Control of Transcription by Cell Size

Chia-Yung Wu1,2, P. Alexander Rolfe3, David K. Gifford2,3,4, Gerald R. Fink1,2*

1 Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts, United States of America, 2 Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, United States of America, 3 Computer Science and Artificial Intelligence Laboratory, Department of Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Cambridge, Massachusetts, United States of America, 4 Broad Institute of MIT and Harvard, Cambridge, Massachusetts, United States of America

Abstract

Cell size increases significantly with increasing ploidy. Differences in cell size and ploidy are associated with alterations in gene expression, although no direct connection has been made between cell size and transcription. Here we show that ploidy-associated changes in gene expression reflect transcriptional adjustment to a larger cell size, implicating cellular geometry as a key parameter in gene regulation. Using RNA-seq, we identified genes whose expression was altered in a tetraploid as compared with the isogenic haploid. A significant fraction of these genes encode cell surface proteins, suggesting an effect of the enlarged cell size on the differential regulation of these genes. To test this hypothesis, we examined expression of these genes in haploid mutants that also produce enlarged size. Surprisingly, many genes differentially regulated in the tetraploid are identically regulated in the enlarged haploids, and the magnitude of change in gene expression correlates with the degree of size enlargement. These results indicate a causal relationship between cell size and transcription, with a size-sensing mechanism that alters transcription in response to size. The genes responding to cell size are enriched for those regulated by two mitogen-activated protein kinase pathways, and components in those pathways were found to mediate size-dependent gene regulation. Transcriptional adjustment to enlarged cell size could underlie other cellular changes associated with polyploidy. The causal relationship between cell size and transcription suggests that cell size homeostasis serves a regulatory role in transcriptome maintenance.

Introduction

The size of cells can vary significantly within an organism, and cells of the same type display pronounced increase in size with increasing ploidy [1,2]. During development, specific cell types in many diploid organisms perform endoreplication and differentiate into polyploid cells that are functionally distinct from their diploid progenitors [2]. Polyploidy also occurs as an intermediate state in aneuploid tumor formation [3] and as a mechanism to create progenitors [2]. Polyploidy also occurs as an intermediate state in aneuploid tumor formation [3] and as a mechanism to create progenitors [2]. Polyploidy also occurs as an intermediate state in aneuploid tumor formation [3] and as a mechanism to create progenitors [2]. Polyploidy also occurs as an intermediate state in aneuploid tumor formation [3] and as a mechanism to create progenitors [2].

Yeast offers a unique advantage in studying the physiological consequences of polyploidy, because it is possible to construct isogenic strains of increasing ploidy. There were two previous analyses that compared transcription between cells of different ploidy. The first analysis of transcription in a yeast ploidy series examined expression of these genes in haploid mutants that also produce enlarged size. Surprisingly, many genes differentially regulated in the tetraploid are identically regulated in the enlarged haploids, and the magnitude of change in gene expression correlates with the degree of size enlargement. These results indicate a causal relationship between cell size and transcription, with a size-sensing mechanism that alters transcription in response to size. The genes responding to cell size are enriched for those regulated by two mitogen-activated protein kinase pathways, and components in those pathways were found to mediate size-dependent gene regulation. Transcriptional adjustment to enlarged cell size could underlie other cellular changes associated with polyploidy. The causal relationship between cell size and transcription suggests that cell size homeostasis serves a regulatory role in transcriptome maintenance.


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Abbreviations: GO, Gene Ontology; MAPK, mitogen-activated protein kinase; qPCR, quantitative PCR; SGD, Saccharomyces Genome Database; WT, wild type; YPD, yeast extract peptone dextrose

* E-mail: gfink@wi.mit.edu
Gene Ontology Analysis Suggests That Cell Size but Not Ploidy Alters Gene Expression in Tetraploids

Interestingly, the genes differentially expressed in tetraploid cells are significantly enriched not for those associated with chromosomes but for those encoding proteins localized to the cell surface (cell wall, extracellular space, and plasma membrane) and for genes that encode regulators of cell surface components (Figure 2; Tables 1 and S2, S3, S4, S5). This compartmental bias suggests that the differential gene expression in tetraploids is not directly caused by an increase in the genome content, but by a difference in cell size/geometry: for a spherical cell, a 4-fold increase in volume corresponds to only a ~2.5-fold increase in surface area. In other words, although tetraploid yeast cells are 4-fold larger in volume than haploid cells [13], the ratio of surface area to volume is smaller in tetraploids than in haploids. Reduction in surface area relative to volume is likely to trigger differential regulation of components associated with the cell surface, where signaling and transport processes take place dynamically. A reduction in relative cell surface area could alter interactions between surface and cytoplasmic signaling pathway components and affect the cell’s ability to transport metabolites across the plasma membrane. Either type of perturbation caused by a reduced surface area relative to volume could alter gene expression in enlarged cells.

