Genome-wide analysis of DNA turnover and gene expression in stationary-phase *Saccharomyces cerevisiae*

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Exponential-phase yeast cells readily enter stationary phase when transferred to fresh, carbon-deficient medium, and can remain fully viable for up to several months. It is known that stationary-phase prokaryotic cells may still synthesize substantial amounts of DNA. Although the basis of this phenomenon remains unclear, this DNA synthesis may be the result of DNA maintenance and repair, recombination, and stress-induced transposition of mobile elements, which may occur in the absence of DNA replication. To the best of our knowledge, the existence of DNA turnover in stationary-phase unicellular eukaryotes remains largely unstudied. By performing cDNA-spotted (i.e. ORF) microarray analysis of stationary cultures of a haploid *Saccharomyces cerevisiae* strain, we demonstrated on a genomic scale the localization of a DNA-turnover marker [5-bromo-2'-deoxyuridine (BrdU); an analogue of thymidine], indicative of DNA synthesis in discrete, multiple sites across the genome. Exponential-phase cells on the other hand, exhibited a uniform, total genomic DNA synthesis pattern, possibly the result of DNA replication. Interestingly, BrdU-labelled sites exhibited a significant overlap with highly expressed features. We also found that the distribution among chromosomes of BrdU-labelled and expressed features deviates from random distribution; this was also observed for the overlapping set. Ty1 retrotransposon genes were also found to be labelled with BrdU, evidence for transposition during stationary phase; however, they were not significantly expressed. We discuss the relevance and possible connection of these results to DNA repair, mutation and related phenomena in higher eukaryotes.

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INTRODUCTION

Prolonged stressful conditions (e.g. high temperature, nutrient depletion or low water availability), which often induce extensive periods completely devoid of DNA replication (and hence, cell division), are constantly encountered by the majority of unicellular micro-organisms in nature (Hohmann & Mager, 1997). Moreover, these stationary periods may be more frequent and prolonged than periods of nutritional plenitude and environmental adequacy, which can be regarded as rare luxuries. Such stress conditions might be lethal or

alternatively, might induce growth arrest, during which the population remains fully viable even for extensive time periods.

The preservation of viability can be achieved by persistent DNA repair activity that maintains an adequate level of genome integrity, and specific modifications of the cellular morphology (Werner-Washburne *et al.*, 1996) that enable the cells to withstand harsh physico-chemical surroundings. Yeast populations (and micro-organisms in general) have the capability of adapting to environmental challenges by transcriptional activation and inactivation of defined sets of genes (Gasch *et al.*, 2000; Causton *et al.*, 2001; Gasch & Werner-Washburne, 2002). In *Saccharomyces cerevisiae*, the transcriptional response to various stressful conditions and the attendant molecular and morphological characteristics required for subsistence during stationary periods are well studied (Fuge & Werner-Washburne, 1997; Gray *et al.*, 2004).

Whereas DNA dynamics in growing cultures and in dividing cells are well studied, very little is known about

Abbreviations: ARS, autonomously replicating sequence(s); BrdU, 5-bromo-2'-deoxyuridine; E, expressed; FDR, false discovery rate; GO, gene ontology; IP, immunoprecipitation; L, labelled; LE, labelled-expressed; SMM, supplemented minimal medium.

A description of the microarray and experiment datasets used in this study are available through ArrayExpress with accession numbers A-MEXP-1465 and E-MEXP-1938, respectively.

Three supplementary figures and seven supplementary tables are available with the online version of this paper.

DNA repair, synthesis and turnover in non-dividing, stationary cultures of micro-organisms, especially eukaryotes. During the last 15 years, stationary-phase bacteria have been studied intensively in connection with the debatable phenomenon of stationary-phase mutation (Cairns *et al.*, 1988). Large amounts of data have been accumulated on the dynamics of DNA turnover, expression and mutation in the stationary phase, providing valuable insights regarding adaptation and evolution (Caporale, 2000, 2003). In contrast, few studies have described DNA dynamics of stationary phase eukaryotes, using *S. cerevisiae* as a model system, rendering this field mostly unstudied. Moreover, none of the studies describing DNA dynamics in stationary-phase model organisms offered a genome-wide view.

The conditions in which yeast may enter the stationary-phase in a natural environment can be mimicked in the laboratory. Upon depletion of the carbon source, *S. cerevisiae* cultures readily enter the stationary-phase and can retain nearly 100 % viability for periods as long as several months (Hohmann & Mager, 1997). When nutrients are eventually replenished, the culture recommences propagation, exhibiting normal, healthy growth characteristics (Hohmann & Mager, 1997). In the absence of cell division, i.e. no replicative DNA synthesis, DNA turnover would be the result of DNA maintenance (e.g. mismatch repair), mutagenic end-joining (Heidenreich *et al.*, 2003; Heidenreich & Eisler, 2004), recombination (and recombinational repair) (Heidenreich *et al.*, 2003; Heidenreich & Eisler, 2004) or other DNA repair activity required for genome integrity. Being a stressful condition in itself, the stationary state can induce genomic responses, such as transposition of mobile DNA elements (Weiner, 2002; Todeschini *et al.*, 2005; Dai *et al.*, 2007; Ebina & Levin, 2007). In addition, we might also observe unscheduled replication, namely, the synthesis of relatively short DNA stretches, which is not strictly controlled and coordinated by the cell cycle (Chevalier & Blow, 1996; Blow & Hodgson, 2002). In order to detect and characterize DNA synthesis and turnover in stationary-phase cultures of yeast, we utilized stationary-phase cultures of a haploid strain (designated E1000), capable of efficient incorporation of very small concentrations ($\sim 50 \mu\text{g ml}^{-1}$) of external 5-bromo-2-deoxyuridine (BrdU; an analogue of thymidine) into its DNA (Lengronne *et al.*, 2001). In practice, the insertion of a nucleotide analogue into the chromosome by the DNA polymerase will mark genomic sites of nucleotide turnover in stationary-phase cells. Such regions can be detected, isolated and identified using the immunoprecipitation (IP) technique, followed by microarray hybridization.

Unlike previous studies of the stationary-phase in yeast, we chose to transfer the cells abruptly to a carbon-deficient medium, and not to cultivate them in rich medium until all carbon sources are fully exhausted. This was done for two reasons. Firstly, since *S. cerevisiae* cells cease to divide almost immediately in a carbon-deficient medium, deter-

mining the stationary-phase of the culture is accurate and does not require cultivation of several weeks to reach the 'real' stationary-phase, beyond the post-diauxic shift. Secondly, cell viability is greater when the culture enters the stationary-phase in a fresh medium, free of dead cells and debris, accumulated toxic substances and other, possibly harmful, secreted metabolites, which are the result of prolonged cultivation in the same medium. Briefly, BrdU-labelled DNA fragments were immunoprecipitated (DNA-IP) using an anti-BrdU antibody, amplified, labelled and hybridized onto a cDNA-spotted microarray containing full-length ORFs of the entire *S. cerevisiae* genome. In parallel, we performed expression analysis of these cultures throughout the entire studied stationary period (Fig. 1). It is important to state that we cannot assume that high transcript abundance, as measured by our expression analysis, is only the result of active transcription, and is not affected by, for example, intrinsically high transcript stability contributing to microarray hybridization signals.

