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Reporter Metabolites Analysis from Inorganic Carbon Acclimation in *Synechococcus elongatus* PCC7942

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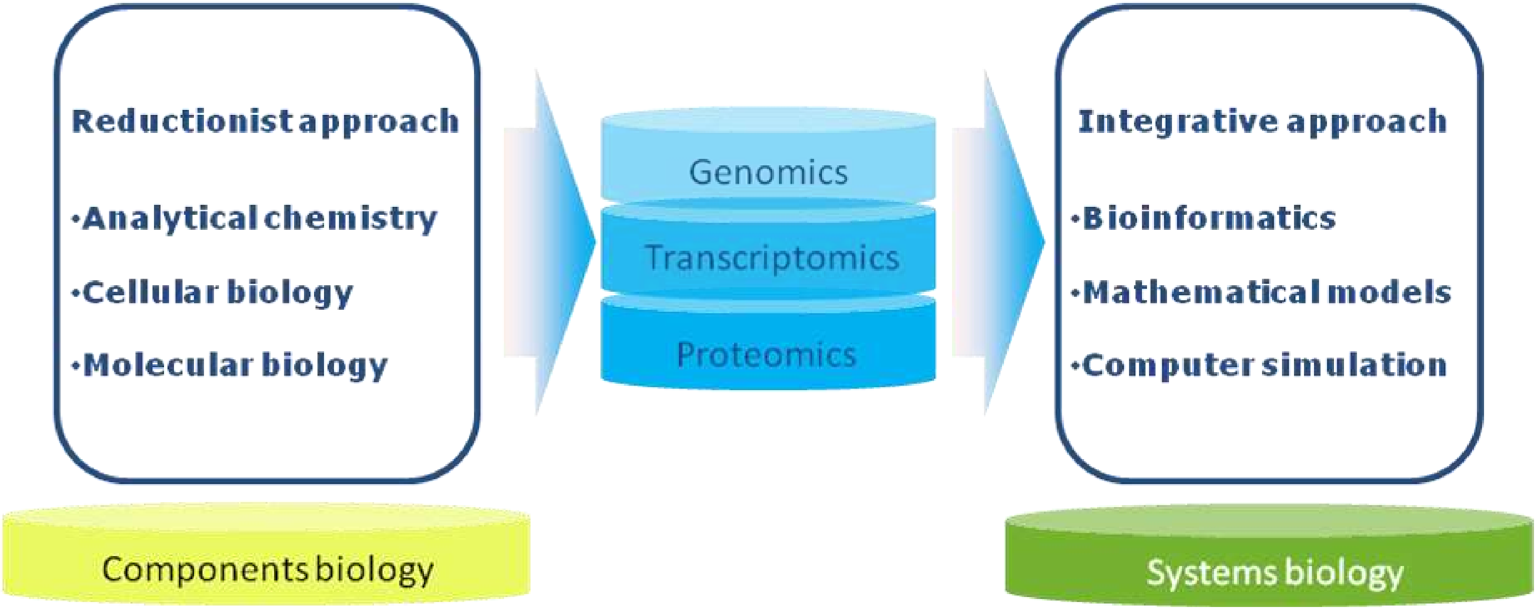
Abstract:

Synechococcus elongatus PCC7942 represents a classical microbial model that has been widely studied. Its genome-scale metabolic network was recently published (*i*Syf715), allowing the integration of high-throughput data with the network connectivity. The high-dimensionality of this analysis paves the way not only for the designing of metabolic engineering strategies but also for the principles elucidation of certain cellular processes functioning. Here we present a reporter metabolites analysis of transcriptional profile of *S. elongatus* PCC7942 under inorganic carbon acclimation by the integration with the connectivity structure of *i*Syf715. The analysis is based on the published transcriptional changes after 6 and 24 hours of cells growth, when the CO_2 concentration was shifted from high to low levels. The *Reporter Features* algorithm was applied to unveil the 52 scattered reporter metabolites ($p<0.05$) across the whole cyanobacterium metabolism, which could represent key regulatory nodes during the CO_2 regime transitions. In addition, clusters of metabolic genes that significantly and coordinately change their expression during this perturbation were identified by using the *Reporter Subnetwork* algorithm. These results could support the subsequent inference of feasible transcriptional regulatory maps as well as the assessment of the metabolic network capabilities of *i*Syf715.

