Temperature Dependent Changes in Glycolysis in the Thermoacidophilic Red Alga *Galdieria sulphuraria*

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Abstract

Galdieria sulphuraria is a thermoacidophilic unicellular red alga found in extreme environments, such as volcanic areas. *G. sulphuraria* can grow at temperatures up to 56oC, with a growth optimum between 35oC and 42oC. To understand adaptation to high temperatures, a temperature jump experiment was performed with RNA-Seq (RNA-sequencing /whole transcriptome shotgun sequencing) at warm (46oC) and cold (28oC) temperatures. The analysis here focuses on the glycolytic pathway, the backbone of cellular metabolism. From existing RNA-Seq data, the temperature dependent changes in transcript levels for genes encoding glycolytic enzymes are determined. Our analysis indicates significant changes in metabolic fluxes.

Keywords: glycolysis, temperature dependence, thermoacidophile, RNA-sequencing

Introduction

Highly acidic, toxic metal environments are not ideal for eukaryotic microorganisms, but this is not the case for *Galdieria sulphuraria*. This extremophile is found in habitats where the pH is between 0-4 and the temperature reaches up to 56 degrees Celsius (Schönknecht 2013). It is able to grow photoautotrophically or heterotrophically in environments that are typically devoid of any photosynthetic organisms. Since it grows on rock, rather than water, it experiences significant temperature changes between day and night (Gross 1997). To acclimate to different temperatures organisms have to change gene expression. A RNA-seq experiment allows one to analyze changes in gene expression. This method takes a snapshot of the organism's mRNAs at a specific moment in time. Once all mRNAs have been sequenced, the organism's gene expression is quantified. The data collected gives information on all processes in the organism. Out of the information for 6,623 protein-coding genes, my task was to look at the 33 genes coding for the enzymes in glycolysis and gluconeogenesis at different temperatures.

Glycolysis is considered the backbone of metabolism and glycolytic enzymes do account for 10 to 20% of total protein in most cells (Liebermeister 2014). Organisms can use the glycolytic pathway in two different directions. One direction is catabolism, which allows the break down of energy rich molecules such as the 6-carbon molecule, glucose, into pyruvate, for ATP biosynthesis. This break down of sugars also leads to amino acid biosynthesis, the production of proteins, and ultimately the production of most of an organism's structures. The reverse metabolic process, gluconeogenesis, is the production of glucose. Both pathways are never fully active at the same time (Ferrier 2014).

Enzymes are protein catalysts that increase the rate of a reaction. Both glycolysis and gluconeogenesis is composed of multienzyme sequences (Ferrier 2014)[refer to Figure 1]. Enzymes do not determine the direction of the reaction, but are simply key components in speeding up the time it takes for a reaction to reach equilibrium. Some reactions have energy profiles that make them almost irreversible. Looking at the how the temperature change affects the expression of genes encoding glycolytic enzymes will help to better understand how the organism responds to temperature changes.

Methods

In previous experiments, as described by Weber (2007) mRNA was isolated from *G. sulphuraria* cultures grown at either 28oC or 46oC for different times (see Figure 2) and sequenced in paired-end mode on two lames with an Illumina HiSeq2000 machine. Culture conditions where *G. sulphuraria* was grown are described in more detail in Schönknecht, G., et al. (2013). RNA counts were normalized for gene size and sample size to obtain Fragments Per Kilobase of Exon per Million mapped reads (FPKM) as calculated by RSEM. Read mapping, read count, and calculation of differentially

