There is a steady-state transcriptome in exponentially growing yeast cells

Vicent Pelechano and José E. Pérez-Ortín*
Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universitat de València, C/Dr. Moliner 50, 46100 Burjassot, Spain

*Correspondence to: José E. Pérez-Ortín, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universitat de València, C/Dr. Moliner 50, 46100 Burjassot, Spain.
E-mail: jose.e.perez@uv.es

#Current address: EMBL, Meyerhofstrasse 1, Heidelberg, Germany.

Received: 29 October 2009
Accepted: 10 February 2010

Abstract
The growth of yeast cells in batches in glucose-based media is a standard condition in most yeast laboratories. Most gene expression experiments are done by taking this condition as a reference. Presumably, cells are in a stable physiological condition that can be easily reproduced in other laboratories. With this assumption, however, it is necessary to consider that the average amount of the mRNAs per cell for most genes does not change during exponential growth. That is to say, there is a steady-state condition for the transcriptome. However, this has not been rigorously demonstrated to date. In this work we take several cell samples during the exponential phase growth to perform a kinetic study using the genomic run-on (GRO) technique, which allows simultaneous measurement of the amount of mRNA and transcription rate variation at the genomic level. We show here that the steady-state condition is fulfilled for almost all the genes during most exponential growth in yeast extract–peptone–dextrose medium (YPD) and, therefore, that simultaneous measures of the transcription rates and the amounts of mRNA can be used for indirect mRNA stability calculations. With this kinetic approach, we were also able to determine the relative influence of the transcription rate and the mRNA stability changes for the mRNA variation for those genes that deviate from the steady state. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: transcription rate; steady state; transcriptome; Saccharomyces cerevisiae; mRNA stability

Introduction

Evaluation of the amount of mRNA (RA) for a given gene by means of northern blot, qPCR or other techniques is a routine in many molecular biology experimental protocols. Comparisons between different physiological situations or between different cell types or mutants are habitually studied. As a significant number of mRNAs can vary their concentrations, even upon subtle changes in internal or external conditions, it is crucial to sample cells in identical situations in order to guarantee repetitiveness and comparative-ness between experiments. This is especially true for batch cultures of free-living microorganisms where the growth rate and environment are continuously changing. This problem can be solved by using continuous culture conditions, but such conditions are more technically demanding. Therefore, most published experiments use batch conditions. For most batch culture analyses, researchers select exponential growth in a rich medium as the default state for comparison, as it is usually thought to be a physiologically constant condition in which most, or all, mRNAs remain unchanged in a steady-state condition. This can facilitate comparisons between experiments done in different laboratories. However, because the emergence of genomic techniques has raised the possibility of simultaneously quantifying most cellular mRNAs at the same time, the
need for a rigorous demonstration of the steady-state condition now extends to thousands of genes.

One of the most studied organisms in functional genomics is the yeast *Saccharomyces cerevisiae*. There are hundreds of experiments on this yeast using cells growing on YPD (yeast extract–peptone–dextrose) complete medium in the ‘exponential growth condition’. It is known, however, that many genes vary during exponential growth when cells approach the diauxic shift (DeRisi *et al.*, 1997) or change in a growth rate-dependent manner (Regenberg *et al.*, 2006). As stated above, the use of chemostats has been described as a more reliable way to avoid such variations because they keep culture conditions stable (Daran-Lapujade *et al.*, 2009; Hayes *et al.*, 2002). However, most yeast researchers have used, and still use, the exponential growth condition in batch cultures for their experiments.