Haploid Size Mutants Also Demonstrate Regulation of Gene Expression by Size

The relationship between cell size and transcription could be assessed by examining gene expression levels in haploid mutants with altered cell size relative to wild type (WT). Cell size mutants (Figure 3A) previously identified in a genome-wide study [14] were selected because they effectively enlarge cell size without significantly affecting fitness or cell shape. In addition, the underlying mutations have no reported functional relationship with the differentially expressed genes identified in tetraploid cells. We considered additional mutations to increase cell size in haploids [15,16] but did not pursue them because of technical concerns that would preclude a clear interpretation of experimental results (Text S1).

If differential expression of a gene in tetraploids is caused by enlarged cell size rather than directly by higher ploidy, the gene should be differentially expressed in haploid size mutants as well. Furthermore, the magnitude of change in the gene’s expression level should, ideally, correspond to the magnitude of change in cell size. This cell size–transcription hypothesis was initially tested by investigating the effect of cell size on expression of FLO11, a gene encoding a cell surface glycoprotein [17,18]. FLO11 showed the highest degree of repression in the tetraploid (Figure 2A), providing a wide range of detection for changes in transcript levels.

Expression levels of FLO11 were measured in WT Σ1278b and isogenic size mutant haploids (Figure 3A) treated with nocodazole for cell cycle arrest in M-phase, when the FLO11 transcript is most abundant (Figure 3B). Because the size mutants manifest an altered cell cycle [1,14], arresting the cell cycle was necessary to separate the transcriptional effect of cell size from that of cell cycle. We analyzed mutant alleles of the CLN3 gene that altered cell size significantly and arrested efficiently in the presence of nocodazole. The CLN3-2 mutant arrested as small cells (66% of WT volume), whereas the cln3Δ mutant arrested as large cells (185% of WT volume) (Figure 3C). In this haploid size series, we found an inverse correlation between FLO11 expression and cell size: FLO11 transcript abundance is highest in the small CLN3-2 haploid and...
Genes repressed in tetraploids:

Pair A candidates

Pair B candidates

significance of overlap: $p << e^{-10}$

Genes induced in tetraploids:

Pair A candidates

Pair B candidates

significance of overlap: $p < e^{-10}$

---

<table>
<thead>
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<th>Read counts in millions</th>
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<tr>
<td></td>
<td>Total</td>
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<td>1n</td>
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<tr>
<td></td>
<td>4n</td>
</tr>
<tr>
<td>Pair B</td>
<td>1n</td>
</tr>
<tr>
<td></td>
<td>4n</td>
</tr>
</tbody>
</table>

Sigma 1278b
MATa cultures
30°C
SC 2% glucose mid-log phase

poly(A)RNA selection, fragmentation

cDNA cloning, sequencing

map reads to reference genome

---

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MATa cultures
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The correlation between cell size and gene expression in haploid and tetraploid cells is demonstrated by the results from the haploid size mutants, which show an inverse relationship with cell size at 124% and 137% of WT volume, respectively (Figure 3D). The expression of FLO11 mimics the down-regulation of genes repressed in the tetraploid. Within each pair of haploid (1n)–tetraploid (4n) RNA-seq datasets, differentially expressed candidate genes were ranked by fold change in expression. Comparison of top-ranking candidates between both pairs, highlighting overlapping genes that were called differentially expressed (see details in Materials and Methods). Cutoffs for top-ranking candidates were selected to obtain a sufficient set of overlapping genes for GO analysis while ensuring that the overlap remained highly statistically significant by hypergeometric test.

To determine whether other genes differentially expressed in tetraploid cells were also influenced by cell size, we used quantitative PCR (qPCR) to compare expression of these genes in WT and cln3Δ haploids. Among the mutants we examined, the cln3Δ haploid displayed the most pronounced change in cell size and arrested in M-phase with efficiency most similar to WT (Figure 3; Table S6). The majority of differentially regulated genes in the tetraploid were regulated in the same direction in the cln3Δ haploid and larger in the WT tetraploid (Tables S7 and S8), especially the top-ranking genes, i.e., those that displayed the strongest differential expression in tetraploids (Figure 2A and 2B). These top-ranking genes likely represent those that respond most robustly to changes in cell size, since the cln3Δ haploid (18% of WT haploid volume) is still much smaller than the tetraploid (400% of WT haploid volume). Notably, the fold changes in expression levels of the top-ranking genes appeared to correlate with the increase in cell size: they were smaller in the cln3Δ haploid and larger in the WT tetraploid (Tables S7 and S8), a trend consistent with a functional relationship between cell size and gene expression.

