A Whole Genome Screen for Minisatellite Stability
Genes in Stationary-Phase Yeast Cells

Bonnie Alver,*1 Peter A. Jauert,*1 Laura Brosnan,* Melissa O’Hehir,* Benjamin VanderSluis,† Chad L. Myers,‡ and David T. Kirkpatrick*1,‡
*Department of Genetics, Cell Biology and Development and †Department of Computer Science and Engineering, University of Minnesota, Minneapolis, Minnesota 55455

ABSTRACT Repetitive elements comprise a significant portion of most eukaryotic genomes. Minisatellites, a type of repetitive element composed of repeat units 15–100 bp in length, are stable in actively dividing cells but change in composition during meiosis and in stationary-phase cells. Alterations within minisatellite tracts have been correlated with the onset of a variety of diseases, including diabetes mellitus, myoclonus epilepsy, and several types of cancer. However, little is known about the factors preventing minisatellite alterations. Previously, our laboratory developed a color segregation assay in which a minisatellite was inserted into the ADE2 gene in the yeast Saccharomyces cerevisiae to monitor alteration events. We demonstrated that minisatellite alterations that occur in stationary-phase cells give rise to a specific colony morphology phenotype known as blebbing. Here, we performed a modified version of the synthetic genetic array analysis to screen for mutants that produce a blebbing phenotype. Screens were conducted using two distinctly different minisatellite tracts: the ade2-min3 construct consisting of three identical 20-bp repeats, and the ade2-h7.5 construct, consisting of seven-and-a-half 28-bp variable repeats. Mutations in 102 and 157 genes affect the stability of the ade2-min3 and ade2-h7.5 alleles, respectively. Only seven hits overlapped both screens, indicating that different factors regulate repeat stability depending upon minisatellite size and composition. Importantly, we demonstrate that mismatch repair influences the stability of the ade2-h7.5 allele, indicating that this type of DNA repair stabilizes complex minisatellites in stationary phase cells. Our work provides insight into the factors regulating minisatellite stability.

Various types of repetitive noncoding DNA exist in abundance within eukaryotic genomes. Categorized by repeat unit size, repetitive elements consist of units that can range from one nucleotide to hundreds of nucleotides in length (reviewed in Debrauwere et al. 1997). Minisatellites (also known as variable number of tandem repeats) are classically defined as repetitive tracts of DNA consisting of repeat units that are specifically 15–100 bp in size (Vergnaud and Denoeud 2000). These repetitive elements are predominantly stable in actively dividing cells yet change in repeat length as well as in composition during meiosis (Jauert et al. 2002; Richard et al. 2008).

Minisatellites have been shown to perform several important biological functions. These functions include regulating gene transcription (Cohen et al. 1987; Krontiris 1995; Spandidos and Holmes 1987) interfering with gene splicing (Turri et al. 1995), acting as chromosomal fragile sites (Lukusa and Fryns 2008; Yu et al. 1997), and influencing chromosomal pairing during meiosis (Ashley 1994; Chandley 1989). A well-characterized example of minisatellite genomic function is that associated with the human HRAS1 oncogene (Capon et al. 1983). This minisatellite is composed of nonidentical repeat units that are each 28 bp in length and have a high GC content (68%). Each repeat, varies with either a C or G at nucleotides +14 and +22 (numbered relative to the first nucleotide in the repeat). The HRAS1 minisatellite is located 3’ of the HRAS1 open reading frame (ORF) and acts as a binding site for the rel/nuclear factor-κB family of transcription factors (Trepicchio and Krontiris 1992). Altered minisatellites exhibit enhancement of HRAS1 transcription (Spandidos and Holmes 1987; Krontiris 1995), indicating minisatellites
can significantly influence the expression of nearby genes. Altered human minisatellites are associated with an increased risk of myoclonus epilepsy (Lafreniere et al. 1997; Virtaneva et al. 1997), diabetes mellitus (Kennedy et al. 1995), asthma (Kirkbride et al. 2001), attention deficit-hyperactivity disorder (Faraone et al. 2001; Yang et al. 2007), and several different types of cancer (Calvo et al. 1998; Krontriris 1995; Rosell et al. 1999; Vega et al. 2001; Weitzel et al. 2000).

The presence of rare altered alleles of the HRA1 minisatellite correlates with tumors of the lung, bladder, ovaries, and brain and have been isolated from the primary tumors of patients with breast cancer (Devlin et al. 1993; Ding et al. 1999; Rosell et al. 1999; Vega et al. 2001; Weitzel et al. 2000).

The majority of human cells exist as a population of nondividing, quiescent cells that are contact and growth inhibited. Cancer formation in eukaryotic organisms requires loss of these inhibitory mechanisms. An initial oncogenic event can result in genomic instability within a quiescent cell, promoting uncontrolled re-entry into the cell cycle, leading to tumorigenesis (Jin et al. 2009; Kim et al. 2005; Suda et al. 2005). At present, little is known about genomic instability events in nondividing cells. The yeast Saccharomyces cerevisiae can serve as a model organism for the study of genomic instability in the context of a quiescent cell population, as yeast can enter a nonmitotic state known as stationary phase (Gray et al. 2004; Werner-Washburne et al. 1993) that mimics several key characteristics associated with mammalian quiescent G0 cells, including a reduced level of gene expression and condensed unreplicated chromosomes.

We previously developed a colony color segregation assay to monitor minisatellite repeat alterations in yeast (Kelly et al. 2007, 2011, 2012). We inserted either a minisatellite consisting of three 20-bp repeat units and a 5-bp linker (the ade2-min3 allele) or a minisatellite consisting of seven-and-a-half variable repeats of the HRA1-associated minisatellite (the ade2-h7.5 allele) into the ORF of ADE2 (Figure 1, A and B). These insertions create frameshifts, resulting in Ade− red colonies that require supplemental adenine for growth. The red pigment is produced as a byproduct of the disruption of the adenine biosynthetic pathway (Smirnov et al. 1967). Loss of a repeat unit or gain of two repeat units within the minisatellite restores the ADE2 reading frame, rendering cells white and Ade+. Minisatellite alterations that occur in stationary phase cells after colony formation is complete lead to the formation of white microcolonies (“blebs”) that arise on the surface of the red colony. This phenotype allows us to easily detect minisatellite alterations that occur in cells within the postmitotic cellular population.

We previously used the ade2-min3 reporter construct to identify mutants that increase stationary-phase minisatellite instability. An ultraviolet (UV) mutagenesis screen identified several mutations in END3 (Kelly et al. 2012) and in the zinc homeostasis genes ZRT1 and ZAP1 (Kelly et al. 2007). Minisatellite alterations were specific to the quiescent population of stationary-phase cells and were independent of adenine auxotrophy or chromosomal context (Kelly et al. 2011). Thus, zinc homeostasis is essential for maintenance of minisatellite stability during the stationary phase possibly due to disruption of zinc-dependent DNA-binding proteins involved in DNA stability, specifically in stationary-phase cells. Minisatellite tract alterations in these mutants require homologous recombination, and multiple pathways act to maintain minisatellite stability in stationary phase cells (Kelly et al. 2011, 2012). Genes involved in these pathways include END3, RAD27, PKC1, ZRT1, and ZAP1. More recently, we used a modified version of the synthetic genetic array (SGA) protocol (Li et al. 2011; Tong and Boone 2006; Tong et al. 2001) to screen all of the genes annotated for checkpoint function and found that a subset of those genes also influence stationary phase minisatellite stability (Alver et al. 2013). Here, we used our modified SGA protocol to screen the entire yeast genome in an effort to (1) determine what factors are involved in regulating minisatellite stability in stationary phase cells and (2) determine whether minisatellites varying in repeat composition and size are regulated by different factors in stationary phase cells.