Although the majority of studies focus only on the determination of RA, it is becoming increasingly clear that gene expression should be studied as a kinetic process in which the amount of mRNA is controlled not only by transcription but also by the influence of mRNA stability (RS, also called mRNA half-life; Pérez-Ortín *et al.*, 2007). In exponential growth a dynamic steady state can be assumed for RA, i.e. the transcription and degradation rates (TR and DR, respectively) are equal. TR follows a zero-order kinetics (does not depend on RA), whereas DR follows first-order kinetics:

\[
DR = k_d \cdot RA
\]

Therefore, in steady state:

\[
TR = DR = k_d \cdot RA
\]

where \( k_d \) is the degradation constant, which is inversely related to RS \( (k_d = \ln 2/RS) \). Thus, in this situation, TR can be calculated from experimentally determined RA and stability data (Holstege *et al.*, 1998); alternatively, DR can be calculated from RA and TR data (García-Martínez *et al.*, 2004). Both approaches are becoming increasingly popular for TR or DR calculations, although it has not yet been established whether the true steady-state condition for gene expression actually applies (Pérez-Ortín, 2007). A steady state defined for RA does not necessarily imply steady states for TR and DR. In fact, it would be theoretically possible for TR and DR to change simultaneously in parallel, keeping RA constant.

In this paper we address the topic of assessing the steady-state condition for mRNA amounts in *S. cerevisiae* cells growing in YPD at the exponential phase. First we performed an experiment to determine whether the steady state applies for yeast in which we simultaneously measure TR and RA for all the genes. We also studied the kinetic behaviour of any gene that seems to deviate from it.

In this way we have demonstrated that the steady-state condition for RA is true for most of the genes studied, but that there are small but significant changes in some groups of genes, which change either their TR or DR as the culture approaches the diauxic shift. Because TR also remains almost invariable, we can say that there is also a steady state for TR and DR. Finally, we reanalysed previously published data (Radonjic *et al.*, 2005) to expand the time window of our study and to determine how long the steady-state condition is maintained. We found that the RA steady state is fulfilled over a long period within the exponential phase.

**Materials and methods**

**Yeast strain and growth conditions**

Yeast strain BQS252 (*MATa, ura3-52;* derived by sporulation from FY1679) was used. Cells were grown in YPD (yeast extract 1%, peptone 2%, and glucose 2%) with agitation (190 rpm) at 28°C. The GRO was done essentially as described in previous publications (Radonjic *et al.*, 2004) to expand the time window of our study and to determine how long the steady-state condition is maintained. Yeast strain and growth conditions.

**Genomic run-on (GRO)**

The GRO was done essentially as described in García-Martínez *et al.* (2004). Briefly, two aliquots of ca. 4 × 10⁸ cells were harvested at each time point. One aliquot was used directly for the GRO protocol, in order to obtain TR data, while the other one was frozen for subsequent total RNA extraction. The cells of the GRO sample were harvested.
permeabilized with 0.5% Sarkosyl and incubated for 20 min on ice. Then the cells were allowed to extend the nascent RNA in the presence of [α-33P]-UTP. Finally, the radioactively labelled RNA was extracted and hybridized onto a nylon macroarray (Alberola et al., 2004). After TR determination, the total RNA sample was labelled by cDNA synthesis with random hexamers and hybridized in the same arrays in order to determine the RA.

Image analysis and data normalization

Images were quantified using Array Vision software, v. 7.0 (Imaging Research Inc.). The signal intensity for each spot was the background subtracted ARM (artifact removed median) density. Only values that were 1.35 times over the corresponding background were taken as valid measurements. The reproducibility of the replicates was checked using Array Stat software (Imaging Research Inc.). We considered the data to be independent and used a minimum number of two valid replicates in order to calculate the mean and standard deviation (SD) values for every gene. Normalization between conditions was done using the global median method.

Data analysis

We used the differential gene expression analysis program from the GEPA suite (Montaner et al., 2006) to analyse whether there was any significantly different slope from 0 for the variation of RA during exponential growth (Radonjic et al., 2005) with regard to a continuous independent variable (time). Similar results were obtained when the culture OD600 was used as a continuous independent variable instead of the time (data not shown).

