Comprehensive Synthetic Genetic Array Analysis of Alleles That Interact with Mutation of the Saccharomyces cerevisiae RecQ Helicases Hrq1 and Sgs1

Elsbeth Sanders,¹ Phoebe A. Nguyen,¹ Cody M. Rogers,² and Matthew L. Bochman³
Molecular and Cellular Biochemistry Department, Indiana University, Bloomington, IN 47405

ABSTRACT

Most eukaryotic genomes encode multiple RecQ family helicases, including five such enzymes in humans. For many years, the yeast Saccharomyces cerevisiae was considered unusual in that it only contained a single RecQ helicase, named Sgs1. However, it has recently been discovered that a second RecQ helicase, called Hrq1, resides in yeast. Both Hrq1 and Sgs1 are involved in genome integrity, functioning in processes such as DNA inter-strand crosslink repair, double-strand break repair, and telomere maintenance. However, it is unknown if these enzymes interact at a genetic, physical, or functional level as demonstrated for their human homologs. Thus, we performed synthetic genetic array (SGA) analyses of hrq1Δ and sgs1Δ mutants. As inactive alleles of helicases can demonstrate dominant phenotypes, we also performed SGA analyses on the hrq1-K318A and sgs1-K706A ATPase/helicase-null mutants, as well as all combinations of deletion and inactive double mutants. We crossed these eight query strains (hrq1Δ, sgs1Δ, hrq1-K318A, sgs1-K706A, hrq1Δ sgs1Δ, hrq1Δ sgs1-K706A, hrq1-K318A sgs1Δ, and hrq1-K318A sgs1-K706A) to the S. cerevisiae single gene deletion and temperature-sensitive allele collections to generate double and triple mutants and scored them for synthetic positive and negative genetic effects based on colony growth. These screens identified hundreds of synthetic interactions, supporting the known roles of Hrq1 and Sgs1 in DNA repair, as well as suggesting novel connections to rRNA processing, mitochondrial DNA maintenance, and lagging strand synthesis during DNA replication.

KEYWORDS
Saccharomyces cerevisiae
HRQ1
SGS1
DNA helicase
yeast

The human genome encodes five RecQ family helicases (RECQL1, BLM, WRN, RECQL4, and RECQL5), all of which are involved in the maintenance of genome integrity (Bochman 2014; Croteau et al. 2014). Two RecQ family helicases exist in Saccharomyces cerevisiae, Hrq1 and Sgs1, which are homologs of the disease-linked human RECQL4 (Barea et al. 2008; Bochman et al. 2014; Rogers et al. 2017) and BLM helicases (Watt et al. 1996; Lillard-Wetherell et al. 2005; Gravel et al. 2008), respectively. However, the discovery of Sgs1 (Gangloff et al. 1994) preceded that of Hrq1, and for many years, Sgs1 was considered the only RecQ family helicase encoded in the S. cerevisiae genome. However, a second DNA helicase with RecQ homology was independently identified several times (Shiratori et al. 1999; Lee et al. 2005), but Hrq1 was never formally named and recognized as a homolog of the RECQL4 helicase until 2008 (Barea et al. 2008), with in vivo and in vitro functional homology to RECQL4 being demonstrated subsequently (Bochman et al. 2014; Rogers and Bochman 2017; Rogers et al. 2017; Nickens et al. 2018; Rogers et al. 2020a).

The known and hypothesized roles of Sgs1 in homologous recombination, DNA replication, meiosis, excision repair, and telomere maintenance were recently reviewed (Gupta and Schmidt 2020). Much less is known about Hrq1, though it is linked to DNA

¹These authors contributed equally to this work.
²Present address: Department of Biochemistry and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229.
³Corresponding author: Matthew L. Bochman; 212 S. Hawthorne Dr., Simon Hall MSB1 room 405B, Bloomington, IN 47405. E-mail: bochman@indiana.edu
RecQ helicases also exist in human RECQL4 (Jin et al. 2008; Ghosh et al. 2011; Ferrarelli et al. 2013; Keller et al. 2014). Contemporaneous work using a multi-omics approach also suggests that Hrrq1 has roles in transcription, chromosome/chromatin dynamics, rRNA processing/ribosomal maturation, and in the mitochondria (Rogers et al.; companion manuscript G3/2020/401864).