MATERIALS AND METHODS

Media and yeast strains

The solid and liquid media used in this study were prepared as stated in (Guthrie and Fink 1991). All media that was used in the SGA analysis was prepared as described in (Tong and Boone 2006; Tong et al. 2001) with the exception of prepsorption media that contains 2% agar, 5% dextrose, 1% Difco yeast extract, and 3% Difco nutrient broth. Media consisting of Geneticin (G418) was prepared by adding 200 mg/L G418 (Cellegro).

The plasmids and S. cerevisiae strains used for this study are listed in Table 1. All strains, except those used to perform the SGA analyses, were derived from DTK271 (MATa his3-1 ura3-0 can1::MFA1pr·spHIS5 ade2-min3 - URA3MX) using a two-step PCR process as previously described (Alver et al. 2013). To summarize, the plasmid pDC369 was used to generate a URA3MX PCR product with flanking sequence using primers 14193004 and 14193005. DTK271 genomic DNA was used as a PCR template to isolate the ade2-min3 allele using primers 14193006 and 14193007. To combine the URA3MX and ade2-min3 PCR products, we performed PCR by using primers 14193007 and 14193008 resulting in the ade2-min3 URA3MX-linked cassette that was transformed into DCY2556. Transformants were selected on solid synthetic media lacking uracil (SD-Ura), resulting in DTK893. DTK893 and DCY2557 were mated, resulting in DTK1175, which was sporulated and dissected. Mating, sporulation, and tetrad dissection were performed as described previously (Jauer et al. 2002). Red spores were selected on solid SD-Ura media. Query strains for the SGA analyses were isolates of DTK1175: DTK1189 5a = MATa derivative for the nonessential SGA and DTK1189 2b = MATa derivative for the essential SGA. pDC369, DCY2556, and DCY2557 are from Dr. Duncan Clarke, University of Minnesota.

To construct the ade2-h7.5 query strain, DTK1624 (MATa his3-1 ura3-0 can1::MFA1pr·spHIS5 ade2-h7.5 - URA3MX), we generated an ade2-h7.5 URA3MX-linked cassette by using a two-step PCR process. In summary, we obtained a URA3MX PCR product bearing a 5′ TEF promoter site and a 3′ TEF terminator site from pDC369 by using primers 14193004 and 14193005. We isolated an ade2-h7.5 PCR product from DTK1188 genomic DNA by using primers 14193006 and 14193007. The two PCR products (URA3MX and ade2-h7.5) were combined by using primers 14193007 and 14193008. The complete ade2-h7.5 URA3MX-linked cassette was transformed into DCY2556. Red Ura+ cells were selected on SD-Ura solid synthetic media, yielding DTK1624.
SGA analysis

Nonessential SGA: For our study, we performed a modified SGA analysis as described in our previous work (Alver et al. 2013; Tong and Boone 2006; Tong et al. 2001) (Figure 1C). To summarize, we inoculated YPD liquid media with a single red colony of query strain DTK1189 5a or DTK1624. Cultures were plated onto YPD solid media after overnight incubation at 30°C. The MATα nonessential Yeast Deletion Strain Haploid Set (Invitrogen; from Dr. Robin Wright, University of Minnesota) was pinned onto the query strain in a 96-well format (Figure 1D). Mated strains were incubated at 30°C overnight. The resulting zygotes were pinned to SD-Ura + G418 solid media and incubated at 30°C overnight. Diploids were then pinned to presporulation solid media, incubated at 30°C overnight, and then pinned to sporulation media and incubated at room temperature (RT) for 6 d. Haploid MATα progeny were selected on synthetic media lacking histidine, arginine, and uracil (SD-His/Arg/Ura) and containing canavanine (US Biological). Strains were incubated at 30°C overnight. This step was repeated. Strains were then pinned to SD-His/Arg/Ura + canavanine + G418 media, and this step was repeated. The haploids were then pinned to YPD and left at RT for 5 d. Each plate was pinned in duplicate, and the screen was repeated three independent times. A positive control consisting of a strong blebbing strain (a MATα zrt1Δ mutant; SCD153) was included on each plate (Kelly et al. 2007). The blebbing produced by the resulting double mutants was rated on a scale of + to ++++ (Figure 1D). Candidate hits were characterized as strains producing a strong degree of blebbing (+++ or ++++). The blebbing phenotype for each hit was verified by restreaking the strain onto YPD and assessing the individual yeast colonies for a blebbing phenotype on a qualitative scale of + to ++++.

Scores for the blebbing phenotypic analysis of the ade2-min3 and ade2-h7.5 nonessential SGA independent screens are listed in Supporting Information, File S1 and File S2, respectively. Duplicate plates from each screen are indicated by an “a” or “b” for each individual screen. The scores are represented numerically instead of by “+”’s.” For example, a “1” indicates that a specific ORF was assigned of score of “+” for a specific screen. A score of “0” indicates that a specific strain did not grow, and a score of “5” indicates that a strain was composed of white cells and therefore could not be assigned a rating for a blebbing phenotype. A hit was defined as a strain (corresponding to a specific ORF) that produced a level of blebbing of “3–4” (+++ to ++++) in both replicates for at least two of the three independent screens performed. A second criteria used to define a hit was a strain that was scored as a “3” or “4” (+++ or ++++) in both replicates for blebbing in combination with a score of “0” (no growth) or a “5” (white patch) for a least two of the three independent screens.

Essential SGA: We followed a similar screening protocol as described previously and in our previous work (Alver et al. 2013). In summary,
the query strain DTK1189 2b was mated to a MATa essential temperature-sensitive (ts) strain set containing 455 ts genes (from Dr. Charles Boone, University of Toronto) (Li et al. 2011) and incubated at RT for 2 d. Zygote selection, presporulation, and sporulation were performed as described previously with the exception that strains were incubated at RT. Haploid and double mutant selection were as described previously with strain incubation being carried out at RT for 2 d. After selection, the double mutant haploids bearing the ade2-min3 allele were pinned to five separate YPD solid media plates, and the plates were incubated for 5 d at a range of temperatures: 26\°, 30\°, 32\°, 34\°, or 37\°. Blebbing was scored as described previously. Strains producing a +++ to ++++ level of blebbing were restruck onto YPD and incubated at the corresponding temperature to verify the blebbing phenotype. Restruck strains were again rated on a scale of + to ++++. As noted previously, a MATa zrt1Δ haploid mutant (SCD202) was used as a positive control (Kelly et al. 2007).

Phenotypic scoring for each independent screen is shown in File S3. As described previously, the scores are represented numerically rather than by “+.” Each score represents the maximum level of blebbing assigned to a specific ORF. Specifically, all of the scores assigned to each individual ts allele of a particular ORF were compared at each individual temperature. The highest blebbing score of all of the alleles and all of the incubation temperatures was assigned to the corresponding ORF for each independent screen. As described previously, a hit was defined as an ORF that produced a level of blebbing of “3-4” (+++ to ++++) for at least two of the three independent screens performed or as an ORF that produced a level of blebbing of “3” or “4” (+++ or ++++) in combination with a score of “0” (no growth) or “5” (solid white patch) for at least two out of the three independent screens.

