The Genetic and Physical Interactomes of the Saccharomyces cerevisiae Hrq1 Helicase

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ABSTRACT The human genome encodes five RecQ helicases (RECQL1, BLM, WRN, RECQL4, and RECQL5) that participate in various processes underpinning genomic stability. Of these enzymes, the disease-associated RECQL4 is comparatively understudied due to a variety of technical challenges. However, Saccharomyces cerevisiae encodes a functional homolog of RECQL4 called Hrq1, which is more amenable to experimentation and has recently been shown to be involved in DNA inter-strand crosslink (ICL) repair and telomere maintenance. To expand our understanding of Hrq1 and the RecQ4 subfamily of helicases in general, we took a multi-omics approach to define the Hrq1 interactome in yeast. Using synthetic genetic array analysis, we found that mutations of genes involved in processes such as DNA repair, chromosome segregation, and transcription synthetically interact with deletion of HRQ1 and the catalytically inactive hrq1-K318A allele. Pull-down of tagged Hrq1 and mass spectrometry identification of interacting partners similarly underscored links to these processes and others. Focusing on transcription, we found that hrq1 mutant cells are sensitive to caffeine and that mutation of HRQ1 alters the expression levels of hundreds of genes. In the case of hrq1-K318A, several of the most highly upregulated genes encode proteins of unknown function whose expression levels are also increased by DNA ICL damage. Together, our results suggest a heretofore unrecognized role for Hrq1 in transcription, as well as novel members of the Hrq1 ICL repair pathway. These data expand our understanding of RecQ4 subfamily helicase biology and help to explain why mutations in human RECQL4 cause diseases of genomic instability.

KEYWORDS DNA helicase RecQ Hrq1 Saccharomyces cerevisiae transcription

A multitude of cellular processes are necessary to ensure the maintenance of genome integrity, including high fidelity DNA replication, recombination and repair, telomere maintenance, and transcription. Among the proteins that are involved, DNA helicases represent one of only a few enzyme classes that are vital to all of these processes (Bochman 2014). Helicases are enzymes that use the power of ATP hydrolysis to drive conformational changes that enable translocation along DNA and unwinding of DNA base pairs (Abdelhaleem 2010; Brosh and Matson 2020). Because these enzymes are involved in so many critical functions in vivo, it is unsurprising that mutations in genes encoding helicases are causative of or linked to numerous diseases of genomic instability such as cancer and aging (Monnat 2010; Suhasini and Brosh 2013; Uchiumi et al. 2015).

Despite their prominent roles in maintaining genome integrity however, we often lack a detailed understanding of why a particular mutation in a helicase is associated with a pathological disorder. In other words, what cellular processes are impacted that eventually precipitate a disease state when a helicase is mutated? Part of the difficulty in answering this question is that many helicases are multi-functional, and a defect in any one of a number of functions could cause genomic instability (Hickson 2003). Another issue is that helicases are numerous, with > 100 predicted to be encoded by typical eukaryotic genomes (Eki 2010), and many helicases share...
partially redundant or backup roles, which complicates identification of phenotypes without thorough genomic or proteomic approaches.

One such under-studied and disease-linked helicase is the human RECQL4 protein. Dozens of mutant alleles of RECQL4 cause three different diseases (Baller-Gerold syndrome (Van Maldergem et al. 1993), RAPADILINO (Vargas et al. 1992), and Rothmund-Thomson syndrome (Liu 2010)) characterized by a predisposition to cancers, but it is unclear why these mutations cause disease. RECQL4 is difficult to study in vivo because it is an evolutionary chimera between a RecQ family helicase and Sld2 (Capp et al. 2010), an essential DNA replication initiation factor in lower eukaryotes (Kamimura et al. 1998). Helicase activity by RECQL4 is not needed for DNA replication, but pleiotropic defects in replication hamper the analysis of the roles of the helicase domain when studying recql4 mutants. Similarly, RECQL4 is difficult to study in vitro because the protein is large (~135 kD) with a natively disordered N-terminus (Keller et al. 2014), making the generation of recombinant protein for biochemistry arduous (Macris et al. 2006; Bochman et al. 2014). Thus, although RECQL4 is reported to be involved in telomere maintenance (Ghosh et al. 2011) and DNA inter-strand crosslink (ICL) repair (Jin et al. 2008; Rogers et al. 2020b), its mechanism of action in these pathways is unknown.

