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ANALYSIS OF GENES REQUIRED FOR QUIESCENT CELL FORMATION IN STATIONARY PHASE CULTURES OF SACCHAROMYCES CEREVISIAE

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By

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B.S. BIOLOGY
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THESIS

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ABSTRACT

Yeast cells in stationary phase cultures, after several days growth in rich, glucose-based medium (YPD), are separable by density-gradient centrifugation into two fractions. The heavier, quiescent cells are mostly virgin daughters whereas the less-dense, non-quiescent cells, are a typical mixture of daughters to aged cells. Quiescent cells can also be separated based on expression of specific GFP-tagged proteins, including many that are localized to the mitochodria. To ask the question, what genes are required for this differentiation process, we used a combination of the diploid, homozygous yeast deletion set, the heterozygous deletion set (carrying one deleted "essential" gene) and a third set designed to reduce mRNA abundance of a number of "essential" genes. Samples from the cultures just prior to the diauxic shift (just prior to glucose exhaustion), stationary phase, and isolated quiescent (Q) and nonquiescent (NQ) cells were harvested and technical and biological replicates analyzed by microarray analysis. The results showed that deletions in more than 500 genes resulted in 2-fold or greater reduction in Q-cell formation. Thus, almost 10% of genes in the yeast genome were important for Q-cell formation. When mutants with a 2-fold in Q vs all other samples were compared, 411 genes were identified that were important for Q cells vs DS, NQ, and SP. These genes encoded proteins involved in mitochondrial function, protein localization, and vesicle transport. We concluded from these results that differentiation of quiescent cells requires a major cellular commitment and that the major functions required are similar to those identified by proteomic and transcriptomic analysis of Q cells done previously in our laboratory, furthering understanding of cell differentiation.

TABLE OF CONTENTS

Acknowledgments

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Finally, my family has been a constant support since I began my graduate studies. I am grateful to have my siblings, parents, and the many other people I have been fortunate enough to have in my life that are now just as close as my family.

LIST OF FIGURES

LIST OF TABLES

Chapter 1

Introduction

Survival of all organisms on earth requires the ability to withstand periods of starvation and stress (De Virgilio, 2011). A model for studying this process and the responses required for survival is the entry of cultures of the yeast *Saccharomyces cerevisiae* into stationary phase (SP) as a result of carbon starvation. Our laboratory has pioneered this work (Werner-Washburne, et.al., 1993) and has discovered important features of cells in SP that suggest this process is analogous to important biogenesis and differentiation processes in other organisms.

Saccharomyces cerevisiae is a unicellular eukaryote that is simple to grow, work with, and manipulate at the molecular level. It is particularly useful for investigating basic cellular processes in eukaryotic cells and was the first eukaryote to be sequenced in 1996 (Goffeau, et. al., 1996). From the genomic data, it has been found that of the 6607 genes in yeast, approximately 2400 genes in the yeast genome have human orthologs. Thus, there is good reason that yeast has been such an important eukaryotic model system.

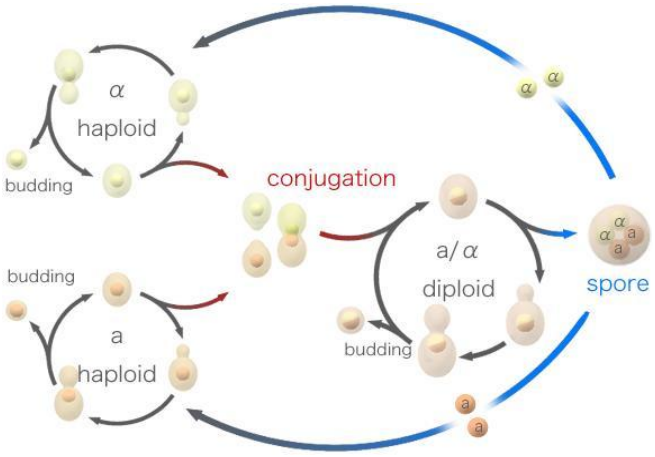


Figure 1.1: Yeast life cycle includes haploid and diploid stages.

Yeast cells may be grown and are stable as either haploid or diploid cells (Figure 1). There are two haploid mating types, MAT a and MAT alpha that can be mated to produce diploid cultures. When

incubated in a poor carbon source and low nitrogen, diploid cells will undergo meiosis and sporulation to produce four haploid spores, two of each mating type, which can be cultured as haploids.

Numerous genome-scale strain sets are available for yeast (Scherens and Goffeau, 2004) including a library of 4200 strains containing the Green Fluorescent Protein (GFP) Gene linked to different yeast genes. The GFP gene, found in the jellyfish *Aequorea victoria,* can be transformed into other species, like yeast, where it works to provide a fluorescent tag onto proteins (Chalfie, et.al., 1994). When the gene for GFP is fused to a gene in the organism, the resultant protein fluoresces when exposed to UV light. The GFP fusion library in yeast contains 4156 strains, each of which contains a separate open reading frame (ORF) in the yeast genome fused, in-frame at the 3' end, to the gene for GFP (Huh, et.al., 2003). This GFP library has allowed identification of protein expression and localization under various treatments by fluorescence detection via microscopy and flow cytometry.

In addition to the GFP library, there are also multiple sets of deletion strains with variations in ploidy, allelic frequency, and mating type. In each, the ORF of interest is replaced by a deletion cassette, which consists of a kanamycin (fungal antibiotic) resistance marker flanked by unique 20mer barcode regions (Winzeler, et.al., 1999). These sets are readily available and can be used to identify genes required for survival under various environmental conditions.

Laboratory yeast are most commonly grown to stationary phase in rich, glucose-based medium. Once glucose in the media is exhausted, at the diauxic shift (Fig. 3),

yeast metabolism changes from fermentation to respiration. Respiration allows oxidation of the carbon-based by-products of fermentation, i.e. ethanol. Once the metabolic shift has occurred, yeast enter the post-diauxic phase and divide once. After the exogenous carbon sources are exhausted via respiration, around 5-7 days post inoculation, the culture enters stationary phase (Pringle and Hartwell, 1981). In stationary phase, the rate at which cells divide is equal to the rate of cell death. This results in no net change in the cultural density, which is why the phase is referred to as stationary (Figure 2).

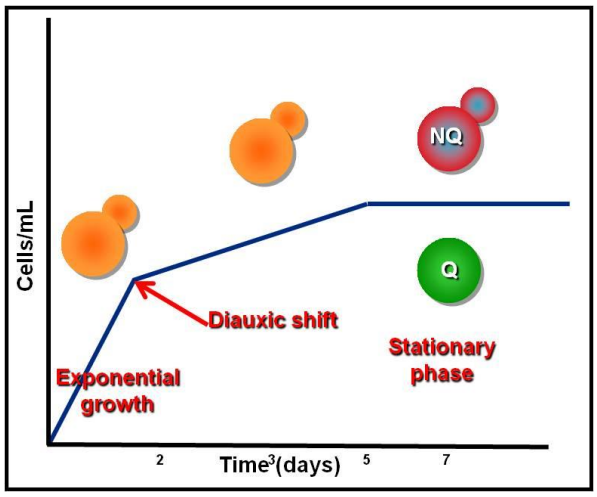


Figure 1.2: Growth of yeast from inoculation to stationary phase.

Most research with yeast has been done with cultures in exponential phase. These cultures are comprised of half daughter and half mother cells at any given point. Although the cell cycle is not synchronous, each cell is capable of budding a new daughter cell every 90 minutes when glucose is available (Gray, et al, 2004). Studies have been done in exponential phase due to the prevailing assumption that stationary phase cultures only contained dead or dying cells and therefore studies of cellular processes would have more impact in exponential phase.

It was previously assumed that the cells in stationary phase cultures were either dead or homogeneously quiescent, (Gray, et. al., 2004) neither of which is true. Research has since shown that in stationary phase, there are two populations of cells separable by density (Figure 3) (Allen, et. al., 2006). This differentiation begins to occur at approximately 14-20 hours after the DS. The denser population is in fact comprised solely of quiescent cells (Q). The less dense population, however, is heterogeneous and is referred to as non-quiescent (NQ). Multiple studies have been conducted by our lab in order to identify further phenotypes that differ between the two populations (Aragon, et. al., 2006, Aragon, et.al., 2008, Davidson, et. al., 2010).

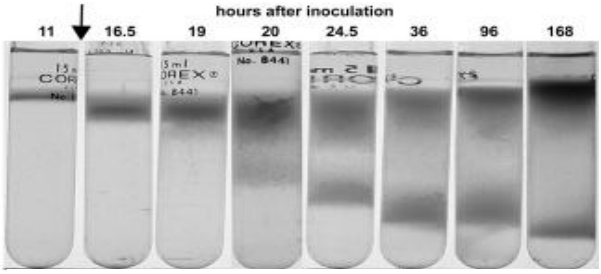


Figure 1.3: Separation of NQ and Q cells in SP cultures by density gradient centrifugation.

The Q population of cells of stationary phase cultures is essentially all (91%) daughter cells, meaning that they have never budded and are not dividing (Allen, et.al., 2006). Upon addition of fermentable carbon sources (i.e. glucose), these cells reenter the cell cycle synchronously. In contrast to NQ cells, nearly 100% of Q cells are able to produce daughter cells. Moreover, the colonies are exquisitely uniform, i.e., none of the colonies that result from growth on glucose rich media are petite, allowing the conclusion that this cell population is genomically stable. They are also thermo-tolerant and stress resistant. Phase contrast microscopy revealed highly refractile cell walls and TEM, the storage of glycogen.

NQ cells, in contrast, exhibit greater heterogeneity. Approximately 50% of the NQ cells are unable to reproduce, i.e. divide to produce daughter cells, and these cells

exhibit high levels of reactive oxygen species (ROS), in contrast to Q cells, which exhibit low or no ROS. Paradoxically, essentially all NQ cells are all metabolically active or viable even if they are incapable of reproducing. The 50% of the population that can reproduce produce a high percentage (40%) of petite colonies, indicating the presence of mitochondrial mutations. By 14 days, 50% of NQ cells exhibit markers indicating they are apoptotic..