Fig. 1. An outline of the experimental strategy for the identification of expressed-labeled sites in stationary-phase yeast cultures. Total genomic, fragmented DNA from stationary and dividing cultures labelled with BrdU was enriched by performing IP using an anti-BrdU antibody; BrdU-containing DNA segments were amplified, labelled and identified by hybridization onto a microarray containing all yeast ORFs.

The study of stationary-phase DNA turnover and metabolism may prove to be very relevant to the study of DNA metabolism, mutation and ageing in various other biological systems (Finch & Goodman, 1997; Ashrafi *et al.*, 1999; Gershon & Gershon, 2000; Chen *et al.*, 2005). The stationary state of a culture or population may contribute more to its survival and adaptation than the state of the cell during periods of propagation. Hence, the systematic study of genome-wide phenomena in stationary-phase micro-organisms, especially eukaryotes, may yet prove to be important to their genetic adaptation and evolution. This representation has similarity to other biological systems in which the majority of the cells are not dividing, e.g. mammalian adult neurons and muscle cells, and other post-mitotic, terminally differentiated cells (Nospikel & Hanawalt, 2002). Stationary-phase yeast may prove to be a simple, yet powerful, applicable system for the study of stationary-phase-related phenomena in these systems as well.

METHODS

Yeast strains and media. In this study, we used the following strains: E1000 (Lengronne *et al.*, 2001), capable of taking up and incorporating exogenous BrdU into its DNA (MATa *ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 GAL URA3::GPD-TK_{7X}*), which contains seven copies of the herpes simplex virus thymidine kinase (TK) gene under control of the GPD promoter at the URA locus; E001, which is auxotrophic for uracil (MATa *ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 GAL*), identical to E1000 but unable to utilize BrdU (Lengronne *et al.*, 2001); and S288c (MATα *SUC2 gal2 mal mel flo1 flo8-1 hap1*). Yeast were grown either in 1% yeast extract/2% peptone/2% glucose (YPD) medium or supplemented minimal medium (SMM), supplemented with adenine sulphate, L-tryptophan and L-histidine (20 mg l⁻¹), and L-leucine (100 mg l⁻¹) (Kaiser *et al.*, 1994).

Yeast growth and maintenance. Frozen (−80 °C) cells from strain E1000 were thawed and plated on SMM agar plates supplemented with all necessary auxotrophic nutrients except uracil (Kaiser *et al.*, 1994; Lengronne *et al.*, 2001). Single colonies were picked and used to inoculate 5 ml SMM starter cultures, grown overnight in an orbital shaker at 30 °C, 200 r.p.m. Cells were then transferred into a 50 ml SMM culture, grown overnight at 30 °C, 200 r.p.m. This culture was used to inoculate a larger, 1 l SMM culture and incubated overnight at 30 °C, 200 r.p.m. Cells (OD₆₀₀ ~1.0) were then pelleted, washed with sterile double-distilled water and transferred into Erlenmeyer flasks containing 500 ml SMM without a carbon source, in order to induce growth arrest and entry into stationary-phase. Cultures were maintained at 30 °C for another 48 h, then BrdU (Acros Organics) was added to a final concentration of 400 µg ml⁻¹ (Lengronne *et al.*, 2001). Cultures were incubated for an additional 4 weeks. Both the OD₆₀₀ and c.f.u. count (by plating on YPD agar plates) were monitored throughout the experiment. BrdU was replenished every 4 days. Dividing yeast cultures, started from single colonies, were grown in SMM supplemented with 400 µg BrdU ml⁻¹ (E1000, E001) or YPD (S288c).

DNA and RNA sampling. Samples were removed by fast vacuum filtration of 10–12 ml yeast culture through sterile nylon filters (pore size 0.45 µm; Whatman). Filters were immediately frozen in liquid nitrogen and stored frozen at −80 °C until further processing. Stationary-phase cultures were sampled for RNA at T₀ (time of

transfer to SMM without glucose), 1, 2, 3, 4, 6, 8 and 24 h, and from 2 to 14 days at 24 h intervals. Stationary-phase samples for DNA extraction and analysis were removed at T₀, 12 h, and from 1 to 14 days at 24 h intervals (see also Fig. 2). The samples used for analysis were from the first 6 days of stationary-phase. Samples for DNA and RNA extraction were also removed from proliferating cultures of E1000, E001 and S288c yeast at mid-exponential phase. Growth phase and viability were monitored throughout the entire time-course of the experiment by measuring the OD₆₀₀ and counting c.f.u. (Fig. 2).

DNA and RNA extraction. The frozen nylon filters were quickly thawed in sterile, cold double-distilled water, and cells were briefly pelleted in a cooled (4 °C) centrifuge. Total RNA was extracted using the MasterPure Yeast RNA kit (Epicentre), according to the manufacturer's instructions. The extracted RNA was treated with RNase-free DNase I to remove any traces of interfering DNA. Total

Fig. 2. C.f.u. count (a) and cell density (OD₆₀₀) (b) of two independent cultures (■ and △) during the experiment's time-course. Genomic DNA and total RNA were sampled from two stationary-phase cultures during a 14 day experiment. The increase in OD₆₀₀ from day 1 to day 2 was the result of transferring the cells to a smaller volume of BrdU-containing medium. Arrows denote sampling of RNA [six time points; lower arrows in (a)] and DNA [three time points; upper arrows in (a)] used for analysis during the selected 6 day period; the asterisk denotes T₀ – the time of addition of BrdU at a final concentration of 400 µg ml⁻¹ to the medium of stationary-phase cultures. Error bars in (a) indicate SEM.

yeast DNA was extracted using the MasterPure Yeast DNA kit (Epicentre), following the manufacturer's instructions. The extracted DNA was treated with DNase-free RNaseA to remove any contaminating RNA. DNA and RNA were dissolved in nuclease-free water and stored frozen until use.