To determine whether the magnitude of change in transcription correlates with the magnitude of change in cell size, we compared expression of the top-ranking size-responsive genes in enlarged haploid mutants and the WT tetraploid. Expression levels in each enlarged strain were measured by qPCR and normalized to those in an isogenic WT haploid. The juxtaposed datasets show a negative correlation (for repressed genes) or a positive correlation (for induced genes) between gene expression levels and cell size (Figures 4 and 5; Table S9). The results indicate that the differential regulation of these genes is enhanced with increasing cell size. This observation strongly supports our cell size–transcription hypothesis and the idea that incremental changes in cell size can be sensed by the cell and lead to incremental transcriptional responses.

Gene Ontology and Transcription Factor Motifs Reveal Pathways That Mediate Size-Dependent Gene Regulation

GO analysis indicated that many of the genes repressed by large cell size are regulated by the mating and the filamentation mitogen-activated protein kinase (MAPK) pathways (Table S3; see complete GO analysis results in Dataset S1). Analysis of transcription factor binding motifs also suggested that these MAPK pathways mediate differential gene regulation in response to cell size: the binding motifs of Dig1 and Ste12, transcription factors that function in both pathways, were significantly enriched (Table S10; Dataset S2). Ste12 is a transcriptional activator crucial for mating and filamentation [19]. Dig1 mediates transcriptional repression in both cellular processes by inhibiting the activity of Ste12 [20,21]. When either of the MAPK pathways is active, Ste12 is phosphorylated by the MAPK and released from inhibition by Dg1.

We compared transcription in haploid and tetraploid cells of a different strain background (S288c) to see whether the mating pathway, which is conserved among different species of yeast [22], was affected by cell size in this background as it is in Σ1278b. The genes downstream of the mating pathway were differentially repressed in the S288c tetraploid (Figure 6A), suggesting that the effect of cell size on the mating pathway could be a general characteristic in yeast. We also constructed isogenic haploid and tetraploid S288c cells expressing FLO11 and found that expression of FLO11 was significantly repressed in this tetraploid strain (Figure 6B), a result consistent with our finding in Σ1278b.

Although genes up-regulated in large cells were significantly enriched for those containing the binding motifs of Ace2, Swi5, Rfx1, and Yap7 in their promoters (Dataset S2), this group gave no obvious clues concerning the molecular pathway causing their differential regulation (Text S2). Moreover, when the transcription of this group of genes in haploids and tetraploids of the S288c background was compared, there was no difference in the levels of their expression (data not shown). This difference between the two yeast strains probably reflects the many regulatory differences between them [9] (Text S2).

The Mating and the Filamentation Pathways Contribute to Differential Gene Expression in Response to Changes in Cell Size

To understand the roles of the mating and the filamentation MAPK pathways in mediating size-dependent gene regulation, we disrupted signaling in these pathways in enlarged cells by making mutations in key pathway components. In the absence of the transcriptional repressor Dig1, several size-repressed genes were less repressed in large cells and became insensitive or much less responsive to size enlargement (Figure 7A). The reduced effect of cell size on gene expression in the absence of Dig1 shows that this transcription factor is involved in gene regulation by cell size. Assessment of the role of the MAPK pathways required a double mutant lacking both Fus3 and Kss1. MAPKs of the mating pathway and the filamentation pathway, respectively. The double mutant was necessary as these MAPKs have partially overlapping functions as transcriptional repressors [23]. The kss1Δ fus3Δ double mutation reduced the effect of enlarged cell size on the transcription of downstream genes (Figure 7B). Results from the dig1Δ and kss1Δ fus3Δ mutants suggest that reduced activities in
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A

note

average fold repression in tetraploids

B

average fold induction in tetraploids

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the mating and the filamentation pathways contribute to
differential gene regulation in large cells.

Discussion

Cell size homeostasis has been intensively studied and shown to
be controlled by a complex coordination of cell growth and cell
division [1,14,24–28]. However, the functional significance of this
intricate and efficient maintenance of cell size has not been
addressed. Moreover, when the genome is duplicated, as in
polyploids, the accompanying increase in cell size is maintained
upon cell division. Such whole genome duplication has been
invoked to explain evolution, development, and diseases [2–5],
with little attention to the transcriptional consequences of the
enlarged cell size that accompanies polyploidy.

To explore the functional relationship between enlarged cell
 size and gene expression, we first profiled the transcriptomes of
isologenic S\textsubscript{1278b} haploid and tetraploid strains by RNA-seq.
This strategy revealed a more complete catalog of the genes influenced
by ploidy than was possible to achieve in a previous study [6].
Genes encoding cell-surface-related proteins were overrepresent-
ed, suggesting an effect of cell size on gene expression. The
top-ranking genes whose transcription was down-regulated by an
increase in genome size were also down-regulated in another
laboratory strain, S\textsubscript{288c}, that has significant physiological
differences from S\textsubscript{1278b} [9]. The fact that these genes behaved
similarly in two different strains supported the hypothesis that cell
size was responsible for the transcriptional effects. This cell size–
transcription hypothesis was plausible because the volume of
yeast cells increases proportionally with ploidy [13]. Moreover,
as the cell surface area relative to cell volume decreases with
increasing cell size, the reduction in cell surface area could alter
gene expression by affecting cell-surface-related signaling mole-
cules and impairing molecular transport across the plasma
membrane.