Despite these advances in yeast RecQ research, little is known about the genetic interactions that occur between HRRQ1 and SGS1 or the physical interactions between Hrrq1 and Sgs1. In humans, some of the RecQ helicases are partially functionally redundant (e.g., BLM and WRN), some display complementarity (e.g., WRN and RECQL5), and others exhibit functional synergism (reviewed in (Croteau et al. 2014)). The latter is exemplified by BLM and RECQL4, where BLM promotes the retention of RECQL4 at DNA double-strand breaks (DSBs), and RECQL4 stimulates BLM activity (Singh et al. 2012). Do such connections exist between their yeast homologs Hrrq1 and Sgs1? Two reports demonstrate that various combinations of hrrq1 and sgs1 alleles display differential responses to DNA damage compared to single mutants (Bochman et al. 2014; Rogers et al. 2020), suggesting that functional interactions among the RecQ helicases also exist in S. cerevisiae.

### Rationale for screen

The study of yeast RecQ homologs has greatly expanded our mechanistic understanding of how these enzymes function in various DNA repair pathways, but the interplay between Hrrq1 and Sgs1 and their roles in other biological processes are not well studied. We sought to identify genes whose mutation affects the growth of hrrq1 and/or sgs1 mutant cells. Because inactive alleles of DNA helicases often act as dominant negatives (Wu and Brosh 2010) and in some cases better represent disease-linked alleles, we utilized both deletion (hrrq1Δ and sgs1Δ) and catalytically inactive mutants (hrrq1-K318A and sgs1-K706A) of the helicases in all combinations (hrrq1Δ, sgs1Δ, hrrq1-K318A, sgs1-K706A, hrrq1Δ sgs1Δ, hrrq1-K318A sgs1-K706A, hrrq1-K318A sgs1-K706A) in our screen. Many genes that encode proteins involved in genome integrity are also essential, so we performed synthetic genetic array (SGA) analysis by mating our query helicase mutant strains to both the S. cerevisiae single-gene deletion collection (Giaever and Nislow 2014) and the temperature-sensitive (TS) collection (Kofoed et al. 2015), the latter of which includes alleles of essential genes not found in the former, to generate a comprehensive set of double and triple mutant strains for SGA analysis.

### MATERIALS & METHODS

#### Screen design

The strains used in this study are listed in Table 1. The HRRQ1 gene was deleted in Y8205 (Table 1) by transforming in a NatMX cassette that was PCR-amplified from the plasmid pAC372 (a gift from Amy Caudy) using oligonucleotides MB525 and MB526 (Table S1). The deletion was verified by PCR analysis using genomic DNA and oligonucleotides that anneal to regions up- and downstream of the HRRQ1 locus (MB527 and MB528). The confirmed hrrq1Δ strain was named MBY639. The hrrq1-K318A allele was introduced into the Y8205 background in a similar manner. First, an hrrq1-K318A(NatMX) cassette was PCR-amplified from the genomic DNA of strain MBY346 (Bochman et al. 2014) using oligonucleotides MB527 and MB528 and transformed into Y8205. Then, genomic DNA was prepared from transformants and used for PCR analyses of the HRRQ1 locus with the same oligonucleotide set to confirm insertion of the NatMX marker. Finally, PCR products of the expected size for hrrq1-K318A(NatMX) were sequenced using oligonucleotide MB932 to confirm the presence of the K318A mutation. The verified hrrq1-K318A strain was named MBY644.

The SGS1 gene was deleted from Y8205 (Table 1) in the same manner as the HRRQ1:NATMX deletion above by transforming in a NatMX cassette that was PCR-amplified using oligonucleotides MB1395 and MB768 (Table S1). The deletion was verified by PCR analysis of genomic DNA and oligonucleotides MB373 and MB374. The confirmed sgs1Δ strain was named MBY640. The sgs1-K706A allele was PCR amplified from plasmid pFB-MBP-Sgs1K706A-his (Cejka and Kowalczykowski 2010) (Table 2) using oligonucleotides MB765 and MB1396. The NatMX cassette was PCR-amplified from pAC372 using oligonucleotides MB1397 and MB768 and fused to the sgs1-K706A PCR product by Gibson assembly (Gibson et al. 2009). The resultant sgs1-K706A(natMX) cassette was reamplified with MB765 and MB768 and transformed into Y8205. Genomic DNA was then prepared from transformants and used for PCR analyses of the SGS1 locus with oligonucleotides MB373 and MB374 to confirm insertion of the cassette. Finally, PCR products of the expected size were sequenced using oligonucleotide MB769 to confirm the presence of the K706A mutation. The verified sgs1-K706A strain was named MBY642.