Transcriptomics of Q and NQ

mRNA transcripts from SP cultures were first detected on a genomic scale by a time-course study of cells in cultures entering (Gasch *et al*., 2000) and exiting the phase (Martinez, et.al., 2004). In the Martinez study, after reintroducing the cells to a glucose rich environment, over 1000 mRNA transcripts exhibited at least a twofold change in expression from when the cultures were initially reintroduced to glucose to 55 minutes after refeeding. These particular mRNAs were involved in aerobic respiration, metabolism and ribosome biogenesis. Also, several of them were involved in mitochondrial function and apoptosis. These high levels of mRNAs involved in mitochondria and metabolism suggest that the cells in SP cultures are either metabolically active or are poised to respond quickly to the introduction of nutrients.

Cells in SP cultures were also found to contain extraction-resistant or "sequestered" mRNAs that are quickly solubilized in response to different stresses (Aragon, et.al., 2008). Microarrays of SP cultures determined that there are more than 2000 transcripts present in cells after exposure to factors that induce stress. These mRNAs were involved in DNA repair, ribosome assembly and processing and oxidative

stress responses, many of the types of mRNAs that were seen to be abundant in non-quiescent cells. Thus, these mRNAs may be part of the switch from Q to NQ states that the cells are capable of to allow a rapid response during reentry into the cell cycle when nutrients are again present.

From the transcriptomics data of these two studies it is clear that cells in SP cultures are definitely not dead or dying and are responsive to environmental changes, such as stressors and nutrients. The mere presence of mRNA transcripts, however, does not indicate that there is correspondingly high protein abundance.

Proteomics of Q and NQ

The GFP library was grown to Exp and SP to identify differences in protein expression between the phases and also to identify strains that had multiple populations of fluorescence in SP (Davidson, et.al., 2011). The library (approximately 4200 strains in triplicate) was grown in 96-well plates overnight for the Exp and for 7 days for the SP samples. The strains were then analyzed by flow cytometry with the FL1 channel to determine the relative abundance of GFP in each population.

Approximately 5% of the strains showed more than a 2-fold difference between the EP and SP samples. The proteins that were found to be abundant in cells in SP cultures were involved in mitochondrial function primarily, while those in cells in EP cultures were involved in biosynthetic processes.

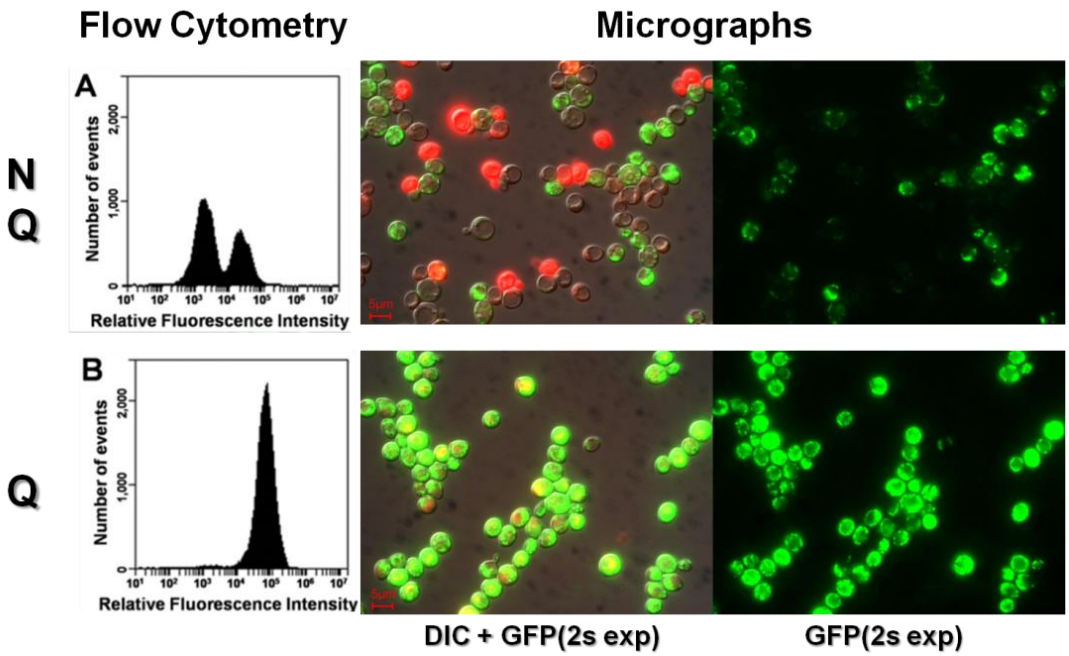


Figure 1.4: Protein abundance of GFP fused citrate synthase (Cit1p:GFP) differentiates NQ and Q cells.

We further examined 38 strains that clearly exhibited two fluorescent populations in the SP sample. One of these strains was Cit1p:GFP (Figure 4), which encodes the citrate synthase protein, the first enzyme of the TCA cycle. All of the Q cells containing this construct express this protein and at a higher level than NQ cells (Figure 4). It is also clear, by function and the above micrographs, that this protein is localized to the mitochondria. Of the strains that showed 2 fluorescent populations in SP, almost 60% of the strains that were examined were involved in mitochondrial function.

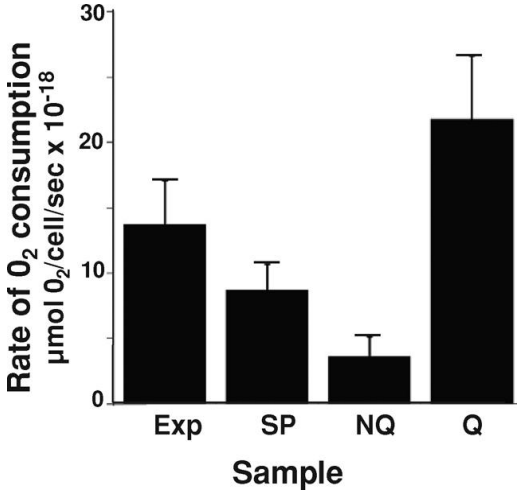


Figure 1.5: Rate of oxygen consumption reveals that Q cells respire at a six fold higher rate than NQ cells.

To determine whether the differences in protein abundance indicated differences in physiological state, we determined the rates of respiration in Q and NQ cells (Figure 5). We found that the Q cells were respiring at a rate six fold that of the NQ cells. The

NQ cells were already known to produce petite colonies, indicating mitochondrial mutations that affected mitochondrial function, but this study showed that Q cells are the only population in SP cultures that are able to use carbon sources available at this time and, although they are not dividing, are respiring at a high rate. This suggests that they are using respiration for maintenance rather than growth. These results further suggest that NQ cells are obtaining any carbon they might have from cataolic metabolism or autophagy.

In yeast, it has been shown that oxidative phosphorylation stops during DNA replication in S phase of the cell cycle to prevent ROS damage (Hu, et. al., 2008). It may be that NQ cells lack some G1 regulation to allow them to stop and, because they cannot respire, become stuck in S-phase. This remains to be tested.

Genomics of Q and NQ

In this study, I used the homozygous and heterozygous diploid deletion sets to identify genes important in Q cell differentiation. These sets have single strains in which one or both alleles for one gene have been deleted. The third tool particularly useful for this study was the hypomorphic Decreased Abundance by mRNA Perturbation (DAmP) set, derived from the deletion sets (Breslow, et.al., 2008). It contains strains that have been disrupted at the 3'UTR with the kanamycin resistance cassette. This results in unstable mRNA transcripts, decreasing the half lives of the transcripts and thereby the cytoplasmic mRNA levels by 4-10 fold. About 970 of the 1033 essential genes were made into DAmP diploids. This set is particularly sensitive for examining the functions of essential genes. The deletions remove the gene from the genome, while the DAmP set

only affects the effectual number of mRNA transcripts. This difference may affect the way that other genes interact to compensate for the deletion.

Until these sets were available, there was no way to determine the relationship between Q and NQ cells at a genetic level in yeast. These two cell types, while a great deal of genomic and biochemical data points to significant differences between them and their etiology, are still controversial for some researchers, especially those who have worked in the yeast cell cycle and are committed to the idea that yeast doesn't have a quiescent state. To know that most Q cells are virgin daughters, that there are such significant differences in physiological state, and that Q cells are not only genomically stable but NQ cells are not seems a reasonable list of differences. However, identifying genes and knowing how many genes are required for the presence of Q cells in SP would be extremely important for understanding the commitment yeast have to the formation and stability of these cells and to begin to understand the evolutionary connection between Q cells and other eukaryotic cell types that might be reltaed, such as stem cells.

Chapter 2

Methods

Strains

Homozygous diploid deletion pools, heterozygous diploid deletion pools and DAmP (Decreased Abundance by mRNA Perturbation) pools were a donation by the Nislow laboratory at the University of Toronto (citation). They were prepared as outlined in Pierce, et. al., 2010, delivered to our lab and placed in a -80 freezer until use.

The individual deletion strains used for the mating were from Invitrogen. (Cat. no. 95400). They are mating type alpha, parental strain BY4742.

For the mating, an individual strain of citrate synthase (Cit1) fused to GFP from the Yeast GFP Fusion Localization Database, constructed from the parental strain ATCC 201388: MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 (S288c) (Brachmann et al., 1998), was used.

Growth Conditions

All strains were initially grown on YPD+A (1% yeast extract, 2% peptone, 2% glucose, 0.04mg/ml adenine) at 30°C for 2 days to isolate colonies.

For the deletion pool separation, 50 ul of approximately 10 OD frozen samples of the homozygous deletion, heterozygous deletion and DAmP sets were grown on YPD+A for 2 days. 10^8 cells from each plate were then grown in liquid 1.1YP+A at 30°C and 250rpm in triplicate. Approximately 20 hours after inoculation, glucose strips were used to determine exit from exponential phase of the cultures. These measure the concentration of glucose upon immersion in solution and indicate the diauxic shift upon glucose

exhaustion. DS samples of all pools were collected. The same cultures were grown to 7 days at which point they were in SP.