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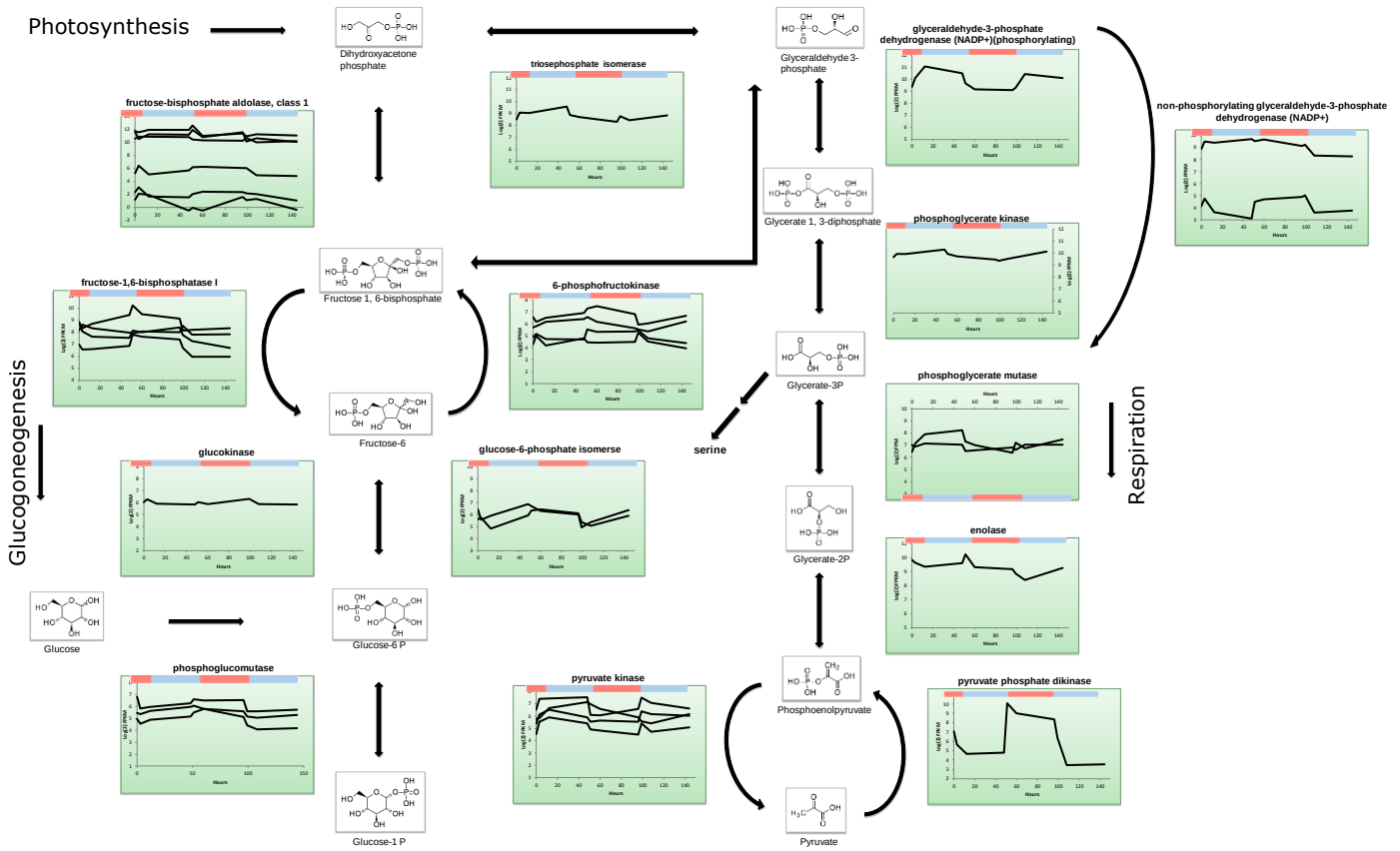

Figure 1 *- The glycolytic pathway indicating temperature- dependent (colored bar) changes in transcription levels (Log(2) FPKM values) of genes encoding glycolytic enzymes. The red (46ºC) and blue (28ºC) bar on each graph indicates the temperatures at which the RNA samples were taken. Arrows on the very left and right indicate directions of gluconeogenesis and respiration sugar breakdown, respectively.*

expressed genes was performed with RSEM (Li and Dewey 2011) and edgeR (Robinson McCarthy and Smyth 2010.)

We analyzed the genes that encode enzymes of the glycolytic pathway. This was done mainly by projecting changes in FPKM values at different conditions (i.e. from different samples) onto KEGG map 00010 (glycolysis/gluconeogenesis)The experimental part (i.e. algal cultures, RNA isolation, RNA sequencing) and the initial bioinformatics analyses (i.e. mapping and calculation which genes were differentially expressed) was performed at the Institute for Plant Biochemistry at the University of Düsseldorf, Germany. For further experimental details see Rossini (2015).

Results

Most reactions in glycolysis are freely reversible, as indicated by arrows going in both directions. Arrows going in one direction only, indicate reactions, with a strongly preferred direction; these control the overall direction of metabolic fluxes. Transcripts levels for specific enzymes increase or decrease depending on heat or cold. The graph allows to draw conclusions about metabolic flux changes with temperature.

Looking at the graph, the arrows pointing in both directions indicate enzymes that are freely reversible. These enzymes, such as phosphoglycerate kinase and fructose-bisphosphate aldolase, class 1, show very little temperature dependent changes in expression. As discussed earlier, most enzymes a not rate limiting, and are seen at comparable expression levels at both temperatures. This means they are active in reactions that occur at both high and low temperatures. As a result, these enzymes, such as phosphoglycerate kinase and fructose-bisphosphate aldolase, class 1, are not expected to show much temperature-dependent fluctuation in expression level.

