Comprehensive synthetic genetic array analysis of alleles that interact with mutation of the Saccharomyces cerevisiae RecQ helicases Hrq1 and Sgs1

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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, transcription, and lagging strand synthesis during DNA replication.
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. 2020).

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 inter-strand crosslink (ICL) repair, telomere maintenance, and the unwinding of noncanonical DNA secondary structures (Bochman et al. 2014; Rogers and Bochman 2017; Rogers et al. 2017; Nickens et al. 2018; Rogers et al. 2020) like human RECQL4 (Jin et al. 2008; Ghosh et al. 2011; Ferrarelli et al. 2013; Keller...
Contemporaneous work using a multi-omics approach also suggests that Hrq1 has roles in transcription, chromosome/chromatin dynamics, rRNA processing/ribosomal maturation, and in the mitochondria (Rogers et al.)².

Despite these advances in yeast RecQ research, little is known about the genetic interactions that occur between HRQ1 and SGS1 or the physical interactions between Hrq1 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 Hrq1 and Sgs1? Two reports demonstrate that various combinations of hrq1 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**

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² Rogers et al., The genetic and physical interactomes of the *Saccharomyces cerevisiae* Hrq1 helicase, submitted as a companion paper to *G3.*
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 Hrq1 and Sgs1 and their roles in other biological processes are not known. We sought to identify genes whose mutation affects the growth of hrq1 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 (hrq1Δ and sgs1Δ) and catalytically inactive mutants (hrq1-K318A and sgs1-K706A) of the helicases in all combinations (hrq1Δ, sgs1Δ, hrq1-K318A, sgs1-K706A, hrq1Δ sgs1Δ, hrq1Δ sgs1-K706A, hrq1-K318A sgs1Δ, and hrq1-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 HRQ1 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 HRQ1 locus (MB527 and MB528). The confirmed hrq1Δ strain was named
MBY639. The hrq1-K318A allele was introduced into the Y8205 background in a similar manner.
First, an hrq1-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
HRQ1 locus with the same oligonucleotide set to confirm insertion of the NatMX marker.
Finally, PCR products of the expected size for hrq1-K318A(NatMX) were sequenced using
oligonucleotide MB932 to confirm the presence of the K318A mutation. The verified hrq1-
K318A strain was named MBY644.

The SGS1 gene was deleted from Y8205 (Table 1) in the same manner as the HRQ1::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
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 hrq1Δ sgs1Δ and hrq1-K318A sgs1Δ strains were generated by deleting SGS1 in strains MBY639 and MBY644, respectively, using a URA3 cassette amplified from pUG72 (GUELDENER 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 hrq1Δ sgs1-K706A and hrq1-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 hrq1Δ sgs1-K706A and hrq1-K318A sgs1-K706A strains were named MBY674 and MBY676, respectively. Further details concerning strain construction are available upon request.

SGA analysis of the hrq1Δ, sgs1Δ, hrq1-K318A, sgs1-K706A, hrq1Δ sgs1Δ, hrq1Δ sgs1-K706A, hrq1-K318A sgs1Δ, and hrq1-K318A sgs1-K706A mutants was performed at the University of Toronto using previously described methods (TONG et al. 2001; TONG et al. 2004). All query strains and the control HO::natMX strain 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.

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. 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 $> |0.08|$.

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 assays to compare the growth of the double or triple mutants to their parental strains and wild-type.
Statistical analysis

Data were analyzed and graphed using GraphPad Prism 6 software. The reported values are averages of ≥ 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 and a description of the other supplementary tables included in Files S2 and S3. File S2 contains Tables S2-S9, and File S3 contains Tables S10-S17.