For the mating, isolated colonies of the deletion strains and Cit1:GFP strain were collected on YPD. Those colonies of the deletion strains were then grown on YPD+A+G418 (YPD+A with 200μg/ml of kanamycin/G418) while the GFP strain was grown on histidine drop out media (synthetic complete media without histidine). Colonies of the deletion strains were mated with colonies of the GFP strain on YPD+A. Following verification of zygotes by microscopic analysis, the yeast were transferred to presporulation media (YPD+A with 4% glucose rather than 2% glucose) overnight and then transferred to KOAc media (potassium acetate) to induce sporulation. The diploids were allowed to sporulate on this media for a week before they were microscopically examined for the presence of tetrads.

The spores that were dissected from the tetrads were grown initially on YPD+A for 2 days. The individual colonies that resulted from each spore were grown on another YPD+A plate for 2 more days and then transferred to the YPD+A+kanamycin plates and histidine drop out plates.

Collection

Five samples, including 3 technical and 3 biological replicates were analyzed at each time point using the homozygous and heterozygous deletion sets and the DAmP set. The first step of analysis was to normalize the data methods. Because the absolute values from the microarray data were lower for quiescent than the other samples, we normalized the samples as a function of percent abundance in the sample. We assumed that, because

we analyzed the same number of cells per sample and quiescent cells are typically more difficult to lyse, that the quiescent results represented a smaller percentage of cells than the other samples and, thus, for abundance comparisons, we should use percentage abundance across the sample.

The deletion pools were collected at the DS and during SP. Approximately 20 hours after inoculation, the DS was determined using glucose detection strips. The density of cells in the cultures was determined using a Z2 Coulter Counter (Beckman). 10^7 cells were collected from each culture into 1.75 ml Eppendorf tubes, pelleted by centrifugation in a microfuge and frozen at -20°C. The SP phase samples were collected in the same way. The SP cultures were also separated into Q and NQ fractions. Percoll (GE Healthcare, Piscataway, NJ) density gradients were made using a solution of one part 1.5M NaCl per 8 parts Percoll by volume (Allen, et.al., 2003). The gradients were formed as 10ml aliquots in 15ml Corex tubes that were centrifuged at 13,400rpm for 15 min at 4°C in a Beckman Coulter (Fullerton, CA) JA-17 rotor. In order to separate the SP cultures, 5ml samples were pelleted by centrifugation at 3270rpm for 3 min at 25°C in a Beckman Allegra tabletop centrifuge. These pellets were resuspended in 500µl of 50mM Tris-HCl buffer (pH 7.5) and overlaid onto the Percoll gradients, which were then centrifuged at 400g for 60 min at 25°C in an Beckman Coulter (Allegra X12-R) tabletop centrifuge with a swinging bucket rotor. The denser Q and less dense NQ fractions were collected by pipette and suspended in 13ml of 50mM Tris buffer. The samples were then pelleted by centrifugation in a microfuge. The pellets were resuspended in 1ml of 50mM Tris buffer and the density of cells in the samples was determined by the Z2 Coulter

Counter. 10^7 cells were collected from each culture into 1.75 ml Eppendorf tubes, pelleted by centrifugation in a microfuge and frozen at -20°C.

Analysis

The pellets were sent overnight on dry ice to the Nislow lab at the University of Toronto. These were analyzed per their yeast genomic method called the HIPHOP assay (Smith, et.al., 2010), the principle of which is similar to the microarray except that it determines presence of DNA rather than mRNA.

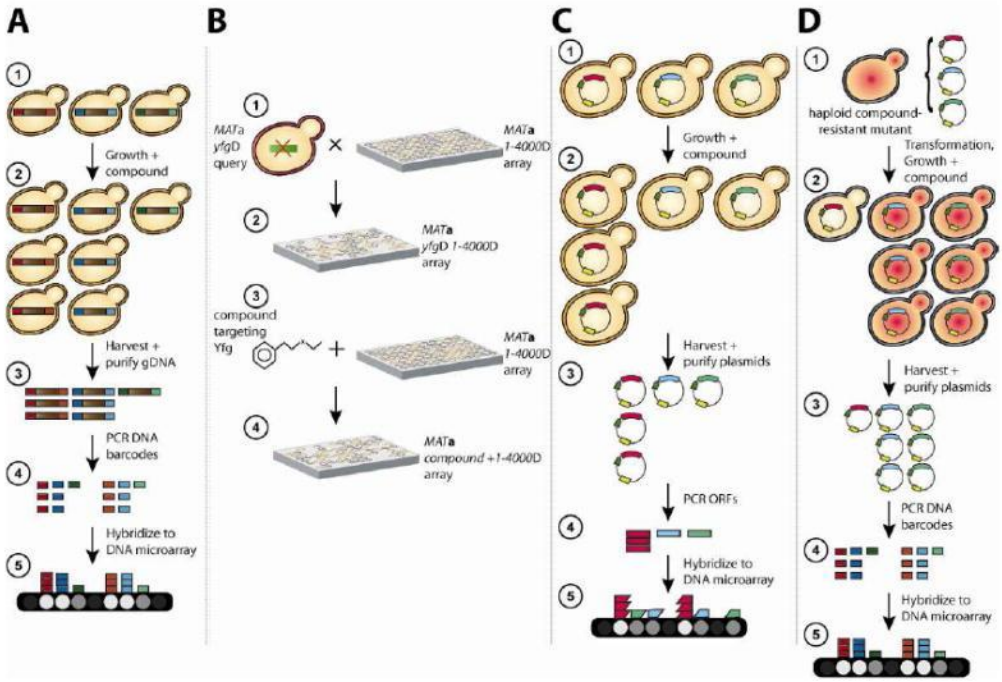


Figure 2.1: HIPHOP assay (Smith, et.al., 2010)

The original datasets comprising the homozygous deletion, heterozygous deletion and DaMP pools contained 13929 strains with measurements for all 5 replicates of DS, SP, NQ, and Q. The uptags and downtags for each ORF were averaged together for a final strain list. The DS timepoint was used as the background control and all strains that had a

measurement of at least 200 in the DS were examined in the SP, Q and NQ datasets. The averages of each strain in the respective sample sets were calculated. The averages of the strains were compared against each other and those that had a fold change of 2-fold or higher between different samples were selected for further analysis.

The 411 deletion strains that were determined to be of interest by these fold comparisons were streaked from the deletion master set onto YPD plates with kanamycin. Strains were grown for 2 days and cells were harvested into 1 ml of 20% glycerol for storage as permanent stocks. Four of the 411 strains did not grow after multiple attempts and were not included in the master set. A master set was also constructed in 96 well plates by placing 200 ul of each deletion strain from the permanent stocks. The samples were only placed in the odd columns to avoid cross contamination of the strains. The first column of each plate was filled with samples of BY4741, the mat a parental strain.

Growth of strains for assays

A pin apparatus was used to inoculate 96-well plates filled with 200ul of YPD and 500ul/100ml of 100x ampicillin from the master sets. Three sets were inoculated for each assay in order to have triplicate results. The plates were sealed with Breathe-Easy membranes (Sigma Aldrich, St. Louis, MO, #380059) to allow gaseous exchange while minimizing desiccation and contamination. The plates were then grown for 7 days at 250rpm and 30°C.

Fungalight

Viability of the deletion strains was determined using the LIVE/DEAD® FungaLight™ Yeast Viability Assay (Invitrogen). The assay contained two vials of 0.033 mM propidium iodide and 0.006 mM Syto9 in PBS. Samples were prepared by pipetting 50 ul of each strain into 96-well plates to which was added 150 ul of working solution. The working solution was mixed directly prior to the experiment and contained 0.25ul of each dye per sample. 150ul was used to ensure that the sample would be diluted enough for flow cytometry analysis. Samples were incubated in a water bath at 30°C for 30 minutes. Samples were then analyzed with an Accuri C6 flow cytometer using 480/500 nm emission/excitation for live samples in the FL1 detector and using 490/635 nm emission/excitation for dead samples in the FL-3 detector. All cells take up the Syto9 dye, so all will emit in the range for detection by the FL1. Only cells with compromised cell walls, i.e. dead cells, will take up the PI dye.

Reactive Oxygen Species

Five mM (Dihydroethidium bromide) DHE in PBS (Invitrogen, Carlsbad, CA) was diluted 1:330 in phosphate-buffered saline (PBS) for a working solution. Samples were prepared by pipetting 50 ul of each strain into 96-well plates to which was added 150 ul of this working solution. Samples were incubated for 10 minutes at room temperature covered with aluminum foil to prevent photobleaching by light pollution. Samples were analyzed with an Accuri C6 flow cytometer using 488 nm excitation and collecting fluorescent emission with filters at 585+/- 40 nm for FL-2 parameter.

Colony Forming Unit (CFU) Reproductive Capacity Assay:

For the CFU assay, strains were inoculated from permanent stocks onto YPD+Gal plates. Colonies from those plates were then inoculated into 100 ml of YPD+A plus 500 ul 100x ampicillin and grown with shaking at 30°C for 7 days to SP. At 7 days, samples were harvested and separations into Q and NQ fractions were obtained by density gradient centrifugation. Mixed SP, Q and NQ samples were counted and 300 cells per sample were plated in triplicate on YPD media plates. Plates were incubated at 30°C for 2 days and colonies were quantified visually.

Mating with Cit1:GFP marker

Colonies of the deletion strains, once grown on YPD+A+G418, were mated with colonies of the GFP strain on YPD+A. Following verification of zygotes by microscopic analysis, the yeast were transferred to presporulation media (YPD+A with 4% glucose rather than 2% glucose) overnight and then transferred to KOAc media (potassium acetate) to induce sporulation. The diploids were allowed to sporulate on this media for a week before they were microscopically examined for the presence of tetrads.

Tetrads were dissected using a light microscope with a 10x objective and 10um needles. 100T zymolyase was diluted to a working solution of 0.5mg/ml in 1M sorbitol. 50ul of this solution was aliquoted into 1.75ml Eppendorf tubes. A swab of the cells from the mating were mixed into this solution and allowed to incubate for 10 minutes. After the incubation period, 800ul of double deionized water was added to the solution and the sample was placed on ice for at least 2 minutes. 20ul of the sample was then spread onto

a YPD+A plate for the dissection. The needle was used to take the tetrads and move them to a clean region of the plate. The spores were broken apart and separated by 5mm to allow visible identification of the colonies.