**Gene Ontology (GO) term analysis**

GO data used ontology version 1.2 with annotations downloaded on January 22, 2012. Enrichment calculations are based on a hyper-geometric overlap test and reported P-values have been Bonferroni-corrected for multiple hypotheses (Boyle et al. 2004).

**Blebbing quantification assay**

Yeast strains used for the quantification assay were streaked onto solid YPD media and incubated at 30° for 2 d. A single red colony was used to inoculate 5 ml of liquid YPD media, and the cultures were incubated at 30° for 4 h. Cultures were diluted and plated onto solid YPD media. Strains were incubated at 30° for 2 d and then left at RT for 6 d to allow for bleb formation. For each strain, blebs were counted on at least 100 colonies ranging in size from 1.26 to 1.32 mm in diameter. This assay was repeated three independent times whereupon the average number of blebs per colony as well as the 95% confidence interval of the mean was calculated.

**RESULTS**

**Genome-wide identification of factors required for maintaining ade2-min3 minisatellite stability in stationary-phase cells**

We previously reported a unique color segregation assay that allows us to monitor minisatellite instability occurring in *S. cerevisiae* cells...
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Table 2 Primers

| Primer | Reference number | Sequence |
|--------|-----------------|----------|
| ADE2 F | 14193008        | GGGTTAGCATTATTCGCCCAATGT |
| ADE2 F | 14193006        | TCCAGTTTTAAGCGCTTCGAAAT |
| RPL22A R | 88529803 | GGCAAAGCGTCTCATAAGCAAC |
| PMS1 F | 2694460 | TAGAAAGCACAGATTAATAC |
| RPL22A R | 88529806 | GCGAAATGCAACTCCATTCC |
| ZAP1 F | 14767981 | ACTGTCGCGGCTACTGGG |
| ZAP1 R | 14193007        | TCGCCTTAAGTTGAAACGGAGTC |
| MLS1 F | 36803545        | CGGTTTGTAGAATCGCGCTAGCA |
| MLH2 F | 4951839         | GCATTATGGTCCCTGCGCAAC |
| MLS3 F | 4951841         | CGGCAATTCACACCCTTGGAT |
| MON1 F | 56866461        | GGTCTAGTATCGCTACCTTTATCC |
| MLS3 R | 4951842         | CGTGAATCGCACAAGCGCAGT |
| MLS2 R | 48201625        | GGGAGATACGTCTTCACGTTCC |
| MLS2 R | 48201625        | CGGAGATACGTCTTCACGTTCC |
| MLS2 R | 48201625        | CGGAGATACGTCTTCACGTTCC |
| MLS2 R | 48201625        | CGGAGATACGTCTTCACGTTCC |
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| MLS2 R | 48201625        | CGGAGATACGTCTTCACGTTCC |
| MLS2 R | 48201625        | CGGAGATACGTCTTCACGTTCC |
| MLS2 R | 48201625        | CGGAGATACGTCTTCACGTTCC |

(Kelly et al. 2007, 2011, 2012). This assay employs the ade2-min3 allele, which consists of a short minisatellite featuring three identical 20-bp repeat units inserted into the gene ADE2 (Figure 1A). Insertion of the minisatellite plus a 5-bp overhang disrupts the ADE2 ORF and results in red colony color. The gain of either two repeat units or the loss of one repeat unit restores the correct reading frame and results in the formation of white cells.

Our assay allows us to distinguish between minisatellite alterations that take place during mitotic growth and those that occur during the stationary phase. Alterations occurring in actively dividing cells lead to a red/white sectoring color segregation phenotype, whereas those occurring in stationary-phase cells result in a novel phenotype known as blebbing (Kelly et al. 2007) in which white microcolonies form on the surface of a red colony. This phenotype allows us to directly identify factors that regulate the stability of a minisatellite allele in stationary phase yeast cells. We recently used a modification of the SGA protocol to examine genes annotated as having a checkpoint function in yeast and determined that a subset of these genes affects the stability of the ade2-min3 minisatellite tract in stationary-phase cells (Alver et al. 2013). This pilot study showed that an automated approach was not possible, as any white Ade+ cells that arose during the procedure rapidly overwhelmed the red Ade− cells. To compensate, we modified the SGA protocols to manually screen for mutants that produced a blebbing phenotype (Figure 1C) (Alver et al. 2013; Li et al. 2011; Tong and Boone 2006; Tong et al. 2001).

Using the ade2-min3 reporter, we identified 102 candidate genes that, when mutated, resulted in a strong blebbing phenotype (Table 3). These included the zinc homeostasis genes ZRT1 and ZAP1 identified in our original UV mutagenesis screen (Kelly et al. 2007), demonstrating that our SGA analysis was working correctly. Also included were the genes PKC1, an essential gene that encodes for a serine/threonine kinase that regulates cell wall modifications (Levin et al. 1990), and RAD27, which encodes for a nuclelease that processes Okazaki fragments (reviewed in Liu et al. 2004a,b). Both genes were shown by our laboratory to be important for maintaining minisatellite stability in stationary phase cells (Kelly et al. 2012). Finally, we previously identified checkpoint-related genes including MRC1, GSM3, and TOFI by SGA analysis (Alver et al. 2013); those genes are marked in Table 3.

To determine whether any enriched GO terms were represented within the 102 candidate hits, we performed GO term analysis using Ontology version 1.2. We find that genes associated with GO terms representing chromosomal maintenance and DNA replication are overrepresented within our list of 102 hits (Table 4), indicating these cellular processes are likely to be important for regulating the stationary phase stability of the ade2-min3 minisatellite.

Of note were three hits from the ade2-min3 SGA analyses that were represented in both the nonessential and the essential mutant strain sets. These hits included the stress response kinase gene DBF2 (Johnston and Thomas 1982), the checkpoint gene SLI15 (Kim et al. 1999; Ruchaud et al. 2007; Sandall et al. 2006), and the SNARE complex gene SEC22 (Liu and Barlowe 2002). Deletion of DBF2 and SLI15 in the nonessential strain set resulted in strains that produced a low level of blebbing. However, ts alleles of both strains were identified as hits in our screen. Our results suggest that aberrant alleles of either gene are more detrimental to minisatellite stability than complete loss of the gene itself. We found the opposite effect for the gene SEC22, which was identified as a hit when deleted but not as a ts allele. Therefore, variations in gene product expression or function appear to differentially affect ade2-min3 minisatellite stability.

Of the 102 genes identified in our screen, only three produced a high level of blebbing (++++) for at least two of the three independent analyses that were performed (Table 3). These included the zinc transporter gene ZRT1 (Zhao and Eide 1996), the mitochondrial transport gene ODC1 (Palmieri et al. 2001), and SAC1, a gene encoding for a lipid phosphatase involved in protein trafficking and cell wall maintenance (reviewed in Strahl and Thorner 2007). These results indicate that aberrant cellular trafficking, such as that involved in regulating intracellular protein and zinc levels, can drastically affect the stability of a minisatellite allele in stationary phase yeast cells.

Identification of factors that maintain the stability of the ade2-h7.5 HRAS1-associated minisatellite allele in stationary-phase yeast cells

Although the ade2-min3 allele allows us to readily assess mutant strains for minisatellite instability, its composition does not mimic most minisatellites found within human cells. The majority of human minisatellites are composed of long repeat units that are variable in sequence composition and are significantly enriched for GC content (Vergnaud and Denoeud 2000). The ade2-min3 minisatellite is comprised of identical repeats with only 50% GC content. Because of these differences, human minisatellites may be regulated by different factors than those associated with the ade2-min3 allele, a short minisatellite with identical repeat units.