DNA processing, immunoprecipitation and labelling. Equimolar amounts of DNA from all samples were processed as follows: total yeast DNA was digested and linked to *Sau3AI* linkers, as described by Kandpal *et al.* (1994). Briefly, 20 µg total yeast DNA was digested overnight at 37 °C with *Sau3AI* (Takara). Digested DNA was cleaned and ligated to the following *Sau3AI* linkers: A, 5'-GCGGTAC-CCGGAAGCTTGG-3', and B, 5'-GATCCCAAGCTTCCGGGTACC-GC-3'. Ligation was verified with PCR using the linkers as specific primers. DNA IP with an anti-BrdU antibody (Beckton Dickinson) was performed as described previously (Cimbora *et al.*, 2000). The resulting DNA was cleaned and linearly amplified with Klenow exo⁻ fragment (New England Biolabs) as described previously (Keshet *et al.*, 2006). The DNA was then cleaned and labelled with either Cy5-dUTP (stationary-phase cultures) or Cy3-dUTP (mid-exponential-phase cultures, reference samples) (GE Healthcare), using the linkers as specific primers (Kandpal *et al.*, 1994). Unincorporated dye was removed using DNA Clean and Concentrator 5 (Zymo Research), and samples were eluted in 7 µl TE buffer (pH 8). The efficiency of dye incorporation was measured using a NanoDrop spectrophotometer (NanoDrop Technologies).

RNA labelling. Total yeast RNA (10 µg per sample) was labelled by reverse transcription, essentially as described by DeRisi *et al.* (1997), with either Cy5-dUTP (stationary-phase cultures) or Cy3-dUTP (exponential-phase cultures, reference samples) (GE Healthcare), using a dT₍₂₀₎VN oligonucleotide as a primer (Sigma Genosys). Unincorporated dye was removed using RNA Clean and Concentrator 5 (Zymo Research), and samples were eluted in 6 µl TE buffer (pH 8). The efficiency of dye incorporation was measured using a NanoDrop spectrophotometer (NanoDrop Technologies). To control for arbitrary loss of mRNA from samples during processing and signal variation arising from differences in labelling efficiency, hybridization and scanning, an external control normalization mix consisting of five different *in vitro*-transcribed *Bacillus subtilis* RNAs [ATCC clones: *LysA* (clone number 87482; final concentration 2 pg µl⁻¹), *Phe* (87483; 8 pg µl⁻¹), *Thr* (87484; 6 pg µl⁻¹), *Trp* (87485; 4 pg µl⁻¹) and *Dap* (87486; 5 pg µl⁻¹)] was added to each of the samples immediately after cell lysis (Arava *et al.*, 2003).

Target preparation, microarray hybridization and scanning. The labelled RNA and DNA were hybridized to glass cDNA microarrays containing full-length ORFs of the entire *S. cerevisiae* genome (both verified and predicted yeast ORFs, each feature is represented once), and 120 features for external control normalization. Printing was spatially even across the entire grid of the array. The microarrays were printed by Dr Yoav Arava, Faculty of Biology, Technion-Israel Institute of Technology, Israel. Three independent repetitions (microarray hybridizations) were done for each selected time point, each from two different cultures. Stationary-phase DNA and RNA were labelled with Cy5-dUTP, whereas mid-exponential phase total RNA and total yeast DNA (taken from a common pool) were labelled with Cy3-dUTP. To quantify the background signal of the DNA IP, total yeast DNA that was not labelled with BrdU was immunoprecipitated, processed as described above, labelled with Cy5-dUTP and hybridized. Slides were washed with 0.1 % SDS, denatured for 3 min in boiling double distilled water, washed with 70 % ethanol and dried. Each slide was then incubated in a filtered 50 ml pre-hybridization solution (10 mg BSA ml⁻¹, 5 × SSC, 0.1 % SDS) at 42 °C for 1 h and washed with double distilled water. Purified targets (10 µl; Cy3 + Cy5) were mixed with 4.5 µl 20 × SSC, 15 µl formamide, 0.3 µl 10 % SDS and 3 µl yeast tRNA (10 mg ml⁻¹; Ambion),

denatured for 3 min at 95 °C and supplemented with 3 µl 10 % BSA. A 25 µl volume of the target mix was placed on each microarray slide and covered with a glass coverslip. Microarray slides were placed in hybridization chambers and incubated for 10–12 h in a water bath at 42 °C. Slides were then washed with 2 × SSC/0.05 % SDS, 1 × SSC and 0.1 × SSC, and scanned with an Axon Instruments Scanner 4000B (Molecular Devices). Data were collected using Axon Instruments GenePix Pro 5.1 program (Molecular Devices).

Data normalization and treatment-control spots differentials.

Data normalization was performed according to Bolstad's quantile procedure for oligonucleotide arrays (Bolstad *et al.*, 2003). Since a significant number of array spots were either control or empty spots, the distribution of high and low biological signals is essentially mixed with the control and 'empty signal' populations. Such a mixture may bias the position of the 'real' experimental signals in the cumulative distribution of the hybridization. Therefore, control and empty spots were filtered out before normalization. In addition, in cDNA-spotted arrays, variation in the amount of probe printed on the glass slide necessarily introduces signal variation across hybridizations. Therefore, the normalization of the signals from the two channels was hybridization-specific. Namely, per hybridization, the cumulative distributions of Cy3 and Cy5 log signals were aligned using the quantile normalization (Bolstad *et al.*, 2003) method with Cy3 (control sample) as the reference. The normalization procedure yielded log differentials (natural logarithm of the signal ratio) of Cy3/Cy5 per spot. The significance of the experiment-control log differential was determined by *t*-test. Spot-specific sds were estimated according to pooled intra-replicate group variability of log differentials of the spot across all hybridizations. In all analyses, the significance of the effect in multiple testing was controlled for by using false discovery rate (FDR; Benjamini & Hochberg, 1995).

Gene ontology (GO) analysis. In order to discover enriched or depleted GO terms and categories in our gene lists, we used the GO-Stats tool of the GO ToolBox gene ontology web application (<http://genome.crg.es/GOToolBox/>) and GO TermFinder (SGD TermFinder: <http://db.yeastgenome.org/cgi-bin/GO/goTermFinder.pl/>). GO Term Finder searches for significant shared GO terms in a given gene list to discover genes that may have common GO categories. The GO-Stats application (Martin *et al.*, 2004) finds statistically significant over- or under-represented (herein enriched or depleted, respectively) GO terms associated with an input gene set. The biases in our input sets were calculated relative to the genomic distribution of GO terms. *P*-values were calculated using a hypergeometric test and corrected for multiple testing using FDR (Benjamini & Hochberg, 1995) with a maximal cut-off of 0.05. Partitioning of features to specific GO terms and categories was performed using GO Term Mapper (SGD GO Slim Mapper web application: <http://db.yeastgenome.org/cgi-bin/GO/goSlimMapper.pl/>). GO Term Mapper maps annotations of a gene set to more general GO terms and then partitions them into broad GO categories. The complete set of enriched and depleted GO terms is given in Supplementary Table S1, available with the online version of this paper.