The cell size–transcription hypothesis was supported by an
independent assessment that measured gene expression in haploid
mutants that make large cells. These large haploid mutants also
showed a causal relationship between cell size and gene
transcription, indicating the existence of a size-sensing mechanism
that alters transcription independently of ploidy. An alternative
model, in which polyploidy and size-altering mutations change
transcription of size-regulated genes, whose altered expression
then enlarges cell size, is unlikely. The set of size-regulated genes
we identified do not themselves regulate cell size [14]. Moreover,
the haploid size mutations and tetraploid enlarge cell size by
different mechanisms [1,13] and affect different cellular processes
[29–32]. The similar transcriptional effects observed in these
physiologically different contexts of increased cell size and the
enhancing of transcriptional changes with increasing cell size
support our hypothesis that cell size sensing is involved in
differential transcriptional regulation.

Our analysis of the transcriptional data suggests that the cell
size signal may be transmitted by the mating and the
filamentation MAPK pathways. Genes regulated by these
pathways in S\textsubscript{1278b} were preferentially down-regulated in large
cells and composed the most significant category in GO analysis.
A previous study did not detect differential regulation of these
genes, as the strains employed (\textit{MATa}/alpha S\textsubscript{288c}) were inactive
for mating and filamentation [8]. We showed that in S\textsubscript{288c} strains of a suitable mating type and with a greater
difference in ploidy, genes downstream of the mating pathway
were also repressed. Genetic disruptions in the mating and the
filamentation MAPKs as well as their common downstream
transcriptional repressor \textit{DIGI} reduced the effect of cell size on
target gene expression. These results confirmed a decrease in
activities of the mating and the filamentation pathways in large
cells. The switch-like dual functions of the MAPKs [23,33] and
the positive feedback loops involving downstream transcription
factors [34–37] likely exacerbate differences in pathway activity
between the active state (in small cells) and the inactive state (in
large cells). These attributes of the pathways may account for a
nonlinear transcriptional response to changes in cell size.
Consequently, the magnitude of change in gene expression did
not appear to correlate with cell size or cell surface area in a
simple fashion (Table S9).

Although the exact signal that initiates a size-dependent change
in transcription is not known, these MAPK pathways have an
architecture that is well suited to transmitting a signal from the cell
surface to the nucleus. In both MAPK pathways, plasma-
membrane-bound G proteins recruit and activate the MAPKKK
upon stimulation by the mating pheromone or by nutrient
starvation. Through a series of further protein–protein interac-
tions, the MAPKKK in turn activates downstream kinases
including the MAPK, which then translocates from the cellular
periphery to the nucleus to induce gene expression [38]. An
enlarged cell size could affect one or more of the molecular events
in the process of pathway activation. Because the nuclear size is
proportional to overall cell size in yeast [39,40], both the nuclear
surface and the cell surface experience a reduction in area relative
to the enclosed volume in large cells. The reduced relative nuclear
surface area could impair translocation of the MAPKs. The
reduced relative cell surface area could affect the interactions
between plasma-membrane-bound and cytoplasmic components
in the pathway.

In summary, we showed that polyploidy-associated differential
gene regulation is largely caused by an increase in cell size. The

Table 1. Cellular compartmental Gene Ontology terms for
genomes differentially regulated in the S\textsubscript{1278b} tetraploid.

<table>
<thead>
<tr>
<th>GO Term</th>
<th>Cluster Frequency</th>
<th>Background Frequency</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell wall</td>
<td>15/65 (23.1%)</td>
<td>85/5,613 (1.5%)</td>
<td>1.5 e \textsuperscript{-14}</td>
</tr>
<tr>
<td>Extracellular</td>
<td>8/65 (12.3%)</td>
<td>22/5,613 (3.9%)</td>
<td>5.8 e \textsuperscript{-11}</td>
</tr>
<tr>
<td>Plasma membrane</td>
<td>11/65 (16.9%)</td>
<td>254/5,613 (4.5%)</td>
<td>1.1 e \textsuperscript{-4}</td>
</tr>
</tbody>
</table>

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A

CLN3-2  WT  cln3Δ

WT tetraploid for reference

bck2Δ  eap1Δ

B

HTA1 (S)

SWI5 (M)

FLO11

ASH1 (M/G1)

min after release from arrest

normalized expression levels (w.r.t. CDC28)