The detailed RA and TR data for exponential growth between OD600 0.36 and 0.47 was calculated as previously described (García-Martínez et al., 2004; for the whole dataset, see Supporting information, Table S1). Briefly, the intensity of the mRNA or GRO hybridization was normalized using a genomic DNA hybridization signal and the U-richness (GRO) or C-richness (mRNA) correction. We computed the slope of RA and TR variations on a log2 scale with regard to time by assuming that the median TR and RA of the gene population remained constant during the experiment (see Supporting information, Table S2). Gene functional analyses were done by analysing either individual genes slopes (using FuncAssociate; Berriz et al., 2003) or gene sets (using Fatiscan from the BABELOMICS suite; Al-Shahrour et al., 2006). To analyse the changes in RS, we computed a theoretical RA variation dataset for all the genes, using the RA amount data obtained for the first time point and the experimentally obtained TR data for the whole experiment. Using these data, it is possible to calculate a theoretical RA for each point by assuming that the RS calculated the first time remains constant during the whole experiment (Pérez-Ortín et al., 2007). We compared the differences between this theoretically computed RA and the actual one, and assumed that the differences found would be due to stability changes during the experiment (see Figure 1A). To obtain a more robust RA variation, we performed a linear regression analysis of the actual and theoretical RA data and computed the predicted RA variation for each gene during our experiment, using all the time points. Finally, we computed the percentage of actual RA variation and the theoretical one (TR-dependent RA variation) for each gene. The difference between the actual and the theoretical RA directly gives the stability-dependent RA variation.

Accession numbers

The genomic data are stored in the Valencia Yeast (VYDbase: http://vydbase.uv.es/) and GEO databases. The GEO Accession No. for the set of different hybridizations is GSE11521.

Results

Verification of the steady state for mRNA on yeast cells using genomic run-on (GRO) analyses

To determine whether the yeast transcriptome was in a steady-state condition during the exponential phase on the YPD medium, we measured the variation of mRNA at five time points during exponential growth. As most of the published experiments start with a very low OD600 after inoculation and reach a middle exponential phase of 0.3–0.6 at OD600, we decided to monitor the mRNA variation under these conditions. We also used our GRO 102...
Figure 1. Relative contributions of the transcription rate (TR) and mRNA stability (RS) to the mRNA amount (RA) change. (A) Scheme of the protocol used to compute the mRNA variation, which is dependent in either TR or RS. The theoretical RA values in the experiment were computed for each gene by taking into account only the TR variations and by assuming a constant RS (in black). When comparing this theoretical TR-dependent RA change with the actual one (experimentally measured, in grey), it is possible to compute the percentage of RA variation for each gene, which is due to changes in either TR or RS. The data shown correspond to a sample gene (YAL004W) that has been chosen to show an important contribution of RS to RA variation. It is shown in natural scale to make the differences more evident. However its RA slope does not reach the significance cut-off for being statistically different from 0 (in either natural or log2 scale; FDR p value > 0.8). (B) Relative contribution of TR (light grey) or RS (dark grey) to the total RA variation (black) for selected gene groups shown in Table 1. Bars represent the median value for the variation for all the genes in each group. The groups with significant differences from 0 (as shown in Tables 1 and S3) are marked with black dots.

We performed an experiment in triplicate for the five time points at OD600 0.36–0.47 (spanning 40 min, at 10 min intervals), using cultures inoculated 16 h beforehand. Under these conditions, the diauxic shift started several hours later at OD600 ≈ 2 (V. Pelechano, unpublished observation). All 15 samples were subjected to the GRO protocol for calculating RA and TR. The repetitiveness of the samples was very good (see Supporting information, Figure S1). Pearson’s correlation was, on average, 0.934 for the TR data and 0.936 for the RA data between the biological replicates. The correlation for the data points for a single growth curve was even better: 0.965 for TR and 0.964 for RA. This suggests that there is more biological variation between growth curves in different experiments than within the time points of a single exponential phase curve. However, as we fused the different replicates, only the time-dependent variations (common for all three experiments) were taken into account.