Recently, we established the Saccharomyces cerevisiae Hrq1 helicase as a functional homolog of the helicase portion of RECQL4, showing that it too is linked to telomere maintenance and ICL repair (Bochman et al. 2014; Rogers and Bochman 2017; Rogers et al. 2017; Nickens et al. 2018; Nickens et al. 2019; Rogers et al. 2020a). However, because Sld2 is a separate protein in S. cerevisiae and recombinant Hrq1 is more amenable to biochemistry, we have been able to delve into the molecular details of Hrq1 in the maintenance of genome integrity. For instance, Hrq1 synergizes with the helicase Pif1 to regulate telomerase activity, likely establishing telomere length homeostasis in vivo (Nickens et al. 2018). In ICL repair, Hrq1 stimulates the translational nuclease activity of Pso2 to aid in removal of the ICL (Rogers et al. 2020a). During the course of these investigations, we have also found that alleles of HRQ1 genetically interact with mutations in the gene encoding the other RecQ family helicase in S. cerevisiae, SGS1 (Bochman et al. 2014), and that Hrq1 may be involved in the maintenance of DNA motifs capable of forming G-quadruplex (G4) structures (Rogers et al. 2017). These facts are mirrored by the interaction of RECQL4 with the human Sgs1 homolog BLM (Singh et al. 2012) and the ability of RECQL4 to bind to and unwind G4 DNA (Keller et al. 2014).

To gain a more comprehensive understanding of the roles of RecQ4 subfamily helicases in genome integrity, we sought to define the Hrq1 interactome in yeast. In a companion manuscript (Sanders, Nguyen et al.; manuscript #401709), we performed synthetic genetic array (SGA) analysis of hrq1Δ and hrq1-K318A (catalytically inactive mutant) cells using the yeast deletion collection and the temperature-sensitive (TS) collection. Here, hundreds of significant positive and negative interactions were identified, with gene ontology (GO) term enrichment for processes such as transcription and RNA processing in addition to expected functions such as DNA repair. Mass spectrometry (MS) analysis of proteins that physically interact with Hrq1 returned similar results. Our initial characterization of the link between Hrq1 and transcription revealed that hrq1 mutant cells are sensitive to the transcription stressor caffeine and that the hrq1Δ and hrq1-K318A mutations affect the transcription of hundreds of genes, many of which are known or hypothesized to be related to transcription, DNA ICL repair, and the cytoskeleton.

MATERIALS AND METHODS

Strain construction

The Hrq1 gene was deleted in Y8205 (Table 1) by transforming in a NatMX cassette that was PCR-amplified from 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. Hrq1 was tagged with a 3xFLAG epitope in the YPH499 genetic background by transformation of a 3xFLAG(His3MX6) cassette that was PCR-amplified from the pFA6a-3xFLAG-His3MX6 plasmid (Funakoshi and Hochstrasser 2009) using oligonucleotides MB1028 and MB1029. Proper integration was assessed by PCR and sequencing as described above for hrq1-K318A(NatMX). The confirmed Hrq1-3xFLAG strain was named MBY520.

SGA analysis

SGA analysis of the hrq1Δ and hrq1-K318A alleles was performed at the University of Toronto using previously described methods (Tong et al. 2001; Tong et al. 2004). The hrq1Δ mutants were crossed 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 mutants for analysis. Quantitative scoring of the genetic interactions was based on colony size. The SGA score measures the extent to which a double mutant colony size deviates from the colony size expected from combining two mutations together. The data include both negative (putative synthetic sick/lethal) and positive interactions (potential epistatic or suppression interactions) involving hrq1Δ and hrq1-K318A. 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|. It should be noted that only top-scoring interactions were confirmed by remaking and reanalyzing the double mutants by hand. The full SGA data are available in our companion manuscript (Sanders, Nguyen, et al.; manuscript #401709).

