Transcriptional Profiling of a Yeast Colony Provides New Insight into the Heterogeneity of Multicellular Fungal Communities

Ana Traven¹, Amrei Jänicke¹, Paul Harrison², Angavai Swaminathan¹, Torsten Seemann², Traude H. Beilharz¹*

1 Department of Biochemistry & Molecular Biology, Monash University, Clayton, Victoria, Australia, 2 Victorian Bioinformatics Consortium, Monash University, Clayton, Victoria, Australia

Abstract

Understanding multicellular fungal structures is important for designing better strategies against human fungal pathogens. For example, the ability to form multicellular biofilms is a key virulence property of the yeast Candida albicans. C. albicans biofilms form on indwelling medical devices and are drug resistant, causing serious infections in hospital settings. Multicellular fungal communities are heterogeneous, consisting of cells experiencing different environments. Heterogeneity is likely important for the phenotypic characteristics of communities, yet it is poorly understood. Here we used colonies of the yeast Saccharomyces cerevisiae as a model fungal multicellular structure. We fractionated the outside colony layers from the cells in the center by FACS, using a Cit1-GFP marker expressed exclusively on the outside. Transcriptomics analysis of the two subpopulations revealed that the outside colony layers are actively growing by fermentative metabolism, while the cells residing on the inside are in a resting state and experience changes to mitochondrial activity. Our data shows several parallels with C. albicans biofilms providing insight into the contributions of heterogeneity to biofilm phenotypes. Hallmarks of C. albicans biofilms – the expression of ribosome and translation functions and activation of glycolysis and ergosterol biosynthesis occur on the outside of colonies, while expression of genes associates with sulfur assimilation is observed in the colony center. Cell wall restructuring occurs in biofilms, and cell wall functions are enriched in both fractions: the outside cells display enrichment of cell wall biosynthesis enzymes and cell wall proteins, while the inside cells express cell wall degrading enzymes. Our study also suggests that noncoding transcription and posttranscriptional mRNA regulation play important roles during growth of yeast in colonies, setting the scene for investigating these pathways in the development of multicellular fungal communities.

Introduction

Unicellular yeasts can associate into multicellular structures such as colonies, flocs, floras, stalks, mats and biofilms [1,2]. Understanding multicellular behaviors of fungi is important for combating human disease caused by fungal pathogens, such as Candida albicans. C. albicans forms biofilms on indwelling medical devices, and these structures are resistant to antifungal treatments [1,2,3]. This makes biofilm-related infections very difficult to treat, resulting in high mortality rates [2,3].

The development of multicellular fungal communities is controlled by complex differentiation pathways [2]. Importantly, the cells growing in the multicellular community differ substantially from their unicellular counterparts. For example, cells from flocs of S. cerevisiae (which form by cells adhering to each other via the action of cell wall adhesins) are more resistant to several forms of stress than non-flocculent cells [4]. Similarly, C. albicans biofilm-derived cells are more resistant to antifungal drugs than their planktonic counterparts [5]. Moreover, transcriptome profiling of S. cerevisiae colonies and biofilms of Candida species revealed that cells growing as a multicellular community display gene expression profiles distinct from single cells growing in liquid media, with one of the main features being metabolic reprogramming [6,7,8,9,10,11,12].

Multicellular fungal communities are structurally complex. The cells in the community have different access to nutrients and oxygen, and overall experience different “neighborhoods”. This means that the cells in the community are heterogeneous, and likely perform distinct physiological roles. An example of such heterogeneity is the presence of a small number of persister cells in C. albicans biofilms, which are more resistant to antifungal drugs than the rest of the biofilm or planktonic cultures [13]. Dissecting the features of the individual subpopulations and their contributions to the phenotypes of the community has the potential to provide new insight into targeting fungal biofilms with therapeutic agents.

Colonies formed by the bakers yeast S. cerevisiae on agar plates have served as a model for understanding multicellular behaviors
to obtain a final colony density of 300 distinct colonies per 10 cm² petri dish.

Microscopy
A to1 strain was stabilized by layering with 1% low-melt agarose to ∼1 mm above the top of the colony. The colony was then sliced in half and the cut-side placed facing down on a coverslip essentially as previously described [10]. The GFP fluorescence was captured using a Leica SP5 inverted microscope with the 10× objective. Leica LAS-AF software was used for analysis.

FACS Analysis and Sorting
Day-4 colonies were washed from the surface of YPAD-plates with ∼5 ml ice-cold dH₂O and stored on ice. For analysis (Figure 1), 30,000 events were captured using a BD Biosciences LSRII flow cytometer. FlowJo software was used in the analysis. To sort the GFP populations a minimum of 1 million GFP+ve cells were captured using a BD Biosciences Influx cell sorter. The gates for sorting were based on the natural separation of the distinct GFP+ve population in the Cit1-GFP strain. The gate for the GFP-ve population was based on the population coordinates of the untagged strain. This meant that dimly fluorescent cells (~25%) within the population are excluded from the analysis. This was performed with 3 biological replicates temporally separated by over one week.

Transcriptome Analysis
Arrays were designed using eArray (Agilent Technologies). We included probes based on transcribed coordinates supplied in supplemental data by Xu et al [8]. This set contains coding and non-coding (CUTs and SUTs) and structural RNA such as snoRNAs. We also included the “YBOX” probe set containing a number of control and normalization probes designed by Pat Brown’s lab at Stanford. Finally, the generic S. cerevisiae probes provided by manufacturer were also included. Each probe was spotted at least twice and provided more than one suitable probe could be designed, multiple probes were designed to each transcript (60,000 spotted probes in total). The array design is freely available via the eArray website [Design ID: 031069, Array Design: Brillhart Sc_01]. Microarray processing was performed by The Ramaciotti Centre for Gene Function Analysis [http://www.ramaciotti.unsw.edu.au] using the low-input kit (Agilent Technologies).

Three biological repeats and one dye-swap were analysed in total. The average log2 intensity of the negative control probes was 4.83, standard deviation 0.32. A log2 average intensity cut off of 5.47 (two standard deviations above mean negative control intensity) was applied. Moderated t-tests were used to look for differential expression, using the limma package in BioConductor, and a FDR cutoff of 0.01. Where multiple probes spanned a single transcribed region the data were averaged.

RNA Extraction and Analysis
RNA was isolated from yeast cells using the hot phenol method, and the concentration estimated using a nanodrop spectrophotometer. For poly(A)tail analysis the ePAT and TVN-PAT assays were performed exactly as previously described [17] using a total of 500 ng of RNA as input into each cDNA synthesis reaction. The primer sequences used were the following: S.c AT01-PAT CCTATA- TAACCCACCAACTAATCGC; S.c ENO2-PAT CCCAC-GGGTGAACAGTTG; S.c HSP30-PAT GCAAGAG-GAATCTGCAAGGAGG; S.c SNR6-PAT GGTCGGAATGCTACCGCC; S.c HP1-PAT GAATGC- CAATACTTGAATGTCTTGG; S.c SUT350-PAT GCCACAA-CACAGGGGCCTAG. Note: Unlike most PAT primers, the SUT350 primer is designed near the start of the annotated SUT350 transcript.

