Biotechnology, College of Science, University of Tehran, Tehran, Iran

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ABSTRACT

Flux coupling analysis is a method for investigating the connections between reactions of metabolic networks. Here, we construct the hierarchical flux coupling graph for the reactions of the *Escherichia coli* metabolic network model to determine the level of each reaction in the graph. This graph is constructed based on flux coupling analysis of metabolic network: if zero flux through reaction *a* results in zero flux through reaction *b* (and not vice versa), then reaction *a* is located at the top of reaction *b* in the flux coupling graph. We show that in general, more important, older and essential reactions are located at the top of the graph. Strikingly, genes corresponding to these reactions are found to be the genes which are most regulated.

1. Introduction

Metabolic networks are certainly among the best-studied biochemical networks (Pfeiffer et al., 2005). The possibility of reconstruction of metabolic network models from genomics (and other omics) data (Thiele and Palsson, 2010), together with successful use of such network models in studying biologically important questions, has made these networks very suitable in systems biology studies (Oberhardt et al., 2009; Kim et al., 2012).

There are different well-established methods to investigate the relationship between fluxes through a pair of reactions of a metabolic network (Xi et al., 2011; Lewis et al., 2012). Flux coupling analysis (FCA) is one of these methods (Burgard et al., 2004). According to this analysis, three types of coupling may exist between a pair of metabolic reactions *i* and *j* (Burgard et al., 2004; Larhlimi and Bockmayr, 2006):

1) Directional coupling: when non-zero flux through reaction *i* implies non-zero flux through reaction *j*, and not vice versa;
2) Partial coupling: when non-zero flux through reaction *i* implies non-zero flux through reaction *j* and vice versa, but the flux ratio of these two reactions is not fixed; and
3) Full coupling: when non-zero flux through reaction *i* implies non-zero flux through reaction *j* and vice versa, and additionally, the flux ratio between the reactions is a fixed value.

The reaction pairs that are not classified in any of these categories are determined to be uncoupled.

In addition to finding the coupling type of a given pair of fluxes and determining blocked reactions in a network, FCA can also help us in studying concepts such as “equivalent knockouts” or “set of affected reactions”. The former is referred to a group of reactions that their removal leads to zero flux of a specific reaction. The latter are all the reactions that cannot have non-zero flux when a particular reaction is removed (Burgard et al., 2004).

Several studies have investigated the link between flux coupling relations of metabolic reactions and different biological properties of the metabolic genes/reactions, including evolutionary history of genes, protein–protein and genetic interactions, position of reactions in the cell, and gene/reaction essentiality (see below).

The connections between horizontal transfer of metabolic genes during evolution and other properties of these genes and their associated reactions were investigated by Pál et al. (2005a). It was shown that most of the changes in the genomes of organisms are due to adaptation to changes in the environmental conditions. In prokaryotes, horizontal gene transfers are responsible for the aforementioned genomic changes and gene duplications have less impact in this process. It was also suggested that peripheral reactions are more susceptible to the horizontal gene transfer while the central parts of metabolism networks are

Abbreviations: FCA, flux coupling analysis; FCG, flux coupling graph.

* Corresponding author.
E-mail address: marashi@ut.ac.ir (S.-A. Marashi).

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relatively more conserved. Additionally, it was shown that evolutionary age of the transferred genes is inversely related to the irregularities in codon usage and the GC contents. Transferred genes are only essential in specific conditions, while more conserved genes are essential in most of the conditions (Pál et al., 2005a).

The genes with coupled reactions are often transferred together and this transfer is more probable in fully coupled reactions compared to directionally coupled reactions (Pál et al., 2005b). A similar study has shown that the most abundant relation among coupled reactions in a cell is asymmetric relation (i.e., directionally coupled reaction pairs). For a given gene pair with asymmetric relation, the independent gene is more essential, more conserved and more expressed compared to the dependent gene (Notebaart et al., 2009). In a recent study by Nam et al. (2012) it was shown that the enzymes catalyzing only one reaction with only one substrate (specialist enzyme) are more essential, carry more flux and are more regulated compared to generalist enzymes. It was suggested that these properties are unchanged, even with changes in the environmental conditions. Another study (Samal et al., 2006) showed that most of the essential genes are among the genes with reactions that uniquely produce (consume) a metabolite. This is because these reactions are the only way to produce (consume) that metabolite and if these metabolites are essential, their removal leads to cell death.

Szappanos et al. (2011) investigated the connection between genetic interactions and flux coupling relations. It was found that although genetic interactions are enriched between the genes associated with fully/partially coupled reactions, a large number of these interactions are among genes with uncoupled reactions (Szappanos et al., 2011). Studying the connection between co-expressivity of genes and their type of flux coupling has proved that genes with coupled reactions have the same expression patterns and are often located in the same operon. In the same study, it has been also shown that the coupling relationships between reactions can better explain the co-regulation of genes compared to other concepts such as reaction distances in the network (Notebaart et al., 2008).