The spores that were dissected from the tetrads were grown initially on YPD+A for 2 days. The individual colonies that resulted from each spore were grown on another YPD+A plate for 2 more days and then transferred to the YPD+A+kanamycin plates and histidine drop out plates. It would be expected that there would be four genetically different spores that we could identify by ability to grow on different kinds of media. The four types would include: capable of growth on His DO, but not G418; capable of growth on G418, but not on His DO; incapable of growth on either media; capable of growth on both media.

Analysis of GFP&Deletion strains

The colonies were allowed to grow on the media for 2 days. They were then analyzed to determine the group of cells resulting from the spore that had the deletion cassette, determined by the ability to grow on G418, and the GFP, determined by the ability to grow in the absence of histidine. Permanent samples were preserved in 20% glycerol in the -80°C freezer. Samples were also grown to SP in liquid YPD+A at 30°C and 250rpm. These samples were separated by density (Allen, et.al., 2006) to determine relative ratio of NQ:Q. The samples were also analyzed by the Accuri C6 flow cytometer using 480/500nm excitation/emission before and after separation. The number of quiescent cells from the density separation was compared to the number of bright cells as determined by the FL1 detector.

Chapter 3

Results

To determine the number of genes involved in differentiation of Q and NQ cells, we examined the diploid homozygous (all "nonessential" genes) and heterozygous (essential genes) deletion sets and the diploid DAmP set, which results in decreased mRNAs for a subset of essential genes (Pierce, et.al., 2007). All genes with abundance at the diauxic shift of 200 or more relative fluorescent units were included in the analysis. Because there is only one cell division after the diauxic shift, this sample was used for a baseline comparison. The percentage of strains in each of the three sets, homozygous, heterozygous, and DAmP, that surpassed the ≥ 200 cutoff was similar, with an average of 84%, including approximately 2/3 of the genes in the yeast genome.

The first step of analysis was to normalize the data methods. Because the absolute values from the microarray data were lower for quiescent than the other samples, we normalized the samples as a function of percent abundance in the sample. We assumed that, because we analyzed the same number of cells per sample and quiescent cells are typically more difficult to lyse, that the quiescent results represented a smaller percentage of cells than the other samples and, thus, for abundance comparisons, we should use percentage abundance across the sample.

Homozygous diploid deletion set results

The technical and biological reproducibility for the measurements were extremely high (> .97 R^2) for all samples: DS, SP, NQ and Q (Fig. 3.1, A and B). Variability between different samples was clearly much greater (Fig. 3.1, C and D). Strains that were 2-fold or greater above the line of regression for all of the sample comparisons were identified.

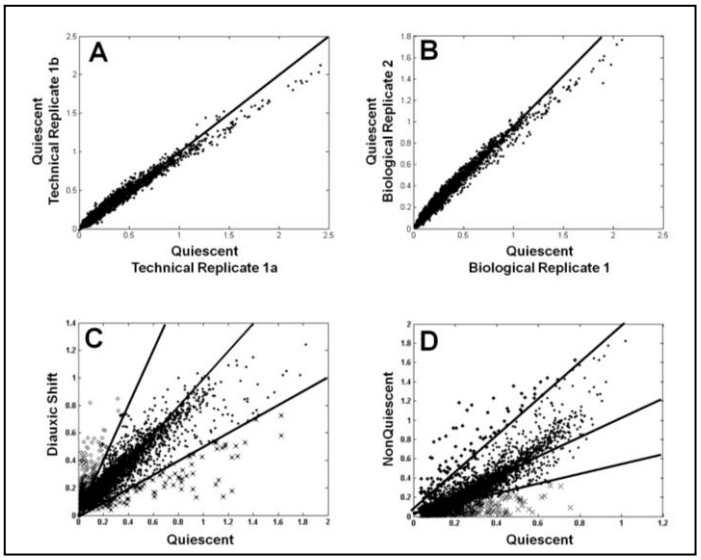


Figure 3.1: HOM data A)Plots of two technical replicates of the Quiescent timepoint; B)Plots of two biological replicates of the Quiescent timepoint; C)Diauxic Shift v Quiescent with lines indicating (from left) 1x2y, 1x1y, 2x1y; D)NonQuiescent v Quiescent with lines indicating (from left) 1x2y, 1x1y, 2x1y

To identify genes important in Q cell formation, strains with a higher abundance in the NQ, DS, and SP samples were identified by comparison (Table 3.1). If genes are important for Q cell formation, we would expect a higher abundance of that deletion strain in the NQ sample, simply because we would not expect those strains to become Q

TABLE I. Ratio of ≥2 fold increase in HOM

COMPARISON	#STRAINS > 2 fold	% of 3849 STRAINS	HIGHEST FOLD CHANGE
NQ/Q	**522**	**13.60%**	**23.5**
SP/Q	**440**	**11.40%**	**22**
DS/Q	**448**	**11.60%**	**22.9**
Q/DS	114	3%	8.3
Q/NQ	144	3.70%	10.8
Q/SP	68	1.80%	4.2
NQ/DS	1	0.00%	2
SP/DS	1	0.00%	3.4
SP/NQ	13	0.30%	2.6
DS/SP	0	0	-
DS/NQ	9	0.20%	3.2
NQ/SP	0	0	-

cells. To determine the samples that showed the most significant genetic effects, we compared DS, SP, NQ, and Q results. DS, SP, and NQ samples showed the least differences between them. The large differences in fluorescence (fold-change) were observed when NQ, SP, or DS samples were compared to Q samples – indicating that Q cell formation is very sensitive to the loss of some genes. Similar relationships were observed in the heterozygous deletion and DAmP sets, although the percentage of strains that exhibited these differences was much smaller. We conclude from this that the influence of specific genes on Q cell differentiation is very strong and that a large part of the yeast genome is involved in this process.

Somewhat fewer strains (~100) were more abundant in Q than in NQ, DS, and SP samples (Table 3.1). This is consistent with the hypothesis that Q cells are very different from other types of cells in yeast cultures. The ratios of these strains were 10-fold or less in the homozygous deletion set and 4-fold in the heterozygous deletion and DAmP sets, further suggesting that gene loss did not lead to greater production of Q cells. Strain abundance in DS, SP, and NQ samples was very similar, with the largest difference (13 strains) between homozygous SP and NQ samples.

We compared gene lists for DS, SP, and NQ vs Q samples and identified a core set of 411 strains that were present in the three lists. When this analysis was carried out with absolute values instead of percent abundance, the number of strains identified in the 3 samples the overlap was higher and the 411 was included in this list. Normalizing the values by calculating percent abundance, therefore, focused our list of core genes. The identity of strain lists between the NQ/Q and DS/Q and SP/Q (p-values for pair wise

overlaps was ~0) provides very strong support for the importance of this particular set of genes for quiescent cell differentiation.

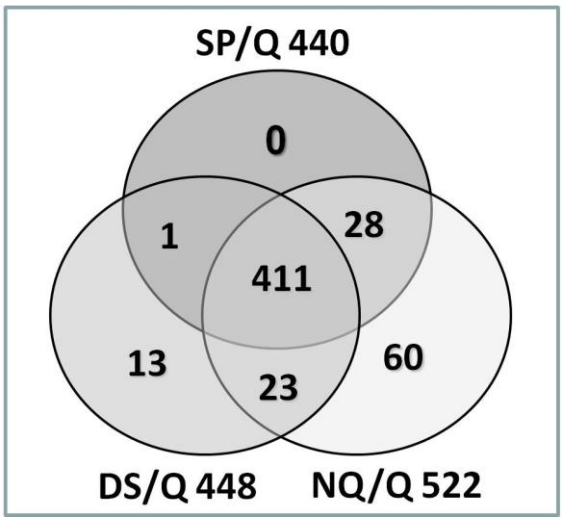


Figure 3.2: Venn diagram of the number of intersecting genes among samples from DS, SP and NQ in comparison to Q. All Genes over 2 fold.

In evaluating the overlap of strains that were higher in NQ, DS, or SP compared with Q, it was clear that not only were the greatest differences (522 strains) found between NQ and Q samples, but that there were 60 strains that were unique to this comparison. The strains with the largest fold change when compared to the Q sample, however, were present in the 411, as well. The most significant results that were found in comparing NQ to Q, therefore, were also highly significant in the other samples and represented in the core set. That there were almost no differences in SP/Q that were not identified in NQ/Q is consistent with NQ and Q comprising SP cultures.

Table II. Significant processes (GO) in DS/SP/NQ vs Q

TERM	NUMBER OF GENES (n=411)	P-Value
mitochondrion organization	63	8.95 E-17
mitochondrial translation	34	3.02 E-11
cellular component organization	169	1.07 E-09
organelle organization	131	1.82 E-08
protein metabolic process	155	2.09 E-08
cellular component organization at cellular level	147	7.82 E-08
cellular protein metabolic process	152	2.57 E-07
protein complex biogenesis	36	1.10 E-06
mitochondrial respiratory chain complex IV biogenesis	11	1.28 E-05
protein localization to organelle	43	4.55 E-05
cellular component organization or biogenesis	174	5.37 E-05
protein localization in Golgi apparatus	7	6.35 E-05
late endosome to vacuole transport	12	7.47 E-05

Genes that were deleted in the 411 strains were analyzed to identify significant GO processes of the proteins they encoded (Table II). For this analysis, the cutoff was $p < E\text{-}05$. Thirteen GO terms that were highly significant involved mitochondrial

organization and function and early protein secretion (protein localization in the Golgi and late endosome to vacuole transport). The numbers of genes and significance of the GO processes suggests that the presence of Q cells in SP cultures is highly dependent on energy production, mitochondrial metabolism, and protein function and stabilization. There are no significant categories that relate to the cell cycle, thus, the differentiation of virgin daughters into Q cells may rely in great part to physiological changes in this particular cell type.