To examine these potential issues, we used a second minisatellite reporter (the ade2-h7.5 allele; Figure 1B), which incorporates a portion of the complex human HRAS1 minisatellite into the ADE2 gene (Kelly et al. 2007, 2011). This tract is composed of seven-and-a-half repeat units that are 28 bp in size, is variable in base content at positions +14nt and +22nt, and has a GC content of 68% (Kelly et al. 2011). Similar to the ade2-min3 assay system, insertion of the tract disrupts the ADE2 ORF, and loss of a 28-bp repeat (or gain of two repeats) will restore the proper reading frame.
To identify the factors involved in regulating the stability of a complex human-associated minisatellite during stationary phase, we used the same SGA methodology described previously, screening the strain set three independent times. A caveat to using the ade2-h7.5 allele as a query strain for this screen is that the overall level of blebbing produced by the deletion allele as a query strain is that the overall level of blebbing observed in this strain. Thus, it is possible that this gene was not identified due to the difficulty in scoring each strain. Deletion of the ORF YGL217C is represented twice in the nonessential deletion haploid set; one deletion strain was identified as a hit in both screens, and the second was not. No other hits were duplicated within our strain sets. PCR analysis revealed that both of the YGL217C isolates from the strain collection and our SGA analyses are correct deletion mutants. Therefore, one or both isolates could contain a secondary mutation that enhances or suppresses the blebbing phenotype.

PCR analysis of the YLR125W deletion mutant from the nonessential haploid strain set as well as of the ade2-min3 YLR125W mutant from the final step of the SGA analysis revealed that each strain was wild-type (WT) at the YLR125W locus. Because both strains are G418R, the KANMX PCR product used to construct the deletion collection parental strain must be located elsewhere in the genome, likely at an area of homology to the YLR125W locus. Future determination of the deletion’s genomic location may give insight into the source of blebbing observed in this strain.

The remaining genes present in each data set are associated with intracellular transport. Included are the vacuolar transport gene MON1 and the zinc-mediating vacuolar transport gene COT1 (Conklin et al. 1992; MacDaid et al. 2000; Meling-Wesse et al. 2002; Wang et al. 2002). The final two overlapping hits are the zinc transport gene ZRT1 and the transcriptional regulator of ZRT1, ZAP1 (Zhao and Eide 1996, 1997) that were previously shown by our laboratory to be important in minisatellite stability (Kelly et al. 2007, 2011). These four may influence minisatellite stability in the same manner, as intracellular zinc is sequestered in vacuoles until needed. Our data suggest that the disruption of cellular transport within stationary phase cells, particularly that associated with zinc transport, results

### Analysis of factors involved in maintaining the stability of both the ade2-min3 and ade2-h7.5 minisatellite alleles in stationary-phase cells

Although the overlap of candidate hits between the ade2-min3 and the ade2-h7.5 screens was statistically significant ($P = 0.047$), only seven genes were identified as hits in both SGA screens (Figure 2A): the genes BUD28, COT1, MON1, YGL217C, YLR125W, ZAP1, and ZRT1. BUD28 and YGL217C are both dubious ORFs that are unlikely to code for a protein. Each ORF, however, overlaps the coding sequence of an adjacent characterized gene. Approximately 98% of the BUD28 ORF overlaps the ribosomal subunit gene RPL22A (Planta and Mager 1998) whereas YGL217C overlaps roughly 10% of KIP3, a gene that encodes for a kinesin-like protein involved in mitotic spindle positioning (Conkling and Hoyt 1997). RPL22A was identified as a hit in our ade2-min3 SGA screen but not in the ade2-h7.5 screen. Nevertheless, this increase in minisatellite stability makes phenotypic scoring more difficult.
Table 4 Enriched GO terms of hits from the ade2-min3 SGA analysis of the yeast nonessential and essential strain sets

| GO ID   | GO term                               | P value     | Genes                                                                 |
|---------|----------------------------------------|-------------|-----------------------------------------------------------------------|
| GO:0006261 | DNA-dependent DNA replication           | 2.25E-08    | ORC2, DPB3, MRC1, DPB4, POL31, POL32, RFC2, RAD27, ORC3, MCM5, POB3, CSM3, TOF1, RFC4 |
| GO:0005657 | Replication fork                        | 6.86E-08    | DPB3, MRC1, DPB4, POL31, POL32, RFC2, MCM5, POB3, CSM3, TOF1, RFC4 |
| GO:0044427 | Chromosomal part                        | 1.26E-07    | STU1, ORC2, SLI15, DPB3, MRC1, MCD1, DPB4, MMS21, CBF2, POL31, POL32, RFC2, NNF1, ASK1, ORC3, MCM5, POB3, CSM3, CEPS, TOF1, SMCS, RFC4, BUB3, IPL1 |
| GO:0043234 | Protein complex                         | 2.15E-07    | SSA1, STU1, SEC17, ORC2, SLI15, ABD1, DPB3, MRC1, MCD1, APC11, COP1, RRI1, TIM22, VPS41, DBF4, TAF12, MMS21, MAK10, TSC11, RSP5, KAP122, RPT6, PAC10, CBF2, RBP3, MET30, POL31, RAV1, POL32, RFC2, NNF1, ASK1, SAC1, ORC3, RIC1, SECC2, MCM5, POB3, CSM3, CEPS, TOF1, SMCS, RFC4, BUB3, RPT4, IPL1 |
| GO:0005694 | Chromosome                              | 1.20E-06    | STU1, ORC2, SLI15, DPB3, MRC1, MCD1, DPB4, MMS21, CBF2, POL31, POL32, RFC2, NNF1, ASK1, ORC3, MCM5, POB3, CSM3, CEPS, TOF1, SMCS, RFC4, BUB3, IPL1 |
| GO:0006260 | DNA replication                         | 2.60E-06    | ORC2, DPB3, MRC1, DPB4, POL31, POL32, RFC2, RAD27, ORC3, MCM5, POB3, CSM3, CEPS, TOF1, RFC4 |
| GO:0043596 | Nuclear replication fork                | 3.85E-06    | DPB3, MRC1, DPB4, POL31, POL32, MCM5, POB3, CSM3, TOF1 |
| GO:0006272 | Leading strand elongation               | 1.03E-05    | DPB3, DPB4, POL31, POL32, RFC2, RFC4 |
| GO:0044422 | Organelle part                          | 3.10E-05    | SSA1, SWH1, SLA1, STU1, ORC2, CCZ1, SLI15, ARL1, AB1, DPB3, MRC1, YCL075W, MCD1, APC11, USO1, COP1, RRI1, TIM22, FA11, VPS41, DPS1, DBF4, YCF1, TAF12, MMS21, YPT31, TSC11, RSP5, KAP122, MON1, DBF2, CBF2, RBP3, MET30, MGA2, POL31, POL32, RFC2, NNF1, ASK1, RAD27, SNU114, SAC1, ORC3, RIC1, RIC2, RPL22A, UPS2, MMRI1, SEC22, MCM5, TEM1, POB3, SEC59, CSM3, CEPS, PEPS, LCBI, TOF1, TGL2, SMCS, RFC4, BUB3, RRS1, COT1, SPC29, ODC1, IPL1 |
| GO:0044446 | Intracellular organelle part            | 3.10E-05    | SSA1, SWH1, SLA1, STU1, ORC2, CCZ1, SLI15, ARL1, AB1, DPB3, MRC1, YCL075W, MCD1, APC11, USO1, COP1, RRI1, TIM22, FA11, VPS41, DPS1, DBF4, YCF1, TAF12, MMS21, YPT31, TSC11, RSP5, KAP122, MON1, DBF2, CBF2, RBP3, MET30, MGA2, POL31, POL32, RFC2, NNF1, ASK1, RAD27, SNU114, SAC1, ORC3, RIC1, RIC2, RPL22A, UPS2, MMRI1, SEC22, MCM5, TEM1, POB3, SEC59, CSM3, CEPS, PEPS, LCBI, TOF1, TGL2, SMCS, RFC4, BUB3, RRS1, COT1, SPC29, ODC1, IPL1 |
| GO:0044454 | Nuclear chromosome part                 | 3.45E-05    | ORC2, DPB3, MRC1, MCD1, DPB4, CBF2, POL31, POL32, NNF1, ASK1, ORC3, MCM5, POB3, CSM3, CEPS, TOF1, BUB3, IPL1 |
| GO:0032991 | Macromolecular complex                  | 1.28E-04    | SSA1, STU1, SEC17, ORC2, SLI15, ABD1, DPB3, MRC1, MCD1, APC11, COP1, RRI1, TIM22, VPS41, DBF4, TAF12, SSD1, MMS21, MAK10, TSC11, RSP5, KAP122, RPT6, PAC10, CBF2, RBP3, MET30, POL31, RAV1, POL32, RFC2, NNF1, ASK1, SNU114, SAC1, ORC3, RIC1, RPL22A, SEC22, MCM5, POB3, CSM3, CEPS, PEPS, LCBI, TOF1, TGL2, SMCS, RFC4, BUB3, RPT4, RRS1, IPL1 |
| GO:0000228 | Nuclear chromosome                      | 1.90E-04    | ORC2, DPB3, MRC1, MCD1, DPB4, CBF2, POL31, POL32, NNF1, ASK1, ORC3, MCM5, POB3, CSM3, CEPS, TOF1, BUB3, IPL1 |