Keywords: *Synechococcus elongatus* PCC7942; Inorganic Carbon Acclimation; Reporter Metabolites; Reporter Features Algorithm; Reporter Subnetwork Algorithm

Introduction

The high-throughput analytical methods have forced changes in classical reductionist analyses towards new integrative approaches.



Reductionist approach

- **Analytical chemistry**
- **Cellular biology**
- **Molecular biology**

Genomics

Transcriptomics

Proteomics

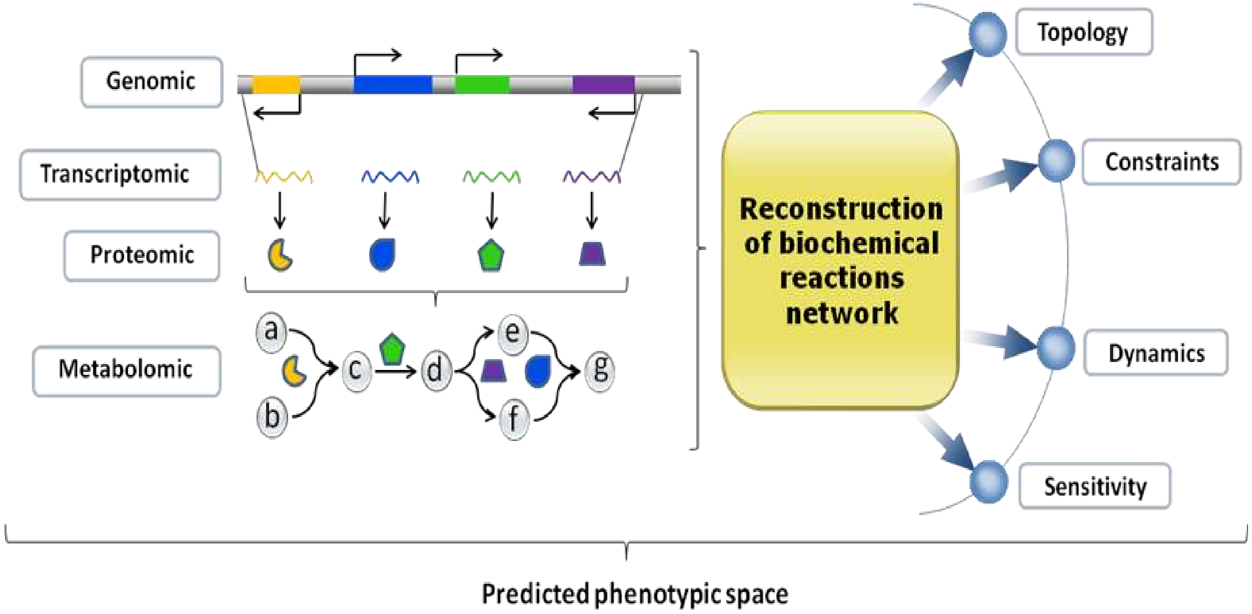
Integrative approach

- **Bioinformatics**
- **Mathematical models**
- **Computer simulation**

Components biology

Systems biology

Introduction

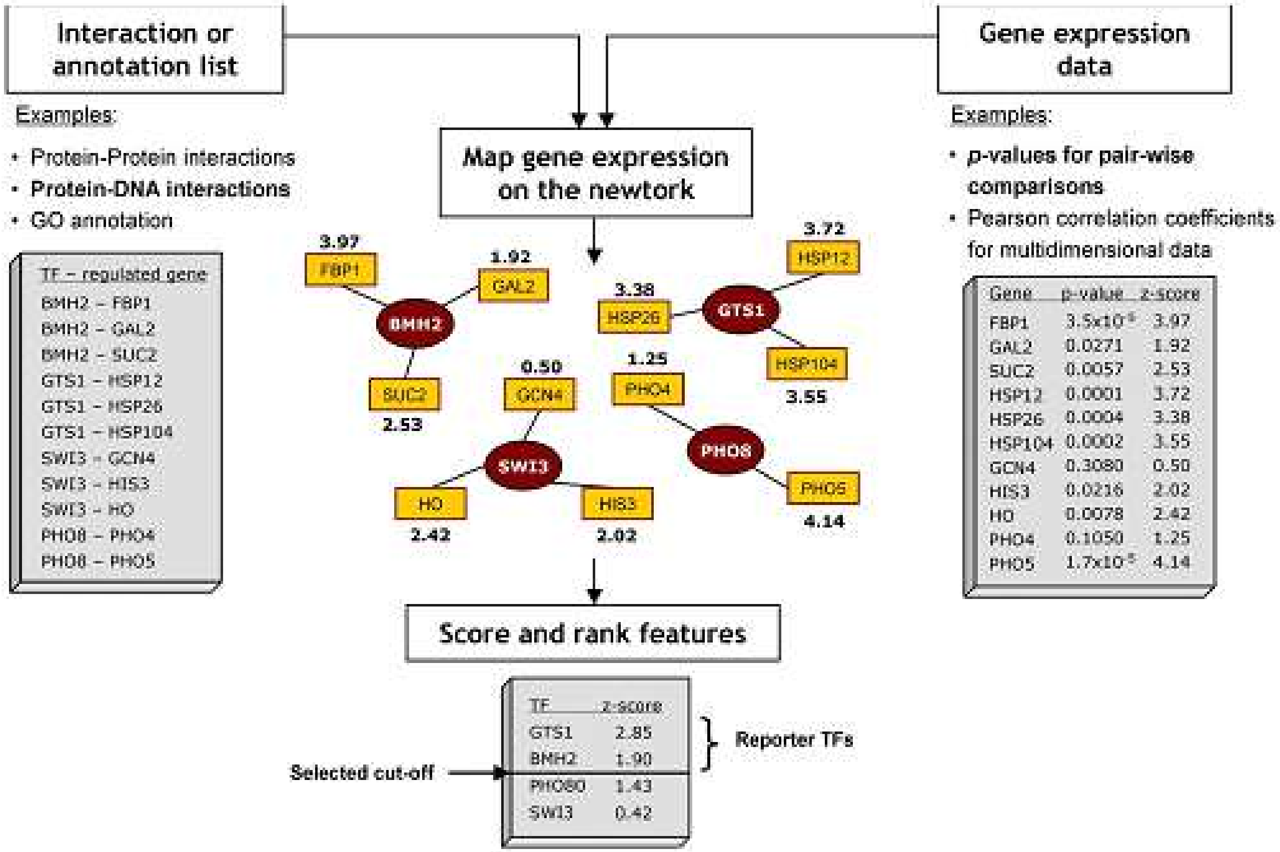


Several techniques have been used in order to analyze and extract knowledge from the vast amounts of *-omics* data, such as transcriptome and metabolome.

However, the problem of integrating these layers of information and elucidate new system's principles is still unresolved.

Introduction

Oliveira *et al.* proposed a new approach that considerably intents to reduce the dimensionality of the data analysis problem (Oliveira *et al.*, 2008)*.