Results
Establishing Methods for FACS Sorting of the Colony Subpopulations
Previously, a GFP-fusion protein has been used to demonstrate that the ammonium exporter Ato1 is differentially expressed within the colony in a spatial manner, with expression evident in the outer layer of the yeast colony, but absent on the inside [10]. Ammonia production is thought to be a central metabolic feature of yeast colonies that drives their development [6,18]. The expression of Ato1-GFP in the outside colony layers coincides with a fully differentiated colony, which is in ammonia-producing phase [10]. The Palkova group has also previously reported the localization of CCR4 and ADE 5,7- LacZ reporter fusions as being predominantly on the inside of colonies [14]. We performed FACS analysis of the untagged BY4741 wild-type strain, and Ato1-GFP, Ccr4-GFP and Ade5,7-GFP strains after 4 days of colony growth on rich media (Figure 1A–D). The Ato1 fusion revealed a small separate GFP positive population of cells (note cells shifted in the B525-A axis) that tended to correspond to larger cells (up shifted in FSC-A axis). Neither Ccr4-GFP nor Ade5,7-GFP expressing colonies revealed clear subpopulations. In these strains the GFP fluorescence was dim and GFP positive cells tended to be the biggest cells in the population. This suggests, contrary to previous findings, that these genes are most abundantly expressed on the colony exterior (see cells shifted to the upper right hand quadrant of the FACS plots relative to the untagged strain). We also tested another gene, CIT1, which is a marker of quiescent cells in stationary phase cultures [19]. This gene was chosen because cells
growing in a colony display several features of quiescence. FACS analysis of Cit1-GFP cells from stationary phase liquid cultures confirmed the distinct quiescent subpopulation previously observed by Davidson et al 2011 (Figure 1E). Moreover, Cit1-GFP cells grown in colonies also demonstrated the presence of two distinct cell populations (Figure 1F). The GFP positive population accounted for approximately 25% of the cells, a further ~25% was dimly fluorescent, while the remaining 50% of cells shared FACS co-ordinates with the untagged wild-type control strain. Again, the fluorescent cells tended to have a higher signal in forwards scatter (y-axis) indicating bigger or budding cells. Confocal fluorescence microscopy showed that Cit1-GFP is indeed expressed on the outside of differentiated yeast colonies, in a pattern analogous to that previously shown for Ato1-GFP (Figure 2, and [10]). Since the Cit1-GFP strain produced the most distinctly separable populations by FACS, we decided to use this strain to sort the two subpopulations of the colony, distinguishing the outside cell layers from those in the inside.

Transcriptome Analysis of the Two Subpopulations within a Yeast Colony

The outside and inside cells from a colony were sorted based on GFP-fluorescence, total RNA was isolated and transcriptome analysis performed using custom 2-colour agilent arrays containing multiple probes for both coding and non-coding RNA. Our arrays were designed to contain probes for the complete set of transcribed coordinates identified by tiling arrays [20] (see Methods). The gene expression of the GFP positive cells was directly compared to the GFP negative cells by competitive hybridization. Genes with elevated expression in each of the colony subpopulations were identified relative to total gene expression in that particular subpopulation, using 1.5 fold enrichment and a false discovery rate of 1% (see Materials and methods). The complete normalised microarray dataset is presented in Dataset S1.

Genes Enriched on the Outside of the Colony

The expression of three hundred and twelve genes was enriched on the outside of the colony. A prominent functional group was genes required for ribosome biogenesis and translation (including 53 genes encoding ribosomal subunits) (Table 1; Gene ontology analysis is shown in Table 2). Prominent metabolic functions included glycolysis and glucose fermentation, ergosterol biosynthesis and fatty acid metabolism, metabolism of amino acids and vitamins, as well as enzymes required for acetyl-coA synthesis and lactate utilization (Tables 1 and 2). Expression of ribosome biogenesis genes and the enzymes of glycolysis and glucose fermentation pathway indicate that the outside of the colony contains actively growing cells that metabolise glucose in the preferred way for yeast fermentation. However, enrichment of...
of the fatty acid oxidation pathway, acetyl-coA synthesis and amino acid metabolism indicates that, while the cells on the outside of the colony are metabolically active, they also exhibit some properties of starved cells.

In addition to metabolism and ribosome biogenesis, several cell wall genes were enriched on the outside of the colony, in particular genes encoding cell wall proteins, and enzymes required for cell wall synthesis, such as β 1,3 glucan synthase \( \text{FKS1} \), chitin synthase \( \text{CHT2} \), and \( \text{KNH1} \) that is required for the synthesis of β 1,6 glucans (Table 1). Several of the cell wall protein genes are known to be regulated by stress responses, such as heat and cold shock, mitochondrial dysfunction, anaerobiosis, and the cell wall integrity pathway (\( \text{TIR1, TIP1, HSP150, PIPI, PIPI3, PST1, YLR194C} \)). These results are consistent with the outside colony layer exhibiting properties of starved and quiescent cells, which are known to remodel their cell walls. Other functional groups enriched on the outside of the colony include transcription factors, such as the \( \text{MED16} \) subunit of Mediator known to be required for stationary phase survival [21], genes involved in cell cycle, polarized growth and DNA replication and repair, and several stress responsive functions, which include the \( \text{AT02} \) and \( \text{AT03} \) ammonium transporters that have long been known to be induced during colony development [6].

Grouping of the genes enriched on the outside of the colony by transcription factors using YEASTRACT (www.yeastract.com) and selecting for “direct evidence” (i.e. transcription factors likely to have a direct effect) found several transcription factors with potential roles in regulating the colony transcriptome. We will list those with more prominent roles (regulation of >10% of the genes in the list). 50.6% of the genes were regulated by \( \text{Ste12} \), a master regulator of pseudohyphal growth in response to nitrogen starvation. Interestingly, the \( \text{ste12} \) mutant displays altered colony morphology [22]. Together, the study by Granek and Magwene (2010) and our results indicate that \( \text{Ste12} \) might be an important colony regulator. As expected, other prominent transcription factors were those involved in ribosome biogenesis (\( \text{Rap1} 36.9\% \) of genes, \( \text{Fhl1} 29.8\% \) of genes, \( \text{Ifh1} 19.2\% \) of genes, \( \text{Sfp1} 15.7\% \) of genes). Moreover, 17.3% of the genes enriched on the outside of the colony are under the control of \( \text{Sok2} \), a known regulator of ammonia production and differentiation in yeast colonies [23]. Several stress-responsive transcription factors, such as \( \text{Skn7} \) (14.4% of the genes), \( \text{Skol} \) (12.5% of the genes) and \( \text{Yap6} \) (11.9% of the genes) were also identified, as were the pseudohyphal regulators \( \text{Tec1} \) (11.5% of the genes) and \( \text{Phd1} \) (13.5% of genes), and the iron-sensing transcription factor \( \text{Yap5} \) (12.2% of the genes).