Despite the lack of a significant cell-cycle category in the G0 analysis of genes required for Q cell differentiation or stabilization, there are cell-cycle related genes that appear in this analysis. So, what is the difference between the arrested, unbudded state of Q cells and the G1 cells in the NQ fraction? To study this, we evaluated the 26 cell cycle genes found in the list of 411 deletion strains (See Appendix) . Interestingly, there was no clear consensus in terms of what cell cycle phase these mutants affected. The list includes genes required for G1, G2, and even G1-S transitions. There are several genes involved in septin formation, sporulation, and even psuedohyphal growth. While this could be a problem with GO assignments, it may also be that this phase of the life cycle when Q and NQ cells differentiate is also a time when diploids sporulate and, under the appropriate conditions, initiate pseudohyphal growth.

To examine what genes might actually inhibit the formation of Q cells, we compared the deletion strains that were higher in Q than DS, SP, and NQ and identified a core set of 68 strains (See Appendix). While most of the 68 strains higher in Q than all other samples had deletions in genes encoding proteins with unknown function (13), others affected cytoplasmic translation (10) and pol II transcription (7). An interesting

group included 4 genes whose encoded proteins are involved in response to DNA damage, including regulation of Ty element transposition: CKA2, EAF3, RAD16, and RRD1. Previous transcriptome analysis showed that the mRNAs involved in DNA recombination and repair and Ty element transposition were significantly enriched in NQ cells (Aragon, et. al., 2006). Four strains carried deletions in genes encoding proteins with mitochondrial localization: TDA5, PUF3, NUM1, and GDS1. None of these genes are well characterized, PUF3 and NUM1 are associated with decreased respiratory growth, although NUM1p is required for nuclear migration and localizes to the mother cell cortex and Puf3p is on the outer mitochondrial surface. These genes are of interest because they may provide insight into different aspects of the regulation of mitochondrial function.

It has previously been thought that the identification of quiescent cells as having a greater density was because they would have an extended G1 phase, during which time glycogen accumulates, and, therefore, genes involved in glycogen metabolism might be among the 618 slow growing strains identified in YPD (Shi, et. al., 2010) However, there was only an overlap of 80 strains between the slow growers in YPD and the 411 strains important for Q cell formation and 11 between the slow growing strains and the 68 strains that showed increased abundance in quiescent samples. That there is only approximately one cell division suggests that this analysis would not be sensitive to strains with a slow-growth phenotype during exponential growth.

Previously, our laboratory had identified more than a thousand mRNAs that were insoluble to typical RNA extraction due to association with ribosomes and proteins, but that were released when aqueous lysates were treated with proteases (Aragon, et. al.,

2008). These mRNAs were also shown to increase in the soluble fraction almost immediately upon the exposure of cells to mild stresses, temperature and oxidative stress, and the mRNAs were released in a stress-specific manner. We found that 50 of the strains identified through the mRNA study overlapped with the 411 strains found to be important for Q cell formation (see Appendix). Of these 50, 8 (16%) are involved in mitochondrial function. It is extremely interesting that so many genes required for Q cells in SP would encode mRNAs that are insoluble in these cells. It suggests that either these mRNAs are soluble and being translated at times before 7 days or that they might be accessible for translation in Q cells.

To identify those strains that were essentially missing from the Q fraction, we examined the 411 strains and found that 68% (281) of the strains were essentially not present in the Q samples. Sixty-two of the 63 genes whose products were localized to mitochondria are in this category. Other significant categories include cellular component organization, single-organism cellular process, organelle organization, and protein complex biogenesis, which were also the most significant GO processes of the 411. The other mitochondrial strain that was included in the 411 was found only marginally above background level in the Q samples. We conclude from this that mitochondrial function is critical for the presence of Q cells in SP cultures. Because there are G1 cells in NQ populations that lack mitochondria, we conclude that the regulation of physiological function in the virgin, daughter Q cells is distinct from that of G1 cells (predominantly older mothers) in the NQ fraction.

Previous studies have shown that both Q and NQ populations are viable or metabolically active, but that NQ cells accumulate ROS (Allen, et. al., 2006). We

therefore used viability and ROS assays to determine if any of the 411 deletion strains that showed reduced or absent Q cell formation would exhibit changes in ROS or viability, as measuresd by FungaLight.

As seen in Table 3.3, 187 deletion strains exhibited increased viability. Fifty of those strains exhibited higher levels of ROS while 131 exhibited lower levels of ROS than the parental control. The major GO categories were mitochondrial and protein related. There were only 41 deletions that decreased the viability of the strains. There were no significant GO categories from the analysis for these strains. It is interesting that the strains the showed higher viability and high ROS carried deletions involved in mitochondrial function, since typically mitochondrial metabolism is thought to be the source of ROS. This suggests that in yeast, in SP, ROS arises from other mechanisms and is confirmed in the literature. Oxygen is not only utilized in aerobic respiration. Oxidation of fatty acids occurs in the peroxisome and protein folding processes in the endoplasmic reticulum require oxygen.

Table III. Results of the Viability and ROS assays

Viability	ROS	# Strains ≥2 Fold	GO Process Categories
Increased (187)	High (50)	39	Mitochondrial organization, mitochondrial translation, mitochondrial resp. chain biogenesis, protein complex biogenesis
	Low (131)	39	Cellular protein complex assembly, mitochondrial protein transporting complex, proton transport complex assembly and biogenesis
Decreased (41)	High	2	No significant ontology categories
	Low	10	No significant ontology categories

Heterozygous diploid deletion and DAmP set results

The heterozygous and DAmP strain sets represent a subset of yeast genes, i.e., those that are essential for cell viability, so the categories of genes that show an increase or decrease in are likely to be different from the homozygous deletion set. The reproducibility of these data sets were comparable to those of the homozygous deletion set (>0.97), but with less variability (Figure 3). There are 34 of the essential strains that were shown to be above background levels and are required for Q cell formation by finding the overlap of the strains that were 2 fold or higher in DS/SP/NQ vs Q as we did for the HOM set. As expected, the most significant GO categories from these strains were not the same as those found in the homozygous set. Also, none of the categories were significant upon further analysis.

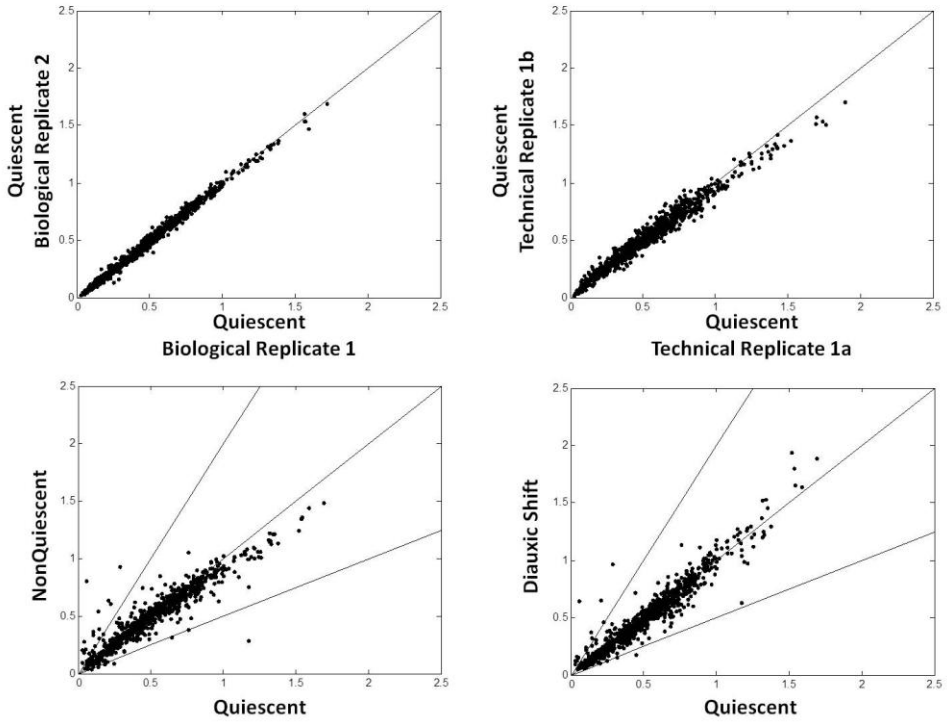


Figure 3: HET DAmP data A)Plots of two technical replicates of the Quiescent timepoint; B)Plots of two biological replicates of the Quiescent timepoint; C)Diauxic Shift v Quiescent with lines indicating (from left) 1x2y, 1x1y, 2x1y; D)NonQuiescent v Quiescent with lines indicating (from left) 1x2y, 1x1y, 2x1y .

Analysis of GFP&Deletion strains

I looked at strains in the homozygous diploid deletion set that were more than 2 fold higher in NQ than Q, in Q than NQ and also at strains that were not differentially present in either fraction. In each of these groups, I selected some mitochondrial strains. Due to potential effects of recombination during mitosis, I also chose genes that were not on the same chromosome as the GFP marker, citrate synthase, chromosome 14. Table IV lists the genes and the processes in which they are involved, as well as the individual fold changes between NQ and Q for each. Any fold change that is below 0.5 is more than 2 fold higher in Q than NQ. The highest fold change for Q/NQ is only about 7, while the highest fold change for NQ/Q is 23.45. I selected the strains with the highest fold change from each comparison for this experiment.

The mating of the citrate synthase (CIT1) GFP strain with the deletion strain resulted in a strain with the GFP and the deletion in a haploid cell. This is why we could only use strains that were in the homozygous diploid deletion set. The haploid deletion has no copies of the allele in the same way that the homozygous diploid deletions do not. If I were to look at an essential gene this way, the cell would not be viable.

The mating and subsequent sporulation of these strains was optimized over many repetitions. The haploid spores were grown on selective media (His DO and Kan+) in order to verify that the GFP and deletion were both present. These strains were then grown to SP. I collected a sample of SP culture and another sample that was separated into NQ and Q fractions. These samples were all analyzed by flow cytometry and compared to each other, as well as the data from the initial screen of the deletion set.