C

D

E

relative expression level

relative expression level

relative expression level

CLN3-2  WT  cln3Δ

WT  bck2Δ

WT  eap1Δ

WT tetraploid for reference

haploids
expression pattern of FLO11. Upon release from alpha-factor arrest in G1, WT haploid cells were harvested at 10-min intervals. Cell cycle stages were assessed using expression profiles of known standard transcripts: HTA1 (S-phase), SWI5 (M-phase), and ASH1 (M/G1 transition) [43]. The expression pattern of FLO11 resembles that of SWI5. It is worth noting that FLO11 was not found to be regulated by the cell cycle in a previous genome-wide study, because of a difference in yeast strain background (W303) [10,43]. (C–E) Abundance of the FLO11 transcript inversely correlates with cell size in haploids. (C) CLN3-based size series (MATalpha strains). (D) WT and bck2Δ MATα strains. (E) WT and eap1Δ MATα strains. CLN3 is the most upstream activator of G1/S transition and maintains the size threshold of mitotic START [1]. CLN3 also regulates vacuolar morphology [29,30]. Bck2 promotes G1/S transition independently of Cln3 [44]. Eap1 regulates translation [31] and has a separate role in chromosome segregation [32].

Cell volume was measured from microscopy images. Gene expression was measured by qPCR. Expression of SWI5 was monitored to rule out the effect of cell cycle in data interpretation. Cell cycle arrest efficiency with nocodazole (70%) was assessed by counting the percentage of cells arrested in metaphase (Table S6). Error bars indicate standard deviation. Statistical significance was calculated using Student’s t test. *, p < 0.05; **, p < 0.01; ***, p < 0.001. n = 50 for volume measurements. n = 3 for transcript quantification.

doi:10.1371/journal.pbio.1000523.g003

Figure 3. Enlarged cell size represses FLO11. (A) Live cell images of WT and size mutant haploids. (B) FLO11 transcript abundance peaks in M-phase during the mitotic cycle. Upon release from alpha-factor arrest in G1, WT haploid cells were harvested at 10-min intervals. Cell cycle stages were assessed using expression profiles of known standard transcripts: HTA1 (S-phase), SWI5 (M-phase), and ASH1 (M/G1 transition) [43].

Table 2. A substantial number of differentially expressed genes in the tetraploid are also differentially expressed in the cln3Δ haploid.

<table>
<thead>
<tr>
<th>Genes Repressed in Tetraploid</th>
<th>Genes Induced in Tetraploid</th>
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<tbody>
<tr>
<td>$ FLO11$</td>
<td>$ DSE1$</td>
</tr>
<tr>
<td>$ YLR042C$</td>
<td>$ DSE2$</td>
</tr>
<tr>
<td>$ MFA1$</td>
<td>$ CT51$</td>
</tr>
<tr>
<td>$ FRE4$</td>
<td>$ SCW11$</td>
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<tr>
<td>$ STE2$</td>
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<td>$ FUS1$</td>
<td>$ YP56$</td>
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<td>$ FUS3$</td>
<td>$ YIL169C$</td>
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<td>$ AGA2$</td>
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<td>$ YNL122C$</td>
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<td>$ YDR379C-A$</td>
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<tr>
<td>$ AGA1$</td>
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<td>$ YDR357C$</td>
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<tr>
<td>$ YPL8$</td>
<td>$ CTL1$</td>
</tr>
<tr>
<td>$ MSB2$</td>
<td>$ SCW10$</td>
</tr>
</tbody>
</table>

Among the genes differentially expressed in the Σ1278b tetraploid, as identified from the RNA-seq experiment (Figure 2A and 2B), listed here are 52 genes that remain expressed and differentially regulated in tetraploid cells cultured in theYPD growth medium. WT and cln3Δ haploids were cultured in YPD plus nocodazole, and equivalent cell cycle arrest was monitored as described in Figure 3. Expression levels were measured by qPCR and analyzed with Student’s t test (n = 3). Genes expressed at significantly different levels (p < 0.05) are labeled as follows: $, genes regulated in the same trend in the cln3Δ haploid and the WT tetraploid; $, gene showing the opposite trend. Quantitative data for gene expression levels and fold changes are summarized in Tables S7 and S8.

doi:10.1371/journal.pbio.1000523.t002

Table 2.

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newly uncovered regulatory relationship between cell size and gene expression suggests that the uniformity of cell size in unicellular organisms and within tissues in multicellular organisms could be necessary to maintain the homeostasis of transcription. Our finding also suggests that cells monitor their geometric properties (i.e., size and shape) and adjust transcription accordingly. These physical features have not been typically considered a regulatory factor in cellular biology, especially in gene expression studies. In metazoans, the control of gene expression by cell size could contribute to the altered development of large or polyploid cells in normal tissues or to the aberrant physiology of tumor cells.