Two recent studies have also considered the link between metabolic networks and the evolution of metabolic genes. In one study, underground reactions, i.e., those reactions which can be seen as weak side metabolic activities of some existing enzyme (with other known activities), and their role in the evolution have been investigated recently. The authors indicated that these reactions give the cell the capability to grow in new environments (Notebaart et al., 2014). In a more recent study, it was shown that there is a negative correlation between the flux carried by reactions of the network, and the dN/dS (nonsynonymous to synonymous substitution rate ratio) of the genes associated with the reactions. The authors suggested that the slower rate of nonsynonymous substitutions for genes with higher fluxes means that they were more constrained during evolution (Colombo et al., 2014).

In the present work, we calculate the flux coupling relations of metabolic reactions and construct a hierarchical flux coupling graph for these reactions. Our goal is to relate some of the biological properties of reactions of the *Escherichia coli* metabolic network model with their location in the flux coupling graph. Reaction essentiality, reaction positions in the cell metabolism, evolutionary age of the reactions and regulatory state of the reactions are studied for the reactions of the network. We also performed gene ontology analysis in order to find the overrepresented biological processes in the graph. We investigated the possible relations between any of the aforementioned properties and the level of each reaction in the graph. The reactions at the top of this graph have independent fluxes and a larger number of reactions are dependent on them (compared to the reactions at the bottom). Therefore, we investigate whether these reactions are more important for cell metabolism, e.g., whether they are more essential, more conserved, or they are involved in more central cell processes.

2. Materials and methods

2.1. Construction of the flux coupling graph (FCG)

There are different algorithms to compute flux coupling relations among metabolic reactions (David et al., 2011; Larhlimi et al., 2012). We utilized F2C2 software for flux coupling analysis (Larhlimi et al., 2012). We then used the flux coupling matrix created by this tool to construct the flux coupling graph with Graphviz software (Gansner and North, 1999). In this graph, which is a directed acyclic graph (Fig. 1A), nodes are the reactions and edges represent directional coupling relations. In other words, a directed edge from reaction A to reaction B shows that $v_A = 0$ implies $v_B = 0$, where v_A and v_B are elements of flux activity vector, v, corresponding to reactions A and B, respectively. More precisely, v includes the reaction fluxes of a metabolic network, which satisfies stoichiometric, thermodynamic and capacity constraints.

Before constructing the graph some modifications were made in the flux coupling matrix:

1- Two reactions which are fully/partially coupled have the same coupling relation with all the other reactions. Therefore, reactions in each of the fully/partially coupled reaction set were merged to one node (Fig. 1B).

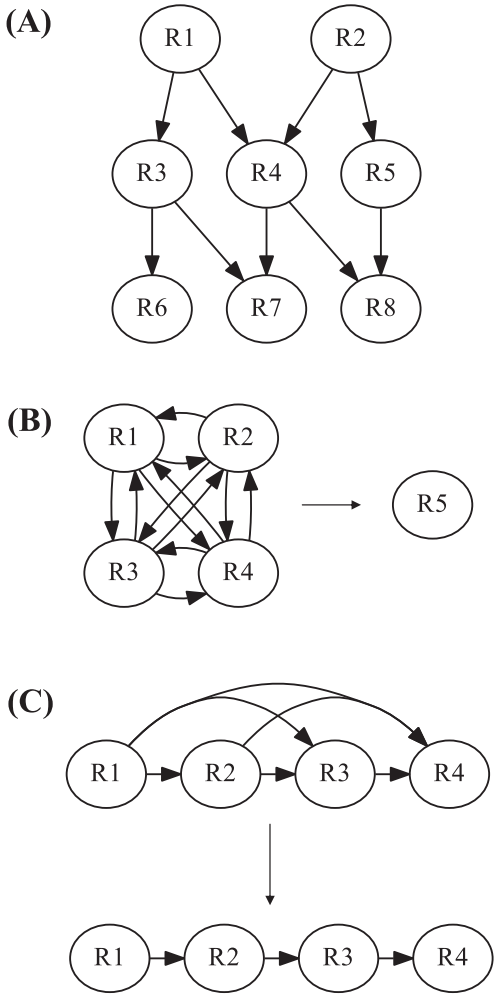

Fig. 1. (A) An example of a flux coupling graph. Here, nodes represent reactions and edges represent directional coupling relation between reactions; (B) Merging a set of fully/partially coupled reactions to a single node; (C) Removing unnecessary edges from the graph (based on transitivity).