The double mutant strains were constructed using similar techniques. Briefly, the hrrq1Δ sgs1Δ and hrrq1-K318A sgs1Δ strains were generated by deleting SGS1 in strains MBY639 and MBY644, respectively, using a URA3 cassette amplified from pUG72 (Guedelert et al. 2002) with oligonucleotides MB1395 and MB355 (Table S1). The strains verified by PCR of genomic DNA and sequencing were named MBY643 and MBY645, respectively. The hrrq1Δ sgs1-K706A and hrrq1-K318A sgs1-K706A strains were constructed by amplifying sgs1-K706A as above, amplifying the URA3 cassette with oligonucleotides MB1397 and MB355, and fusing the PCR products via Gibson assembly. The sgs1-K706A(URA3) cassette was then transformed into strains MBY639 and MBY644, and transformants were confirmed for proper integration by PCR and Sanger sequencing. The verified hrrq1Δ sgs1-K706A and hrrq1-K318A sgs1-K706A strains were named MBY674 and MBY676, respectively. Further details concerning strain construction are available upon request.

 SGAs analysis of the hrrq1Δ, sgs1Δ, hrrq1-K318A, sgs1-K706A, hrrq1Δ sgs1Δ, hrrq1-K318A sgs1Δ, hrrq1-K318A sgs1-K706A sgs1-K706A mutants was performed at the University of Toronto using previously described methods (Tong et al. 2001; Tong et al. 2004). All query and control strains were crossed in quadruplicate to both the S. cerevisiae single-gene deletion collection (Giaever and Nislow 2014) and the TS alleles collection (Kofoed et al. 2015) to generate double or triple mutants for analysis. For the double mutant screens, the control strain (Y8835) contained the NatMX marker inserted into the benign ura3 locus (MATa ura3Δ: natMX4 can1Δ:STET2pr-Sp_his5 hp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 lys2+). For the triple mutant screens, the control strain was Y10906 (MATa ura3Δ: natMX4 his3Δ1:KURA3 can1Δ:STET2pr-Sp_his5 hp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 lys2+), as described previously (Kuzmin et al. 2018).

#### Phenotypes

Quantitative scoring of the genetic interactions was based on colony size. The SGA score measures the extent to which the size of a double or triple mutant colony differs from the colony size expected from combining the query and tester mutations together (Baryshnikova et al. 2013; Ferrarelli et al. 2013; Keller et al. 2014). The contem-
et al. 2010). The data includes both negative (putative synthetic sick/lethal) and positive interactions (potential epistatic or suppression interactions) (Tables S2-17). The magnitude of the SGA score is indicative of the strength of the interaction. Based on statistical analysis, it was determined that a default cutoff for a significant genetic interaction is $P < 0.05$ and SGA score $> |0.08|$ (Costanzo et al. 2010).

### Verification of mutants

The top five negative and positive interactions for each query strain were confirmed by remaking and reanalyzing the double and triple mutants by hand, followed by spot dilution (Andis et al. 2018) and/or growth curve (Ononye et al. 2020) assays to compare the growth of the double or triple mutants to their parental strains and wild-type. Examples are shown in Figure S1.

### Statistical analysis

Data were analyzed and graphed using GraphPad Prism 6 software. The reported values are averages of $\geq 3$ independent experiments, and the error bars are the standard deviation. $P$-values were calculated as described in the figure legends, and we defined statistical significance as $P < 0.01$.

### Data availability

Strains, plasmids, and other experimental reagents are available upon request. File S1 contains Table S1, a description of the other supplementary tables included in Files S2-S4, and Figures S1-S4. File S2 contains Tables S2-S9, File S3 contains Tables S10-S17, and File S4 contains the complete SGA data for all screens in the form of Tables S18-33. Supplemental material available at figshare: https://doi.org/10.25387/g3.13157519.