Materials and Methods

Yeast Growth Conditions

Yeast strains used in this study are listed in Table 3. Strains L6437 (WT MATα haploid) and L6440 (WT MATα tetraploid) were grown in synthetic complete medium plus 2% glucose at 30°C until mid-log phase for transcriptome profiling by RNA-seq. Cells were handled with caution to minimize passaging in order to avoid aneuploidy in the tetraploid. For cell cycle arrest using nocodazole, cultures were inoculated at low density in yeast extract peptone dextrose (YPD) plus 1% DMSO from overnight precultures and incubated at 30°C. After a few hours, the cultures in exponential phase were diluted to ~0.15 O.D.600 in prewarmed fresh medium and incubated for another 30 min. Nocodazole was added to a final concentration of 15 μg/ml to arrest cell cycle for 3 h. Enrichment of cells in M-phase was monitored by SWI5 RNA transcript abundance and by counting the percentage of large budded cells with DAPI-stained nuclei at the mother–bud junction. To compare gene expression levels in isogenic strains at different ploidies, cells were cultured in YPD at 30°C until mid-log phase. Cells were collected by centrifugation for RNA extraction and microscopy.

Preparation of cDNA Libraries for Sequencing

Total RNA was extracted from yeast cultures in mid-log phase with acidic phenol. After enrichment of poly(A) RNA (Qiagen Oligotex mRNA kit), the resultant mRNA was processed for cDNA library construction and sequencing as previously described [11]. The libraries were sequenced for 36 cycles on Illumina Genome Analyzer 2 using the standard protocol.

Mapping Algorithm for RNA-Seq Reads

Reads were mapped to the Σ1278b genome using the Bowtie alignment software (version 0.10.0; http://bowtie-bio.sourceforge.net/index.shtml). Reads were either mapped uniquely (bowtie –solexa-quals -k 100 -m 100 –best –strata -p 2 –strandfix) or multiply (bowtie –solexa-quals -k 100 -m 100 –best –strata -p 2 –strandfix). We used unique mappings only to look at differential gene...

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Yeasts Control of Transcription by Cell Size...
expression. Multiple mappings were used to assess how many reads aligned to TY elements, the rDNA cluster, and other repetitive sequences; a read that mapped to n genomic locations was assigned a weight of 1/n, and the "number of reads" mapping to a repetitive element was the sum of the weights of the hits in that element. The complete RNA-seq data are available at the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) repository with the accession number GSE19685.

Enriching Differentially Expressed Candidate Genes in the RNA-Seq Data

Mapped reads were stored in the David K. Gifford group’s in-house ChIP/RNA-seq database and analyzed with the code provided in DifferentialExpression.java. This code performs the following procedure on each annotated ORF in the Σ1278b genome. (1) Determine the total number of uniquely mapped reads in the haploid and tetraploid experiments. (2) Determine the number of uniquely mapped reads on both strands in the ORF in the haploid and tetraploid experiments. (3) Compute frequency_haploid = (haploid count for gene)/(total haploid count) and frequency_tetraploid = (tetraploid count for gene)/(total tetraploid count). (4) Use frequency_haploid to compute a p-value for the observed reads in the tetraploid experiment using a binomial model given the frequency in the haploid experiment. That is

\[ 1 - CMF(\text{tetraploid count for gene} \mid \text{total tetraploid count, frequency_{haploid}}) \]

The CMF is the cumulative mass function (the discrete equivalent of a cumulative distribution function) and is the sum of the probabilities for all counts less than or equal to the observed count. This is the p-value for the haploid observation given the tetraploid observation. (5) Compute the p-value for the tetraploid observation given the haploid observation. (6) Retain genes with p<0.001.

The worksheets “sample pair A” and “sample pair B” in Dataset S1 show the results.

Identification of Genes Transcriptionally Affected by Ploidy in the RNA-Seq Data

Based on read counts of known silenced genes (hypoxia response and sporulation specific), a threshold of 15 was set as the minimal expressed level. In total, 5,613 genes were considered expressed and constituted the “background gene list” for subsequent GO analysis on Saccharomyces Genome Database (SGD). The list of differentially expressed genes with read count of 15 or greater provided the set of candidate genes.

The differentially regulated candidate genes were sorted by fold change after gene read counts had been normalized by the total number of reads in each dataset. Equal numbers of the top-ranking candidates from the two haploid–tetraploid replicates were compared, and the overlapping candidates were identified as differentially regulated. The numbers of top-ranking candidates from the replicates were selected to obtain a sufficient number of overlapping genes for GO analysis while ensuring that the overlap between replicates was highly statistically significant (p<e−10) by hypergeometric test using MATLAB (MathWorks).

Quantifying Expression Levels of Specific Genes by PCR

Total RNA extracted using acidic phenol was processed for cDNA synthesis using the QuantiTect Reverse Transcription Kit (Qiagen). Expression levels were measured on an Applied Biosystems 7500 Real-Time PCR System with SYBR Green in Absolute Quantification mode following manufacturer’s procedure. Unless specified, we used the abundance of ACT1 transcript to normalize expression levels of genes of interest. The representation of ACT1 transcript in total RNA is constant in all strains used in this study. Statistical treatment (unpaired t test for two-tailed p-values) of qPCR data was performed using GraphPad Prism (GraphPad Software).