(continued)
| GO ID      | GO term                                           | P value    | Genes                                                                 |
|------------|--------------------------------------------------|------------|-----------------------------------------------------------------------|
| GO:0005634 | Nucleus                                          | 4.64E-04   | SSA1, SWH1, SLA1, STU1, PKC1, ORC2, TEC1, SLJ15, ABD1, DPB3, MRC1, YCL075W, MCD1, APC11, RR11, FAL1, PDS1, DPB4, TAF12, SSD1, MMS21, RSP5, MOB2, KAP122, RPT6, CBF2, RBP3, MET30, MGA2, ZAP1, POL31, POL32, RFC2, NNF1, ASK1, RAD27, SNU114, ORC3, COF1, RCI1, MCM5, POB3, CSM3, CEP3, ERG8, PSE1, TOF1, SMCM5, RFC4, BUB3, RPT4, RRS1, SPC29, IPI1 |
| GO:0005634 | Cellular component organization at cellular level | 4.70E-04   | SSA1, SLA1, STU1, SEC17, PKC1, ORC2, CC21, MRC1, MCD1, APC11, USO1, TIM22, VPS41, PDS1, TAF12, SSD1, TSC11, RSP5, MOB2, RPT6, PAC10, DBF2, CBF2, RFC2, NNF1, ASK1, SNU114, ORC3, COF1, UP52, MMR1, SEC22, MCM5, GAS2, TEM1, POB3, CSM3, CEP3, ERG8, PeP5, LC81, PSE1, ADE12, TOF1, ATG3, TGL2, SMCM5, RFC4, BUB3, RPT4, RRS1, COT1, ERG10, SPC29, ODC1, IPI1 |
| GO:0006271 | DNA strand elongation involved in DNA replication | 4.86E-04   | DPB3, DPB4, POL31, POL32, RFC2, RAD27, RFC4                           |
| GO:0007059 | Chromosome segregation                          | 4.98E-04   | SLJ15, MRC1, MCD1, PDS1, CBF2, RFC2, NNF1, ASK1, CSM3, TOF1, SMCM5, RFC4, IPI1 |
| GO:000793  | Condensed chromosome                            | 5.66E-04   | STU1, SLJ15, MCD1, MMS21, CBF2, NNF1, ASK1, CEP3, SMCM5, BUB3, IPI1   |
| GO:0032993 | Protein–DNA complex                             | 6.30E-04   | ORC2, DPB3, DPB4, POL31, POL32, ORC3, MCM5, POB3                     |
| GO:0039987 | Cellular process                                 | 6.65E-04   | SSA1, SWH1, SLA1, STU1, URA7, SEC17, PKC1, ORC2, TEC1, CC21, SLJ15, ARL1, AB1, DPB3, MRC1, YCL075W, RMD1, MCD1, APC11, USO1, COPI, RR11, TIM22, VPS41, PDS1, DPB4, RFC4, TAF12, SSD1, MFA1, MMS21, MAK10, YPT31, TSC11, RSP5, MOB2, KAP122, RPT6, VGL114W, MON1, ZRT1, NUP1, PAC10, DBF2, CBF2, RBP3, MET30, MGA2, ZAP1, POL31, RAV1, POL32, RFC2, NNF1, ASK1, RAD27, SNU114, SAC1, ORC3, COF1, RCI1, RPL22A, UPS2, MMR1, SEC22, MCM5, STT4, GAS2, TEM1, POB3, CSM3, CEP3, ERG8, PeP5, LC81, PSE1, ADE12, TOF1, ATG3, TGL2, SMCM5, RFC4, BUB3, RPT4, RRS1, COT1, ERG10, SPC29, ODC1, IPI1 |
| GO:0006281 | DNA repair                                       | 7.21E-04   | DPB3, MRC1, MCD1, PDS1, MMS21, RPT6, POL31, POL32, RFC2, RAD27, MCM5, POB3, CSM3, TOF1, SMCM5, RFC4, RPT4 |
| GO:0022616 | DNA strand elongation                            | 7.93E-04   | DPB3, DPB4, POL31, POL32, RFC2, RAD27, RFC4                         |
| GO:0044428 | Nuclear part                                     | 8.03E-04   | SWH1, ORC2, AB1, DPB3, MRC1, YCL075W, MCD1, APC11, RR11, FAL1, DPB4, TAF12, MMS21, KAP122, CBF2, RBP3, MET30, POL31, POL32, NNF1, ASK1, RAD27, SNU114, ORC3, COF1, MCM5, POB3, CSM3, CEP3, TOF1, BUB3, RRS1, IPI1 |
| GO:0006996 | Organelle organization                          | 8.60E-04   | SSA1, SLA1, STU1, SEC17, PKC1, ORC2, CC21, MRC1, MCD1, APC11, USO1, TIM22, VPS41, PDS1, TAF12, SSD1, TSC11, RSP5, MOB2, RPT6, DBF2, CBF2, RFC2, NNF1, ASK1, COF1, UP52, MMR1, SEC22, MCM5, TEM1, POB3, CSM3, CEP3, PeP5, TOF1, ATG3, TGL2, RFC4, IPI1 |
| GO:0000777 | Condensed chromosome kinetochore                 | 1.16E-03   | STU1, SLJ15, CBF2, NNF1, ASK1, CEP3, BUB3, IPI1                      |
| GO:0000775 | Chromosome, centromeric region                   | 1.18E-03   | STU1, SLJ15, MCD1, CBF2, NNF1, ASK1, CEP3, BUB3, IPI1               |
| GO:0043228 | Nonmembrane-bounded organelle                   | 1.40E-03   | SLA1, STU1, PKC1, ORC2, SLJ15, DPB3, MRC1, MCD1, USO1, FAL1, PDS1, DPB4, MMS21, DBF2, CBF2, POL31, POL32, RFC2, NNF1, ASK1, RAD27, ORC3, COF1, RPL22A, MCM5, TEM1, POB3, CSM3, CEP3, TOF1, SMCM5, RFC4, BUB3, RRS1, SPC29, IPI1 |
| GO:0043232 | Intracellular nonmembrane-bounded organelle     | 1.40E-03   | SLA1, STU1, PKC1, ORC2, SLJ15, DPB3, MRC1, MCD1, USO1, FAL1, PDS1, DPB4, MMS21, DBF2, CBF2, POL31, POL32, RFC2, NNF1, ASK1, RAD27, ORC3, COF1, RPL22A, MCM5, TEM1, POB3, CSM3, CEP3, TOF1, SMCM5, RFC4, BUB3, RRS1, SPC29, IPI1 |
| GO ID       | GO term                                      | P value | Genes                                                                 |
|------------|---------------------------------------------|---------|----------------------------------------------------------------------|
| GO:0005488 | Binding                                      | 1.98E-03| SSA1, SWH1, SLA1, STU1, URA7, SEC17, PKC1, ORC2, TEC1, ARL1, ABD1,   |
|            |                                             |         | DPB3, YCL075W, MCD1, APC11, USO1, COP1, RRI1, TIM22, FA1, VPS41,    |
|            |                                             |         | PDS1, DBP4, YCF1, TAF12, SSD1, MFA1, MMS21, YPT31, TSC11, RSP5,    |
|            |                                             |         | CAP122, RPT6, PAC10, DBF2, CBF2, RPB3, MET30, ZAP1, POL31, RFC2,   |
|            |                                             |         | ASK1, RAD27, SNU114, ORC3, COF1, SEC22, MCM5, SST4, GAS2, TEM1,    |
|            |                                             |         | POB3, CEP3, ERP8, PEP5, LCB1, PSE1, ADE12, TLG2, SMC5, RFC4, BUB3, |
|            |                                             |         | BPT4, ERG10, ODC1, IPL1                                             |
| GO:0000779 | Condensed chromosome, centromeric region    | 2.03E-03| STU1, SLI15, CBF2, NNF1, ASK1, CEP3, BUB3, IPL1                     |