**Genes Enriched in the Inside of the Colony**

Two hundred and thirteen genes displayed enriched expression on the inside of the colony. The transcriptome profile of the inside of the colony was very different to that of the cells residing on the outside (Table 3, GO analysis is shown in Table 2). Most notably, there was no enrichment in ribosome biogenesis or glycolysis, consistent with the inside cells having less capacity for growth. Instead, the expression of the key enzyme of gluconeogenesis \( \text{PCK1} \) was enriched. However, somewhat counter intuitively, so were three negative regulators of gluconeogenesis: \( \text{UBC8, GID8} \)
Table 1. List of selected functional categories, genes and non-coding transcripts enriched on the outside of the colony.

| FUNCTION                                      | GENES                                                                 |
|-----------------------------------------------|------------------------------------------------------------------------|
| Ribosome biogenesis and translation           | 53 genes encoding subunits of the cytoplasmic ribosome; ribosome and 35S rRNA translation factors (ECM1, ECM16, EGD2, LOC1, MAK21, RIA1, RIX1, RR51, RR53, URB1, URB2, UTP2, UTP10, UTPP21); translation factors and rRNA synthetases (ETF1, ETF2, SES1, CDC60, DED1, FR51, RBG2, GCN20, AC51) |
| Enzymes of glycolysis and gluconeogenesis, glucose fermentation pathway | PGK1, ENO1, ENO2, TDH2, TDH3, FBA1, TPI1, PDC1, ADH1, ADH2, ALD4 |
| Fatty acid oxidation and biogenesis           | Fatty acid oxidation enzymes (POT1, POX1, FOX2, FAA2, FAA4, DC1, EC1); other factors involved in the beta-oxidation pathway (TES1, ANT1, CTA1, PCD1); fatty acid biosynthesis enzymes (FAS1, ELO1); AC81 (Acyl-CoA binding protein; transports newly synthesized acyl-CoA esters to acyl-CoA-consuming processes) |
| Ergosterol biosynthesis                       | Ergosterol biosynthesis enzymes (ERG1, ERG5, ERG6, ERG11, ERG25, ERG13; ERG10); other factors required for ergosterol biosynthesis and transport (ERG28, DET1) |
| Metabolism of amino acids and nucleotides      | Amino acid catabolism (ARO10, ARO9, IC2); amino acid biosynthesis (ARO4, HIS7, ILV3, ARO9, ME1, MR1, AAT1, AC01); pentose phosphate pathway (TKL2); purine and pyrimidine biosynthesis (ADE6, ADK1, URA2, URA7, RNR2, RNR4); transporters (TAT1, ALP1) |
| Biosynthesis of vitamins and NAD              | Biotin biosynthesis (BIO2, BIO4); folate biosynthesis (FOL3, MIS1, GCV2); riboflavin biosynthesis (RIB2); NAD biosynthesis enzymes (NMA1, NPT1); nicotinic acid permease TNA1 |
| Mitochondria                                  | Respiratory chain and ATP synthase (CYB2, NCA2, NCA3, STF1, SUE1); putative mitochondrial proteins (YDL157C, FMP37, YER077C); heme biosynthesis (HEM15); mtDNA maintenance (CLU1, MGM101, RIM1); Other (SHH3, MCK1, CIR1, DIC1) |
| Other metabolism                              | Acetyl-CoA synthesis (AC51, AC52); coenzyme A synthesis (CA81); TCA cycle (AC01, LSC2); utilization of lactate (DLD2) |
| Cell wall                                     | Cell wall proteins (FIT3, TR1, PIR1, PIR3, CIS3, HSP150, PST1, TPI1, YLR194C, CW22, CW14, RW1, AGA2); cell wall biosynthesis: glucan and chitin (FX61, CH52, RH1); mannose transferase (PMT4) |
| Cell cycle, DNA replication and repair         | Cell cycle and polarized growth (CDC10, RGA2, CLA4, GIC2, CLN1, NKP1, APC5, SMC2, SW16, MSA2); DNA replication and repair (RNR2, RNR4, POL2, POL4, POL3, POL30, DNA2, SMC5, RFC1, DMC1, ECL1, CIN1, ABF1) |
| Transcription                                 | Mediator subunit SIR4/MED16 required for stationary phase survival; SAGA subunit SIT7 (mutant has stress responsive and cell cycle phenotypes); THID subunit TAF2; Cell cycle (SW16, MSA2, ABF1) |
| Stress response                               | Nutrient stress, starvation and stationary phase (ATO2, ATO3, SNZ22, TOS3, SIN4); Oxidative stress (SOD2, TSA1, YPL108W, ALO1, OLA1, STF2); Osmotic stress (GPD1, STL1, GRE1, SP18, FPZ1, YWC1); Heat shock proteins and general stress response (HSP26, SSA3, HMFI1, UBC5) |
| ER and Golgi                                   | Protein glycosylation (WBP1); ER and nuclear pore complex association (PER33); Protein folding in the ER (EMC4); Vessel trafficking (ERV29, SED4, ERP1); Protein targeting to the ER (SRP68, SEC63, SEC65); Other (PFP2, IRC22); Golgi (COY1, COQ5, VPS54, GGA2) |
| Noncoding transcripts (SUTs and CUTs)         | CUT406, CUT410, CUT525, CUT775, CUT866, SUT058, SUT121, SUT330, SUT565, SUT660, SUT664, SUT761 |

The complete list of genes enriched on the outside of the colony is shown in Dataset S1. CUT-cryptic unstable transcript; SUT-stable unannotated transcript.

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and VHS2, which are involved in proteasome-dependent degradation of the gluconeogenesis enzyme fructose-1,6-bisphosphatase in response to switching from a non-fermentable carbon source to glucose [24,25]. Several regulators of glucose-dependent pathways were enriched in the colony center, but again, both gluconeogenesis activators (such as the transcription factor CAT8) and transcription factors that mediate glucose repression such as MIQ1 and NRG1, were identified (Table 3) [26]. The inside cells displayed features of nitrogen starvation, as indicated by an enrichment in the expression of several genes under the control of nitrogen catabolite repression (e.g. ammonium permeases MEP2 and MEP5, the allantoate permease DAL5, the allantoicase DAL2 and the proline oxidase PUT1), as well as genes required for biosynthesis and transport of amino acids (Table 3).