We reasoned that the slight changes occurring during growth in the culture composition would affect the cells’ physiology, leading to a continuous change in RA or TR, which would be visible as positive or negative slopes in their respective plots. In this way, artifactual or random changes would not be accounted for. However, when we analysed these data using the GEPAS suite (a differential expression analysis tool; Montaner et al., 2006), no individual slope of any gene differed significantly from zero (FDR > 0.6). This result confirms that the steady-state condition applies for all (at least most of) the yeast genes. Therefore, no important error is associated when taking samples for mRNA quantification from cells growing in the early exponential phase. Thus, either TR or RS can be calculated in these growth conditions from the RA experimental data and either from the respective RS data, measured by conventional methods (Grigull et al., 2004), or from the TR data, measured by GRO by means of mathematical calculations (Pérez-Ortín, 2007).
However, the behaviour of genes can be too subtle to be discovered from studying single genes tendencies only. Therefore, in order to analyse this result in more depth, we used a tool from the BABELOMICS suite (Fatiscan algorithm; Al-Shahrour et al., 2006), which scans the whole set of gene data to discover any common trends in the GO categories. Specifically, this algorithm does a segmentation test that checks whether there are any significant asymmetries in the distribution of each GO. In this way, minor changes that are often too minor to be significant for individual genes can be statistically stressed by the common GO group behaviour. We scanned all the GO categories at all the possible levels. With this analysis, we found that some groups significantly increased or decreased during the experiment (Table 1A). Moreover, as we simultaneously measured RA and TR, we were able to distinguish between the different regulatory strategies.

Some group changes were detected in both TR and RA, but others were observed in only one of them. For instance, the translation-related categories were seen to lower RA and TR, which is in agreement with the decrease noted in the cell duplication rate that the culture would undergo several hours afterwards. Moreover, the differences between RA and TR behaviours illustrate the possibility of detecting the regulatory strategies followed for the mRNA changes: are the changes in RA due to changes in transcription or to changes in mRNA stability? If we assume that the steady-state condition applied for this experiment, then we could calculate the respective influence of TR and RS on the RA change (for a detailed explanation, see Figure 1A and Materials and methods). With this protocol, we were able to calculate their relative influence on the changes noted in each GO category, as seen in Table 1 and Figure 1B.

We analysed the slope of the RA variation that could be explained by RS changes using Fatiscan. Then we selected the groups with either a significant positive contribution (meaning increased stability) or a significant negative one (meaning decreased stability) (Table 1B). The fact that the number of statistical significant groups found for the RS changes is smaller than the ones found in the direct measures could be explained mainly by two factors; because it only takes into account the part of the variation for the RA, due to stability

Table 1. Functional groups with a significant deviation from the steady state during exponential growth