Interaction or annotation list

Examples:

- Protein-Protein interactions
- **Protein-DNA interactions**
- GO annotation

TF – regulated gene
BMH2 – FBP1
BMH2 – GAL2
BMH2 – SUC2
GTS1 – HSP12
GTS1 – HSP26
GTS1 – HSP104
SWI3 – GCN4
SWI3 – HIS3
SWI3 – HO
PHO8 – PHO4
PHO8 – PHO5

Gene expression data

Examples:

- *p*-values for pair-wise comparisons
- Pearson correlation coefficients for multidimensional data

Gene	p-value	z-score
FBP1	3.5×10^{-5}	3.97
GAL2	0.0271	1.92
SUC2	0.0057	2.53
HSP12	0.0001	3.72
HSP26	0.0004	3.38
HSP104	0.0002	3.55
GCN4	0.3080	0.50
HIS3	0.0216	2.02
HO	0.0078	2.42
PHO4	0.1050	1.25
PHO5	1.7×10^{-5}	4.14

Map gene expression on the newtork

Score and rank features

TF	z-score
GTS1	2.85
BMH2	1.90
PHO80	1.43
SWI3	0.42

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*Oliveira AP, Patil KR, Nielsen J. (2008) **Architecture of transcriptional regulatory circuits is knitted over the topology of bio-molecular interaction networks**. *BMC Syst Biol*, **2**:17.