The enzymes that are only seen in one way reactions are signified by the arrows pointing in one direction. The figure indicates that during a decrease in temperature transcripts for certain enzymes increased. Looking at the graph going from glucose to pyruvate, there is not a significant increase in a particular enzyme before the triosephosphates, which are dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. It is observed that in the cold, triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase (NADP+)(phosphorylating), and phosphoglycerate kinase, which channel triosephosphates into amino

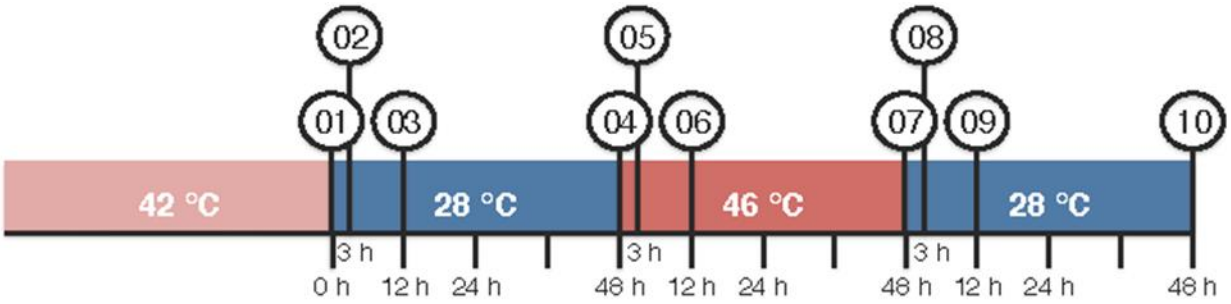

Figure 2 *- The temperatures and hours at which RNA samples were drawn. This bar matches the bar on each graph in Figure 1.*

acid biosynthesis or to pyruvate for ATP production, increase transcripts in the cold. Additional information from outside studies, support the evidence that serine biosynthesis increases in the cold. The graph shows a significant increase in the enzyme, pyruvate kinase, which contributes to pyruvate for ATP production. This indicates that ATP production and amino acid biosynthesis increase at lower temperatures.

In much the same way, genes coding for certain enzymes were observed in the increasing temperatures. Pyruvate phosphate dikinase and fructose-1,6-bisphosphatase I, which are both key enzymes for the production of glucose from pyruvate increased in the warm environment. The graph indicates at elevated temperatures significant change in gene expression occurs in favor of gluconeogenesis, which is the production of sugars. From this study it is observed that some enzymes are neither temperature dependent, nor rate limiting. Transcript levels that code for specific enzymes revealed that in decreasing temperatures, amino acid biosynthesis and pyruvate production is prevalent. In the reverse direction, gluconeogenesis is dominant in increasing temperatures.

Discussion

Analyzing the graphs of transcript levels of glycolytic enzymes we observed that in the cold, the break down of stored sugars (glucose) into pyruvate for ATP biosynthesis by respiration and for amino acid biosynthesis dominates. In the warm environment, metabolic flux is reversed and production of glucose prevails. We also saw that some enzymes that catalyze freely reversible reactions, and are not rate limiting, show little temperature dependent transcription changes, as expected. Last, we saw that in the cold, there is an increased flux of triose phosphates from photosynthesis into serine biosynthesis. These findings indicate that 28°C induce cold stress in *G. sulphuraria*, resulting in mobilization of stored sugars and increased protein biosynthesis. While at 46°C, closer to the optimum growth temperature, photosynthetic activity allows for gluconeogenesis, i.e. the net production of sugar reserves. Obviously, *G. sulphuraria* not only tolerates high temperatures, but is well adapted to them, and is cold stressed at 28°C.

References

Ferrier, D. R., 2014. *Biochemistry*. Lippincott Williams & Wilkins, PA, USA.

Gross, W. J. Kuver, G. Tischendorf, N. Bouchaala, and W. Busch. 1997. Cryptoendolithic growth of the red alga Galdieria sulphuraria in volcanic areas. *European Journal of Phycology*. 33:25-31.

Li, B. and C Dewey. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics, 12(1):323

Liebermeister, W., E. Noor, A. Flamholz, D. Davidi, J. Bernhardt, and R. Milo, R. 2014. Visual account of protein investment in cellular functions. *Proceeding of the National Academies of Science Of the United States of America* 111: 8488-8493.

Robinson, M.D., D. J. McCarthy, and G. K. Smyth. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics, 26(1):139–140,

Rossoni, A. 2015. Master's Thesis 'systems biology of the cold response in extremophilic red algae', University of Düsseldorf, Germany.

Schönknecht, G. et al. 2013. Gene Transfer from bacteria and archaea Facilitated Evolution of an extremophilic eukaryote. *Science* 339:1207-1209.

Weber, A.P.M., K. L. Weber, K. Carr, C. Wilkerson, and, J. B. Ohlrogge. 2007. Sampling the Arabidopsis transcriptome with massively parallel pyrosequencing. *Plant Physiology*. 144, 32-42.