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 $hrq1-K318A$, 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
sgs1Δ and sgs1-K706A. This is consistent with the generally more modest phenotypes of
hrq1Δ and hrq1-K318A strains compared to sgs1Δ and sgs1-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-K318A sgs1Δ mutant, which generated 880 synthetic interactions, a nearly twofold
increase over the additive effect of the 132 hrq1-K318A and 312 sgs1Δ interactions individually
(compared to ~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 hrq1Δ and hrq1-K318A
mutants had the fewest number of synthetic interactions by a factor of > 2.1 compared to
sgs1Δ and sgs1-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 synthetic genetic interactions, with outliers denoted as single points, for the single-gene deletion collection (Fig. 1A-C) and the TS collection (Fig. 1D-F). The outliers represent the mutants with the strongest synthetic phenotypes. As shown in Figures 1A and 1D, most synthetic phenotypes were mild decreases or increases in the growth of the double and triple mutant colonies. There were no significant differences in the distribution of the SGA scores among any of the mutant sets generated by crossing the query strains to the single-gene deletion collection. However, several significant differences were found in the distributions of positive SGA scores for the mutant sets yielded from the crosses to the TS collection. These includes mild differences between hrq1-K318A vs. hrq1Δ sgs1Δ (p = 0.0123) and sgs1Δ vs. hrq1-K318A sgs1Δ (p = 0.0303), intermediate differences for sgs1Δ vs. hrq1Δ sgs1Δ (p = 0.0016) and sgs1-K706A vs. hrq1-K318A sgs1-K706A (p = 0.0070), and strong differences between sgs1-K706A and hrq1Δ sgs1Δ, hrq1Δ sgs1-K706A, and hrq1-K318A sgs1Δ (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 simplicity, only the negative genetic interactions are discussed in further detail below.

hrq1Δ interactions
The deletion of \textit{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 \textit{et al.})\textsuperscript{1}. For instance, RECQL4 is the only human RecQ found in both the nucleus and mitochondria (Croteau \textit{et al}. 2014), and Hrq1 likewise localizes to both organelles (Koh \textit{et al}. 2015) and physically interacts with mitochondrial proteins (Rogers \textit{et al.})\textsuperscript{1}. Here, we found strong negative synthetic genetic interactions between \textit{hrq1}Δ and mutation of \textit{MRM2}, a mitochondrial 2' O-ribose methyltransferase whose deletion results in mitochondrial DNA (mtDNA) loss (Pintard \textit{et al}. 2002), and \textit{YSC83}, a mitochondrial protein of unknown function (Sickmann \textit{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 \textit{hrq1}Δ with mutation of \textit{SPO16}, which is involved in the meiotic cell cycle (Shinohara \textit{et al}. 2008), and \textit{RAD14}, a nucleotide excision repair protein (Guzder \textit{et al}. 2006) and regulator of transcription (Chaurasia \textit{et al}. 2013). Deletion of \textit{HRQ1} also negatively interacted with mutation of \textit{SLX9}, an rRNA processing factor (Bax \textit{et al}. 2006) that additionally binds G-quadruplex (G4) DNA structures (Gottz \textit{et al}. 2019). This is provocative in light of the connection of Hrq1 to rRNA processing and ribosome biogenesis (Rogers \textit{et al}.\textsuperscript{1}, as well as the fact that G4 structures are preferred substrates for Hrq1 \textit{in vitro} (Rogers \textit{et al}. 2017). Finally, mutations in \textit{YEF3}, \textit{YUR1}, \textit{MUP3}, and \textit{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), TCO89, 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 importer (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-Soety 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.

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), 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 (Olmez et al. 2015). Thus, multiple different mms4 alleles were hits in the screen, again acting as positive controls for our approach.

In addition to known effects, we also discovered three new negative interactions with sgs1Δ. These include the deletions of SWC4 and SWC5, 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 (Mondoux and Zakian 2005). As with hrq1Δ and hrq1-K318A, the yef3-f650s TS allele was also a negative genetic interactor with sgs1Δ (Table 5).
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, SLX4, SLX9, SLX1, SWC5, WSS1, MMS4, 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 (*RNH203, SLX8, RNH202*, and *MUS81*) are previously reported negative interactors with *sgs1Δ* (see: [https://www.yeastgenome.org/locus/S000004802/interaction](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 *hrq1* 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.* 2008; *Izumikawa et al.* 2008; *Saponaro et al.* 2014), Sgs1 is involved in transcription, a hypothesis also put forth for Hrq1 (*Rogers et al.*). In support of this, the remaining interactor *ASK10* encodes a glycerol channel regulator (*Beese et al.* 2009) that also associates with RNA polymerase II (*Page et al.* 1996).
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, SAE2, BUL1, CAT8, YMR031W-A, ICY1, RPL6B, DSK2, RIT1, SWI4, COX7, RGM1, TRM732, ROY1, YMR265C, ELG1, YMR194C-A, OCA5, RTT107, RAD27, YJR084W, INP1, FPR2, YDR186C, YMR245W, KAP114, DNA2, NSE4, MOB2, SMT3, ROT1, 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, SWI4*, 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 nucleases involved in Okazaki fragment processing (*Kao et al. 2004*), Cdc2 is the catalytic subunit of DNA polymerase δ (*Johnson et al. 2015*), and Pri2 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; 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, ICY1, YMR245W, YMR194C-A, and YMR031W-A gene products and RecQ biology in S. cerevisiae.