### RESULTS AND DISCUSSION

#### Overall results of the screen

Hundreds of synthetic interactions were detected for all query strains screened through both the single-gene deletion (Table 2) and TS mutant (Table 3) collections (Tables S2-17). For the single-gene deletion collection screen, the numbers of negative and positive genetic interactions were generally the same for all query strains, except $hrq1\Delta$ and $hrq1\Delta-K318A$, which yielded approximately twice as many negative as positive interactions (Table 2). These mutants also had the fewest number of synthetic interactions by a factor of $>2.3$ compared to $sgs1\Delta$ and $sgs1\Delta-K706A$. This is consistent with the generally more modest phenotypes of $hrq1\Delta$ and $hrq1\Delta-K318A$ strains compared to $sgs1\Delta$ and $sgs1\Delta-K706A$ for DNA damage sensitivity (Bochman et al. 2014). The double mutant query strains yielded a greater than additive number of synthetic genetic interactions than the single mutant parental query strains, indicating that mutating both RecQ helicases had a synergistic effect. This synergism was strongest for the $hrq1\Delta-K318A$ $sgs1\Delta$ mutant, which generated 880 synthetic interactions, a nearly twofold increase over the additive effect of the 132 $hrq1\Delta-K318A$ and 312 $sgs1\Delta$ interactions individually (compared to $\sim 1.5$- to $1.6$-fold increases for the other combinations).

For the TS allele collection screen, the numbers of negative and positive genetic interactions were again generally similar for all query strains (Table 3). As above, the $hrq1\Delta$ and $hrq1\Delta-K318A$ mutants had the fewest number of synthetic interactions by a factor of $>2.1$ compared to $sgs1\Delta$ and $sgs1\Delta-K706A$. In this case, however, the double mutant query strains yielded approximately an additive number of synthetic genetic interactions compared to the single mutant parental query strains and thus did not display the synergism described for the single-gene deletion SGA analysis. It should also be noted that the numbers of synthetic genetic interactions listed in Table 3 are inflated because several different TS alleles of the same ORF are included in the collection for many individual genes (Kofoed et al. 2015).

Figure 1 shows the frequency distribution of all of the SGA scores as violin plots and separate box plots of the negative and positive interactions individually (compared to $\sim 1.5$- to $1.6$-fold increases for the other combinations).
The deletion of hrq1 pairwise combinations of triple mutants screens are available in sgs1 actively following up on phenotypic difference among all of the mutants, especially compared to the synthetic genetic interactions signiﬁcant differences between the sgs1-K706A and hrq1 vs. hrq1-K318A sgs1 vs. hrq1-K318A sgs1 (P = 0.0030), intermediate differences for sgs1 vs. hrq1α sgs1α (P = 0.0016) and sgs1-K706A vs. hrq1-K318A sgs1-K706A (P = 0.0070), and strong differences between sgs1-K706A and hrq1α sgs1α, hrq1α sgs1-K706A, and hrq1-K318A sgs1α (all P < 0.0001). It is currently unclear why the strength of the positive synthetic genetic interactions signiﬁcantly varied among these mutants, especially compared to the sgs1-K706A query strain, but we are actively following up on phenotypic difference among all of the hrq1 and sgs1 alleles. Regardless, as mutants giving the strongest growth effects, the outliers in Figures 1B, C, E, and F are summarized in Tables 4 and 5. For instance, only the negative genetic interactions are discussed in further detail below. Comparisons between the full SGA datasets for the hrq1Δ/hrq1-K318A, sgs1Δ/sgs1-K706A, and all pairwise combinations of triple mutants screens are available in Figures S2 and S3.

hrq1Δ interactions

The deletion of HRQ1 displayed strong negative interactions with mutations in 10 genes (Tables 4 and 5), many of which correspond to the recently described Hrq1 interactome (Rogers et al.1). For instance, RECQL4 is the only human RecQ found in both the nucleus and mitochondria (Croteau et al. 2014), and Hrq1 likewise localizes to both organelles (Koh et al. 2015) and physically interacts with mitochondrial proteins (Rogers et al.1). Here, we found strong negative synthetic genetic interactions between hrq1Δ and mutation of MRM2, a mitochondrial 2’-O-ribose methyltransferase whose deletion results in mitochondrial DNA (mtDNA) loss (Pintard et al. 2002), and YSC83, a mitochondrial protein of unknown function (Sickmann et al. 2003). It is still unclear what the role of Hrq1 is in the mitochondria, but it is tempting to speculate that it is involved in mtDNA maintenance in a similar fashion to its maintenance of the nuclear genome.