(A) Direct measures

<table>
<thead>
<tr>
<th>Groups in which Transcription Rate (TR) increases</th>
<th>Functional group</th>
<th>Adjusted p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.35 × 10⁻³</td>
<td>Protein modification</td>
<td>3.36 × 10⁻⁴</td>
</tr>
<tr>
<td>7.26 × 10⁻³</td>
<td>Post-translational protein modification</td>
<td>5.71 × 10⁻⁴</td>
</tr>
<tr>
<td>1.66 × 10⁻²</td>
<td>Cation transporter activity</td>
<td>1.27 × 10⁻²</td>
</tr>
<tr>
<td>2.53 × 10⁻²</td>
<td>Meiosis I</td>
<td></td>
</tr>
<tr>
<td>3.39 × 10⁻²</td>
<td>Oxidoreductase activity; oxidizing metal ions</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups in which transcription rate (TR) decreases</th>
<th>Functional group</th>
<th>Adjusted p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.82 × 10⁻¹⁰</td>
<td>Cytosolic part</td>
<td>2.52 × 10⁻⁸</td>
</tr>
<tr>
<td>1.33 × 10⁻⁶</td>
<td>Ribosome</td>
<td>2.78 × 10⁻⁷</td>
</tr>
<tr>
<td>8.91 × 10⁻⁵</td>
<td>Ribosome biogenesis and assembly</td>
<td>4.00 × 10⁻⁶</td>
</tr>
<tr>
<td>2.32 × 10⁻⁴</td>
<td>Structural constituent of ribosome</td>
<td>7.23 × 10⁻⁵</td>
</tr>
<tr>
<td>6.35 × 10⁻³</td>
<td>Translation</td>
<td>1.09 × 10⁻⁴</td>
</tr>
<tr>
<td>1.18 × 10⁻²</td>
<td>Cellular biosynthetic process</td>
<td>5.29 × 10⁻³</td>
</tr>
<tr>
<td>2.15 × 10⁻²</td>
<td>Cytoplasmic exosome (RNase complex)</td>
<td>4.16 × 10⁻²</td>
</tr>
<tr>
<td>2.15 × 10⁻²</td>
<td>Histidine biosynthetic process</td>
<td></td>
</tr>
<tr>
<td>3.39 × 10⁻²</td>
<td>Riboflavin metabolic process</td>
<td></td>
</tr>
<tr>
<td>3.39 × 10⁻²</td>
<td>Response to unfolded protein</td>
<td></td>
</tr>
<tr>
<td>3.39 × 10⁻²</td>
<td>Response to protein stimulus</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups in which mRNA amount (RA) increases</th>
<th>Functional group</th>
<th>Adjusted p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.36 × 10⁻⁴</td>
<td>Biopolymer metabolic process</td>
<td></td>
</tr>
<tr>
<td>5.71 × 10⁻⁴</td>
<td>Regulation of cellular process</td>
<td></td>
</tr>
<tr>
<td>1.27 × 10⁻²</td>
<td>Regulation of transcription</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups in which mRNA amount (RA) decreases</th>
<th>Functional group</th>
<th>Adjusted p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.52 × 10⁻⁸</td>
<td>Structural constituent of ribosome</td>
<td></td>
</tr>
<tr>
<td>2.78 × 10⁻⁷</td>
<td>Ribosome</td>
<td></td>
</tr>
<tr>
<td>4.00 × 10⁻⁶</td>
<td>Cytosolic part</td>
<td></td>
</tr>
<tr>
<td>7.23 × 10⁻⁵</td>
<td>Large ribosomal subunit</td>
<td></td>
</tr>
<tr>
<td>1.09 × 10⁻⁴</td>
<td>Translation</td>
<td></td>
</tr>
<tr>
<td>5.29 × 10⁻³</td>
<td>Ribosome biogenesis and assembly</td>
<td></td>
</tr>
<tr>
<td>4.16 × 10⁻²</td>
<td>Monocarboxylic acid metabolic process</td>
<td></td>
</tr>
</tbody>
</table>

Copyright © 2010 John Wiley & Sons, Ltd. Yeast 2010; 27: 000–000. DOI: 10.1002/yes
changes, and because it is an indirect measure. The fact of being an indirect measure depending on the TR and RA data makes it more noisy (in fact the SD for the RS-dependent RA slopes is larger (0.0074) than that from TR or RA direct measures (0.0058 and 0.0057, respectively). It can be seen that both TR and RS cooperate to either lower or increase RA for most categories; that is to say, homodirectional changes occurred, although in variable proportions. In some groups, however, the changes observed operated in opposite senses. RiBi genes had a decreased RA, due to a large effect on TR that compensated slight mRNA stabilization, whereas unfolded protein response mRNAs increased because of strong mRNA stabilization, despite a certain decrease in TR. It is important to note that all these changes were very subtle, and could be taken in account only for those GOs in which significant changes for the kinetic parameters had been previously detected. These results illustrate the variable methods used by the yeast cell to change the mRNA level (Pérez-Ortín et al., 2007).