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 Hrq1 and Sgs1 RecQ family helicases. This data set improves upon the existing sets of known hrq1Δ and sgs1Δ interactions and expands the genetic interactome landscape of hrq1 and sgs1 mutants by including interactions with the inactive hrq1-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 hrq1 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 hrq1 cells are sensitive to the general transcription inhibitor caffeine and that hrq1 mutations alter the S. cerevisiae transcriptome (Rogers et al.)¹. Similarly, it will be exciting to discover why double hrq1 sgs1 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 hrq1 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 incorporate physical interactomes, to fully establish the roles of these enzymes in genome integrity.
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FOOTNOTES

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Table 1. Strains used in this study.

| Name  | Genotype                                                                 | Source                           |
|-------|---------------------------------------------------------------------------|----------------------------------|
| Y8205 | MATα can1Δ::STE2pr-Sp_his5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 ura3Δ0        | (TONG et al. 2001)               |
| MBY346| MATα ura3-52 lys2-801_amber ade2-101_ochre trp1Δ63 his3Δ200 leu2Δ1 hxt13::URA3 hrq1::hrq1-K318A-NatMX | (BOCHMAN et al. 2014)            |
| MBY639| MATα can1Δ::STE2pr-Sp_his5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 ura3Δ0 hrq1::natMX | This study                       |
| MBY640| MATα can1Δ::STE2pr-Sp_his5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 ura3Δ0 sgs1::natMX | This study                       |
| MBY642| MATα can1Δ::STE2pr-Sp_his5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 ura3Δ0 sgs1::sgs1-K706A(natMX) | This study                       |
| MBY643| MATα can1Δ::STE2pr-Sp_his5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 ura3Δ0 hrq1::natMX sgs1::URA3 | This study                       |
| MBY644| MATα can1Δ::STE2pr-Sp_his5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 ura3Δ0 hrq1::hrq1-K318A(natMX) | This study                       |
| MBY645| MATα can1Δ::STE2pr-Sp_his5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 ura3Δ0 hrq1::hrq1-K318A(natMX6) sgs1::URA3 | This study                       |
| MBY674| MATα can1Δ::STE2pr-Sp_his5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 ura3Δ0 sgs1::sgs1-K706A(natMX6) hrq1::URA3 | This study                       |
| MBY676| MATα can1Δ::STE2pr-Sp_his5 lyp1Δ::STE3pr-LEU2 his3Δ1 leu2Δ0 ura3Δ0 hrq1::hrq1-K318A(URA3) sgs1::sgs1-K706A(natMX6) | This study                       |
Table 2. Results of the SGA analyses for all query strains crossed to the single-gene deletion collection.

| Query strain          | No. negative genetic interactions | No. positive genetic interactions | Total |
|-----------------------|-----------------------------------|-----------------------------------|-------|
| *hrq1Δ*               | 76                                | 41                                | 117   |
| *hrq1-K318A*          | 84                                | 48                                | 132   |
| *sgs1Δ*               | 164                               | 148                               | 312   |
| *sgs1-K706A*          | 189                               | 172                               | 361   |
| *hrq1Δ sgs1Δ*         | 361                               | 333                               | 694   |
| *hrq1Δ sgs1-K706A*    | 392                               | 396                               | 788   |
| *hrq1-K318A sgs1Δ*    | 442                               | 438                               | 880   |
| *hrq1-K318A sgs1-K706A* | 400                           | 396                               | 796   |

Table 3. Results of the SGA analyses for all query strains crossed to the temperature-sensitive allele collection.