This role in genome integrity is highlighted by the negative interactions of hrq1Δ with mutation of SPO16, which is involved in the meiotic cell cycle (Shinozaka et al. 2008), and RAD14, a nucleotide excision repair protein (Guzder et al. 2006) and regulator of transcription (Chaurasia et al. 2013). Deletion of HRQ1 also negatively interacted with mutation of SLX9, an RNA processing factor (Bax et al. 2006) that additionally binds G-quadruplex (G4) DNA structures (Gott et al. 2019). This is provocative in light of the connection of Hrq1 to RNA processing and ribosome biogenesis (Rogers et al.1), as well as the fact that G4 structures are preferred substrates for Hrq1 in vitro (Rogers et al. 2017). Finally, mutations in YEF3, YUR1, MUP3, and PHO5 (encoding a translation elongation factor, protein glycosylase, methionine permease, and acid phosphatase, respectively), as well as the dubious open reading frame (ORF) YDR455C (Fisk et al. 2006), also negatively interacted with hrq1Δ.

hrq1-K318A interactions

Mutations in only two genes, RAD14 and YEF3, are shared between the lists of strong negative interactors with hrq1Δ and hrq1-K318A. This is not unexpected based on the ability of Hrq1-K318A to phenocopy wild-type in some pathways (Bochman et al. 2014). However, mutations in genes encoding proteins involved in processes shared between both sets are evident. This includes HAP2 and HAP3, which are activators of transcription (Xing et al. 1993), TC089, a member of the TOR complex and global regulator of histone H3 K56 acetylation (Chen et al. 2012), and RAD14 as described above. Similarly, TOM70 encodes a subunit of the mitochondrial protein import receptor (Brix et al. 2000), which is likely important for localizing Hrq1 to the mitochondria where it may be involved in mtDNA maintenance. Genome integrity is also highlighted by CBC2, which encodes an RNA binding and processing factor involved in telomere maintenance (Lee-Soey et al. 2012). Hrq1 is known to regulate telomerase activity at both DSBs and telomeres (Bochman et al. 2014; Nickens et al. 2018; Nickens et al. 2019). Mutation of the gene encoding the Vps41 vacuolar membrane protein (Nakamura et al. 1997) also negatively interacted with hrq1-K318A.

The overall genetic interactome of HRQ1

In a companion manuscript, we present proteomic and transcriptomic data related to Hrq1 and Hrq1-K318A, highlighting similarities and differences between the wild-type and mutant to generate a wholistic picture of Hrq1 biology (Rogers et al.; manuscript #401710). We also touch on the SGA data included herein. Thus, it is useful to compare some of the hrq2Δ and hrq1-K318A data. In total, 117 signiﬁcant (P < 0.05) genetic interactions (76 negative and 41 positive) were identiﬁed between hrq1Δ and the single-gene deletion collection, and 119 (65 negative and 54 positive) were identiﬁed between hrq1Δ and the TS alleles collection (Table S2). Similarly, 132 signiﬁcant (P < 0.05) genetic interactions (84 negative and 48 positive) were identiﬁed between hrq1-K318A and the single-gene deletion collection, and 102 (41 negative and 61 positive) were identiﬁed between hrq1-K318A and the TS alleles collection (Table S3). When comparing the hrq1Δ and hrq1-K318A data sets in aggregate, there was ~39% overlap between the negative genetic interactions (Figure 2A) and > 30% overlap
between the positive genetic interactions (Figure 2B). However, there was very little overlap when comparing negative to positive genetic interactions and vice versa (Figure 2C,D), consistent with these hrq1 alleles having similar effects in vivo and the wild-type and mutant proteins displaying similar activities in vitro, though often of different magnitudes (Bochman et al. 2014; Rogers and Bochman 2017; Rogers et al. 2017; Nickens et al. 2018; Nickens et al. 2019; Rogers et al. 2020b).

sgs1Δ interactions

Over 500 genetic interactions with sgs1 alleles have been reported (see: https://www.yeastgenome.org/locus/S000004802/interaction), including most of the hits from our screen, such as the genome integrity genes MMS4, RRM3, SLX1, SLX4, SRS2, and WSS1 (Fisk et al. 2006), as well as SLX9 (see above) and EFB1, which encodes a translation elongation factor (Hiraga et al. 1993). These hits serve as internal positive controls. It should also be noted that: 1) YBR099C is a dubious ORF that completely overlaps MMS4 (Fisk et al. 2006), as well as SLX9 (see above) and EFB1, which encodes a translation elongation factor (Hiraga et al. 1993). These hits serve as internal positive controls. It should also be noted that: 1) YBR099C is a dubious ORF that completely overlaps MMS4 (Fisk et al. 2006), 2) YBR100W was an originally misannotated ORF and more recently merged with an adjacent ORF such that the coding region is now named MMS4 (Xiao et al. 1998), and 3) the pby1Δ strain in the single-gene deletion collection is actually a deletion of MMS4 (ölmez et al. 2015). Thus, multiple different mms4 alleles were hits in the screen, again acting as positive controls for our approach.
Table 4 Genes whose deletion cause the strongest growth phenotypes when combined with the hrq1 and sgs1 mutants