| GO:0003887 | DNA-directed DNA polymerase activity        | 2.23E-03| DPB3, YCL075W, DPB4, POL31, POL32                                 |
| GO:00030894| Replisome                                    | 2.25E-03| DPB3, DPB4, POL31, POL32, POB3                                    |
| GO:00043601| Nuclear replisome                           | 2.25E-03| DPB3, DPB4, POL31, POL32, POB3                                    |
| GO:0000776 | Kinetochore                                  | 2.32E-03| STU1, SLI15, CBF2, NNF1, ASK1, CEP3, BUB3, IPL1                    |
| GO:0016043 | Cellular component organization             | 3.46E-03| SSA1, SWH1, SLA1, STU1, SEC17, PKC1, ORC2, TEC1, ARL1, ABD1,       |
|            |                                             |         | DPB3, YCL075W, MCD1, APC11, USO1, COP1, RRI1, TIM22, FA1, VPS41,   |
|            |                                             |         | PDS1, TAF12, SSD1, TSC11, RSP5, CAP122, RPT6, PAC10, DBF2, CBF2,   |
|            |                                             |         | RFC2, ZAP1, POL31, RFC2, ASK1, RAD27, SNU114, ORC3, COF1, SEC22,   |
|            |                                             |         | MCM5, SST4, GAS2, TEM1, POB3, CEP3, ERP8, PEP5, LCB1, PSE1, ADE12,  |
|            |                                             |         | TLG2, SMC5, RFC4, BUB3, BPT4, ERG10, ODC1, IPL1                    |
| GO:0031981 | Nuclear lumen                               | 3.85E-03| STU1, MRC1, MCD1, PAL1, SSD1, MOB2, CBF2, MET30, NNF1, ASK1, CEP3, |
|            |                                             |         | MCM5, TEM1, CSM3, TOF1, BUB3, IPL1                                 |
| GO:0034061 | DNA polymerase activity                     | 3.90E-03| DPB3, YCL075W, DPB4, POL31, POL32                                 |
| GO:0000278 | Mitotic cell activity                        | 3.93E-03| STU1, MRC1, MCD1, PAL1, SSD1, MOB2, CBF2, MET30, NNF1, ASK1, CEP3, |
|            |                                             |         | MCM5, TEM1, CSM3, TOF1, BUB3, IPL1                                 |
| GO:0031298 | Replication fork protection complex         | 4.09E-03| MRC1, MCM5, POB3, CSM3, TOF1                                        |
| GO:0006259 | DNA metabolic process                        | 4.13E-03| ORC2, DPB3, MRC1, MCD1, PAL1, DBP4, MMS21, RPT6, POL31, POL32, RFC2, |
|            |                                             |         | RAD27, ORC3, MCM5, POB3, CSM3, TOF1, SMC5, RFC4, RPT4              |
| GO:0000280 | Nuclear division                            | 4.90E-03| STU1, MRC1, MCD1, PAL1, SSD1, MOB2, DBF2, NNF1, ASK1, TEM1, CSM3, |
|            |                                             |         | TOF1, BUB3, IPL1                                                    |
| GO:0000087 | M phase of mitotic cell cycle               | 5.63E-03| STU1, MRC1, MCD1, PAL1, SSD1, MOB2, DBF2, NNF1, ASK1, TEM1, CSM3, |
|            |                                             |         | TOF1, BUB3, IPL1                                                    |
| GO:0051716 | Cellular response to stimulus               | 6.38E-03| PKC1, CC21, ARL1, DPB3, MRC1, MCD1, RRI1, HBT1, SSD1, MFA1, MMS21, |
|            |                                             |         | YPT31, TSC11, RSP5, RPT6, MGA2, ZAP1, POL31, POL32, RFC2, RAD27, |
|            |                                             |         | MCM5, SST4, TEM1, POB3, CSM3, TOF1, ATG3, SMC5, RFC4, RPT4          |
| GO:0005819 | Spindle                                      | 6.45E-03| STU1, SLI15, PDS1, DBF2, CBF2, ASK1, TEM1, SPC29, IPL1             |
| GO:0042575 | DNA polymerase complex                       | 6.93E-03| DPB3, DPB4, POL31, POL32                                           |
| GO:0007049 | Cell cycle                                   | 7.00E-03| STU1, PKC1, MRC1, RMD1, MCD1, PAL1, SSD1, MOB2, DBF2, CBF2, MET30, |
|            |                                             |         | RFC2, NNF1, ASK1, MMM1, MCM5, TEM1, CSM3, TOF1, SMC5, RFC4, BUB3, |
|            |                                             |         | SPC29, IPL1                                                        |
| GO:0006974 | Response to DNA damage stimulus             | 7.21E-03| DPB3, MRC1, MCD1, PDS1, MMS21, RPT6, POL31, POL32, RFC2, RAD27, |
|            |                                             |         | MCM5, POB3, CSM3, TOF1, SMC5, RFC4, RPT4                           |
| GO:0048285 | Organelle fission                           | 8.96E-03| STU1, MRC1, MCD1, PAL1, SSD1, MOB2, DBF2, NNF1, ASK1, TEM1, CSM3, |
|            |                                             |         | TOF1, BUB3, IPL1                                                    |
| GO:0071840 | Cellular component organization or biogenesis| 9.43E-03| SSA1, SWH1, SLA1, STU1, SEC17, PKC1, ORC2, TEC1, CC21, MRC1, MCD1, |
|            |                                             |         | PAL1, SSD1, TSC11, RSP5, RPT6, MGA2, ZAP1, POL31, RFC2, ASK1,        |
|            |                                             |         | RAD27, SNU114, ORC3, COF1, SEC22, MCM5, SST4, GAS2, TEM1, POB3, CSM3, |
|            |                                             |         | CEP3, ERP8, PEP5, TOF1, ATG3, TLG2, SMC5, RFC4, RPT4, RRS1, SPC29, |
|            |                                             |         | IPL1                                                               |
| GO:0030174 | Regulation of DNA-dependent DNA replication initiation | 1.22E-02| MRC1, MET30, MCM5, CSM3, TOF1                                      |