Determination of the time window when assuming the steady state for mRNAs in yeast cells growing in the exponential phase

As our experiment covered only a relatively small time window of exponential phase growth in yeast (OD$_{600}$ = 0.3–0.5), we decided to assess whether this steady state for the transcriptome is maintained in later phases of exponential growth. To determine this, we reanalysed the data from the comprehensive study of Radonjic et al. (2005). In their experiment, the authors analysed the transcriptome of wild-type yeast (S288c background) growing in a batch culture in YPD since the inoculation on fresh medium to the late stationary phase. They use OD$_{600}$ = 0.5 as the starting condition, which precludes a direct comparison with our conditions. The growth curve included nine experimental points in the exponential phase in the range 3.9–9 h after inoculation (see Figure 1A from Radonjic et al., 2005). This study measured only the RA changes, but this is enough to check the steady-state condition. First, we analysed this data using the GEPAS suite (a differential expression analysis tool; Montaner et al., 2006). Probably because the analysed time window in this experiment is longer, covering more physiological variations, the number of genes with a slope different from zero (FDR <0.01) was higher. We obtained 304 genes showing a positive slope, and 271 showing a negative slope when their log$_2$ RA data were plotted against the time course (Figure 2A). They were enriched in some GO categories. Specifically, the mRNA levels of those categories related with respiration and protein catabolism increased, unlike the mRNA levels of those categories related to ribosome biogenesis, which decreased (Figure 2B). Both results fitted the expected changes in cell metabolism, which would take place when entering the diauxic shift some hours later (DeRisi et al., 1997; Radonjic et al., 2005), and they are in agreement with the slight changes detected in our previous experiment. A close inspection of the results, however, showed that most of the changes noted in relation to the initial exponential phase at 3.9 h in those genes took place at the last two time points analysed.
Steady-state transcriptome in exponentially growing yeast cells

Figure 2. mRNA amount variation during exponential growth. (A) Histogram of slopes for the mRNA amount (RA) variation (in log₂ scale for arbitrary units) as a function of time (min) for the 3.9–9 h interval, according to Radonjic et al. (2005). The distribution of all the gene slopes is shown as a black line. Bars represent the genes with slopes that significantly differ (positively in dark grey and negatively in light grey) from 0 (FDR < 0.01). The y axis represents the percentage of genes, with regard to the total number analysed, belonging to each class. (B) The gene groups that are significantly enriched in genes that increase or decrease RA. Significance was calculated using FuncAssociate (Berriz et al., 2003) Only the most representative functional groups are shown.

Table 2. Change in the mRNA amount slope during exponential growth

<table>
<thead>
<tr>
<th>Functional group</th>
<th>3.9–4.75 h</th>
<th>4–5.25 h</th>
<th>4.25–5.75 h</th>
<th>4.75–6.5 h</th>
<th>5.25–7.25 h</th>
<th>5.75–9 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytoplasm</td>
<td>2.29 × 10⁻⁸</td>
<td>9.53 × 10⁻⁸</td>
<td>2.76 × 10⁻⁵</td>
<td>2.05 × 10⁻⁷</td>
<td>3.35 × 10⁻⁶</td>
<td>1.14 × 10⁻⁶</td>
</tr>
<tr>
<td>Mitochondrial part</td>
<td>3.42 × 10⁻⁴</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Oxidoreductase activity</td>
<td>2.31 × 10⁻⁶</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Alcohol metabolic process</td>
<td>6.73 × 10⁻³</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Response to unfolded</td>
<td>4.67 × 10⁻³</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Integral to membrane</td>
<td>—</td>
<td>1.34 × 10⁻⁵</td>
<td>1.00 × 10⁻⁷</td>
<td>6.59 × 10⁻⁴</td>
<td>4.44 × 10⁻⁴</td>
<td>—</td>
</tr>
<tr>
<td>Ribosome biogenesis and assembly</td>
<td>↓ 7.85 × 10⁻¹²</td>
<td>↓ 4.75 × 10⁻⁶</td>
<td>↓ 1.21 × 10⁻³</td>
<td>5.56 × 10⁻²⁴</td>
<td>1.05 × 10⁻⁰⁸</td>
<td></td>
</tr>
<tr>
<td>Structural constituent of ribosome</td>
<td>↓ 6.20 × 10⁻³</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Translation</td>
<td>6.09 × 10⁻⁴</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Nucleus</td>
<td>↓ 8.04 × 10⁻¹⁴</td>
<td>↓ 2.04 × 10⁻⁶</td>
<td>—</td>
<td>3.13 × 10⁻⁶</td>
<td>1.83 × 10⁻⁶</td>
<td></td>
</tr>
</tbody>
</table>

