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Genome-wide expression analyses of the stationary phase model of ageing in yeast

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ABSTRACT

Ageing processes involved in replicative lifespan (RLS) and chronological lifespan (CLS) have been found to be conserved among many organisms, including in unicellular Eukarya such as yeast *Saccharomyces cerevisiae*. Here we performed an integrated approach of genome wide expression profiles of yeast at different time points, during growth and starvation. The aim of the study was to identify transcriptional changes in those conditions by using several different computational analyses in order to propose transcription factors, biological networks and metabolic pathways that seem to be relevant during the process of chronological ageing in yeast. Specifically, we performed differential gene expression analysis, gene-set enrichment analysis and network-based analysis, and we identified pathways affected in the stationary phase and specific transcription factors driving transcriptional adaptations. The results indicate signal propagation from G protein-coupled receptors through signaling pathway components and other stress and nutrient-induced transcription factors resulting in adaptation of yeast cells to the lack of nutrients by activating metabolism associated with aerobic metabolism of carbon sources such as ethanol, glycerol and fatty acids. In addition, we found STE12, XBP1 and TOS8 as highly connected nodes in the subnetworks of ageing yeast.

1. Introduction

Ageing is considered to be a complex and regulated cellular process that is defined as a decline in the ability to protect against cellular stress and damage, or repair (or counteract) some of the its consequences (Sharon et al., 2009). Different theories of aging have been put forward, for example those that are the so-called "programmed" theories and those that are "damage and/or error" theories (Jin, 2010). Not all of the proposed theories apply to the yeast model but some do, such as the theory of free radicals (Balaban et al., 2005) and the programmed longevity theory (Davidovic et al., 2010; Longo et al., 2005). The discovery of genes and pathways mediating lifespan extension supports the presence of lifespan-regulatory programs in many organisms. Ras, protein kinase A (PKA), Sch9 and the target of rapamycin (TOR) have been found to be evolutionarily conserved from yeast to multicellular eukaryotes. Ras2, Tor1 and Sch9 are yeast orthologues of mammalian Ras, mTOR, Akt and S6 kinase (S6K), respectively (Longo and Fabrizio, 2012).

Yeast *Saccharomyces cerevisiae* is one of the preferred eukaryal model organisms, due to many experimental advantages and large number of knowledge- and data-collections. Many yeast genes have mammalian orthologues and many metabolic pathways are conserved between yeast and mammal cells so it is possible to translate discoveries from yeast to other cells, including human cells.

S. cerevisiae has been used as a model organism to study two ageing paradigms in *Eukarya*: replicative ageing, measured by the replicative lifespan (RLS) and chronological ageing measured by the chronological lifespan (CLS) (Longo and Fabrizio, 2012). The RLS is determined by the total number of daughter cells produced by a mother cell before it stops dividing. The extrachromosomal ribosomal DNA circles (ERCs) are considered as toxic species associated with the RLS (Longo and Fabrizio, 2012). The protein Sir2p is known to regulate ERCs production by inhibiting rDNA recombination and subsequently reducing ERCs accumulation (Longo et al., 2012). Mutation of SIR2 gene, thereby, decreases replicative lifespan. Sirtuin 1 (SIRT1) is the mammalian orthologue of the Sir2p and it is a promising target against ageing-related diseases (Wang et al., 2012). The yeast CLS measures survival time of non-dividing cells. Several methods to measure CLS have been established see (Longo et al., 2012) for review. Under standard conditions (syn-

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Table 1
List of R packages for gene expression analyses.

Name	Description	Reference
Piano	Basic microarray analyses and gene set analyses (See online documentation: http://bioconductor.org/packages/release/bioc/vignettes/piano/inst/doc/piano-vignette.pdf)	(Varemo et al., 2013)
Affy	Include functions for processing Affymetrix probe-level data to expression values e.g. loading CEL file, background correction, normalization and probe set annotation	(Gautier et al., 2004)
PLIER	Affymetrix algorithm to estimate expression values	(Hubbell et al., 2005)
Limma	Linear model fitting procedure for differential expression analysis	(Smyth, 2005)
Reporter features	Hypothesis-driven method for gene-set enrichment analysis	(Varemo et al., 2013)
BioNet	Integrated analysis of gene expression data in the context of biological networks to identify functional modules	(Beisser et al., 2010)

thetic medium SD with 2% glucose), the cells stop growing and dividing when nutrients are depleted and they adapt to nutrient depletion through signal transduction that alters transcriptional responses and expression of downstream genes (Galdieri et al., 2010). The yeast CLS has been considered to be a model for ageing of non-dividing metazoan cells.