| Query strain          | No. negative genetic interactions | No. positive genetic interactions | Total |
|-----------------------|-----------------------------------|-----------------------------------|-------|
| *hrq1Δ*               | 65                                | 54                                | 119   |
| *hrq1-K318A*          | 82                                | 61                                | 143   |
| *sgs1Δ*               | 155                               | 197                               | 352   |
| *sgs1-K706A*          | 138                               | 172                               | 310   |
| *hrq1Δ sgs1Δ*         | 156                               | 246                               | 402   |
| *hrq1Δ sgs1-K706A*    | 238                               | 260                               | 498   |
| *hrq1-K318A sgs1Δ*    | 200                               | 268                               | 468   |
| *hrq1-K318A sgs1-K706A* | 223                           | 232                               | 455   |
Table 4. Genes whose deletion cause the strongest growth phenotypes when combined with the hrq1 and sgs1 mutants.

| Query strain | Negative interactions<sup>a</sup> | Positive interactions |
|--------------|----------------------------------|-----------------------|
| hrq1Δ        | SPO16, RAD14, YSC83, MRM2, SLX9, YUR1, YDR455C, MUP3, PHO5 | SHE1                  |
| hrq1-K318A   | HAP3, TCO89, VPS41, HAP2, RAD14, CBC2, TOM70 | VAM7, SHE1, MRM2, EAP1, ARP8 |
| sgs1Δ        | YBR099C, PBY1, YBR100W, RRM3, SLX4, MMS4, SRS2, SLX9, WSS1, SLX1, SWC5 | MKS1, EAP1, YLR235C, HXT2, RPS10A, AIM14, EOS1, RTG1, BUD31, OCA5, YIL206C-A, RPL20B, RTG2, GRR1, RTG3, YGL214W, UGA1, YNL046W, MIR1, HAP2, LDH1 |
| sgs1-K706A   | SRS2, SLX4, SLX9, SLX1, SWC5, RNH203, WSS1, SPO16, SLX8, ASK10, RNH202, MUS81, YBR099C, PBY1, YBR100W, MMS4, YSC83, HAP3, ELG1 | EMC5, RPL20B, ITC1, RIM101, BUD31, SPP1, RAD54, YGL214W, SHU1 |
| hrq1Δ sgs1Δ  | MUS81, MMS4, PBY1, YBR100W, YBR099C, CAT2, SLX1, WSS1, SLX4, SRS2, AEP2, SAE2, RRM3, BUL1, CAT8, YMR031W-A, ICY1, RPL6B, DSK2, SLX8, RIT1, SWI4, COX7, RGM1, ASK10, TRM732, ROY1, YMR265C | MUB1, SNZ1, YMR102C, UBP1, MFG1, HBT1, EOS1, MIR1, AIP1, PTP1, BRE4, GCS1, RPO41, SSB1, MRM2, OST4, HAP2, KCH1 |
| hrq1Δ sgs1-K706A | SLX1, MUS81, SRS2, RRM3, BUL1, SLX8, AEP2, ASK10, YMR031W-A, SLX4, ELG1, WSS1, YSC83, TRM732, SAE2, ROY1, YMR194C-A, YBR099C, OCA5, SWI4 | YMR075C-A, IRC21, EOS1, CSM2, YKR040C, SAM1, SNZ1, RIM9, RPO41, HBT1, RPL20B, YMR102C, SEG1, MFG1, AIP1, HAP3, STV1, OST4, BUD31, KCH1, BRE4, YMR087W, PTP1, GCS1, TKL1, YJR120W, SSB1, WWM1 |
| hrq1-K318A sgs1Δ | MUS81, SRS2, WSS1, SLX4, RTT107, SLX1, CAT2, ASK10, YMR194C-A, PBY1, TRM732, RRM3, CAT8, YBR099C, | UBP1, RRI1, WHI4, UBX4, GYP7, YMR075C-A, ENV10, MUB1, SNZ1, HBT1, MIR1, EOS1, YMR087W, SNO1,
| Negative interactors | Positive interactors |
|----------------------|----------------------|
| MMS4, YBR100W, SGS1, RIT1, BUL1, ICY1, RAD27 | FMP45, SEG1, RPS10A, IMP2, YDR161W, SRT1, MFG1, BUD31, RPL20B, YLR346C, SHU1, YMR102C, MBR1, SPO16, RPO41, AIP1, PTP1, GCS1, YDR514C, SCY1, BRE4, SSB1, OST4 |
| hrr1-K318A sgs1-K706A | MUS81, YJR084W, SLX1, RRM3, CAT2, SWI4, WSS1, YBR100W, SLX8, YBR099C, INP1, YMR194C-A, SLX4, TRM732, FPR2, YDR186C, YMR245W, MMS4, PBY1, KAP114, SRS2, OCA5 | SNZ1, YDL211C, EOS1, APJ1, IGO1, SRT1, MBR1, MFG1, YJR120W, YLR235C, RIM9, IRC21, BUD31, HBT1, YDR008C, CSM2, RII1, BRE4, YMR087W, YMR102C, RPO41, GCS1, PTP1, SSB1, HAP3 |