A striking feature of the cells that reside in the inside of the colony was an enrichment of genes (both nuclear and mitochondrially-encoded) required for the activity of the mitochondrial respiratory chain, as well as the expression of the transcription factor HAP4 (a subunit of the HAP2/3/4/5 complex that is a central regulator of genes required for respiration), and genes required for the biogenesis and assembly of iron-sulfur (Fe-S) clusters, iron transporters and transcription factors responsive to iron (Tables 3 and 2). These changes indicate that the cells residing in the inside of the colony are attempting to maintain mitochondrial activity even in the absence of growth. The cells on the inside of the colony also displayed changes to genes with functions in cell wall metabolism, however this was notably different from the changes observed in the outside cell layers. The inside cells did not show an enrichment in the expression of genes encoding cell wall proteins (with the exception of two genes, one of which SPI1 is induced at the diauxic shift in planktonic cultures) [27]. Moreover, the inside cells did not express cell wall biosynthesis enzymes (such
as glucan and chitin synthases, which were enriched on the outside of the colony, but rather they expressed cell wall degrading enzymes, such as glucanases (EGT2, DSE2 and DSE4) and the chitinase CTS1 (Table 3). We speculate that this provides a mechanism to mobilize carbohydrate stored in the cell wall.

Analysis of the transcription factors for which direct evidence exist for regulation of the genes enriched in the inside of the colony using YEASTRACT showed a very similar picture as for the outside cells. Again, only the transcription factors with control over 10% of the genes will be mentioned. A large proportion of genes (49.3%) are known to be regulated by Ste12, the colony regulator Sok2 exhibits control over 27.1% of the genes, and the stress responsive factors Skn7, Cin5, Sko1, Yap6 and Hsf1 control 15.8%, 13.8%, 11.8%, 11.3%, 10.8% and 10.3% of genes respectively. Also identified were the generalist transcription factors Rap1 and Fhl1 (34.5% and 21.7% of genes respectively), the pseudohyphal regulators Phd1 (16.7% of the genes) and Tec1 (13.8% of the genes), and the cell cycle regulators Swi4 (10.8% of the genes).

Table 2. Gene ontology analysis of differential gene expression within the colony.

| Functions enriched on the outside of the colony | GO category | p value   |
|-----------------------------------------------|-------------|-----------|
| structural constituent of ribosome [GO:0003735] |             | 4.41E-11  |
| translation [GO:0006412]                      |             | 7.12E-11  |
| rRNA export from nucleus [GO:0006407]         |             | 1.12E-05  |
| maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA) [GO:0000462] | | 3.02E-05  |
| snRNP export from nucleus [GO:0006608]        |             | 7.1E-03   |
| snRNA export from nucleus [GO:0006408]        |             | 7.09E-03  |
| ergosterol biosynthetic process [GO:0006696]  |             | 4.08E-05  |
| fatty acid metabolic process [GO:0006631]     |             | 1.1E-04   |
| glucoseogenesis [GO:0006094]                  |             | 1.9E-04   |
| reactive oxygen species metabolic process [GO:0072593] | | 4E-04    |
| lactate metabolic process [GO:0006089]        |             | 6.5E-03   |
| acetyl-CoA biosynthetic process [GO:0006085]  |             | 2.2E-03   |
| alcohol fermentation [MIPS functional classification 02.16.01] | | 0.2E-03   |
| C-2 compound and organic acid catabolism [MIPS functional classification 01.05.06.07] | | 0.5E-03   |
| propionate fermentation [MIPS functional classification 02.16.11] | | 2.2E-03   |
| metabolism of derivatives of dehydroquinic acid, shikimic acid and chorismic acid [MIPS functional classification 01.20.15] | | 4.9E-03   |
| structural constituent of cell wall [GO:0005199] | | 4.76E-07  |
| peroxisomal matrix [GO:0005782]               |             | 7.1E-03   |

| Functions enriched on the inside of the colony | GO category | p value   |
|-----------------------------------------------|-------------|-----------|
| electron carrier activity [GO:0009055]        |             | 4.3E-03   |
| respiratory electron transport chain [GO:0022904] | | 1.05E-3   |
| proton-transporting ATP synthase complex, coupling factor F(o) [GO:0045263] | | 9.3E-03   |
| mitochondrial electron transport, cytochrome c to oxygen [GO:0006123] | | 7.5E-03   |
| ammonium transmembrane transport [GO:0072488] | | 3.31E-05  |
| detoxification of cadmium and copper ion [GO:0071585; 0010273] | | 1.03E-3   |
| cytokinesis, completion of separation [GO:0007109] | | 1.4E-03   |
| negative regulation of glucoseogenesis [GO:0045721] | | 2.4E-03   |
| diacetyl reductase ((R)-acetoin forming) activity [GO:0052587] | | 1.03E-03  |
| cellular response to water deprivation [GO:0042631] | | 9.7E-03   |
| response to pH [GO:0009268]                   |             | 9.7E-03   |
| nitrogen utilization [GO:0019740]             |             | 2.4E-03   |
| general transcription activities [MIPS classification 11.02.03.01] | | 7.3E-03   |
| degradation of polyamines [MIPS classification 01.05.01.02] | | 5.9E-03   |
|cation transport (H+, Na+, K+, Ca2+, NH4+, etc.) [MIPS classification 20.01.01.01] | | 0.3E-03   |
| cAMP/cGMP mediated signal transduction [30.01.09.07] | | 5.9E-03   |
| inorganic chemical agent resistance (e.g. heavy metals) [32.05.01.03.03] | | 1.05E-03  |

Only minimally overlapping GO terms are shown. The full GO analysis is presented in Dataset S1.
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Noncoding Transcription in a Yeast Colony

Our transcriptome analysis identified several noncoding transcripts that were enriched either on the inside, or on the outside of the colony (Tables 1 and 3). These include cryptic unstable transcripts (CUTs), stable un-annotated transcripts (SUTs), small nucleolar RNA genes (snoRNA, SNR genes), the RNA component of RNaseP and the RNA component of the Signal Recognition Particle. Many more noncoding transcripts were enriched of the inside than on the outside of the colony (53 versus 12). All of the snoRNA genes were enriched on the inside [20]. In regards to the CUTs and SUTs, 31 were enriched on the inside than on the outside of the colony (53 versus 12). All of the snoRNA genes were enriched on the inside, versus 12 on the outside. The snoRNAs are enriched for rRNA biogenesis. Ribosome biogenesis was strongly induced on the outside of the colony, while, counter intuitively, the snoRNAs were enriched on the inside (Tables 1 and 3). As part of the decay mechanism, the snoRNAs are adenylated [28], which would make them more prone to be captured by reverse transcription. We suggest that the apparent enrichment of the snoRNAs on the inside of the colony might actually be due to increased activity of the decay pathway on this set of genes in the absence of active ribosome biogenesis. The CUTs and SUTs were mapped onto the genome to identify the neighboring genes, using the Steinmetz lab web-site (http://steinmetzlab.embl.de/NFRsharing/) that accompanies the manuscript by Xu et al (2009). In 16 cases the expression of the neighboring genes was enriched in the same subpopulation of cells as the noncoding transcript. Some examples are shown in Figure 3. Several noncoding transcripts could be originating from bidirectional promoters, which drive expression of a gene that is also enriched (for example CUT343 and RGS2 in Figure 3). In some cases, the non-coding transcript was placed upstream of the gene that was also enriched in expression (such as SUT243 and PCK1, Figure 3), suggesting that active transcription of the noncoding transcript could be contributing to the expression of the downstream gene. In other cases the opposite was true, i.e. the noncoding transcript was downstream of a highly expressed gene, suggesting its higher levels might be a byproduct of active transcription of the upstream gene (CDC60 and CUT410 in Figure 3). Several examples included noncoding transcripts overlapping the expressed genes transcribed in the opposite direction on the other strand. There were also more complex situations, such as the case of PIR1 and PIR3 (two cell wall genes enriched on the outside) and SUT660 (a noncoding