Table IV. Fold changes of strains selected for mating with GFP

ORF	Name	GO Process	NQ/Q Fold Change
YPR100W	MRPL51	mitochondrial translation(IC) | aerobic respiration(IMP)	23.45
YBR003W	COQ1	ubiquinone biosynthetic process(IMP)	14.92
YMR244C-A	YMR244C-A	biological_process(ND)	13.53
YDR518W	EUG1	protein folding(IGI)	13.52
YCR017C	CWH43	fungal-type cell wall organization(IGI) | GPI anchor biosynthetic process(IMP)	11.96
YCR003W	MRPL32	mitochondrial translation(IC)	9.69
YBR172C	SMY2	ER to Golgi vesicle-mediated transport(IPI)	1.03
YDL057W	YDL057W	biological_process(ND)	1.03
YDR063W	AIM7	actin filament debranching(IDA) | negative regulation of Arp2/3 complex-mediated actin nucleation(IDA)	1.03
YLR077W	FMP25	mitochondrial respiratory chain complex III assembly(IMP)	1.03
YFL014W	HSP12	cellular response to oxidative stress(IMP) | cell adhesion(IDA) | plasma membrane organization(IMP) | cellular response to osmotic stress(IMP) | cellular response to heat(IMP)	1.01
YDL011C	YDL011C	Unknown(Unknown)	1.01
YDR281C	PHM6	biological_process(ND)	0.43
YOR355W	GDS1	aerobic respiration(IMP)	0.41
YJR066W	TOR1	G1 phase of mitotic cell cycle(IMP) | response to DNA damage stimulus(IMP) | mitochondria-nucleus signaling pathway(IMP) | TOR signaling cascade(IMP)	0.41
YDR516C	EMI2	ascospore formation(IMP) | positive regulation of transcription from RNA polymerase II promoter(IMP)	0.34
YGL043W	DST1	transcription initiation from RNA polymerase II promoter(IMP) | transcription elongation from RNA polymerase II promoter(IDA)	0.34
YBL089W	AVT5	transport(ISS)	0.33
YMR238W	DFG5	fungal-type cell wall biogenesis(IDA) | pseudohyphal growth(IMP) | budding cell bud growth(IGI)	0.32
YGR163W	GTR2	microautophagy(IMP) | positive regulation of transcription from RNA polymerase II promoter(IDA)	0.28
YKR007W	MEH1	microautophagy(IMP) | vacuolar acidification(IMP)	0.26
YFL056C	AAD6	cellular aldehyde metabolic process(ISS)	0.14

My findings from this experiment are not consistent with the deletion screen in with the strains were grown together. The fold changes for NQ/Q are summarized in Table V and are in the same order as the strains in Table IV for quick comparison. Some of the strains exhibited the same patterns as in the screen of the homozygous deletion set in that there were more NQ cells than Q cells or vice versa. The ratios were not similar, at all, however. Also, there were a few strains that actually showed the opposite results.

This indicates that there may be a difference between growing deletion strains in pools and individually in culture.

Table IV. GFP & Deletion Strains Fold Changes

ORF	Name	NQ/Q Fold Change
YPR100W	MRPL51	2.23
YBR003W	COQ1	0.19
YMR244C-A	YMR244C-A	8.27
YDR518W	EUG1	1.01
YCR017C	CWH43	2.38
YCR003W	MRPL32	1.32
YBR172C	SMY2	3.01
YDL057W	YDL057W	2.01
YDR063W	AIM7	0.31
YLR077W	FMP25	7.20
YFL014W	HSP12	0.89
YDL011C	YDL011C	2.35
YDR281C	PHM6	0.92
YOR355W	GDS1	1.63
YJR066W	TOR1	7.06
YDR516C	EMI2	0.94
YGL043W	DST1	1.08
YBL089W	AVT5	4.26
YMR238W	DFG5	0.40
YGR163W	GTR2	0.98
YKR007W	MEH1	0.92
YFL056C	AAD6	2.72

The other part of this experiment was to compare the mixed SP samples to the separated samples to determine if adding the citrate synthase GFP fluorophore that is such a specific marker for Q cells would allow us to identify the relative proportion of Q cells in a given sample. I found that the Q cells were bright green, as expected, in the separated sample and the NQ cells contained a mixed population. Approximately 22% of the NQ cells were as bright as the Q cells in the parental strain. Therefore, upon examination of the mixed SP sample, the results were skewed to find more Q cells than were actually present in the sample.

Chapter 4

Discussion

Our whole genome study of yeast identified 445 essential and nonessential genes that are required for the formation of quiescent cells in yeast and thereby the differentiation of stationary phase cultures. This number represents approximately 7.5% of all of the yeast genome, suggesting that this process is required and programmed. The primary processes in which these genes were involved were mitochondrial.

Previous research on the different phenotypes of NQ and Q cells corroborate these findings. We found that there is limited mitochondrial function in the NQ cells due to the fact that the respiration levels were lower (Davidson, et.al., 2011). Also, approximately 40% of the cells form petite colonies when plated on glucose rich media. This slow growth is indicative of a mitochondrial mutation that does not allow the cells to produce enough energy for proliferation. Such mitochondrial defects are not found in the Q cells. Also, the 68 strains that were found more highly expressed in the Q cells than the NQ cells were not involved in mitochondrial processes at all, but instead were involved in processes such as transcription, translation and cell and organelle structure.

There were 63 strains found in the 411 that were involved in mitochondrial processes, which is over 15% of the genes required for Q cell formation. In the whole yeast genome, we find less than 5% are involved in mitochondrial processes. This is a significant finding, with a p-value on the order of 10^{-16}. There were 415 mitochondrial strains in these data sets, including the overlap of the DAmP and HET sets. Of the 415 mitochondrial strains, only 282 were above background levels in DS and were therefore further analyzed. 188 of these were found in the HOM set while the other 94 were

essential. This increases the significance of the number of mitochondrial strains that we discovered in the 411. Also, the finding that 62 of those 63 strains with mitochondrial deletions that were part of the 411 did not produce any Q cells detectable above background noise further corroborates the conclusion that mitochondrial function is required for formation of Q cells.

Comparison of transcriptomic, proteomic and genomic data

There is minimal overlap between the processes that were identified in the trancriptome, proteome and genome studies. We did find an abundance of mitochondrial proteins involved in the screening of the GFP library (59%) and in this genomic data, but only one of those proteins and genes were the same. We can conclude from this that just because a gene is required for the formation of Q cells does not necessarily mean that the protein will be abundantly expressed and vice versa. The same may be said for mRNA transcripts. The resources may be developed before they are needed as proteins in order for the cell to be poised to respond to varying environmental stimuli.

The GO categories, however, were found to be listed in a similar order to those that we found significant in the 411 strains from this genomic screen. A lot of the genes that were required for formation of Q cells are not highly expressed at the mRNA or protein levels. It is possible that these genes are sufficient when expressed at low levels. Also, the abundance of protein or mRNA transcripts does not allow us to conclude that these are required or even important for Q cell formation. A prevailing hypothesis has been that Q cells are poised to respond to a variety of potentially stressful environments and to exit from stationary phase once nutrients are again available (Narayanaswamy, et.al., 2009). The abundance of proteins and mRNA transcripts we have found in this

population of cells support that hypothesis. Therefore, the fact that we did not see significant overlap among the lists does not diminish the significance of the results that we have reported from the latest genomic study.

We also found that there were many more strains involved in the formation of Q cells than in NQ cells. The 2-fold comparisons revealed a significantly higher number of strains when DS/SP/NQ were compared against the Q sample. There are more genes that are involved in the regulation of Q cell formation than the others. The comparison of SP and DS samples exhibited very little variability, with a correlation coefficient similar to that of the replicates. This indicates that the variability we found among the strains is due to the Q population of cells. If we had not separated the SP culture into the two populations, we would have seen very little difference between the genes required for SP and cultures just prior to the DS. The formation of Q cells is clearly a highly regulated process.

Cells in Exp cultures are known to divide asymmetrically (Neumuller and Knoblich, 2009) in order to maintain nutrient levels in the culture. There is clear heterogeneity in these cultures, as well. As was introduced, the culture is heterogeneous based on the reproductive and chronological ages of the cell. There are probably other characteristics that contribute to the heterogeneity of this culture, as well, due to the inherent asymmetry of division. We do not see this asymmetric division in Q and NQ cells, at least with respect to protein expression (Davidson, et.al., 2011). Once the differentiation process has occurred, asymmetry of division does not seem to be as important. Even though Exp cultures are not homogenous, the defining characteristics

such as density, cell wall refractility, protein accumulation, etc., of Q and NQ cells do not appear until starvation.

The GO categories that were found to be most significant in the genes required for Q cell formation also included organization of the cell and its organelles at various levels. Not only is metabolism important for Q cells, but the organization of the cell itself is clearly vital. This may be associated with the increasing number of punctae that are found in cells in SP cultures (Narayanaswamy, 2009).

Stem cells and Cellular differentiation

There are three types of stem cells in the human body. These include pluripotent embryonic stem (ES) cells, multipotent cells and the precursor cells. ES cells have the potential to differentiate into any kind of body cell. Multipotent cells are found in adult tissues and can only differentiate into certain cell types. Precursor cells are those cells that are already committed to a certain pathway of differentiation. Stem cells can produce a daughter cell that is also a stem cell or, under some conditions, a daughter that differentiates.

Arguably, the most important question that is yet to be answered about stem cells is how differentiation is regulated. This process of cellular differentiation may clearly be modeled by the differentiation we see in SP cultures of yeast. The only way that it is possible for stem cells to maintain their lineage as well as produce differentiated cells is to asymmetrically divide (Thorpe, et. al., 2008). Once a stem cell region is formed in a specific tissue, the stem cells stay there (Fuch, et.al., 2004). Physical interactions between the stem cells are required to maintain the stem cells and keep them in these niches.

There are certain proteins expressed by these stem cells to anchor them to the niches. As they mature, they lose these adherent proteins and are able to migrate from the niche and differentiate.

Adult stem cells are kept in a quiescent state in order to maintain a stem cell lineage. They are able to exit quiescence and differentiate in response to environmental stressors. This quiescent state is important for long-term maintenance of the stem cell compartment as well as the long-term reproductive capacity. Stem cells are kept quiescent by both internal mechanisms and environmental signals.