*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.*
Table 5. Temperature-sensitive alleles that cause the strongest growth phenotypes when combined with the \textit{hrq1} and \textit{sgs1} mutants.

| Query strain          | Negative interactions$^a$ | Positive interactions                                                                 |
|-----------------------|---------------------------|---------------------------------------------------------------------------------------|
| \textit{hrq1}\textbar | \textit{ye}f3-f650s       | \textit{mps3-1, sli}x9, \textit{act1-105, arp3-g302y, arp3-31, c}rm1-1                 |
| \textit{hrq1-K318A}   | \textit{ye}f3-f650s       | \textit{cse2, dbp5-2, c}rm1-1, \textit{mps1-1}                                      |
| \textit{sgs1}\textbar | \textit{ye}f3-f650s, \textit{efb1-4, swc4-4} |                                                                                      |
| \textit{sgs1-K706A}   | \textit{yrp086w-ph, ye}f3-f650s, \textit{swc4-4} | \textit{brn1-9, ydr331w-ph, \textit{mps3-1, arc40-ph, cdc20-3, dpb11-1, arp3-g302y, c}rm1-1} |
| \textit{hrq1}\textbar \textit{sgs1}\textbar | \textit{dna2-1, nse4-ts2, mob2-38, smt3-331, rot1-ph, mob2-22} | \textit{kch1, pol1-17, nuf2-ph, pse1-41, ndc1-4, tor2-29, \textit{mps1-1, nsl1-6, prp6-ts, ypr086w-ph, mps3-1, rad5}} |
| \textit{hrq1}\textbar \textit{sgs1}-\textit{K706A} | \textit{nse4-ts2, dbf4-1, rot1-ph, cdc2-1, mob2-28, cep3-1, nse4-ts1, mob2-22, smc6-9, mob2-38, mob2-14, prl2-1, nse1-16, mob2-8} | \textit{arf1, ala1-1, gna1-ts, gle1-4, sfi1-7, nut1, tim22-19, prp6-ts, cdc23-1, cdc20-1, cdc13-1, rad54, nse5-ts1, yol102c-ph} |
| \textit{hrq1-K318A}   | \textit{dbf4-1, dna2-1, sgs1, nse4-ts2} |                                                                                      |
| \textit{hrq1-K318A}   | \textit{nse4-ts2, rot1-ph, cdc2-1, mob2-38} | \textit{lip1-ph, ala1-1, gle1-4, srv2-2, sfi1-1, nut1, pse1-41, sth1-3, cdc28-td, gpi17-ph, tim22-19, arp3-31, yol102c-ph, prp43-ts2} |

$^a$ 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.
Figure 1. Analysis of the distribution of the magnitudes of the synthetic genetic interactions.

Violin plots of the synthetic genetic interactions with the single-gene deletion collection (A) and TS collection (D). The median values are denoted with dashed lines, and the quartiles are shown as solid lines. The SGA data are also shown in separate box and whisker plots drawn using the Tukey method for the negative (B) and positive (C) interactions with the deletion collection, as well as for the negative (E) and positive (F) interactions with the TS collection. The individually plotted points outside of the inner fences represent outliers (i.e., interactions with mutants yielding the strongest SGA scores) and correspond to alleles whose SGA score is less than the value of the 25th quartile minus 1.5 times the inter-quartile distance (IQR) for negative interactions and alleles whose SGA score is greater than the value of the 75th quartile plus 1.5IQR for positive interactions. The significant differences between SGA data sets discussed in the main text were calculated using the Kruskal-Wallis test and Dunn’s multiple comparisons test.