Table 3. List of selected functional categories, genes and noncoding transcripts enriched on the inside of the colony.

| FUNCTION  | GENES |
|-----------|-------|
| Mitochondria | Respiratory chain subunits encoded on the mtDNA (ATP6, ATP8, COB1, OLI1, COX2, COX3); CYC7 (cytochrome C expressed under hypoxic conditions); cytochrome C oxidase assembly factors (PET100, COA2, COX19): DLD3 (D-lactate dehydrogenase activated by mitochondrial dysfunction); transcriptional regulators of mitochondrial biogenesis and respiratory growth (HAP4, RFS1); other (APJ1) |
| Fe-S clusters and iron | Fe-S clusters biogenesis and assembly (SSQ1, ISU2, NBP35, DRE2); iron transporters (FTR1, FET14); transcription factors responsive to iron (CTH1, CAD1, MSN1) |
| Amino acid metabolism, Nitrogen starvation | Transporters, uptake (MEP2, MEP3, MUP3, DAL5, LST8, AVT6); STP1 (transcription factor, activator of amino permease genes); AOR1 (plasma membrane transporters required for excretion of excess amino acids); metabolic enzymes required for amino acid biosynthesis and utilization of alternative nitrogen sources (SER3, TMT1, DAL2, MET14, MET2, CPA1, PUT1, SAM2) |
| Glucose-regulated pathways | Transcription factors (MIG1, NRGI, HAP4, CAT8, IMP2); signal transduction: CAMP-PKA pathway (PDE1, PDE2); negative regulators of glucose signaling (MTH1, RGS2); SKS1 (putative kinase, adaptation to low glucose); CSRI2 (proposed to regulate utilization of non-fermentable carbon sources) |
| Gluconeogenesis | Enzymes (PKC1); negative regulators of gluconeogenesis (UBC8, GID8, VD24) |
| Lipid metabolism and regulation | Sphingolipids (YPF1, SUR1, SUR2, YNL194C); phospholipids (OPD3, PAH1, CLD1, FPX1, PLB3, INO4); fatty acids (FAA1) |
| Butanediol biosynthesis (fermentation of pyruvate) | BDH1, BDH2 |
| Ion homeostasis and transport | Detoxification (CUP1-1, CUP1-2, BSD2); transport (PHO89, ENA1, SMF2, SAT4); transcription factor ZAP1 (responsive to zinc); HEF3 (translation factor expressed in zinc deficient cells) |
| Cell wall | Cell wall proteins (SPI1, PRY3); cell wall modifying enzymes: glucanases (EFT2, DSE2, DSE4), chitinase (CTS1); regulators (NIC1 kinase required for cell wall integrity, ZEO1 membrane protein, regulates the PKC-dependent cell wall stress pathway) |
| Stress response | Nutrient deprivation, stationary phase (HSP30, MOH1, RBA50, SSA4, YL144W); other stresses (GRX6, YDL012C, HSP42, AHA1, CA1, SSA4, YER130C, SKN7, ROX3, SPS1, CMK2, ZEO1, MSN1, YGK3) |
| Noncoding transcripts (CUTs, SUTs and snoRNAs) | 20 SNR genes (snoRNAs); RPR1 (RNA component of nuclear RNaseP); SCR1 (RNA component of Signal Recognition Particle); CUTs (CUT419, CUT420, CUT428, CUT438, CUT439, CUT600, CUT643, CUT672, CUT673, CUT734, CUT807, CUT843, CUT917, CUT918); SUTs (SUT024, SUT032, SUT098, SUT102, SUT161, SUT174, SUT178, SUT185, SUT243; SUT285, SUT308, SUT326, SUT329, SUT350, SUT409, SUT650) |

The complete list of differentially expressed genes is shown in Dataset S1. CUT-cryptic unstable transcript; SUT-stable un-annotated transcript; snoRNA-small nucleolar RNA.

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transcript enriched on the outside), in which SUT660 is upstream of PIR1 transcribed in the same direction, but antisense to PIR3. Another such complex example includes SUT329, the ammonium permease MEP2 and CUT807 that were all enriched in the inside of the colony (see Table 3). SUT329 is upstream of MEP2, while CUT807 is antisense (Figure 3). These results collectively suggest that noncoding transcription could be contributing to the shaping of the transcriptome of a yeast colony in a complex manner.

Posttranscriptional mRNA Regulation in the Developmental Differentiation of a Yeast Colony