Measurement of Cell Size

Cells were fixed in 3.7% formaldehyde at 4°C overnight and digested with a mixture of zymolyase and glusulase in the presence of 1.2 M sorbitol citrate to relieve aggregation. Microscopy images...
Figure 5. Differential regulation of the top-ranking size-induced genes correlates with cell size. See legend for Figure 4.
doi:10.1371/journal.pbio.1000523.g005
of more than 50 cells per strain were analyzed using ImageJ (United States National Institutes of Health). In experiments involving cell cycle arrest with nocodazole, cell size was calculated from the measured width and length of both mother and bud of large budded cells, assuming rotational symmetry about the long axis. For actively cycling cultures, cell size was calculated from the measured width and length of the mother of budded cells. Statistical comparison of cell size was performed using Student’s t test.

Figure 6. Differential down-regulation of the top-ranking size-repressed genes also occurs in the S288c strain background. (A) Expression levels of the top-ranking size-repressed genes were examined in the WT MATa haploids and tetraploids of the S288c strain background. MFA1, FUS1, STE2, and FUS3 are genes regulated by the mating MAPK pathway. The function of YLR042C is unknown, but the presence of Dig1 and Ste12 motifs in its promoter (Table S10) suggests that it could be regulated by the mating pathway. FRE4, not involved in the mating response, was included in the analysis because it is one of the most size-regulated genes in S1278b. FLO11 is not expressed in this background [10] and thus was omitted. (B) Transcript levels of FLO11 in S288c strains expressing functional Flo8, a transcriptional activator required for FLO11 expression [10]. All strains were cultured asynchronously in YPD until mid-log phase, and transcript abundance was measured by qPCR. Error bars indicate standard deviation. Statistical significance was calculated using Student’s t test with n = 3. **, p < 0.01; ***, p < 0.001.

doi:10.1371/journal.pbio.1000523.g006
Figure 7. Cell size alters gene expression through the mating and the filamentation pathways. (A) The transcriptional repressor Dig1 in the mating and the filamentation pathway is involved in gene repression by cell size. In the absence of Dig1, several size-repressed genes became less responsive to an increase in cell size. The fold changes in expression between the dig1Δ and the dig1Δ cln3Δ mutants were smaller than those...
between the WT and the ctn3A mutant. All strains were haploid and MATa in the S1278b background. As described in Figure 3, cells were cultured in the presence of nocodazole prior to subsequent processing, and SW5 transcript was quantified to verify comparable cell cycle arrest. Error bars indicate standard deviation. Statistical significance was calculated using Student’s t test; n = 50 for volume measurements; n = 3 for transcript quantification. *, p < 0.05; **, p < 0.01; ***, p < 0.001. (B) The mating and the filamentation MAPks (Fus3 and Kss1) contribute to differential regulation of downstream genes in large cells. In the kss1A fus3A double mutants, the effect of enlarged cell size on gene expression was reduced. The fold changes in expression between the mutant haploid and diploid strains were smaller than those between the WT haploid and diploid strains. Although all genes showed a reduction in fold change between the mutant haploids and diploids, expression levels of individual genes were affected differently by the double mutation. The unique response of each gene likely reflects gene-specific, additive effects of the single mutations. All strains were MATa S1278b and were cultured asynchronously in YPD until mid-log phase. Statistical treatment was performed as in (A).

doi:10.1371/journal.pbio.1000523.g007

### Table 3. Yeast strains used in this study.

<table>
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<tr>
<th>Strain ID</th>
<th>Genotype/Description</th>
<th>Source</th>
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<tr>
<td>L6437</td>
<td>MATa WT</td>
<td>Gerald Fink lab</td>
</tr>
<tr>
<td>L6440</td>
<td>MATaaaa isogenic to L6437</td>
<td>Gerald Fink lab</td>
</tr>
<tr>
<td>yCW478</td>
<td>MATa WT, can1A::STE2pr-SpfI5S, lyp1A::STE3pr-LEU2</td>
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<tr>
<td>yCW784</td>
<td>yCW478 with bck2::kanMX</td>
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</tr>
<tr>
<td>yCW802</td>
<td>yCW478 with esp1::kanMX</td>
<td>Charles Boone lab</td>
</tr>
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<td>MATalpha CLN3, leu2::hisG::LEU2</td>
<td>Gerald Fink lab</td>
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<td>MATalpha cln3::LEU2</td>
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<tr>
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<td>This study</td>
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<td>MATa dig1::kanMX, cln3::LEU2</td>
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<td>David Pellman lab</td>
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<td>Gerald Fink lab</td>
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<tr>
<td>yCW834</td>
<td>MATaaaa, isogenic to BYC0037</td>
<td>This study</td>
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All strains are in the S1278b background with the genotype ura3-52, leu2::hisG, his3::hisG unless noted otherwise. 
doi:10.1371/journal.pbio.1000523.s003