Introduction

(Oliveira *et al.*, 2008)

- These authors propose the use of genome-scale bio-molecular interaction information, such as physical or functional interactions between metabolites in reconstructed metabolic networks, constraining the solution space and revealing regulatory principles under certain physiological conditions.

- The developed algorithm, named Reporter Features, allows for the identification of cellular regulatory focal points (*i.e. reporter features*). In the case of the metabolic network, the algorithm would unveil R*eporter Metabolites*, which can represent regulatory hubs of the metabolism.

Introduction

- *Synechococcus elongatus* PCC7942 as photo-biological production platform of value-added metabolites.
- Genome-scale metabolic network model of *Synechococcus elongatus* PCC7942 (*i*Syf715) (Triana *et al*., 2014)*.

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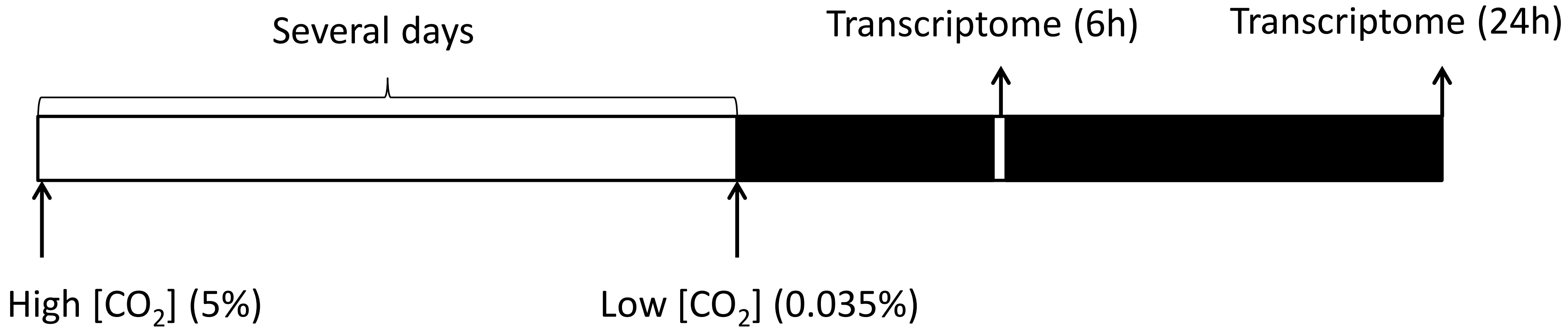
*Triana J, Montagud A, Siurana M, Fuentes D, *et al*. (2014) **Generation and evaluation of a genome-scale metabolic network model of *Synechococcus elongatus* PCC7942**. *Metabolites*, **4(3):**680-698.

Introduction

Transcriptional changes in *S. elongatus* PCC7942 cells, that had been shifted from high to low CO_2 levels in growth conditions, were reported by Schwarz *et al.* (Schwarz *et al.*, 2011)[*].



Several days

Transcriptome (6h)

Transcriptome (24h)

High [CO_2] (5%)

Low [CO_2] (0.035%)

Objective: perform analyses that help to identify around which metabolites (reporter metabolites) the transcriptional changes are significantly concentrated; and thus, to identify cellular regulatory focal points.

[*] Schwarz D, Nodop A, Hüge J, *et al.* (2011) **Metabolic and transcriptomic phenotyping of inorganic carbon acclimation in the cyanobacterium *Synechococcus elongatus* PCC 7942**. *Plant Physiol*, **155**:1640-1655.

Methodology

Transcriptome data analysis

- Gene expression data from the comparison of two inorganic carbon acclimation conditions (Schwarz *et al.*, 2011) was integrated in the metabolic reconstruction.

- Reporter Features algorithm (Patil and Nielsen, 2005; Oliveira *et al.*, 2008), currently available online at Biomet Toolbox (Cvijovic *et al.*, 2010) (http://www.sysbio.se/BioMet), was used for this purpose.

Methodology

Transcriptome data analysis

The algorithm works with three kinds of information:

(a) *p-values* for genes, resulting from, for example, Student’s *t*-test or the Mann-Whitney *u*-test run on transcriptomic data.

(b) *interaction file,* where genes/reactions are connected to the corresponding features, in this case the corresponding substrates and products.