Validation of microarray expression data using comparative (relative quantification) real-time PCR. cDNA was synthesized from total RNA samples of all time points (six samples) using a Verso cDNA synthesis kit (Thermo-Fisher Scientific), according to the manufacturer's instructions. In addition, cDNA was synthesized from the green microarray channel (one sample for all microarray hybridizations; used as calibrator). For relative quantification of transcript levels in our stationary-phase and calibrator samples, we used a standard curve composed of a mix of all RNA samples (including the microarray green channel as the calibrator sample) in equal parts. This standard curve was used in all PCRs. Based on our microarray data, we selected a normalizer gene that exhibited very

small fluctuations in expression level across the entire time-course of the experiment ($SD=0.228$; $CV\%=2.8$). Real-time PCR amplification of cDNA was performed using an ABsolute Blue QPCR SYBR green low ROX mix (ABgene) on a Corbett Research Rotor-Gene 3000 PCR machine. Data acquisition and analysis were performed using Rotor-Gene 6000 software (Corbett Research).

RESULTS

During the 14 day stationary-phase period, both the OD_{600} and the number of c.f.u. remained largely stable (Fig. 2), and no budding was observed (data not shown). From the entire time-course of the experiment, we selected and analysed the first 6 day period of the 14 day stationary-phase, based on three DNA-IP samples and six expression time points (in biological duplicates, each in triplicate; a total of 36 expression microarray hybridizations and 18 DNA-IP microarray hybridizations). The microarray description and experiment datasets are available through ArrayExpress with accession numbers A-MEXP-1465 and E-MEXP-1938, respectively.

Our analysis revealed, for the first time to our knowledge, genome-wide occurrence of DNA synthesis during the stationary-phase in yeast, totalling 728 ORFs, classified as BrdU-labelled (L) ORFs (see Supplementary Table S2). In contrast, we obtained a uniform, total-genome BrdU labelling signal when we performed the same analysis using exponentially growing E1000 cultures, resulting from BrdU that is incorporated into the DNA during replication. These cultures were used as positive controls (see Methods).

Initially, we performed IP of both dividing (exponential-phase) and stationary-phase BrdU-incorporating and non-incorporating yeast strains (E1000 and E001, respectively), grown in SMM, in order to detect non-specific binding of the anti-BrdU antibody to normal DNA and any 'noise' that might be introduced by our IP procedure. Whereas strain E1000 incorporates external BrdU that is present in the medium, strain E001 lacks such capability (due to the absence of the herpes simplex virus TK construct present in the E1000 strain; see Methods) and we therefore tested the specificity of our IP technique to BrdU-containing DNA by differential IP of both strain cultures. IP DNA fragments were amplified by PCR, separated on agarose gels and measured by UV spectroscopy (260 nm). We did not observe the presence of immunoprecipitated DNA either in E001 or in E1000 grown in SMM without BrdU, in exponential- and stationary-phase cultures. This signifies a low level of non-specific binding or other sources of background signal, originating from amplified DNA fragments that were not labelled with BrdU (Fig. 3).

GO term mapping

Next, we performed GO profiling of the BrdU-labelled set of features (L) for GO root terms: 'Biological process', 'Molecular function' and 'Cellular component'. The GO

Fig. 3. IP of both exponential-phase and stationary-phase BrdU-incorporating and non-incorporating yeast strains (E1000 and E001, respectively), grown in SMM. In order to observe the background level of DNA-IP (i.e. IP of unlabelled DNA) immunoprecipitated DNA from both stationary-phase and exponentially growing cultures, grown in SMM with or without BrdU, was collected and amplified by PCR. Reaction products were run on agarose gel (1.7% w/v) and analysed with a spectrophotometer (OD_{260}). No labelled DNA was present either in E1000 (a; lanes 2 and 4) or in E001 (b; lanes 1 and 2), signifying low levels of background signal. SP, Stationary-phase cultures; Exp, exponential-phase cultures; M, size marker.

mapping profile is given in Supplementary Fig. S1. A similar analysis was also performed for our expression data (see Supplementary Fig. S2a). As can be seen in Supplementary Figs S1 and S2(a), many features are related to various metabolic activities rather than to stress response. GO mapping of the LE set according to the root term 'Biological process' revealed a profile similar to that of the L and E sets; however, different proportions of genes were assigned to each shared GO category (Supplementary Fig. S2b).

Enrichment of GO terms

GO TermFinder analysis under root term 'Biological process' revealed significant enrichment of metabolism-related features in our expression set (E), whereas stress-related features were indeed expressed, but not enriched (Table 1). This enrichment is evident in several GO categories, among which are alcohol and carboxylic acid metabolic processes, glycolysis and glucose catabolic processes. GO-Stats analysis of the E set according to root term 'Molecular function' revealed significant enrichment of DNA helicases (16 genes) and peroxidase activity (5 genes; Table 2).

GO-Stats analysis of enriched and depleted GO terms under root term 'Biological process' of the L set revealed the enrichment of GO terms associated mainly with metabolism, response to stress (61 genes), cell growth (19 genes)

Table 1. Enrichment of metabolism-related features in the expressed (E) set

SGD TermFinder results for the expression (E) set. The table displays the frequency of gene ontology identifier (GOID)-associated features in the obtained dataset versus the frequency of these features in the database (background frequency). FDR-corrected *P*-value cut-off is 0.01. GO, Gene ontology.

GOID	GO term	Frequency in E set	Database frequency	<i>P</i> -value
6066	Alcohol metabolic process	49/735 genes, 6.7 %	179/7158 genes, 2.5 %	4.39e−08
19752	Carboxylic acid metabolic process	72/735 genes, 9.8 %	333/7158 genes, 4.7 %	2.56e−07
6082	Organic acid metabolic process	72/735 genes, 9.8 %	333/7158 genes, 4.7 %	2.56e−07
6096	Glycolysis	13/735 genes, 1.8 %	22/7158 genes, 0.3 %	2.12e−05
6006	Glucose metabolic process	22/735 genes, 3.0 %	64/7158 genes, 0.9 %	0.00013
32787	Monocarboxylic acid metabolic process	34/735 genes, 4.6 %	135/7158 genes, 1.9 %	0.00037
6091	Generation of precursor metabolites and energy	42/735 genes, 5.7 %	189/7158 genes, 2.6 %	0.00068
6007	Glucose catabolic process	14/735 genes, 1.9 %	32/7158 genes, 0.4 %	0.00084
42254	Ribosome biogenesis	73/735 genes, 9.9 %	409/7158 genes, 5.7 %	0.00087
46165	Alcohol biosynthetic process	19/735 genes, 2.6 %	57/7158 genes, 0.8 %	0.00155
5975	Carbohydrate metabolic process	43/735 genes, 5.9 %	210/7158 genes, 2.9 %	0.00496
19318	Hexose metabolic process	22/735 genes, 3.0 %	78/7158 genes, 1.1 %	0.00565
5996	Monosaccharide metabolic process	24/735 genes, 3.3 %	90/7158 genes, 1.3 %	0.00635
19320	Hexose catabolic process	14/735 genes, 1.9 %	37/7158 genes, 0.5 %	0.00663
46364	Monosaccharide biosynthetic process	14/735 genes, 1.9 %	37/7158 genes, 0.5 %	0.00663

and reproduction (23 genes; Table 3). A similar analysis under root term ‘Molecular function’ revealed a significant enrichment of genes associated with DNA helicase activity (19 genes) and regulation of transcription (47 genes; Table 4). The smaller LE set was found to be enriched with categories associated with establishment of protein localization and nitrogen compound metabolic process under root term ‘Biological process’ (Table 5). GO categories under root term ‘Molecular function’, similar to the L set,

were associated with nine genes (*YBL113C*, *YRF1-2*, *YIL177C*, *YLL066C*, *YRF1-4*, *YRF1-5*, *YML133C*, *YRF1-6* and *YPR204W*) that have helicase activity (Table 6).