Microarray technology is useful to identify the genome-wide transcriptional changes which help to establish which cellular processes are vital during chronological ageing of yeast, at different stages. Gene expression assays have been extensively used to study lifespan extension in yeast, particularly under calorie restriction. Several studies commonly highlight that Ras, Tor1 and Sch9 pathways inhibit Rim15p and stress response transcription factors such as Msn2p,4p and Gis1p (Cheng et al., 2007; Wei et al., 2008; Wei et al., 2009). Under calorie restriction, the Ras, Tor1 and Sch9 pathways are inhibited, which in turn promotes changes in gene expression of stress-responsive genes, such as mitochondrial superoxide dismutase (MnSOD) and heat shock proteins (HSPs). This enhances cellular protection and prolongs the lifespan (Wei et al., 2008). The Sp1-transcription factor (Gis1p orthologue) was found to be responsive to cellular redox state in mammals (Wei et al., 2008). Additionally, Gis1p and Rph1p regulate genes in acetate and glycerol metabolism (Orzechowski Westholm et al., 2012).

A number of computational approaches have been implemented for gene expression analyses; for instance, the identification of differentially expressed genes from a static or temporal experiment (Storey et al., 2005), gene clustering for pattern finding (Zhang et al., 2009), gene-set enrichment analysis (Varemo et al., 2013) and network-based analysis for functional modules (Lorenz et al., 2009). One goal of microarray studies is to find genes whose expression levels change differentially between two samples that are being compared. Resulting genes lists can provide hypotheses for the molecular basis of conditional adaptations. The problem with differential gene expression analysis is that it usually results in very large genes lists (hundreds or thousands), even when using very stringent cut-offs (such as a p value <0.001). The gene-set enrichment analyses help with biological interpretation of such gene lists by adding known biological information such as biological processes and metabolic pathways. Mapping transcriptome data to the network scaffold can contribute to identification of functional modules which then help with our interpretation of how pathways (or proteins) orchestrate specific processes under a particular condition (Berger et al., 2013).

We grew wild type yeast and sampled the RNA during the exponential phase, 2 days, 6 days and 10 days after inoculation, and we performed a genome-wide transcriptional analysis, using DNA microarrays. Different gene expression analyses were used in order to obtain not only the list of significantly changed genes, but to identify relevant transcription factors, biological processes and metabolic pathways which transcriptionally change during the ageing process. Table 1 contains the list of computational packages used in this study, and their brief descriptions. This integrated approach could assist understanding of the transcriptional changes during chronological ageing in yeast, and thus could guide further studies in this field.

2. Materials and methods

2.1. Microarray data acquisition and analyses

S. cerevisiae wild type strain 113-7D (haploid, mating type A) without selection markers, was grown in synthetic complete medium (SD) (Nijkamp et al., 2012) and cell samples were collected during the exponential growth phase (log phase (Log)), 2 days (D2), 6 days (D6) and 10 days (D10) of incubation. CEN.PK113-7D is a laboratory strain derived from ENY.WA-1A and MC996A strains, and has been used previously in systems biology studies. Each sampling was performed in triplicate. The microarray dataset that was generated was deposited in the GEO database (Accession number GSE55508).

First, the microarray data were preprocessed using the Piano R package (Varemo et al., 2013) which is integrated with the Affy R package (Gautier et al., 2004). Preprocessing includes the scanned array image files (CEL file) loading, background correction, expression annotation, estimation and probe set annotation, normalization (with PLIER) (Gautier et al., 2004). Such processed data were used for pairwise analyses (PA) to compare changes in gene expression of stationary yeast cells (D2, D6 or D10) versus logarithmic growth phase (Log). The PA were based on the linear model fitting procedure with the limma package (Smyth, 2005) and the obtained p-values were adjusted for multiple testing with a False discovery rate (FDR) of 5%. Table 1 contains the list of programs used in this study.

For each PA, we conducted the following gene-set enrichment analyses (GSA): biological processes (BP), transcription factors (TF) and metabolic pathways (PTW). The BP gene sets are based on gene ontology (GO) annotations for yeast genes, as defined by the SGD (Saccharomyces Genome Database, http://www.yeastgenome.org). Transcriptional regulation interactions were collected from YEASTRACT (Abdulrehman et al., 2011; Harbison et al., 2004), and the PTW gene sets are based on the genome-scale metabolic model iTO977 (Osterlund et al., 2013). The GSA were performed using the reporter features method in the Piano package (Patil and Nielsen, 2005; Varemo et al., 2013). The adjusted p-values from each PA were used as the input to the reporter features.

In addition to pairwise comparisons, the gene expression data were analyzed in a time-series fashion to find genes differentially expressed based on their temporal expression profiles. In the following, $S(t-1)$ and $S(t)$ denote two sequential observations for a

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