(continued)
in the destabilization of a minisatellite regardless of tract length or repeat unit sequence composition.

To determine whether any sets of genes were overrepresented in both the ade2-min3 and ade2-h7.5 hit lists, we performed GO term analyses on the combined results of each screen (Table 6). Each enriched GO term was primarily associated with genes identified in the ade2-min3 screen rather than those of the ade2-h7.5 screen. Therefore, the GO terms represented general terms associated with cellular chromosomes and DNA replication as discussed previously. Based upon the low number of overlapping hits between each screen and the results from our GO term analyses, we conclude that each minisatellite tract is regulated by a distinct set of genes that do not share many overlapping functions or processes.

To verify the results of the SGA analysis, we deleted these genes in a separate genetic background—our well-characterized ade2-min3 (DTK271) and ade2-h7.5 (DTK1188) strain background (Alver et al. 2013; Kelly et al. 2007, 2011). We then quantified the blebbing phenotype in the resulting mutants. As previously reported, deletion of ZAP1 and ZRT1 in both ade2-min3 and ade2-h7.5 strain backgrounds resulted in a high level of blebbing compared with the WT parental strains (Figure 2, B and C) (Kelly et al. 2007, 2011). However, unlike the results from the SGA analyses, strains bearing a deletion of COT1, MON1, or RPL22A did not result in a dramatic increase in blebbing in either strain background. The deletion of COT1, MON1, or RPL22A in a strain bearing the ade2-min3 allele produced a level of blebbing that, although significantly greater than that of the WT strain, was only 30% of that displayed by the zrt1Δ strain (cot1Δ at 8.8 blebs/colony; mon1Δ at 9.7 blebs/colony; rpl22aΔ at 6.7 blebs/colony vs. WT at 4.7 blebs/colony and zrt1Δ at 24.5 blebs/colony). An ade2-h7.5 strain bearing a deletion of COT1, MON1, or RPL22A did not produce a significant increase in blebbing compared with the WT strain (cot1Δ at 2.6 blebs/colony; mon1Δ at 2.6 blebs/colony; rpl22aΔ at 0.3 blebs/colony vs. WT at 2.0 blebs/colony). We suspect that these results could be due to a secondary mutation in the SGA strain background that could act as an enhancer, or in our laboratory strain background which could act as a suppressor of the blebbing phenotype.

Mismatch repair regulates ade2-h7.5 minisatellite stability in stationary-phase cells

Previous work in actively dividing cells suggested that mismatch repair is associated with preventing microsatellite, rather than minisatellite, alterations (Sia et al. 1997). However, the deletion of PMS1 in the ade2-h7.5 strain resulted in a strong blebbing phenotype, indicating a potential role for mismatch repair in stationary-phase cells (Table 5). PMS1 encodes a mismatch repair protein that, together with Mlh1p, repairs multiple forms of damaged DNA (Proli et al. 1994a, b). To verify the results of our screen, we deleted PMS1 in our laboratory strain background (Kelly et al. 2011) and quantified the average number of blebs/colony. Deletion of PMS1 confirmed the results from the SGA analysis, as pms1Δ produced a significantly higher level of blebbing (13.4 blebs/colony) compared with the WT strain (2.0 blebs/colony; Figure 3).

To determine whether other well-characterized mismatch repair genes (reviewed in Marti et al. 2002) were involved in maintaining stationary-phase minisatellite stability, we quantified the level of blebbing in several mismatch repair mutant strains bearing the ade2-h7.5 allele (Figure 3). Strains with a deletion of MLH1 (10.0 blebs/colony), MSH2 (13.8 blebs/colony), or MSH6 (14.1 blebs/colony) produced notably greater levels of blebbing compared with the WT strain. Deletions of EXO1, MLH2, MLH3, or MSH3 did not result in a level of blebbing significantly different from that of the WT strain. Together our results indicate that a specific subset of mismatch repair genes maintains the stationary-phase stability of the variable-repeat HRASJ-associated minisatellite. The MLH1, MSH2, and MSH6 results also demonstrate that our SGA screens may not have identified all genes that contribute to minisatellite stability, possibly due to issues of sensitivity or strain background.

DISCUSSION

We used a modified version of the SGA procedure (Alver et al. 2013; Li et al. 2011; Tong and Boone 2006; Tong et al. 2001) to identify genes involved in maintaining minisatellite stability in stationary-phase cells. We performed two individual screens; the first screen used a query strain bearing a minisatellite consisting of three identical
20-bp repeats (ade2-min3 allele) (Kelly et al. 2007, 2011, 2012), whereas the second screen utilized a query strain containing a minisatellite consisting of seven-and-a-half 20-bp repeats of the HRAS1-associated minisatellite allele (ade2-h7.5 allele) (Kelly et al. 2007, 2011). Each screen incorporated analysis of approximately 4800 nonessential genes consisting of seven-and-a-half 28-bp repeats of the ade2-min3 allele (Kelly et al. 2007, 2011). Replication factor C is a clamp loader or clamp loader subunit or clamp loader subunits (Araki et al. 1994; Araki 1998; Sugimoto et al. 1995) and base excision repair (Wang et al. 1993), repair of UV-damaged DNA (Torres-Ramos et al. 1997), and template switching after DNA damage occurs (Vanoli et al. 2010). Polh has been implicated in nucleotide excision repair (Shivi et al. 1993), Polh has been shown to be involved in base excision repair (Blank et al. 1994), repair of UV-damaged DNA (Torres-Ramos et al. 1997), and template switching after DNA damage occurs (Vanoli et al. 2010). Polh has been implicated in nucleotide excision repair (Shivi et al. 1995), base excision repair (Wang et al. 1993), and double-strand break repair (Holmes and Haber 1999). GO term analysis of the 102 hits from the ade2-min3 screen revealed that the majority of enriched GO terms were associated with chromosomal regulation and DNA replication. Although it might seem surprising to find such strong evidence of DNA replication in stationary-phase cells,

20-bp repeats (ade2-min3 allele) (Kelly et al. 2007, 2011, 2012), whereas the second screen utilized a query strain containing a minisatellite consisting of seven-and-a-half 20-bp repeats of the HRAS1-associated minisatellite allele (ade2-h7.5 allele) (Kelly et al. 2007, 2011). Each screen incorporated analysis of approximately 4800 nonessential genes with an additional 450 essential genes screened using the ade2-min3 allele. We identified 102 genes that are involved in regulating the stability of the ade2-min3 minisatellite and 157 genes that regulate the stability of the ade2-h7.5 minisatellite in stationary phase cells. Only seven hits overlapped both screens. Finally, we demonstrated that mismatch repair genes regulate ade2-h7.5 minisatellite stability.