Comparison of gene expression and DNA labelling

Next, we compared the data for the L set with features that are exclusively expressed (E; see Supplementary Table S3),

Table 2. Enrichment and depletion of molecular-function-associated GO terms in the expressed (E) set of features

GO ToolBox analysis of all features of the E set (735 features), and the enrichment and depletion of specific GO terms and GOIDs associated with GO root term ‘Molecular function’. GO level cut-off is 3; FDR-corrected *P*-value cut-off is 0.05.

GOID	GO term	Number of features in E set	Frequency in E set	<i>P</i> -value	Enriched/Depleted
0016491	Oxidoreductase activity	59	0.087	9.46e−09	E
0022857	Transmembrane transporter activity	44	0.0649	0.003963	E
0004386	Helicase activity	16	0.0236	0.005106	E
0016740	Transferase activity	90	0.1327	0.005684	E
0016829	Lyase activity	16	0.0236	0.006994	E
0019208	Phosphatase regulator activity	6	0.0088	0.010767	E
0022892	Substrate-specific transporter activity	43	0.0634	0.013901	E
0004601	Peroxidase activity	5	0.0074	0.025489	E
0046906	Tetrapyrrole binding	2	0.0029	0.029419	E
0005199	Structural constituent of cell wall	5	0.0074	0.030987	E
0005215	Transporter activity	46	0.0678	0.044472	E
0016787	Hydrolase activity	83	0.1224	0.048791	D
0030695	GTPase regulator activity	3	0.0044	0.021379	D
0030234	Enzyme regulator activity	12	0.0177	0.019986	D
0005515	Protein binding	51	0.0752	0.018849	D
0008639	Small protein conjugating enzyme activity	2	0.0029	0.017946	D
0003676	Nucleic acid binding	54	0.0796	0.002408	D

Table 3. Enrichment and depletion of biological-process-associated GO terms in the labelled (L) set of features

GO ToolBox analysis of all features of the L set (728 features), and the enrichment and depletion of specific GO terms and GOIDs associated with GO root term 'Biological process'. GO level cut-off is 3; FDR-corrected *P*-value cut-off is 0.05.

GOID	GO term	Number of features in L set	Frequency in L set	<i>P</i> -value	Enriched/ Depleted
7154	Cell communication	40	0.0627	0.000276	E
50789	Regulation of biological process	99	0.1552	0.00052	E
40008	Regulation of growth	6	0.0094	0.000748	E
16043	Cellular component organization and biogenesis	254	0.3981	0.00093	E
50794	Regulation of cellular process	94	0.1473	0.001241	E
6810	Transport	118	0.185	0.002298	E
16049	Cell growth	19	0.0298	0.002602	E
9653	Anatomical structure morphogenesis	37	0.058	0.002716	E
48856	Anatomical structure development	37	0.058	0.002716	E
51234	Establishment of localization	119	0.1865	0.003333	E
19222	Regulation of metabolic process	68	0.1066	0.004979	E
7155	Cell adhesion	4	0.0063	0.007027	E
65008	Regulation of biological quality	36	0.0564	0.008151	E
6950	Response to stress	61	0.0956	0.008155	E
33036	Macromolecule localization	49	0.0768	0.009711	E
7163	Establishment or maintenance of cell polarity	18	0.0282	0.01512	E
65009	Regulation of a molecular function	9	0.0141	0.020532	E
22402	Cell cycle process	52	0.0815	0.022559	E
45184	Establishment of protein localization	37	0.058	0.022975	E
51649	Establishment of cellular localization	69	0.1082	0.022994	E
32506	Cytokinetic process	14	0.0219	0.023883	E
51301	Cell division	31	0.0486	0.028452	E
19953	Sexual reproduction	17	0.0266	0.030214	E
746	Conjugation	17	0.0266	0.030214	E
7049	Cell cycle	52	0.0815	0.033517	E
42221	Response to chemical stimulus	44	0.069	0.036014	E
51236	Establishment of RNA localization	12	0.0188	0.043317	E
22414	Reproductive process	23	0.0361	0.043587	E
6091	Generation of precursor metabolites and energy	23	0.0361	0.043587	E
9628	Response to abiotic stimulus	16	0.0251	0.044502	E
7059	Chromosome segregation	16	0.0251	0.048412	E
6914	Autophagy	1	0.0016	0.045797	D
9056	Catabolic process	37	0.058	0.040936	D
44238	Primary metabolic process	309	0.4843	0.022034	D
43170	Macromolecule metabolic process	264	0.4138	0.013788	D
9058	Biosynthetic process	81	0.127	9.89e-07	D

namely, that display high transcript levels at all six time points. Basically, such analysis could have been done for each time point separately, followed by combining the results across six time points. However, a more robust approach would be first to filter out noise resulting from inevitable stochastic variation between time points (i.e. hybridization data per time point), and only then to compare expression and DNA turnover data. To do that, we chose to co-analyse DNA turnover and expression data only for features displaying significant (FDR-controlled for multiple testing) levels of signal at all six tested time points. Based on the proportions of expressed and labelled features, the number of overlapping features (both expressed and labelled) that deviate from the expected under the assumption of independence can be tested by a

simple analysis of the corresponding 2×2 contingency table. We found a significant (albeit very low) positive association of these two features, manifested as an excess of labelled and expressed features ($C=0.088$, $P<5.10^{-7}$; C = contingency coefficient between labelled and expressed features measured as:

$$C = \sqrt{\frac{\chi^2}{N + \chi^2}} \cdot \sqrt{\frac{k-1}{k}}; k=2).$$

The same conclusion was reached when we used a different approach to evaluate the labelling-expression association. Under the assumption of 'no-association' between BrdU labelling events and gene expression, the probability of a

Table 4. Enrichment and depletion of molecular-function-associated GO terms in the labelled (L) set of features

GO ToolBox analysis of all features of the L set (728 features), and the enrichment and depletion of specific GO terms and GOIDs associated with GO root term 'Molecular function'. GO level cut-off is 3; FDR-corrected *P*-value cut-off is 0.05.