In addition to transcriptional regulation, posttranscriptional mechanism of mRNA control, such as control of poly(A) tail length, stability, translation rates and alternative 3' UTR usage, contribute significantly to gene expression [29,30,31,32]. Previous work which used promoter-lacZ fusions suggested that the major cytoplasmic mRNA deadenylase Ccr4 is more highly expressed on the inside than on the outside of the colony [14]. Our transcriptome analysis did not find changes in CCR4 gene expression (Dataset S1), and using a Ccr4-GFP fusion we did not observe clear subpopulations of GFP-expressing and non-expressing cells, unlike in the case of Ato1-GFP or Cit1-GFP (Figure 1C). However, we did find that the expression of another putative mRNA deadenylase, NGL3, was enriched in the colony center (Dataset S1). This prompted us to test for differential posttranscriptional mRNA regulation in colonies (inside versus outside), versus planktonic cells (stationary, which metabolically resemble colonies, and logarithmically growing cells). These comparisons allowed us to differentiate regulatory events that likely occur due to metabolic changes and nutrient starvation (these should be evident in the logarithmic versus stationary planktonic cultures), from those specific for colonies (which should only be evident in the planktonic versus colony comparisons). For these experiments, we chose several genes that showed altered gene expression on the outside or inside of colonies in our transcriptome analysis (Figure 4). To simultaneously confirm the differential expression identified by the array experiments, and to probe 3'UTR dynamics, we applied the semi-quantitative TVN-PAT and ePAT methods [17]. These methods report an invariant short poly(A)-tail or the full poly(A)-tail respectively. Both methods also detect alternative polyadenylation site usage. ATO1 showed increased expression on the outside of the colony, and the mRNA in these cells showed an overall longer poly(A)-tail suggesting better translation (note longer smear of increasing amplicon size in ePAT reaction). ATO1 is strongly expressed with alternate 3'UTRs in both 4-Day colony and planktonic stationary cultures, but expression is low in log phase cultures. However, a higher PCR cycle number revealed that the low level of ATO1 that is expressed in logarithmic cultures has a significantly longer 3'UTR (see ATO1 ↑ cycles panel). The glycolytic enzyme ENO1 is also clearly enriched on the outside of the colony whereas NGL3, HSP30, and the U6 spliceosomal RNA, SNR6 are enriched in the cells representing the colony interior, confirming the array data.
To confirm the co-ordinate expression of regulated genes and nearby non-coding RNA as illustrated in Figure 3, we monitored the expression of SUT350 and HPFI, which likely share a bi-directional promoter. SUT350 was among the most enriched transcripts of the colony interior whereas HPFI is a cell wall mannoprotein expressed preferentially on the outside of the colony. HPFI is also expressed in log phase cultures. SUT350 is expressed as transcripts of different sizes depending on the metabolic state of cells, consistent with previous data suggesting heterogeneous transcription initiation and termination sites [33]. Cells on the outside of the colony and logarithmically growing cells express predominantly an intermediate-sized SUT350 transcript (see 2* in Figure 4). On the other hand, cells of the colony interior and stationary phase cultures express a distribution of transcripts, including very short and very long forms (see 1* and 3*) that were under-represented on the outside layer of the colony and in log cultures.

Discussion

Metabolism in the Yeast Colony

Our analysis of differential regulation of gene expression in the center (inside) and margin (outside) of a yeast colony revealed profound differences between the two subpopulations in regards to growth and metabolism. The outside cells displayed higher levels of genes required for ribosome biogenesis and expressed enzymes required for the fermentation of glucose, suggesting active fermentative growth. The inside cells appeared not to be growing actively: they did not display enrichment in ribosome biogenesis genes, and several functions which were enriched are a hallmark of nutrient deprivation, such as genes with functions in gluconeogenesis and those responding to nitrogen catabolite repression. As noted in the results, we observed that expression of both activators and repressors of gluconeogenesis and non-fermentative growth was present in the inside cells. For example the transcription factors CAT8 (activator of gluconeogenesis) and MIG1 (repressor of gluconeogenesis) were both expressed, as were the key enzyme of gluconeogenesis, the phosphoenolpyruvate carboxykinase PCK1, and three genes required for the turnover of another key gluconeogenesis enzyme, the fructose 1, 6 biphosphatase FBP1 (Table 3). This could mean that longer term survival of the cells on the inside of the colony requires careful balancing of metabolic functions to ensure viability, but not exceed the capacity for growth. Of note, although generally the outside cells were expressing functions consistent with active growth, they also expressed genes which are hallmark of nutrient deprivation, most notably those required for fatty acid oxidation and acetyl-CoA synthesis (Table 1).

Mitochondrial functions were enriched in both subpopulations (Tables 1 and 3), but a closer inspection revealed that the cells residing in the inside of the colony expressed a larger proportion of genes required for the activity and assembly of the respiratory chain. The inside cells also expressed genes related to Fe-S cluster biogenesis and iron availability, which was not observed in the outside cell layers. The two key pathways requiring iron, Fe-S biogenesis and heme biosynthesis, both require mitochondrial biogenesis in the inside of the colony, which in our case was confined to the respiratory chain and iron-sulfur cluster biogenesis genes, such as SSQ1 and ISU2, while Cap et al reported enrichment of a large number of mitochondrial ribosomal proteins and chaperons (2012). These subtle differences could be due to somewhat different experimental set-ups and population isolation methods.

Noncoding Transcription and Posttranscriptional Gene Regulation of Colony Development

Our results suggest that, in addition to the expression of protein-coding genes, noncoding transcription also shapes the transcriptome of the outside and inside colony layers. We found several CUTs and SUTs enriched in the two subpopulations of colony cells. Mapping these noncoding transcripts showed that often the genes in their neighborhood were also differentially regulated. As shown in Figure 3, several scenarios were observed, including expression from bidirectional promoters, the sense transcription of the noncoding transcript and the coding gene, and antisense transcription. At the present, it is not clear if or how the noncoding transcripts regulate the expression of the coding genes in the colony, but it is possible, and likely, that our observations reflect true regulatory relationships. An exciting question for the future is to test the noncoding-coding transcript pairs or trios in some cases, to understand this fascinating regulation.

Our data further indicates that posttranscriptional mRNA regulation contributes to gene regulation in the distinct metabolic and developmental state of cells growing in a colony. We observed differences in mRNA poly(A) tail length distribution and 3' UTR usage between colony and planktonically grown cells for several genes which displayed altered expression levels in the colony (Figure 4). The stress protein HSP30 for example, is expressed in all tested conditions and the presence of two distinct PCR amplicons indicates alternative 3'UTR usage. Cells on the inside of the colony, and stationary phase cells utilize predominantly the short 3'UTR isoform. Cells on the outside of the colony additionally express a longer 3'UTR isoform, which is the main form expressed in log phase cultures. Intriguingly, most transcripts show a smear of amplicons reflecting the distribution of poly(A)-tails of new and aging mRNA transcripts. The poly(A)-tail of HSP30 in log phase cultures is universally long for both UTR isoforms. We speculate that this reflects an inactive/stored RNA population in these cells, whereas the mRNA within the colony and in stationary phase cells is actively translated and undergoes translation and age-associated deadenylation. These data, together with data showing differential adenylation site usage within the SUT350 transcriptional locus support our suggestion that the two populations within the yeast colony reflect distinct developmental states, opening a new field of study into the control of 3' UTR dynamics in the development of colonies and other multicellular fungal structures.
Parallels between S. cerevisiae Colonies and Candida Biofilms: Insight into How Heterogeneity of Growth and Metabolic States Shapes Biofilm Phenotypes

Several metabolic changes in yeast colonies that we observed in our transcriptome analysis have been previously reported to occur during biofilm growth of C. albicans and also Candida parapsilosis [7,8,9,12]. These include expression of ribosome biogenesis and translation-related functions, glycolysis genes, genes required for ergosterol biosynthesis and fatty acid metabolism, amino acid metabolism and iron-related functions, as well as genes encoding cell wall functions. This is quite striking, as C. albicans biofilms are morphologically very different to S. cerevisiae colonies of lab strains. For example, unlike colonies of laboratory S. cerevisiae strains, C. albicans biofilms contain both yeast and filamentous cells and depend on adherence. Moreover, C. albicans biofilms are grown under very different conditions than what we used for our study. Using “the awesome power of yeast genetics” in the study of multicellular biofilms would be very beneficial, as pathogens such as C. albicans are genetically much less tractable then S. cerevisiae. In regards to lab strains, growth as a mat on semi-solid substrate of Sigma1278b was previously suggested to be a model for biofilms, mainly due to the requirement for adherence [36]. Our result, that growth of S. cerevisiae in a colony resembles Candida biofilms in regards to transcriptome changes, suggests that colony growth of laboratory strains, such as BY4741 that was used in our study, could be a good model for fungal biofilms. Therefore, the multitude of functional genomics tools in lab strains of S. cerevisiae could be explored for understanding biofilm formation in greater detail.