<table>
<thead>
<tr>
<th>Query strain</th>
<th>Negative interactors</th>
<th>Positive interactors</th>
</tr>
</thead>
</table>

In addition to known effects, we also discovered three new negative interactions with sgs1Δ. These include the deletions of SWC4 and SWCS, which encode subunits of the SWR1 complex that replaces histone H2A with H2A.Z (Mizuguchi et al. 2004), preventing the spread of silent heterochromatin (Meneghini et al. 2003). This interaction could be connected to the role of Sgs1 in telomere maintenance (Huang et al. 2001; Johnson et al. 2001; Azam et al. 2006) because telomeric DNA is also silenced via the telomere position effect (Mondou and Zakian 2005). As with hrq1Δ and hrq1-K318A, the yef3-f650s TS allele was also a negative genetic interactor with sgs1Δ (Table 5).

sgs1-K706A interactions

Unlike sgs1Δ, much less is known about the genetic interactome of the catalytically inactive sgs1-K706A allele. We found that the strong negative interactors were mutations in genes that completely overlap with the sgs1Δ set (SRS2, SLS4, SLY9, SLY1, SWCS, WSS1, MSS4, ELG1, YEF3, and SWC4). However, the sgs1-K706A interactors also included mutations in genes that were not ranked as causing the strongest negative effects with sgs1Δ. Nevertheless, alleles of some of these genes (RNI203, SLY8, RNI202, and MUS81) are previously reported negative interactors with sgs1Δ (see: https://www.yeastgenome.org/locus/S000004802/interaction).

Mutations in the remaining genes have not previously been reported to negatively interact with sgs1Δ, but three of them (SPO16, YSC83, and HAP3) overlap with the hrq1Δ interactors described above, perhaps suggesting some overlap in function between Hrq1 and Sgs1 in the pathways related to these genes. That leaves only two genes, SUA7 and ASK10, as unique interactors here. The SUA7 gene product is the yeast transcription factor TFIIB that is needed for RNA polymerase II transcriptional start site selection (Pinto et al. 1992). This may indicate that like the human RECQL5 helicase (Aygun et al. 1992), this may have a role in transcription. A possible explanation for this interaction is that like the human RECQL5 helicase, Sgs1 is involved in transcription, a hypothesis also put forth for Hrq1 et al. 2015). However, none of these genes overlap with the catalytically inactive sgs1-K706A interactions (see: https://www.yeastgenome.org/locus/S000004802/interaction).

Negative genetic interactions with the hrq1 sgs1 double mutants

The sets of synthetic negative genetic interactions for the hrq1 sgs1 double mutants shown in Tables 4 and 5 generally contain the strong interactors from the single-mutant parental strains, but they also include many new interactions, evident of the synergistic effect of mutating both RecQ family helicases in S. cerevisiae. These genes (CAT2, AEP2, SAE1, BUL1, CAT8, YMR031W-A, ICY1, RPL6B, DSK2, RIT1, SW14, COX7, RGM1, TRM732, ROY1, YMR265C, ELG1, YMR194A-C, OCA5, RTT107, RAD27, YJR084W, INP1, PBP2, YDR186C, YMR245W, KAP114, DNA2, NSE4, MOB2, SMT3, ROY1, DBF4, CDC2, CEP3, SMC6, PRI2, and NSE1) are enriched for gene ontology terms related to genome integrity, including DNA repair (DNA2, ELG1, NSE1, NSE4, POL3, PRI2, RAD27, RTT107, SAE2, and SMC6), DNA replication (DBF4, DNA2, ELG1, CDC2, PRI2, and RAD27), and transcription by RNA polymerase II (CAT8, CEP3, RGM1, SW14, and YJR084W) among others.

The links to DNA replication are notable because the negative genetic interactions preferentially occur with genes encoding lagging strand synthesis machinery. Dna2 and Rad27 are both nuclease involved in Okazaki fragment processing (Kao et al. 2004). Cdc2 is the catalytic subunit of DNA polymerase δ (Johnson et al. 2015), and Prib2 is the large subunit of DNA primase (Foiani et al. 1989). It is also
known that both Hrq1 (Bochman et al. 2014; Nickens et al. 2018) and Sgs1 (Wagner et al. 2006) interact with the Pif1 helicase, an enzyme involved in the two-nuclease Okazaki fragment processing pathway (Rossi et al. 2008; Pike et al. 2009). Therefore, combinatorial mutations of both yeast RecQ helicases are strongly deleterious when lagging strand synthesis is also disrupted by mutation. It is tempting to speculate that hindered Okazaki fragment maturation may yield DNA structures or lesions that require the repair activities of Hrq1 and Sgs1 for processing.