GOID	GO term	Number of features in L set	Frequency in L set	<i>P</i> -value	Enriched/ Depleted
4386	Helicase activity	19	0.0298	0.000168	E
3676	Nucleic acid binding	92	0.1442	0.000429	E
16564	Transcription repressor activity	12	0.0188	0.001906	E
30528	Transcription regulator activity	47	0.0737	0.002208	E
22857	Transmembrane transporter activity	42	0.0658	0.003787	E
22892	Substrate-specific transporter activity	42	0.0658	0.009372	E
3702	RNA polymerase II transcription factor activity	20	0.0313	0.010974	E
5515	Protein binding	69	0.1082	0.015807	E
5215	Transporter activity	48	0.0752	0.017026	E
8047	Enzyme activator activity	11	0.0172	0.023498	E
5199	Structural constituent of cell wall	5	0.0078	0.025153	E
3712	Transcription cofactor activity	8	0.0125	0.030609	E
4871	Signal transducer activity	8	0.0125	0.044803	E
42910	Xenobiotic transporter activity	2	0.0031	0.0473	E
16787	Hydrolase activity	75	0.1176	0.044543	D
3735	Structural constituent of ribosome	16	0.0251	0.030213	D
16740	Transferase activity	61	0.0956	0.029348	D
5198	Structural molecule activity	26	0.0408	0.018032	D

gene with high DNA label index being present in a segment of a list of genes under any sorting is proportional just to the segment length and is independent of the position of that segment in the list. The same is true for the list of genes sorted by decreasing expression level. Thus, the probability of having $\geq k$ BrdU labelling events in any segment of length n (including the starting segment of the sorting—highest expression levels) is calculated according to the binomial distribution formula:

$$P(\geq k|n) = 1 - \sum_{i=0}^{k-1} \frac{n!}{i!(n-i)!} p^i (1-p)^{n-i}, \quad (1)$$

where p is the fraction of features with high DNA BrdU label index. Since P is small (~ 0.014) and the number of

features in each array is large (~ 7000), the probability (1) can be approximated by the following expression, based on Poisson distribution:

$$P(\geq k|n) < \sum_{i=k}^{\infty} \frac{\lambda^i}{i!} e^{-\lambda} < \frac{\lambda^k}{k!} e^{-\lambda} \cdot \left(1 + \frac{\lambda}{k+1} + \frac{\lambda^2}{(k+1)^2} + \dots\right) \\ = \frac{\lambda^k}{k!} e^{-\lambda} \frac{1}{\left(1 - \frac{\lambda}{k+1}\right)}, \quad (2)$$

where $\lambda = p \cdot n$.

For each starting segment of the expression decreasing gene sorting of size n ($n=1, \dots, 7000$) we can calculate the real number of genes (k) with high BrdU label index within this segment, and then the probability $P(\geq k|n)$ under the hypothesis of 'no-association'. If k is large and the

Table 5. Enrichment and depletion of biological-process-associated GO terms in the labelled-expressed (LE) set of features

GO ToolBox analysis of all features of the LE set (122 features), and the enrichment and depletion of specific GO terms and GOIDs associated with GO root term 'Biological process'. GO level cut-off is 3; FDR-corrected *P*-value cut-off is 0.05.

GOID	GO term	Number of features in LE set	Frequency in LE set	<i>P</i> -value	Enriched/ Depleted
45184	Establishment of protein localization	9	0.0849	0.030086	E
6807	Nitrogen compound metabolic process	8	0.0755	0.031397	E
44238	Primary metabolic process	48	0.4528	0.047071	D
44237	Cellular metabolic process	49	0.4623	0.033015	D
43170	Macromolecule metabolic process	36	0.3396	0.009125	D

Table 6. Enrichment and depletion of molecular-function-associated GO terms in the labelled-expressed (LE) set of features

GO ToolBox analysis of all features of the LE set (122 features), and the enrichment and depletion of specific GO terms and GOIDs associated with GO root term 'Molecular function'. GO level cut-off is 3; FDR-corrected *P*-value cut-off is 0.05.

GOID	GO term	Number of features in LE set	Frequency in LE set	<i>P</i> -value	Enriched/Depleted
0004386	Helicase activity	9	0.0849	5.23e−06	E
0004133	Glycogen debranching enzyme activity	1	0.0094	0.016371	E
0016564	Transcription repressor activity	3	0.0283	0.039231	E
0003674	Molecular function	76	0.717	0	D

probability [$P(\geq k|n)$] of finding k BrdU events among n most expressed genes is sufficiently small, the overpopulation of the most expressed genes by the k BrdU events (hence, the association) is significant. This means that, the starting segment of highly expressed genes is markedly overpopulated by features with high DNA label index. For the 655 genes with the highest average expression (the first 655 genes of the sorting), the overpopulation with features with high BrdU label index was the biggest and, indeed, highly significant [$P(\geq k|n)=0.000004$], thereby falsifying the initial assumption of independence. Fig. 4 plots minus log probability [$-\ln(P(\geq k|n))$] against the length of the starting segment. Obviously the biggest deviation from the 'no-association' hypothesis is for the starting segment of 655 genes ($n=655$) enriched by 122 BrdU labelling events.

Since the distributions of signals of the two microarray channels [i.e. Cy3 (green) and Cy5 (red)] are dissimilar, we have also recalculated the normalization according to right tails of each channel's distribution (25 % of tail; highest Q25 of Cy3 and Cy5 distributions). Following this quantile normalization of the higher signal data, we could conclude that the association between DNA-labelling and total expression reproduces the previous estimate well: the new

overpopulation maximum is at 512 whereas earlier it was 655 (see Supplementary Fig. S3).

We composed a list of overlapping LE features, comprising 122 features (see Supplementary Table S4). Next, we analysed the positional pattern of E and LE sites in the yeast genome. Log-linear analysis revealed a highly significant paucity and excess of LE sites in chromosomes 2 and 8, respectively (Fig. 5 and Table 7); weaker deviations were observed in other chromosomes as well. In addition, the distribution of BrdU-labelled sites among chromosomes significantly deviates from the proportions expected based on the yeast genome map [Fig. 5, Ensembl KaryoView (http://www.ensembl.org/Saccharomyces_cerevisiae/karyoview) and Table 7]. Namely, several chromosomes are over- or under-represented with respect to the occurrence of L sites. We also found that several *Ty1* retrotransposon elements (*YAR010C*, *YDR316W-A*, *YGR109W-B*, *YMR046C*, *YMR050C*, *YPR158C-D*) were significantly labelled with BrdU. These genes, located across several chromosomes, indicate possible retrotransposition during stationary-phase. Although they have a poly(A) tract, these *Ty1* elements were not significantly expressed and were therefore

Fig. 4. Highly significant overpopulation of the set of highly expressed features with features having a high DNA labelling index. The graph shows the $-\ln$ of the probability of having $\geq k$ labelling events within the first segment of length n in the ordered list of expressed features, assuming no association (H_0 hypothesis) between expression and labelling; n , number of features of starting segment in equation 1 (Methods); P , Poisson probability; k , number of genes with high BrdU label index.

