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Transcriptional Profiling of Stress Response or Slow Growth?

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Abstract

Transcriptomic profiling analyses are frequently used for the study of cells in response to environmental stress factors that often impede cell optimal growth. Given that transcriptional profiles of optimally growing cells differ significantly from those of sub-optimally growing cells, stress-induced differentially-transcribed genes are thus inevitably mixed with slow growth genes. It is therefore necessary to separate the stress-specific response genes from slow growth genes. Methodologies used to deconvolute stress-specific response from non-specific response such as slow growth are discussed in this editorial.

Keywords: Microarray; Next-generation sequencing; Transcriptomic profiling; Stress response; Slow growth

Introduction

Transcription regulation is one of the major ways to control gene activity in cells. Hence, transcription levels of genes are thought to be linked largely to the levels of gene activity. Serial Analysis of Gene Expression (SAGE) was the technology that for the first time allows the study of a transcriptome or a majority of transcripts in eukaryotic cells [1]. Instead of sequencing the entire Expressed Sequence Tag (EST) derived from cDNA libraries [2], the SAGE method takes only a ~15 bps tag from end of each cDNA fragment and ligates to chain multiple short cDNA tags for PCR amplification and sequencing analysis. By comparing cDNA tag sequences against EST databases (http://ncbi.nlm.nih.gov/nucest), a transcriptomic profile can be thus obtained [1]. Nevertheless, this technique is a bit tedious and expansive, which limited its wide applications.

Nearly at the same time, DNA microarray technologies, especially the two-colour DNA microarrays, have been developed for the study of transcriptomes without involvement of DNA sequencing [3]. In this case, a DNA microarray is made by spotting cDNA fragments or pre-synthesized ORF-specific oligomers of 50-80 nucleotides in length on glass slides or synthesizing oligomers in situ. Fluorescence cyanine Cy3 and Cy5 dyes are coupled with cDNA derived from treated and control samples (respectively) and co-hybridized with the microarray in a chamber. Transcription levels of individual ORFs are acquired through the scanned image of the chip. Due to saturation of hybridization signal on chip, it is believed that the dynamic range of transcription levels in a transcriptome is underestimated by hybridization-based methodologies when compared to DNA sequencing-based technologies. Regardless of this concern, DNA microarray technologies are still widely used in studies including but not limited to transcriptomes due to its affordability and easy handling.

Nowadays, high-throughput sequencing technologies that are also known as Next-Generation Sequencing (NGS) technologies are getting more and more popular for transcriptomic studies in organism with or without complete genome sequences. Next-generation sequencing such as HiSeq2500 platform is based on sequencing by synthesis method (http://www.illumina.com) that runs 300 million to 2 billion sequence reads of 50-500 bps in length simultaneously. Assembly of massive short sequence reads requires information of complete genome sequences. However, development of the de Bruijn graph-based sequence-alignment algorithms has permitted transcriptome assembly without reference genome or *de novo* [4]. Both microarray and NGS methods are frequently applied for the study of genome-wide transcriptional profiling of cells in response to various stress factors, besides others.

Common Environmental Stress Response Genes

Transcriptomic profiling analysis of cells in response to various environmental stress factors is thought to be a convenient way to annotate biological function of many novel genes discovered through genome sequencing projects. Early genome-wide transcriptional profiling studies have found that majority of the stress response genes in yeast are common to all tested environmental stress factors such as heat shock, osmotic shock, oxidative stress, and nutrient depletion and so on [5,6]. It is therefore that these genes are designated as Environmental Stress Response (ESR) genes [5] or Common Environmental Response (CER) genes [5]. Approx. 10% of the yeast genome are found to be ESR or CSR genes [5,6]. It is assumed that stress responsive genes are required for growth fitness under the respective stress condition. However, functional analysis of a large set of barcoded deletion strains in yeast indicated that CSR genes have shown no correlation with requirement of growth fitness under the respective stress conditions. It is thus puzzling why cells alter transcription levels of ESR or CSR genes upon treatment with various stress factors.

Slow Growth Genes

However, it is difficult to distinguish between the primary effects caused by the addition of stress factors and the secondary effects arising from growth inhibition. Analysis using chemostat cultures of wild type and mcm1 in yeast showed that most of the differentially transcribed genes in mcm1 are a result of slow growth, because they are not differentially transcribed after cells adjusting growth rate [7]. Careful analysis of transcriptomic profiles of yeast cells under various nutrients has revealed the distinct transcriptomic profiles of cells under different growth rates. The results reveal that majority (80%) of ESR or CSR genes are growth-rate related genes or slow growth genes [8]. This is further supported by the study showing connections among growth rate, metabolism, stress, and the cell cycle [9]. It is inevitable that addition of stress factors reduces the growth rate of cells. Hence,

identification of the primary effects by stress factors requires deconvolution of transcriptional changes caused by growth inhibition, the secondary effects by stress factors.

Deconvolution of Stress Response from Slow Growth

In fission yeast, transcription factor Atf1p is known to be a key regulator involved in oxidative stress response [10]. Analyses show that some but not all oxidative stress induced genes whose promoter is bound by Atf1p [11]. Phenotypic assessment of Atf1p-bound genes regardless of differential transcription in response to oxidative stress indicates a tight association between Atf1p-bound genes and requirement for growth fitness under oxidative stress. On the other hand, Atf1p-unbound oxidative response genes appear to be unrelated to growth fitness [11]. Based on genes that are controlled by the major regulator for oxidative stress response in fission yeast, it is possible to deconvolute between primary and secondary effects on differentially transcribe genes. However, this approach is unsuitable for studies in organisms whose major regulators involved in stress responses are unknown.

Dunaliella has an ability to survive under the wide ranges of salinity with the optimal growth from 0.5 M NaCl to 2 M NaCl [12,13]. Hence, it becomes a popular model for study of cellular adaptation to salinity changes [14]. To explore transcriptional changes upon salinity changes in Dunaliella, Kim et al. [15] discovered 112 differentially transcribed genes upon salinity increase from 1.5 M NaCl to 4.5 M NaCl and 85 differentially transcribed genes upon salinity decrease from 1.5 M NaCl to 0.08 M NaCl. It was found that 58 differentially transcribed genes were common to both salinity increase and decrease. However, none of them shows inversely correlated transcriptional changes upon salinity increase and decrease, suggested that the slow growth genes dominated when cell exposure to extreme salinities. Indeed, all 58 common genes are found to be both up-regulated or both down-regulated upon salinity increase and decrease.

Transcription profiling analysis of Dunaliella in response to reciprocal salinity changes within the optimal growth conditions between 0.5 M to 2 M NaCl, Fang et al. [16] showed that majority (100 out of 130) of the differentially transcribed common genes appeared to be inversely correlated in response to salinity increase and decrease, suggesting the salinity-specific responses. Analysis of transcriptional level changes of enzymes involved in glycerol and its potential carbon sources metabolisms permit predicting metabolic flux of Dunaliella cells in response to salinity changes.

Conclusion

Transcriptomic profiling analysis is a useful method to study cellular response to treatment of environmental stress factors. However, deconvolution of the primary effects caused by stress factors from the secondary effects resulting from slow growth is essential. Profiling analysis of cells in response to reciprocal alterations of stress and stress-free conditions can facilitate identification of inversely-correlated differentially-transcribed common genes, the bonafide stress factor-specific response genes.

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