Also intriguing are the genes of unknown function (YMR265C, ICY1, and YMR245W) and those categorized as dubious ORFs (YMR194C-A and YMR031W-A) (Fisk et al. 2006). For instance, even though it is a dubious ORF, deletion of YMR031W-A yields cells with short telomeres (Askree et al. 2004), and Hrq1 (Bochman et al. 2014; Nickens et al. 2018) and Ssg1 (Wagner et al. 2006) are known to interact with the PIH1 helicase, an enzyme involved in the two-nuclease Okazaki fragment processing pathway (Rossi et al. 2008; Pike et al. 2009). Therefore, combinatorial mutations of both yeast RecQ helicases are strongly deleterious when lagging strand synthesis is also disrupted by mutation. It is tempting to speculate that hindered Okazaki fragment maturation may yield DNA structures or lesions that require the repair activities of Hrq1 and Sgs1 for processing.

### Figure 2
Venn diagrams of the shared synthetic genetic interactions displayed by Hrq1 and Hrq1-K318A. A) Sixty-one alleles negatively interact with both the Hrq1 and Hrq1-K318A mutations. B) Thirty-five alleles positively interact with both the Hrq1 and Hrq1-K318A mutations. C) Very few of the negative genetic interactors with Hrq1 are shared by the set of positive genetic interactors with Hrq1-K318A. D) Likewise, only ten of the positive genetic interactors with Hrq1 are shared by the set of negative genetic interactors with Hrq1-K318A.

#### Table 5 Temperature-sensitive alleles that cause the strongest growth phenotypes when combined with the Hrq1 and Sgs1 mutants

<table>
<thead>
<tr>
<th>Query strain</th>
<th>Negative interactions</th>
<th>Positive interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hrq1Δ</td>
<td>yeF3-f650s</td>
<td>mps3-1, stx9, act1-105, ap3-g302y, ap3-31, cm1-1</td>
</tr>
<tr>
<td>Hrq1-K318A</td>
<td>yeF3-f650s</td>
<td>cse2, dbp5-2, cm1-1, mps1-1</td>
</tr>
<tr>
<td>Sgs1Δ</td>
<td>yeF3-f650s, efb1-4, sqc4-4</td>
<td>stt3-1, ap2-14, ydl003w-ph, sf1-7, nse5-ts1, rad54, nsf1-6, act1-105, mps3-1, smc2-8, hpr1-7, cm1-1</td>
</tr>
<tr>
<td>Sgs1-K706A</td>
<td>ypo086w-ph, yeF3-f650s, sqc4-4</td>
<td>brn1-9, ydr331w-ph, mps3-1, arc40-ph, cd20-3, dbp11-1, ap3-g302y, cm1-1</td>
</tr>
<tr>
<td>Hrq1Δ Sgs1Δ</td>
<td>DNA2-1, nse4-ts2, mps1-2, sqc4-4</td>
<td>kch1, pol1-17, nut2-ph, pse1-41, ndc1-4, tor2-29, mps1-1, nsf1-6, prp6-ts, ypr06w-ph, mps3-1, rad54</td>
</tr>
<tr>
<td>Hrq1-K318A Sgs1Δ</td>
<td>DNA2-1, nse4-ts2</td>
<td>arf1, ala1-1, gna1-ts, gie1-4, sfl-7, nut1, tim22-19, prp6-ts, cd23-1, cd20-1, cd13-1, rad54, nse5-ts1, yol106c-ph</td>
</tr>
<tr>
<td>Hrq1-K318A Sgs1-K706A</td>
<td>DNA2-1, nse4-ts2</td>
<td>yhl122w-ph, nut1, sfl-7, pse1-41, yjl174w-ph, cab1-ph, sm1-ts, kch1, tim22-19, sfl-1-3, yj011c-ph, prp6-ts, bemi1, yol106c-ph, act1-105</td>
</tr>
</tbody>
</table>

Negative interactors are listed from largest absolute value of their SGA score to the smallest, but positive interactors are listed from the smallest absolute value of their SGA score to the largest.
2014; Rogers et al. 2017; Nickens et al. 2018) and Sgs1 (Watt et al. 1996; Huang et al. 2001; Johnson et al. 2001; Azam et al. 2006) are both involved in telomere maintenance. Further research should be devoted to uncovering the links between the YMR265C, JCY1, YMR245W, YMR194C-A, and YMR031W-A gene products and RecQ biology in *S. cerevisiae*.