Fig. 5. Site distribution of feature sets. Schematic representation of chromosomal locations of labelled (green arrows), expressed (brown arrows) and LE (red lines) features.

excluded from the expressed (E) set, and, consequently, from the LE set as well.

Real-time PCR validation of expression data

Finally, we performed real-time PCR validation of our microarray data, using eight transcripts exhibiting high microarray expression ratios (Cy5/Cy3): *YCR013C*, *YDR033W*, *YDR037W*, *YGR159C*, *YGR234W*, *YGR254W*, *YKL060C* and *YLR249W*. We validated the selected genes in one of our two biological replicates, and analysed one or two genes per expression time point. As a normalizer, we selected gene *YHR071W*, which exhibited a relatively low level of variation in expression across time points ($SD=0.228$; $CV\%=2.8$). *YHR071W* is a cyclin and negatively regulates Gcn4 transcription factor. Also, this gene may be a sensor of the cellular protein biosynthetic capability and is essential for response to amino acid limitation. As such, its expression in the stationary phase is indeed expected. Moreover, its stable level of expression, as indicated by our microarray data, seems to reflect the constant stressful state during our stationary-phase experiment. Relative quantification of selected transcripts was performed against the RNA sample from our reference sample, which was used for microarray hybridization as the green (Cy3) channel (calibrator sample).

Consistent with our microarray data, all selected genes showed expression levels which were markedly higher than those of the calibrator sample (the green channel of the microarray; for the full dataset see Supplementary Tables S5 and S6, and Table 8), and exhibited between 7.87- and 24.86-fold changes. Although we observed differences in

the expression level of the normalizer gene between the calibrator sample and the experimental stationary-phase samples, the normalized values clearly demonstrated higher transcript levels in our stationary-phase samples (Table 8). Interestingly, among these stationary-phase samples are genes *YGR254W*, *YGR234W* and *YKL060C*, which appear to reflect the stressful state of the cells and are likely to be highly expressed during stationary-phase. *YGR254W* is enolase I, and functions in both glycolysis and gluconeogenesis, albeit catalysing reverse reactions. Its expression is repressed in response to glucose. Similarly, *YKL060C* functions in both glycolysis and gluconeogenesis, and is translocated to the mitochondrial outer surface upon oxidative stress. *YGR234W* is a nitric oxide oxidoreductase involved in nitric oxide detoxification and is associated with oxidative and nitrosative stress responses.

DISCUSSION

BrdU is a readily available DNA building block, widely used to probe genome dynamics in mammalian cells (Lengronne *et al.*, 2001). Although BrdU is a mutagen in itself, it is reported that the use of a maximal concentration of $400\ \mu\text{g ml}^{-1}$ has no observed detrimental effect on normally growing yeast (Lengronne *et al.*, 2001). BrdU may still prove harmful to stationary-phase yeast, whereas it does not damage dividing yeast cells. However, our preliminary tests and the data displayed in Fig. 2 suggest that the E1000 system is indeed suitable for our purpose. Genotoxic effects of BrdU are highly likely to result in apoptotic (or other) cell death, manifested as a steady decrease in the c.f.u. count throughout the experiment. Moreover, simple microscopy would immediately reveal apoptotic or dead cells. However, such cells were not evident.

Most importantly, our study revealed genome-wide DNA synthesis activity in stationary-phase yeast. Remarkably, it appears that approximately 10 % of yeast ORFs exhibited DNA synthesis during a 6 day period in stationary-phase, whereas budding was not observed. To the best of our knowledge, this finding has not been described in any study of stationary-phase yeast. This cross-genome DNA synthesis can be the result of several processes, or their

Table 7. Analysis of distribution among chromosomes of labelled (L), expressed (E) and LE feature sets

$ML\chi^2$, Maximum-likelihood log-linear test of between-chromosomes homogeneity of feature distribution (for $2 \times n$ table).

	LE	E	L
$ML\chi^2$	33.16	106.46	35.98
χ^2 P-value	0.0045	$<10^{-6}$	0.0029

Table 8. Real-time PCR values of selected, highly expressed genes in stationary-phase

To calculate the fold change in gene expression, GOI concentrations were divided by the corresponding normalizer concentrations, to obtain a normalized value. Then, normalized values of stationary-phase samples were divided by the corresponding calibrator sample values to yield the fold change in expression. cal, Calibrator; C_T , threshold cycle; gm, geometric mean; GOI, gene of interest; SP, stationary-phase.

GOI	Sample	Average C_T	GOI conc. (gm)	Normalizer concentration	Normalized concentration	Fold change (SP/cal)
YLR249W	t3	19.1	364.0	435	0.84	8.09
	cal	23.14	3.0	29	0.10	
YGR159C	t3	23.84	142.0	141	1.00	8.74
	cal	33.31	0.7	6	0.11	
YDR033W	t5	21.78	287.0	214	1.34	13.41
	cal	29.43	2.0	20	0.10	
YCR013C	t6	15.99	289.0	210	1.38	22.02
	cal	23.93	1.0	16	0.06	
YGR234W	t8	21.87	289.0	208	1.39	7.87
	cal	28.88	3.0	17	0.18	
YDR037W	t10	19.46	320.9	223	1.44	24.86
	cal	28.03	0.9	15	0.06	
YGR254W	t13	21.78	287.0	211	1.36	12.24
	cal	29.43	2.0	18	0.11	
YKL060C	t13	15.99	148.6	106	1.40	16.49
	cal	25.23	0.5	6	0.09	

combinations: DNA repair, recombination, mutagenic end-joining, transposition of mobile DNA elements or unscheduled replication. Apart from the latter, these processes have been shown to be associated with stress response (Heidenreich & Wintersberger, 2001; Nouspikel & Hanawalt, 2002; Heidenreich *et al.*, 2003; Heidenreich & Eisler, 2004; Ebina & Levin, 2007). Indeed, we observed Ty1 labelling, indicative of possible retrotransposition during stationary-phase, which was probably the result of the stationary-phase-exerted stress (Ebina & Levin, 2007). Such response was clearly reflected in our expression data (see Results). In addition, the distribution of labelled DNA features among yeast chromosomes slightly (albeit significantly) deviated from a random distribution, whereas the distribution of the expressed features exhibited a much more pronounced deviation from randomness.