Confirmation of top SGA hits

The top five positive and negative interactors with hrq1Δ and hrq1-K318A from the single-gene deletion and TS arrays were reanalyzed by hand to confirm their phenotypes. Briefly, the SGA query strains MBY639 and MBY644 (NatP) were mated to MATa tester strains from the arrays (CanP), sporulated, and then analyzed by random spore analysis (Lichten 2014), spot dilution (Andis et al. 2018), and/or growth curve (Ononye et al. 2020) assays and/or growth curve analyses of NatR KanR spore clones compared to the parental single-mutant strains and wild-type.
Hrq1-3xFLAG affinity pulldown

To immunoprecipitate Hrq1-3xFLAG and its associated proteins, strain MBY520 was grown to an optical density at 600 nm (OD600) of ~1.5 in YPD medium at 30°C with shaking. The cells were harvested by centrifugation at 4°C, washed with 50 mL of sterile ice-cold H2O, and harvested as before. The cell pellet was then resuspended in 100 μL/g of cells resuspension buffer (20 mM Na-HEPES, pH 7.5, and 1.2% w/v PEG-8000) supplemented with 10 μg/mL DNase I and protease inhibitor cocktail (600 nM leupeptin, 2 μM pepstatin A, 2 mM benzamidine, and 1 mM phenylmethanesulfonyl fluoride). This cell slurry was slowly dripped into liquid nitrogen to generate frozen yeast "popcorn", which was stored at -80°C until use. To cryo-lyse the cells, the popcorn was ground in a freezer mill with dry ice. The resultant powder was collected into 50-mL conical tubes that were loosely capped and stored at -80°C. To perform the Hrq1 pull down, the cell powder was resuspended in 2.5 g powder per 25 mL lysis buffer (40 mM Na-HEPES, pH 7.5, 10% glycerol, 350 mM NaCl, 0.1% Tween-20, and protease inhibitor cocktail) with gentle agitation. Then, 100 U DNase I and 10 μL of 30 mg/mL heparin were added, and the sample was incubated for 10 min at room temperature with gentle agitation. Cellular debris was pelleted by centrifugation at 14,000 g for 10 min at 4°C. Then, 100 μL of anti-FLAG agarose slurry was washed and equilibrated with lysis buffer, and the clarified lysate and anti-FLAG resin were added to a fresh 50-mL conical tube. This suspension was incubated at 4°C overnight on a nutator. The resin and lysate were subsequently placed in a 30-mL chromatography column, and the lysate was allowed to flow through the resin by gravity. The anti-FLAG agarose was washed with 30 mL lysis buffer, and the beads were then resuspended in 150 μL lysis buffer and transferred to a 1.5-mL microcentrifuge tube. At this point, the sample could be used for proteinase digestion and MS analysis, or proteins could be eluted from the resin and examined by SDS-PAGE and Coomassie staining.

Label-free quantitative proteomics interactome analysis

For on-bead digestion, 500 μL of trypsin digestion buffer (50 mM NH4HCO3, pH 8.5) was used to resuspend the FLAG resin. To this slurry, 10 μL of 0.1 μg/μL Trypsin Gold (Promega) was added and allowed to incubate overnight at 37°C with shaking. After digestion, the FLAG resin was separated from the digested peptides via spin columns and centrifugation. Formic acid (0.1% final concentration) was added to the supernatant to quench the reaction. After digestion, the peptide mix was separated into three equal aliquots. Each replicate was then loaded onto a microcapillary column. Prior to sample loading, the microcapillary column was packed with three phases of chromatography resin: reverse phase resin, strong cation resin, and reverse phase resin, as previously described (Florens and Washburn 2006; Mosley et al. 2011; Mosley et al. 2013). An LTQ Velos Pro with an in-line Proxeon Easy nLC was utilized for each technical replicate sample, with a 10-step MudPIT method. In MS1, the 10 most intense ions were selected for MS/MS fragmentation, using collision induced dissociation (CID). Dynamic exclusion was set to 90 s with a repeat count of one. Protein database matching of RAW files was performed using SEQUEST and Proteome Discoverer 2.2 (Thermo) against a FASTA database from the yeast Uniprot proteome. Database search parameters were as follows: precursor mass tolerance = 1.4 Da, fragment mass tolerance = 0.8 Da, up to two missed cleavages were allowed, enzyme specificity was set to fully tryptic, and minimum peptide length = 6 amino acids. The false discovery rate (FDR) for all spectra was <1% for reporting as PSM. Percolator, within Proteome Discoverer 2.2, was used to calculate the FDR (Käll et al. 2007).