Our transcriptome profiling of the two subpopulations within a yeast colony provides insight into the heterogeneity of fungal multicellular structures, and the contributions of the different cell populations to the phenotypes associated with growth in communities. Most of the metabolic and growth reprogramming observed in both biofilms and colonies occurs in the outside colony layers (e.g., enrichment of genes with functions in ribosome biogenesis, glycolysis, ergosterol biosynthesis, fatty acid metabolism). Enrichment in amino acid metabolism genes is a hallmark of C. albicans biofilms, and is observable in both the outside and inside colony layers. A notable difference is in the expression of genes with functions in the sulfur assimilation pathway, which are strongly induced in C. albicans biofilms [7,8], but not in a colony. However, some genes in this category were enriched in the inside colony layers, such as the adenylysulfate kinase MET14 and the S-adenosylmethionine synthetase SAM2 (Table 3). This indicates that the changes in sulfate assimilation in biofilms could be due to the metabolic state of the cells in the center. The cell wall undergoes profound changes in a C. albicans biofilms compared to the planktonic state, for example the cell wall proteome is substantially remodelled (reviewed in [2,3]). Another characteristic of C. albicans biofilms is production of extracellular matrix, which is important for biofilm cohesiveness and antifungal drug resistance [35,37]. The extracellular matrix in C. albicans biofilms consists largely of β1,3 glucans, the main carbohydrate component of the fungal cell wall, and matrix production is linked to pathways that regulate cell wall functions [35,39]. Our data suggests that the different colony environments to which the outside and inside cells are exposed impinge in a distinct manner on cell wall functions. The cell wall proteome is remodelled in the outside cells, and also the expression of the FKS1 glucan synthase is enriched, while the cells in the center of the colony express cell wall degrading enzymes such as glucanases and chitinases. It is interesting that the homolog of the only C. albicans gene expression regulator of matrix production reported so far, the zinc-responsive transcription factor Zap1 [39], is also enriched on the inside of S. cerevisiae colonies (Table 3). An exciting area for the future is to decipher how this differential regulation of cell wall functions in the different subpopulation links into the pathways controlling cell wall remodeling and matrix production in fungal biofilms. An important contribution in this context could be from changes to mitochondrial activity, which are observed within the colony. The biofilm environment is likely hypoxic, and hypoxia has been suggested to be responsible for several biofilm characteristics, such activation of genes required for ergosterol biosynthesis and glycolysis [9,40]. Hypoxia would impact on mitochondrial function. Mitochondrial function is important for cell wall integrity in several fungal species, including S. cerevisiae, C. albicans and C. glabrata [41,42,43,44,45]; reviewed in [46]). In some cases, links between mitochondrial activity and cell wall β-glucans have been reported [41,43]. It could therefore be envisaged that changes to mitochondrial function in a fungal community of cells could be contributing to cell wall restructuring and production of the extracellular matrix.

Supporting Information
Dataset S1 CIT 1 sorted by fold change. (XLS)

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Author Contributions
Conceived and designed the experiments: AT THB PH TS. Performed the experiments: THB AJ AS. Analyzed the data: AT THB PH TS. Wrote the paper: AT THB.

References
1. Bruckner S, Mosch HU (2012) Choosing the right lifestyle: adhesion and development in Saccharomyces cerevisiae. FEMS Microbiol Rev 36: 25–58.
2. Finkel JS, Mitchell AP (2011) Genetic control of Candida albicans biofilm development. Nat Rev Microbiol 9: 109–118.
3. Fanning S, Mitchell AP (2012) Fungal biofilms. PLoS Pathog 8: e1002585.
4. Smukalla S, Caldara M, Pochet N, Beauvais A, Guadagnini S, et al. (2008) Genome-wide transcription profiling of the early phase of biofilm formation by Candida albicans. Eukaryot Cell 4: 1562–1573.
5. Garcia-Sanchez S, Aubert S, Iraqui I, Jambon G, Ghigo JM, et al. (2004) Candida albicans biofilms: a developmental state associated with specific and stable gene expression patterns. Eukaryot Cell 3: 536–545.
6. Murillo LA, Newport G, Lan CU, Habelitz S, DunGAN J, et al. (2005) Genome-wide transcription profiling of the early phase of biofilm formation by Candida albicans. Eukaryot Cell 4: 1562–1573.
7. Rossignol T, Ding C, Guida A, d’Enfert C, Higgins DG, et al. (2009) Correlation between biofilm formation and the hypoxic response in Candida parapsilosis. Eukaryot Cell 8: 550–559.
8. Vaychovska O, Chernyavskyi M, Strachova D, Bianchini P, Bardinova Z, et al. (2009) Architecture of developing multicellular yeast colony: spatio-temporal expression of Ato1p ammonium exporter. Environ Microbiol 11: 1066–1077.
9. Nett JE, Lepak AJ, Marchillo K, Andes DR (2009) Time course global gene expression analysis of an in vivo Candida biofilm. J Infect Dis 200: 307–313.
12. Yeater KM, Chandra J, Cheng G, Mulkherjee PK, Zhao X, et al. (2007) Temporal analysis of Candida albicans gene expression during biofilm development. Microbiology 153: 2373–2383.

13. Khot PD, Suci FA, Miller RL, Nelson RD, Tyler BJ (2006) A small subpopulation of blastospores in candida albicans biofilms exhibit resistance to amphotericin B associated with differential regulation of ergosterol and beta-1,3-glucan pathway genes. Antimicrob Agents Chemother 50: 3708–3716.

14. Minarikova L, Kuthan M, Ricicova M, Forstova J, Palkova Z (2001) Differentiated gene expression in cells within yeast colonies. Exp Cell Res 271: 296–304.

15. Howson R, Hul WK, Ghaemmaghami S, Falvo JV, Bower K, et al. (2003) Construction, verification and experimental use of two epitope-tagged collection of budding yeast strains. Comp Funct Genomics 6: 2–16.