A cautious assumption is that the stress itself may, at least in part, dictate the distribution of DNA synthesis in stationary-phase yeast. If this is the case, then different stress conditions may induce corresponding (specific) shifts in the distribution of BrdU labelling events. Such an experiment is a necessary continuation of the current study. In addition, if we assume that the majority of the DNA synthesis in stationary-phase yeast is the result of DNA repair activity, which is essential for maintaining genome integrity, then the majority of BrdU-labelled sites can be regarded as damaged regions. Damaged regions, harbouring DNA repair activity, could be divided roughly into two types: (i) sites that were damaged stochastically, for which the probability of a lesion is equal to any other site, and (ii) sites that are more predisposed to damage due to a susceptible conformation of the DNA, i.e. unpaired

bases in ssDNA secondary structures, possibly the result of transcription (Wright *et al.*, 2004). These unpaired bases are thermodynamically unstable such that their intrinsic instability in itself is responsible for their increased mutability (Singer & Kuśmierk, 1982).

It is acknowledged that transcription-associated factors and processes can place the DNA in a damage-susceptible conformation that increases the probability of a lesion to a level that is significantly higher than spontaneous (Wright *et al.*, 1999, 2004; Wright, 2004). Such factors or processes could be associated with transcription initiation or the advancement of the RNA polymerase holoenzyme along the DNA, the binding of certain transcription factors, or even the conformation assumed by the DNA when it is unpacked from nucleosomes prior to transcription, generating exposed base-pairs (Lindahl, 1993; Wright *et al.*, 2004). These sites can be justly regarded as mutable sites, and in the scope of the current study, it may be that what we observe as BrdU-labelled sites are in part inherently mutable sites. The last assumption can be tested by utilizing repair-deficient, BrdU-incorporating yeast strains, in which the observed labelling pattern will be the result of processes other than DNA repair. The degree of attenuation in the BrdU-labelling and its pattern will signify the extent to which DNA repair contributes to DNA synthesis during stationary-phase. In yeast and bacteria, it has already been found that the mutation rate is directly proportional to the level of transcription (Datta & Jinks-Robertson, 1995; Wierdl *et al.*, 1996; Wright *et al.*, 1999, 2004; Kim *et al.*, 2007), and the coupling of transcription and DNA repair was shown to elicit repair preferentially at the transcribed strand (Selby & Sancar, 1994; Nouspikel

et al., 2006). However, genome-wide evidence for this phenomenon has not been clearly demonstrated, and the clarification of this issue requires extensive study.

In addition, we found an overlap between expressed features (E) and BrdU labelling events (L features) in stationary-phase, constituents of the small LE set, which consists of 122 features. This overlap is approximately 35 % larger than what is expected at random, i.e. in the absence of association. Although the correlation between the two overlapping sets is small (yet highly significant), the possibility that this correlation may reflect a valid functional connection between transcription-related processes and DNA synthesis cannot be readily dismissed. The expression–DNA turnover (and possibly DNA repair) interface has earned much attention throughout the last 15 years, due to its possible connection to mutations in the stationary phase in bacteria and yeast (Caporale, 1999; Foster, 1999; Caporale, 2000, 2003). Therefore, it is worthwhile to suggest a thorough genome-wide investigation of this problem, utilizing the advantages of the BrdU-incorporating yeast system. It is important to mention, however, that our study would have benefited from the use of whole-genome microarrays, such as Affymetrix yeast tiling arrays, which would allow a more comprehensive view of DNA turnover and gene expression during stationary-phase that may lie beyond ORFs. Such microarray platforms will indeed be used in our planned continuation of the described study.

The LE set was found to be significantly enriched with DNA helicases. Several DNA helicases in yeast are encoded by Y' elements, which are present in many sites in the genome. Therefore, the detection of Y' helicases can be simply the result of microarray cross-hybridization and not a true observation. However, if this were the real situation, we would have detected enrichment of DNA helicases in the L and E sets as well, necessarily adding up to helicase enrichment in the LE set, which was not the case. Therefore, it is highly likely that the discovery of DNA labelled–expressed DNA helicase loci is a valid finding.

Our GO analysis of expressed features revealed a prominent, statistically significant connection to metabolism, whereas a naive point of view may suggest that stress-related genes should be more pronounced in the E set. However, the expression of abundant metabolism-related genes in stationary-phase may not only prove vital for the stressed cells but also actually define, in terms of the expression profile, the stationary-phase, as shown in several studies on *S. cerevisiae* (Gasch *et al.*, 2000; Gasch & Werner-Washburne, 2002) and *Candida albicans* (Uppuluri & Chaffin, 2007). Similarly, our gene expression results are roughly comparable to those of Andalis *et al.* (2004): in both studies, increased expression of several genes related mostly to metabolism (*YOL126C*, *YKR097W*, *YKL148C*, *YAL054C*, *YDR171W*, *YGR043C*, *YIL125W*, *YPR026W*, *YMR096W*, *YNL117W*, *YFR015C* and *YPR184W*) was observed, among which is *SNZ1*, a stationary-phase-induced gene, involved in vitamin B6 synthesis. Although our experimental setup

differs from previous systems used for studying stationary-phase yeast (see Introduction), it appears that the obtained expression data are partially congruent with some previously published data (Gasch *et al.*, 2000; Gasch & Werner-Washburne, 2002; Andalis *et al.*, 2004). Other studies of stationary-phase yeast indeed showed a greater proportion of stress-related genes compared with other processes. Accordingly, our data also demonstrate that 54 of 735 genes (7.3 % of the E set) and 63 of 728 genes (8.7 % of the L set), are related to stress response, a significant enrichment compared with the entire genome. However, the predominant enrichment for metabolism-associated genes is maintained and appears as a major characteristic of stationary-phase yeast (Gasch *et al.*, 2000; Gasch & Werner-Washburne, 2002; Gray *et al.*, 2004).

Conclusions

By utilizing a BrdU-incorporating yeast strain, and DNA-IP coupled to cDNA microarray analysis, we demonstrated, for the first time to our knowledge, considerable stationary-phase DNA turnover in yeast. We discovered that stationary-phase yeast exhibit massive genome-wide synthesis of DNA, without evidence of budding, and remain fully viable without noticeable cell death. This widespread DNA turnover involves approximately 10 % of the genome, is distributed non-randomly across the genome, and might be associated with specific biological processes and functions during stationary-phase, as suggested by the GO analysis. In addition, retrotransposition of *Ty1* elements contributed to the observed DNA turnover during stationary-phase. Furthermore, we observed a significant overlap between labelled sites and stable, highly expressed sites during stationary-phase. By extending the current study it may be possible to (i) identify cellular processes involved in DNA turnover during stationary-phase and (ii) define the connection between expression and DNA turnover in the stationary-phase as putative factors in stationary-phase mutation and possibly in evolution.

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