We investigated the hits of each screen independently to characterize candidate genes associated with each individual minisatellite. Several hits identified in the ade2-min3 screen were genes associated with checkpoint function and were described previously (Alver et al. 2013). Other identified genes involve DNA replication and repair. These included POL31, a subunit of Polh (Giot et al. 1997; Hashimoto et al. 1998; Sugimoto et al. 1995) and the Polh subunits DBP3 or DBP4 (Araki 1994; Araki et al. 1991; Lou et al. 2008) (Table 3). Also included were the RFC2 and RFC4 subunits of replication factor C (Cullmann et al. 1995; Noskov et al. 1994; Yao et al. 2003). Replication factor C is a clamp loader of the proliferating cell nuclear antigen, a sliding clamp for Polh and Polh (Chilkova et al. 2007). Both Polh and Polh have been implicated in DNA repair mechanisms. Polh has been shown to be involved in base excision repair (Blank et al. 1994), repair of UV-damaged DNA (Torres-Ramos et al. 1997), and template switching after DNA damage occurs (Vanoli et al. 2010). Polh has been implicated in nucleotide excision repair (Shivi et al. 1995), base excision repair (Wang et al. 1993), and double-strand break repair (Holmes and Haber 1999). GO term analysis of the 102 hits from the ade2-min3 screen revealed that the majority of enriched GO terms were associated with chromosomal regulation and DNA replication. Although it might seem surprising to find such strong evidence of DNA replication in stationary-phase cells,
components of DNA replication and repair mechanisms prevent ade2-min3 minisatellite alterations in stationary phase cells.

GO term analysis of candidate hits from the ade2-h7.5 SGA analysis showed that only one term (carboxy-lyase activity) was overrepresented within our data set. This finding could be a consequence of factors that regulate the stability of the ade2-h7.5 minisatellite representing a wide range of cellular functions, or the inherent low level of blebbing associated with the ade2-h7.5 allele (Kelly et al. 2011) complicating the accuracy of scoring this screen. Also, it is possible that uncharacterized candidate hits could be specific to stationary phase gene expression, but a comparison of the uncharacterized hits with genes known to be expressed in stationary phase cells revealed no strong correlation (Aragon et al. 2008; Davidson et al. 2011).

Interestingly, only seven gene hits overlapped between the ade2-min3 and ade2-h7.5 screens (Figure 2A): BUD28, COT1, MON1, YGL217C, YLR125W, ZAP1, and ZRT1. Deletion of each gene in our laboratory strain background revealed that, like the SGA analyses, deletion of ZRT1 or ZAP1 resulted in a dramatic increase in minisatellite instability in both the ade2-min3 and ade2-h7.5 alleles (Kelly et al. 2007, 2011). Deletion of COT1, MON1, or RPL22A (BUD28) led to a moderate increase in ade2-min3 instability but did not affect ade2-h7.5 instability. Thus, differences in strain backgrounds, such as the presence of a secondary enhancer or suppressor mutation, can significantly affect minisatellite stability in stationary phase cells. In support of this, the nonessential gene deletion strain collection previously has been shown to harbor secondary mutations (Lechner et al. 2007). The small degree of overlap between the screens further suggests that sequence differences between the ade2-min3 and ade2-h7.5 minisatelites may govern repeat tract stability and alteration. Our laboratory, as well as others, has previously shown that tract length and sequence variability within a minisatellite allele can greatly affect repeat stability (Denoeud et al. 2003; Jauert and Kirkpatrick 2005; Legendre et al. 2007). This study supports these findings and extends it to stationary phase cells, suggesting that the composition and size of the minisatellite affect which cellular components and mechanisms govern tract stability.

Surprisingly, a strong candidate hit identified in the ade2-h7.5 screen was the mismatch repair gene PMS1 (Prolla et al. 1994a,b). The mismatch repair system has usually been associated with instability in microsatellite tracts, rather than minisatellite tracts (Johnson et al. 1996; Strand et al. 1993, 1995). Minisatellite instability in actively dividing cells was not significantly affected by mutations in mismatch repair mutant strains (Sia et al. 1997), in an analysis that used a minisatellite tract identical to the ade2-min3 allele. Deletion of PMS1 affected only the stability of the ade2-h7.5 minisatellite allele rather than the ade2-min3 allele. Only a subset of the mismatch repair components (PMS1, MLH1, MSH2, and MSH6) affected the stability (Figure 3) of the ade2-h7.5 minisatellite tract. Previous work in actively growing cells demonstrated that mutating the mismatch repair genes MLH1, MSH2 or PMS1 resulted in a high degree of microsatellite instability, while mutating MSH3 or MSH6 had a less drastic effect (Johnson et al. 1996; Sia et al. 1997; Strand et al. 1993, 1995). This and other work suggested that yeast contain distinct mismatch repair complexes; one complex contains the Mhl1-Pms1 and Msh2-Msh6 heterodimers, whereas another incorporates Mlh1-Pms1 and Msh2-Msh3 (Johnson et al. 1996; Marsischky et al. 1996). Each complex has been implicated in the repair of different substrates; the Msh2-Msh6 heteromer is involved in basebase mismatch repair as well as repair of single-base loops generated by insertion or deletion mispairing (Alani 1996; Marsischky et al. 1996) whereas Msh2-Msh3 primarily targets small loops (Habranka et al. 1996; Marsischky et al.
Based upon our data, we predict that the Mlh1-Pms1, Msh2-Msh6 complex prevents the instability of the ade2-h7.5 minisatellite by targeting and repairing mismatches that can occur at two positions within a repeat. If there is misalignment of repeat units during recombinational repair (shown to be required for tract alterations) (Kelly et al. 2011), there will be potential C/C or G/G mismatches at nucleotides 14 and 22 in each repeat; these would be substrates for mismatch repair that could also affect tract length during the repair process. Our results lend further support to the idea that the composition of a minisatellite may dictate which factors are involved in preventing tract alterations within stationary phase cells.

In summary, we have conducted the first whole-genome screens to identify factors that regulate minisatellite stability in stationary phase cells, uncovering more than 250 genes that strongly affect the stability of minisatellite tracts in stationary-phase cells. We provide evidence that factors involved in regulating stationary-phase minisatellite stability are affected by minisatellite repeat length or sequence, as we found only a small overlap in detected genes when using significantly different minisatellite tracts. We find that disruption of DNA replication and repair components result in a dramatic increase in instability of a simple minisatellite tract, whereas loss of a subset of mismatch repair proteins specifically influences variable-repeat minisatellite instability. Thus, our work lends support to the argument that the composition of the repeat tract within a minisatellite greatly affects minisatellite stability and regulatory mechanisms.

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