Multiple genes are involved in this internal regulatory system. Like quiescent cells in yeast, stem cells are also particularly adept at maintaining low levels of ROS, indicating mitochondrial involvement. Negative regulators of the mTOR (mammalian target of rapamycin) pathway are required for preserving the quiescent state, as well. In yeast, TOR proteins are only activated when grown on nitrogen rich media to allow division. In the absence of nutrients, the TOR pathway is downregulated. This pathway seems to be evolutionarily conserved to ensure that cells only divide when nutrients are available.

Metabolism and energy levels of a cell are also important in maintaining quiescence. In stem cells, it has been shown that low levels of glucose or insulin limit the activity of mTOR, thereby preventing proliferation. Aside from glucose levels, low ATP levels activate the protein kinase AMPK. This kinase indirectly affects activity of mTOR, preventing cellular proliferation when energy levels are low (Hay and Sonenberg, 2004). Stem cells have been found to respire at lower rates than those of differentiated cells,

have lower levels of ATP and secrete more lactate suggesting that these cells produce energy by glycolysis. However, they also upregulate genes involved in mitochondrial processes, the reasons for which are not yet clearly understood (Lonergan, et.al., 2007).

Cancer and Quiescence

Quiescence is also particularly relevant in the study of cancer. Cancerous stem cells are capable of resisting stressors like chemotherapy and maintaining viability and reproductive capacity. There are many intrinsic and extrinsic mechanisms required to maintain quiescence, as discussed in the previous section. Drug resistance of cancerous stem cells may be potentially weakened by targeting these mechanisms.

The NQ cells in yeast give us another potential model for cancer research. There is a subpopulation of NQ cells that are still proliferating in SP. In the absence of nutrients, we understand that mechanisms for cellular proliferation like the TOR pathway are downregulated. The Warburg effect is a phenomenon that characterizes the metabolism of most cancer cells (and potentially stem cells). These cells do not rely on the mitochondria and oxidative phosphorylation for the production of energy. Instead, they use aerobic glycolysis, which is known to be less efficient in production of energy (Vander Heiden, et.al., 2009). In the environment of a NQ cell in a SP culture, there is no more glucose available. However, in our proteomic study using GFP, we found that NQ cells are not respiring, therefore they must be obtaining the energy required for proliferation through glycolysis instead. Once again, disruption of the metabolism of cancerous cells may allow more effective treatment.

Finally, whole genome sequencing of tumors versus normal cells has allowed identification of commonly mutated genes. The genes for isocitrate dehydrogenase isozenzymes 1 and 2 were frequently found to be mutated. These proteins were among those found in our proteomic study to be more highly expressed in the Q fraction. These particular enzymes are required for converting isocitrate to alpha-ketoglutarate in the TCA cycle. A number of various studies have focused on the role of IDH mutations in cancer (Mardis, 2010).

Role of Mitochondria

Clearly, mitochondrial function is integral in understanding cellular differentiation in stem cells and cancer cells. In fact, mitochondrial mutations are responsible for a number of human diseases (Detmer and Chan, 2007). Most mitochondrial research has been done outside of the cell. This has narrowed our understanding of the role that mitochondria play as part of a larger system, not simply as producers of energy. The genetic research in reference to mitochondria has also largely focused on the nuclear genome. Most of the the 1500 proteins involved in mitochondrial-related functions in human cells are encoded in the nuclear genome (Lonergan, et.al., 2007), suggesting a high level of regulation of this organelle based on cellular demand. Mitochondria are found to have a perinuclear arrangement in stem cells, possibly due to this interaction. Energy transfer may also be required for transport across the nuclear pores and this arrangement may make that more efficient. Also, it is suggested that mitochondria act as a buffer against an influx of small molecules to the nucleus (Lonergan, et.al., 2007).

Based on our understanding of the abundance of mitochondrial-related protein expression in quiescent cells as well as its related role in stem cells and cancer cells, we conducted a study of the whole yeast genome in order to determine what was required for the formation of Q cells. To further enhance our understanding of the role that the mitochondria play in cell differentiation, it would be of interest to study the mitochondrial genome on the same level that we did the nuclear genome in this study.

We know from this study and others that Q cells are poised to respond to environmental changes. They have an abundance of mRNAs and proteins and are highly regulated in the genome. The GFP library in particular could help us produce a sort of biosensor to determine the relatively immediate effects of different environmental stimuli. For example, the Cit1p:GFP that is highly expressed in Q cells may allow us to identify the signals that promote differentiation after glucose exhaustion. The results from this study were promising in this potential application. Although the results were skewed, there is a definite correlation between the level of fluorescence and the number of Q cells in the sample. As long as a baseline fluorescence measurement is established prior to exposing the sample to various treatments/signals, the data collected from this combination of samples could have a momentous impact on our understanding of cell differentiation.

Appendix

Core Set of Genes (411)

YMR072W	ABF2
YJR083C	ACF4
YDL203C	ACK1
YLR304C	ACO1
YMR064W	AEP1
YER017C	AFG3
YER093C-A	AIM11
YJL046W	AIM22
YMR157C	AIM36
YBR194W	AIM4
YPL227C	ALG5
YOR002W	ALG6
YOR067C	ALG8
YNL219C	ALG9
YNL094W	APP1
YLR370C	ARC18
YHR013C	ARD1
YDR127W	ARO1
YGL148W	ARO2
YFR021W	ATG18
YNL242W	ATG2
YLR431C	ATG23
YJL178C	ATG27
YNR007C	ATG3
YPL149W	ATG5
YHR171W	ATG7
YBL078C	ATG8
YLR393W	ATP10
YML081C-A	ATP18
YNR020C	ATP23
YBR039W	ATP3
YER167W	BCK2
YLR412W	BER1
YNL271C	BNI1
YNR051C	BRE5
YPL084W	BRO1
YDL037C	BSC1
YBR290W	BSD2
YOR276W	CAF20
YHL038C	CBP2
YBR120C	CBP6
YDL069C	CBS1
YDR197W	CBS2
YKL208W	CBT1
YGR150C	CCM1
YCR002C	CDC10
YCR094W	CDC50
YER061C	CEM1
YMR138W	CIN4
YPR119W	CLB2
YKL137W	CMC1
YLR218C	COA4
YNL041C	COG6
YBR003W	COQ1
YLR201C	COQ9
YPL132W	COX11
YJL003W	COX16
YLL009C	COX17
YNL052W	COX5A
YHR051W	COX6
YLR395C	COX8
YDL142C	CRD1
YPL181W	CTI6
YMR264W	CUE1
YCR017C	CWH43
YOR065W	CYT1
YKL087C	CYT2
YNL314W	DAL82
YML113W	DAT1
YKL149C	DBR1
YKR035W-A	DID2
YKL002W	DID4
YKL213C	DOA1
YDR069C	DOA4
YBR278W	DPB3
YMR287C	DSS1
YGR146C	ECL1
YHR132C	ECM14
YJL201W	ECM25
YBR078W	ECM33
YPL037C	EGD1

YDR512C	EMI1
YDR414C	ERD1
YGL054C	ERV14
YOR051C	ETT1
YDR518W	EUG1
YLR300W	EXG1
YJL157C	FAR1
YNL325C	FIG4
YBL013W	FMT1
YNL135C	FPR1
YLR047C	FRE8
YBR020W	GAL1
YEL042W	GDA1
YCL036W	GFD2
YCL039W	GID7
YMR135C	GID8
YEL003W	GIM4
YML094W	GIM5
YDR507C	GIN4
YLR094C	GIS3
YCR098C	GIT1
YMR311C	GLC8
YOR371C	GPB1
YDL021W	GPM2
YHR104W	GRE3
YKL109W	HAP4
YMR293C	HER2
YMR207C	HFA1
YBR009C	HHF1
YDR317W	HIM1
YOL095C	HMI1
YOR258W	HNT3
YJR075W	HOC1
YLR113W	HOG1
YHL002W	HSE1
YBR133C	HSL7
YDR533C	HSP31
YHR067W	HTD2
YJR122W	IBA57
YER078C	ICP55
YOL081W	IRA2
YMR073C	IRC21
YPR067W	ISA2
YJL073W	JEM1
YPR061C	JID1
YMR294W	JNM1
YER110C	KAP123
YCL055W	KAR4
YPL269W	KAR9
YJL094C	KHA1
YDR483W	KRE2
YHR082C	KSP1
YPL055C	LGE1
YJR070C	LIA1
YOR196C	LIP5
YFL018C	LPD1
YCL051W	LRE1
YJL124C	LSM1
YKL029C	MAE1
YBR298C	MAL31
YDL056W	MBP1
YOR221C	MCT1
YLR368W	MDM30
YPR083W	MDM36
YOL027C	MDM38
YOL111C	MDY2
YPR070W	MED1
YJL102W	MEF2
YPL060W	MFM1
YJR144W	MGM101
YOR330C	MIP1
YBR084W	MIS1
YOR350C	MNE1
YHR204W	MNL1
YER001W	MNN1
YJL183W	MNN11
YBR015C	MNN2
YKL064W	MNR2
YOR274W	MOD5
YJR074W	MOG1
YOL088C	MPD2
YGR084C	MRP13
YPR166C	MRP2
YKL167C	MRP49
YDR116C	MRPL1
YKR006C	MRPL13