16. Knop M, Siegers K, Pereira G, Zachariae W, Wissow B, et al. (1999) Epitope tagging of yeast genes using a PCR-based strategy: more tags and improved practical routines. Yeast 15: 963–972.

17. Janické A, Vancovenbergen J, Boag PR, Traven A, Brilhaz TH (2012) ePAT: A simple method to tag adenylated RNA to measure poly(A)-tail length and other 3’ RACE applications. RNA 18: 1289–1295.

18. Palkova Z, Janderova B, Gabriel J, Zikanova P, Propisek M, et al. (1997) Ammonia mediates communication between yeast colonies. Nature 390: 532–536.

19. Davison GS, Joe RM, Roy S, Meirelles O, Allen CP, et al. (2011) The proteomics of quiescent and nonquiescent cell differentiation in yeast stationary-phase cultures. Mol Biol Cell 22: 9881–9890.

20. Xu Z, Wei W, Gageur J, Perocchi F, Clauer-Munster S, et al. (2009) Bidirectional promoters generate pervasive transcription in yeast. Nature 457: 1033–1037.

21. Chang YW, Howard SC, Budowskaya YV, Rine J, Herman PK (2001) The rye transcription factor is involved in adaptive program relevant for long term survival of Saccharomyces cerevisiae colonies. J Biol Chem 276: 37973–37981.

22. Schiefele K, Mathes S, Jenecke TH, Beilharz TH, Djordjevic JT, et al. (2009) Mitochondrial sorting RACE applications. RNA 18: 1289–1295.

23. Cap M, Stepanek L, Harant K, Vachova L, Palkova Z (2012) Cell differentiation within a Yeast Colony: Metabolic and Regulatory Parallels with a Tumor-Affected Organism. Mol Cell 46: 436–448.

24. Nett JE, Sanchez H, Cain MT, Andes DR (2010) Genetic basis of Candida albicans resistance to drug-sequestering matrix glucan. J Infect Dis 202: 171–175.

25. Reynolds TB, Fink GR (2001) Bakers’ yeast, a model for fungal biofilm formation. Science 291: 870–871.

26. Nett JE, Crawford K, Marchillo K, Andes DR (2010) Role of Fks1p and matrix glucan in Candida albicans biofilm resistance to an echinocandin, pyrimidine, and polyene. Antimicrob Agents Chemother 54: 3505–3508.

27. Bonhomme J, Chauvel M, Guyard S, Roux P, Rossignol T, et al. (2011) Contribution of the glycolytic flux and hypoxia adaptation to efficient biofilm formation by Candida albicans. Mol Microbiol 80: 995–1013.

28. Neill DH, Beilharz TH, Marguerat S, Mata J, Watt S, et al. (2007) A network accentuates expression of the yeast transcriptome. RNA 13: 982–997.

29. Goldstrohm AC, Wickers M (2008) Multifunctional deadenylase complexes diversify mRNA control. Nat Rev Mol Cell Biol 9: 337–344.

30. Lackner DH, Brilhaz TH, Marguerat S, Mata J, Watt S, et al. (2007) A network of multiple regulatory layers shapes gene expression in fusion yeast. Mol Cell 26: 143–155.

31. Quenault T, Lithgow T, Traven A (2011) PUF proteins: repression, activation and mRNA localization. Trends Cell Biol 21: 104–112.

32. Neil H, Malabat C, d’Aubenton-Carafa Y, Xu Z, Steinmetz LM, et al. (2009) Widespread bidirectional promoters are the major source of cryptic transcripts in yeast. Nature 457: 1033–1042.

33. Cap M, Stepanek L, Harant K, Vachova L, Palkova Z (2012) Cell Differentiation within a Yeast Colony: Metabolic and Regulatory Parallels with a Tumor-Affected Organism. Mol Cell 46: 436–448.

34. Nett JE, Sanchez H, Cain MT, Andes DR (2010) Genetic basis of Candida albicans resistance to drug-sequestering matrix glucan. J Infect Dis 202: 171–175.

35. Reynolds TB, Fink GR (2001) Bakers’ yeast, a model for fungal biofilm formation. Science 291: 870–871.

36. Nett JE, Crawford K, Marchillo K, Andes DR (2010) Role of Fks1p and matrix glucan in Candida albicans biofilm resistance to an echinocandin, pyrimidine, and polyene. Antimicrob Agents Chemother 54: 3505–3508.

37. Bonhomme J, Chauvel M, Guyard S, Roux P, Rossignol T, et al. (2011) Contribution of the glycolytic flux and hypoxia adaptation to efficient biofilm formation by Candida albicans. Mol Microbiol 80: 995–1013.

38. Neill DH, Beilharz TH, Marguerat S, Mata J, Watt S, et al. (2007) A network accentuates expression of the yeast transcriptome. RNA 13: 982–997.

39. Goldstrohm AC, Wickers M (2008) Multifunctional deadenylase complexes diversify mRNA control. Nat Rev Mol Cell Biol 9: 337–344.

40. Lackner DH, Brilhaz TH, Marguerat S, Mata J, Watt S, et al. (2007) A network of multiple regulatory layers shapes gene expression in fusion yeast. Mol Cell 26: 143–155.

41. Dagley MJ, Gentle J, Brilhaz TH, Petronino FA, Djordjevic JT, et al. (2011) Cell wall integrity is linked to mitochondria and phospholipid homeostasis in Candida albicans through the activity of the post-transcriptional regulator Ccr4-Pop2. Mol Microbiol 79: 986–989.

42. Qu Y, Jelicic B, Petronino F, Perry A, Lo TL, et al. (2012) Mitochondrial sorting and assembly machinery subunit Sam37 in Candida albicans: insight into the roles of mitochondria in fitness, cell wall integrity, and virulence. Eukaryot Cell 11: 532–544.

43. Zhong Q, Gvozdenovic-Jeremic J, Webster P, Zhou J, Greenberg ML (2005) Loss of function of KRE5 suppresses temperature sensitivity of mutants lacking mitochondrial anionic lipids. Mol Biol Cell 16: 665–675.

44. Batova M, Borecka-Melkusova S, Simkova M, Dzugasova V, Goffa E, et al. (2008) Functional characterization of the CgPGS1 gene reveals a link between mitochondrial phospholipid homeostasis and drug resistance in Candida glabrata. Curr Genet 53: 313–322.

45. Singh-Babak SD, Babak T, Diezmann S, Hill JA, Xie JL, et al. (2012) Global analysis of the evolution and mechanism of echinocandin resistance in Candida glabrata. PLoS Pathog 8: e1002718.

46. Shingu-Vazquez M, Traven A (2011) Mitochondria and fungal pathogenesis: drug tolerance, virulence, and potential for antifungal therapy. Eukaryot Cell 10: 1376–1383.