### Construction of Isogenic MATaa Diploids

Haploid strains with suitable genotypes were transformed with a plasmid encoding the HO endonuclease inducible by galactose to enable mating-type switching. Transformed strains were pregrown overnight in synthetic complete drop-out medium supplemented with 0.1% glucose. After sufficient washing with water, cells were resuspended in synthetic complete drop-out medium plus 2% galactose and grown for approximately one doubling time to enable mating-type switching before plating on YPD agar. Mating-type-switched candidates were passaged multiple times on YPD to ensure loss of the HO-encoding plasmid prior to mating-type assessment by auxotrophic marker complementation or a pheromone-dependent growth inhibition assay.

### Supporting Information

#### Dataset S1 Results for the RNA-seq comparison between the transcriptomes of haploid and tetraploid cells. In the worksheets “sample pair A” and “sample pair B,” read counts of annotated ORFs in the two pairs of RNA-seq datasets are listed. Also included are p-values for calling differentially expressed genes and the sample total read counts. The worksheet “gene lists” shows genes in the “background list” for GO analysis and the lists of genes differentially regulated in tetraploids sorted by average fold change in the RNA-seq datasets. The remaining worksheets show the complete GO term search results from SGD for genes differentially expressed in tetraploids. GO terms for biological process, molecular function, and cellular compartment are listed separately. Found at: doi:10.1371/journal.pbio.1000523.s001 [2.33 MB XLS]

#### Dataset S2 Overrepresented transcription factor motifs in the promoters of differentially regulated genes. Worksheet “Dig1, Ste12, Mcm1” shows the likelihood of these transcription factors binding to the promoters of interest. The remaining worksheets show motifs found to be significantly enriched in specific gene sets. Found at: doi:10.1371/journal.pbio.1000523.s002 [0.05 MB XLS]

#### Table S1 Regulation of disproportionally expressed genes in the tetraploid is not correlated with stages in the mitotic cell cycle. Found at: doi:10.1371/journal.pbio.1000523.s003 [0.07 MB DOC]
Table S2  Cellular compartment GO terms for genes repressed in the tetraploid.
Found at: doi:10.1371/journal.pbio.1000523.s004 (0.03 MB DOC)

Table S3  Biological process GO terms for genes repressed in the tetraploid.
Found at: doi:10.1371/journal.pbio.1000523.s005 (0.03 MB DOC)

Table S4  Molecular function GO terms for genes repressed in the tetraploid.
Found at: doi:10.1371/journal.pbio.1000523.s006 (0.03 MB DOC)

Table S5  GO terms for genes induced in the tetraploid.
Found at: doi:10.1371/journal.pbio.1000523.s007 (0.03 MB DOC)

Table S6  Mitotic arrest efficiency, measured as percentages of arrested cells, in experiments shown in Figure 3C–3E.

Table S7  Expression levels of genes down-regulated in the WT tetraploid in the cln3Δ haploid.
Found at: doi:10.1371/journal.pbio.1000523.s008 (0.03 MB DOC)

Table S8  Expression levels of genes up-regulated in the WT tetraploid in the cln3Δ haploid.
Found at: doi:10.1371/journal.pbio.1000523.s009 (0.06 MB DOC)

Table S9  Expression levels of genes up-regulated in the WT tetraploid in the cln3Δ haploid.
Found at: doi:10.1371/journal.pbio.1000523.s010 (0.06 MB DOC)

Table S10  Expression levels of genes down-regulated in the WT tetraploid in the cln3Δ haploid.
Found at: doi:10.1371/journal.pbio.1000523.s011 (0.05 MB DOC)

Table S11  The binding motifs of Dig1 and Ste12 are overrepresented in the promoters of genes repressed in the Σ1278b tetraploid.
Found at: doi:10.1371/journal.pbio.1000523.s012 (0.05 MB DOC)

Text S1  Cell size mutants incompatible with cell size-transcription analysis.
Found at: doi:10.1371/journal.pbio.1000523.s013 (0.03 MB DOC)

Text S2  Discussion on genes up-regulated in large cells.
Found at: doi:10.1371/journal.pbio.1000523.s014 (1.08 MB DOC)

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Author Contributions
The author(s) have made the following declarations about their contributions: Conceived and designed the experiments: CYW. Performed the experiments: CYW PAR. Analyzed the data: CYW PAR. Contributed reagents/materials/analysis tools: DKG GRF. Wrote the paper: CYW GRF.

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44. Epstein CB, Cross FR (1994) Genes that can bypass the *CLN* requirement for *Saccharomyces cerevisiae* cell cycle START. Mol Cell Biol 14: 2041–2047.