Gene-set enrichment analysis using Fatiscan (Al-Shahrour et al., 2006) according to the RA slopes at different intervals during exponential growth (Radonjic et al., 2005). The categories which increase RA (↑, enriched in the positive slopes) are shown in bold type, and the categories which decrease RA (↓, enriched in the negative slopes) are depicted in normal type. All the slopes were computed using four time points. Only some representative functional groups are shown.

1 i.e. at 7.25 and 9 h after inoculation. When we considered only the first seven time points, from 3.9 to 6.5 h, no gene’s slope significantly differed from zero when we used the same criteria. This result confirms that the time window in which a steady state for the transcriptome can be widely assumed is extended to up a couple of hours before the diauxic shift.

In order to confirm the small deviations from the steady state that we were able to calculate in our previous experiment, we used the same gene set enrichment analysis (Fatiscan algorithm; Al-Shahrour et al., 2006) to detect the groups of the related genes showing slight but significant changes in their RA in the Radonjic et al. (2005) data. Table 2 shows how the RA of some GO...
categories tended to increase (↑, bold numbers) or
decrease (↓) along the growth curve. As expected,
the respiration and stress response categories were
significantly represented in the RA increase, while
the translation categories were significantly repre-
sented in the RA decrease.

Therefore, although we conducted a kinetically
detailed study of the transcriptome only during
mid-exponential growth, these results confirm that
our conclusions can be extrapolated to most of the
exponential growth phase.

Discussion

It is commonly assumed that mRNAs are approxi-
mately in a steady state during exponential growth
in a free-living microorganism. For instance, we
have used this assumption in order to calculate
the mRNA stabilities in the yeast *S. cerevisiae*
by means of GRO experiments (García-Martínez
et al., 2004). However, an experimental demonstra-
tion is currently lacking.

Here we report a detailed study of the gene
expression in yeast during exponential growth. In
this experiment we simultaneously checked both
RAs and TRs. The general conclusion is that a
steady state for the transcriptome and transcription
rates can be assumed, and that small deviations
from it can be detected, but only when looking
at all the genes from a given GO at the same
time. This last analysis procedure is much more
sensitive because it detects common tendencies
for a group of related genes. However, when we
analysed them individually, each yeast gene was
within the steady-state condition for its mRNA.

In addition, our kinetically orientated approach
enables us to determine the respective contributions
of TR and RS to the putative changes in RA.
For most cases, both changes work in the same
direction. However, certain exceptions indicate
how the cell uses mRNA stability as an additional
controller of the gene expression. Moreover, the
demonstration of steady-state for TR allows the
conclusion that the cells keep the RA values
constant by also maintaining constant turnover, not
by coordinately changing TR and DR — a more
complicated option but theoretically possible.

To extend our results and to determine how
long the steady state for the transcriptome can
be assumed during exponential growth, we used
data from a comprehensive microarray study by
Radonjic et al. (2005), which is representative for
many others. Using these data, we verified that the
steady-state condition is met by a sample series
over a long period (for at least 3 h and up to 2 h
before the diauxic shift). There is nevertheless a
significant tendency of the mRNAs related with
the growth rate (translation) to decrease and the
genes related to the diauxic shift (mitochondria)
to increase. This probably reflects the cell’s early
response to the forthcoming changes to take place
in metabolism, and corroborates the suggestion that
yeast cells use a feed-forward strategy, anticipating
growth changes with gene expression changes
(Levy and Barkai, 2009).