YLR312W-A	MRPL15
YNL252C	MRPL17
YNL177C	MRPL22
YOR150W	MRPL23
YDR462W	MRPL28
YCR003W	MRPL32
YKL170W	MRPL38
YPR100W	MRPL51
YGR220C	MRPL9
YPL013C	MRPS16
YMR188C	MRPS17
YIR021W	MRS1
YOR334W	MRS2
YML128C	MSC1
YDR205W	MSC2
YOR354C	MSC6
YOL033W	MSE1
YDR335W	MSN5
YHR091C	MSR1
YLR203C	MSS51
YPL097W	MSY1
YJL123C	MTC1
YHR168W	MTG2
YGR023W	MTL1
YKL074C	MUD2
YBR057C	MUM2
YGR206W	MVB12
YMR004W	MVP1
YDR493W	MZM1
YDL040C	NAT1
YNL119W	NCS2
YPL226W	NEW1
YLR138W	NHA1
YDL213C	NOP6
YIL038C	NOT3
YEL062W	NPR2
YHL023C	NPR3
YOR209C	NPT1
YNL091W	NST1
YPR031W	NTO1
YKL055C	OAR1
YDR316W	OMS1
YJR073C	OPI3
YKR035C	OPI8
YER154W	OXA1
YDR071C	PAA1
YGR078C	PAC10
YJL128C	PBS2
YER178W	PDA1
YBR221C	PDB1
YOR360C	PDE2
YJL053W	PEP8
YMR257C	PET111
YER058W	PET117
YOR158W	PET123
YJL023C	PET130
YPL159C	PET20
YLR067C	PET309
YGR222W	PET54
YKL197C	PEX1
YGL153W	PEX14
YOL044W	PEX15
YDR329C	PEX3
YJL179W	PFD1
YKL127W	PGM1
YNL097C	PHO23
YBL022C	PIM1
YBL051C	PIN4
YDR276C	PMP3
YOR266W	PNT1
YPL144W	POC4
YJR043C	POL32
YNL055C	POR1
YDR435C	PPM1
YLR014C	PPR1
YPL148C	PPT2
YGR135W	PRE9
YBL046W	PSY4
YBL056W	PTC3
YCR079W	PTC6
YHR189W	PTH1
YPR191W	QCR2
YFR033C	QCR6
YLR204W	QRI5
YDL104C	QRI7
YMR274C	RCE1

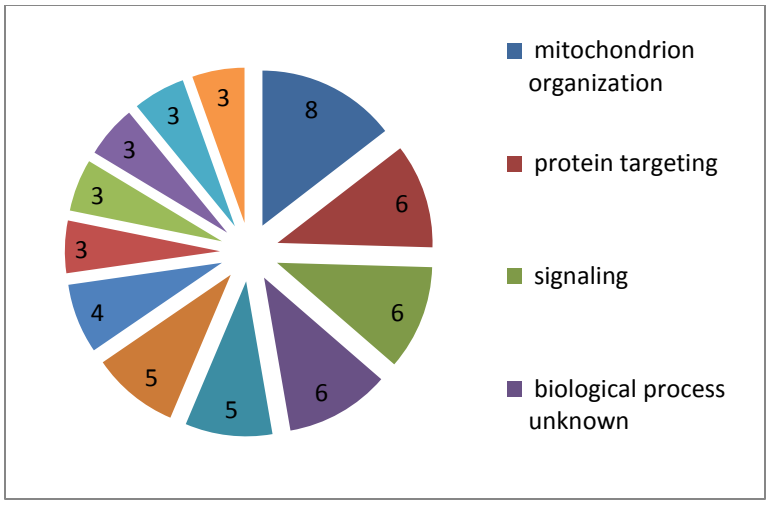
YNL090W	RHO2
YCR028C-A	RIM1
YMR063W	RIM9
YDR255C	RMD5
YPL123C	RNY1
YLR371W	ROM2
YIL119C	RPI1
YMR121C	RPL15B
YDR500C	RPL37B
YIL148W	RPL40A
YGL147C	RPL9A
YDL130W	RPP1B
YOL121C	RPS19A
YDL061C	RPS29B
YOR305W	RRG7
YNL213C	RRG9
YCR045C	RRT12
YER050C	RSM18
YNR037C	RSM19
YKL155C	RSM22
YIL093C	RSM25
YDR494W	RSM28
YOL138C	RTC1
YPL183W-A	RTC6
YOR216C	RUD3
YCR009C	RVS161
YDR388W	RVS167
YDL076C	RXT3
YMR263W	SAP30
YBL052C	SAS3
YMR214W	SCJ1
YLR292C	SEC72
YHR207C	SET5
YCL010C	SGF29
YGL066W	SGF73
YBL031W	SHE1
YOR035C	SHE4
YOL110W	SHR5
YGR112W	SHY1
YHR206W	SKN7
YDL033C	SLM3
YCR024C	SLM5
YHR030C	SLT2
YHR050W	SMF2
YGR229C	SMI1
YOR290C	SNF2
YDL194W	SNF3
YGL115W	SNF4
YDR006C	SOK1
YJL192C	SOP4
YDR392W	SPT3
YLR055C	SPT8
YLR119W	SRN2
YLR006C	SSK1
YNR031C	SSK2
YNL025C	SSN8
YLR369W	SSQ1
YDR169C	STB3
YCL032W	STE50
YDL048C	STP4
YPL032C	SVL3
YNL081C	SWS2
YDR395W	SXM1
YGL232W	TAN1
YJL052W	TDH1
YMR313C	TGL3
YOR297C	TIM18
YPR040W	TIP41
YOR091W	TMA46
YNL079C	TPM1
YPL176C	TRE1
YLR425W	TUS1
YHR111W	UBA4
YEL012W	UBC8
YLL039C	UBI4
YER151C	UBP3
YBR006W	UGA2
YPL139C	UME1
YJR049C	UTR1
YLR386W	VAC14
YOR068C	VAM10
YOR332W	VMA4
YDR049W	VMS1
YGR106C	VOA1
YLL040C	VPS13
YKL041W	VPS24

YNR006W	VPS27
YPL120W	VPS30
YJL154C	VPS35
YOR069W	VPS5
YDR484W	VPS52
YDR486C	VPS60
YOL129W	VPS68
YDR372C	VPS74
YNL197C	WHI3
YNL283C	WSC2
YER123W	YCK3
YCR059C	YIH1
YMR152W	YIM1
YER005W	YND1
YDR057W	YOS9
YGR270W	YTA7
YNR039C	ZRG17
YBL083C	YBL083C
YBR027C	YBR027C
YBR284W	YBR284W
YBR292C	YBR292C
YCR043C	YCR043C
YCR085W	YCR085W
YCR087W	YCR087W
YEL007W	YEL007W
YHL005C	YHL005C
YHR078W	YHR078W
YIL060W	YIL060W
YIR024C	YIR024C
YKL023W	YKL023W
YDL068W	YDL068W
YDL073W	YDL073W
YDR114C	YDR114C
YDR431W	YDR431W
YGL072C	YGL072C
YGL235W	YGL235W
YGR022C	YGR022C
YGR054W	YGR054W
YGR071C	YGR071C
YGR164W	YGR164W
YGR250C	YGR250C
YJL022W	YJL022W
YJR008W	YJR008W
YJR054W	YJR054W
YJR111C	YJR111C
YJR120W	YJR120W
YJR142W	YJR142W
YLR111W	YLR111W
YLR149C	YLR149C
YLR171W	YLR171W
YLR202C	YLR202C
YLR232W	YLR232W
YLR352W	YLR352W
YLR374C	YLR374C
YMR124W	YMR124W
YMR158W-B	YMR158W-B
YMR244C-A	YMR244C-A
YMR245W	YMR245W
YMR295C	YMR295C
YNL120C	YNL120C
YNL203C	YNL203C
YNL296W	YNL296W
YNR040W	YNR040W
YOL050C	YOL050C
YOR200W	YOR200W
YOR277C	YOR277C
YOR333C	YOR333C
YPL182C	YPL182C
YPR003C	YPR003C
YPR084W	YPR084W
YKL033W-A	YKL033W-A
YLR391W	YLR391W

Q vs. All	
YFL056C	AAD6
YBL089W	AVT5
YOR061W	CKA2
YLR381W	CTF3
YAL012W	CYS3
YMR238W	DFG5
YIL103W	DPH1
YGL043W	DST1
YPR023C	EAF3
YKL048C	ELM1
YDR516C	EMI2
YMR219W	ESC1
YPL262W	FUM1
YDR024W	FYV1
YOR355W	GDS1
YDL035C	GPR1
YML121W	GTR1
YGR163W	GTR2
YIL110W	HPM1
YGL203C	KEX1
YKL176C	LST4
YGR057C	LST7
YKR007W	MEH1
YDR144C	MKC7
YDR150W	NUM1
YNL099C	OCA1
YNL056W	OCA2
YCR095C	OCA4
YHL029C	OCA5
YHR034C	PIH1
YOL100W	PKH2
YLL013C	PUF3
YDR496C	PUF6
YBR114W	RAD16
YCR036W	RBK1
YMR075W	RCO1
YJL121C	RPE1
YDR418W	RPL12B
YIL133C	RPL16A
YBR084C-A	RPL19A
YBL027W	RPL19B
YPL079W	**RPL21B**
YBL087C	RPL23A
YGR034W	RPL26B
YMR194W	RPL36A
YLL045C	RPL8B
YNL067W	RPL9B
YIL153W	RRD1
YHL034C	SBP1
YNL032W	SIW14
YBR077C	SLM4
YER161C	SPT2
YKR092C	SRP40
YLR372W	SUR4
YDR457W	TOM1
YGR166W	TRS65
YML124C	TUB3
YBL104C	YBL104C
YBR226C	YBR226C
YHL044W	YHL044W
YHR033W	YHR033W
YLL007C	YLL007C
YLR252W	YLR252W
YLR426W	YLR426W
YMR193C-A	YMR193C-A
YOR309C	YOR309C
YPL080C	YPL080C
YDR349C	YPS7

Overlap mRNA & Q (50)

YCL032W
YCR079W
YCR094W
YDL203C
YDR197W
YDR205W
YDR372C
YDR435C
YDR483W
YGL054C
YGR071C
YGR106C
YGR146C
YHL038C
YHR091C
YHR132C
YHR171W
YIL148W
YJL003W
YJR008W
YJR122W
YKL002W
YKL137W
YKL149C
YKR035W-A
YLL040C
YLR006C
YLR119W
YLR149C
YLR304C
YLR352W
YLR425W
YML128C
YMR072W
YMR152W
YMR207C
YMR287C
YMR295C
YNL213C
YNL219C
YNL325C
YNR006W
YOL044W
YOL121C
YOR069W
YOR209C
YPL123C
YPL149W
YPL183W-A
YPR084W



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