**Comparisons to previous screens**

A comprehensive set of SGA data already exists for all non-essential *S. cerevisiae* gene deletion strains (Costanzo et al. 2010; Costanzo et al. 2016), including the *hrlq1Δ* and *sgs1Δ* alleles screened in this work. Indeed, these strains were included as internal controls for the catalytically inactive helicase allele screens. Comparing our data to the previously published screens, there is good overlap between the significant positive and negative genetic interactors with *hrlq1Δ* and *sgs1Δ* (Fig. S4). However, the overlap is far from complete, despite the screens being performed using strains of the same genetic backgrounds and on the same equipment in the Donnelly Centre (University of Toronto). This underscores the importance of re-preforming such control screens when new alleles such as *hrlq1-K318A* and *sgs1-K706A* are investigated.

**CONCLUSIONS AND PERSPECTIVES**

Here, we have reported a comprehensive set of synthetic genetic interactions between most of the genes in the *S. cerevisiae* genome and deletion and catalytically inactive alleles of the Hrql and Sgs1 RecQ family helicases. This data set improves upon the existing sets of active and inactive double mutants. As with the previously published screens, there is good overlap between the significant positive and negative genetic interactors with *hrlq1Δ* and *sgs1Δ* (Fig. S4). However, the overlap is far from complete, despite the screens being performed using strains of the same genetic backgrounds and on the same equipment in the Donnelly Centre (University of Toronto). This underscores the importance of re-preforming such control screens when new alleles such as *hrlq1-K318A* and *sgs1-K706A* are investigated.

Here, we have reported a comprehensive set of synthetic genetic interactions between most of the genes in the *S. cerevisiae* genome and deletion and catalytically inactive alleles of the Hrql and Sgs1 RecQ family helicases. This data set improves upon the existing sets of known *hrlq1Δ* and *sgs1Δ* interactions and expands the genetic interactome landscape of *hrlq1* and *sgs1* mutants by including interactions with the inactive *hrlq1-K318A* and *sgs1-K706A* alleles, as well as all combinations of the null and inactive double mutants. As with the five human RecQ helicases (Croteau et al. 2014), it is clear that *HRQ1* and *SGS1* genetically interact in yeast, and perhaps they may also physically interact.

These SGA analyses have also generated testable hypotheses to drive on-going and future research. The genetic interactomes of *hrlq1* and *sgs1* suggest links to transcription, much like the functional interaction between human RECQL5 and RNA polymerase II (Aygun et al. 2008; Izumikawa et al. 2008; Saponaro et al. 2014). Indeed, we have already shown that *hrlq1* cells are sensitive to the general transcription inhibitor caffeine and that *hrlq1* mutants alter the *S. cerevisiae* transcriptome (Rogers et al.) Similarly, it will be exciting to discover why double *hrlq1 sgsl* mutations are particularly deleterious to defects in lagging strand synthesis during DNA replication.

Obviously, our focus on the strongest negative synthetic genetic interactions in the SGA data set reported here is far from all encompassing. There are certainly important conclusions to be drawn from more subtle negative effects, considering the positive genetic interactions, and comparing the genetic interactomes between the various *hrlq1* and *sgs1* mutants analyzed. It is our hope that these data will spur additional research in the field, both with the yeast RecQs and their human homologs RECQL4 and BLM, as well as with proteomic investigations to identify complex physical interactomes, to fully establish the roles of these enzymes in genome integrity.

**ACKNOWLEDGMENTS**

We thank Amy Caudy and Stephen Kowalczykowski for sharing plasmids, the University of Toronto for performing the SGA analyses, Michael Costanzo and members of the Boone lab for help with data collection and interpretation, and members of the Bochman lab for critically reading this manuscript. This research was supported by the College of Arts and Sciences, Indiana University (to MLB), the Indiana University Collaborative Research Grant fund of the Office of the Vice President for Research (to MLB), the American Cancer Society (RSG-16-180-01-DMC to MLB), and the National Institutes of Health (1R03GM133437 to MLB).

**LITERATURE CITED**


Costanzo, M., B. VanderSluis, E. N. Koch, A. Baryshnikova, C. Pons et al., 2016 A global genetic interaction network maps a wiring diagram of


Communicating editor: G. Brown