Having established that the steady-state condi-
tion during exponential growth had been fulfilled,
we can say that our protocol to calculate mRNA
half-lives indirectly, as well as that used by other
authors to calculate TR from the RA and RS data,
have been verified. The correlations for direct and
indirect computed TRs are about 0.5 (Pérez-Ortín,
2007). This is a significant correlation but is not as
high as expected. There may be several reasons for
this: first, the mathematical error associated with
indirect calculations; second, the error associated
with the direct measurement of the mRNA half-
life (see below). Both problems are unavoidable
and will mostly introduce random noise into the
indirect data and, therefore, decreased correlation.

Another reason for the low correlation obtained
could be that indirect TR measures the appear-
ance of mature mRNA in the cytoplasm, whereas
GRO (or other methods to estimate the TR, such
as RPCC (Pelechano et al., 2009), measures the
density of RNA polymerases. Density can only be
converted into TR by assuming a constant RNA
*pol-1* speed (Hirayoshi and Lis, 1999). Direct
methods measure ‘nascent TR’, which can differ
from ‘mature TR’ (increase of mature, cytoplasmic
mRNAs over time) if the proportion of produc-
tive transcription (finished mRNAs) is not the same
for all the genes, or if the transport of mRNAs is
not equally efficient for them all. This discrepancy,
however, is potentially interesting because it opens
a way to determine the differences in transcription
elongation or mRNA processing between different
groups of genes.

The calculation of the RS data indirectly from
the RA and TR data can also be compared with 101
those calculated by direct methods. In this case, 102
no correlation exists (see Pérez-Ortín, 2007). This result is surprising and suggests potential confounding influences when mathematically operating with variables that have already been correlated. For instance, TR and RA correlate positively, and both correlate negatively with RS (García-Martínez et al., 2007). Thus, when computing TR with the RA and RS data (TR $\alpha$ RA/RS), we obtain a new dataset by dividing one dataset that correlates positively with the TR by one that is inversely correlated. The result is, therefore, a new dataset that should mathematically correlate positively with the TR, as is in fact the case. However, when we attempted to compute RS using the TR and RA data (RS $\alpha$ RA/TR), we divided two datasets that negatively correlated to RS. Thus, this negative correlation in the new dataset decreased, due to a confusing effect of the different variables. Another source of discrepancy is the dilution effect caused by the continuous growth of the culture when calculating indirect RS that contributes to mRNA concentration reduction besides the mRNA degradation itself (Alon, 2006), whereas it does not affect indirect methods because the growth of the culture is stopped due to the transcription stop. Additional limitations in the calculations are related to the use of nascent TR (see above) and the well-known problems brought about by the stressing situation caused to cells because of the transcription stop that they require (Grigull et al., 2004; Pérez-Ortín et al., 2007).

Finally, the confirmation of a transcriptional steady state during the exponential phase means that the functional analyses of gene expression done in yeast to date are reliable, as this steady state confirms that the different time points within that phase can be considered as identical with regard to all mRNA levels.

Acknowledgements

We wish to thank Dr S. Chávez and Albert Sorribas for critically reading the manuscript, and all the members of the Yeast Functional Genomics Group for discussions. We are especially grateful to José García-Martínez (DNA-chip service at the SCSIE) for performing the DNA macroarrays. V.P. was partially funded by a FPI fellowship from the Generalitat Valenciana (Valencian Regional Government). This work was supported by Grant No. BU07-07575-C03-01/BMC from the Spanish Ministry of Science and Innovation and Grant No. ACOMP/2009/368 from the Generalitat Valenciana (to J.E.P.-O.).

Supporting information on the internet

The following supporting information may be found in the online version of this article:

Table S1. TR and RA values for the experiment
Table S2. Relative contribution of TR to RA changes
Table S3. Complete lists of functional groups with a significant deviation from the steady state during exponential growth

Figure S1. Correlation between the different time points for the amount of mRNA (RA) and the transcription rate (TR)

References


QUERIES TO BE ANSWERED BY AUTHOR

IMPORTANT NOTE: Please mark your corrections and answers to these queries directly onto the proof at the relevant place. Do NOT mark your corrections on this query sheet.

Queries from the Copyeditor:
AQ1 Please spell out MEC in full
AQ2 Insert CITY of publisher