(c) *association file*, where genes are linked to the corresponding reactions, either by coding for the enzyme or by regulating the gene that codes for the enzyme.

$$z_{gene\ i} = \mathrm{cdf}^{-1}\left[1 - p_{gene\ i}\right] \longrightarrow z_{featurej} = \frac{1}{\mathrm{N_j}} \sum_{k=1}^{Nj} \mathrm{z_{gene\,k}} \longrightarrow z_{featurej}^{corrected} = \left(\frac{\mathrm{z_{feature\ j}} - m_N}{s_N} \right)$$

$p_{gene\ i}$: *p-value* for a given node

cdf^{-1} : inverse normal cumulative distribution function

Nj: neighbor nodes (genes)

m_N: mean of random aggregates of size *N*

S_N: standard deviation of random aggregates of size *N*

Results and discussion

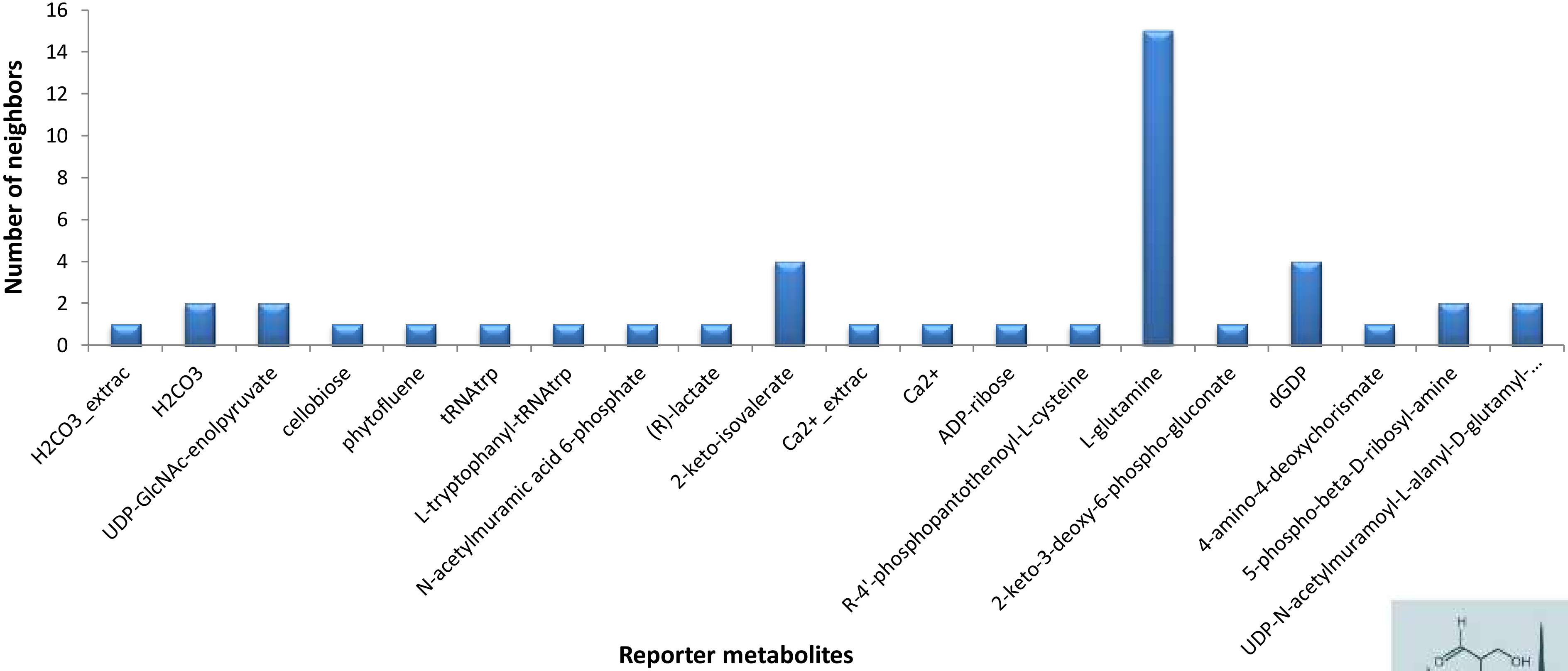
1. Reporter Metabolites from Reporter Features Approach

*i*Syf715 metabolic model file together with the p-values for differential expression under the two conditions (see methods) was used to rank the *reporter metabolites*.

Results and discussion

1. Reporter Metabolites from Reporter Features Approach

The top 20 ranking metabolites out of 52 significant hits ($p<0.05$) are shown in the figure.



Results and discussion

1. Reporter Metabolites from Reporter Features Approach

- During growth on low ambient CO_2 condition, HCO_3^- transporters together with carbonic anhydrase, form the central component of the CO_2 concentrating mechanism that support the production of the biomass precursors.
- *UDP-GlcNAc-enolpyruvate*, *N-acetylmuramic acid 6-phosphate* and *(R)-lactate* are important intermediates in aminosugars metabolism (implicated in redox reaction).
- *Cellobiose* which is hydrolyzed to beta-D-glucose (as an alternative carbon source in response to low carbon fixation flux).
- *Phytofluene* participate in carotenoid biosynthesis, such as zeta-carotene (protecting against potentially lethal photooxidative damage).

Results and discussion

1. Reporter Metabolites from Reporter Features Approach

- *tRNAtrp* and *L-tryptophanyl-tRNAtrp* are metabolites involved in protein biosynthesis, (L-tryptophan is activated).
- *2-keto-isovalerate* is a 2-keto acid implicated in several pathways, such as: L-valine, L-leucine and coenzyme A biosynthesis (decarboxylation processes).
- Ca^{2+}*_extrac* and Ca^{2+} correspond to the uptake of this cofactor (related with nitrogen deprivation in *S. elongatus* PCC7942).
- *ADP-ribose* is involved in purine metabolism and is a precursor of D-ribose-5-phosphate and D-ribulose-5-phosphate (physiological response of the system to a reduction in carbon sequestration).
- *R-4'-phosphopantothenoyl-L-cysteine* which is related with coenzyme A biosynthesis (pantetheine 4'-phosphate decarboxylation)

Results and discussion

1. Reporter Metabolites from Reporter Features Approach

- The *L-glutamine* is an intermediate in several pathways, like: amino acids metabolism, pyrimidine, purine and folate biosynthesis, cobalt, non-cobalt and coenzyme B12 pathway, glutathione and aminosugars metabolism (Conversely, it seems that only some reactions of these pathways, picture a scenario of global regulation).
- *2-keto-3-deoxy-6-phospho-gluconate* is a reporter from pentose phosphate pathway (involved pyruvate decarboxylations).
- *dGDP* is an intermediate of purine metabolism.
- *4-amino-4-deoxychorismate* and *5-phospho-beta-D-ribosyl-amine*, which constitute intermediates in folate biosynthesis and purine metabolism, respectively (chorismate and 5-phosphoribosyl 1-pyrophosphate are probably mostly used as a carbon skeletons for subsequent nitrogen assimilation).

Results and discussion

1. Reporter Metabolites from Reporter Features Approach

- *UDP-N-acetylmuramoyl-L-alanyl-D-glutamyl-meso-2,6-diaminoheptanedioate* constitutes a metabolite into the peptidoglycan biosynthesis pathway (peptidoglycan synthesis could be affected).

Results and discussion

1. Reporter Metabolites from Reporter Features Approach

Hot spots within the metabolic network (each of 52 reporter metabolites in a whole metabolic map). Some of the metabolites (yellow points) are repeated in the graph.

Results and discussion

2. Reporter Subnetwork Approach

Sets of genes that significantly change their expression coordinately

184 ORFs

- 88 ORFs
  - 89% Carbohydrate metabolism
  - 11% Amino acids metabolism
- 96 ORFs
  - 42% Nucleotide metabolism
  - 39% Metabolism of cofactors
  - 18% Nitrogen metabolism
  - 1% Carotenoids metabolism

Conclusions

- Reporter Features was applied to study the inorganic carbon acclimation in *Synechococcus elongatus* PCC7942.
- Several metabolites (52) that could represent regulatory hubs when metabolism is turned on from HC to LC for 6 and 24 h, were identified.
- To acquire a better overview of the hot spots within the metabolic network, the 52 reporter metabolite in a whole metabolic map were visualized.
- By using the Reporter Subnetwork algorithm, we uncover sets of genes that significantly change their expression coordinately during the perturbation.

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