SAINT probability scores were calculated as outlined in the Contaminant Repository for Affinity Purification (CRAPome) website (Mellacheruvu et al. 2013) and other publications ((Breitkreutz et al. 2010; Choi et al. 2011; Choi et al. 2012; Kwon et al. 2013).

Caffeine sensitivity

The sensitivity of hrq1 mutant cells to caffeine was assessed both qualitatively and quantitatively. In the first method, cells of the indicated strains were grown overnight in YPD medium at 30°C with aeration, diluted to OD600 = 1 in sterile H2O, and then serially diluted 10-fold to 10^-4. Five microliters of these dilutions were then spotted onto YPD agar plates and YPD agar plates supplemented with 10 mM caffeine. The plates were incubated at 30°C for 2 days before capturing images with a flatbed scanner and scoring growth. In the second method, the overnight cultures were diluted to OD600 = 0.01 into YPD or YPD supplemented with various concentrations of caffeine. They were then treated as described in (Ononye et al. 2020) with slight modifications. Briefly, 200 μL of each culture was placed in duplicate into wells in 96-well plates, and each well was overlaid with mineral oil to prevent evaporation. The plates were incubated (30°C with shaking) in a Synergy1H microplate reader (BioTek), which recorded OD600 measurements at 15-min intervals for 24 h. The mean of the OD600 readings for each strain was divided by the mean OD600 of the same strain grown in YPD.

RNA-seq

Cells were harvested from mid-log phase cultures grown in YPD medium, and total RNA was prepared using a YeastStar RNA kit (Zymo

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Table 1 Strains used in this study

| Name    | Genotype                                                                 | Source          |
|---------|--------------------------------------------------------------------------|-----------------|
| Y8205   | MATa can1::STE2pr-Sp_his5_ymy1::STE3pr-LEU2 his3Δ1 leu2Δ0 ura3Δ0        | (Tong et al. 2001) |
| YPH499  | MATa ura3-52 lys2-801_amber ade2-101_ochre trp1Δ63 his3Δ200 leu2Δ1         | (Sikorski and Hieter 1989) |
| MBY346  | MATa ura3-52 lys2-801_amber ade2-101_ochre trp1Δ63 his3Δ200 leu2Δ1 hxt1Δ::URA3 hrl1::hrq1-K318A-NatMX | (Bochman et al. 2014) |
| MBY520  | MATa ura3-52 lys2-801_amber ade2-101_ochre trp1Δ63 his3Δ200 leu2Δ1 HQR1::3xFLAG-His3MX6 | This study |
| MBY639  | MATa can1::STE2pr-Sp_his5_ymy1::STE3pr-LEU2 his3Δ1 leu2Δ0 ura3Δ0 hrl1::hrq1-NatMX | This study |
| MBY644  | MATa can1::STE2pr-Sp_his5_ymy1::STE3pr-LEU2 his3Δ1 leu2Δ0 ura3Δ0 hrq1::hrq1-K318A(NatMX) | This study |
Research). Sequencing libraries were prepared, and Illumina sequencing was performed by, Novogene Corporation. Data analysis was then performed by the Indiana University Center for Genomics and Bioinformatics. The sequences were trimmed using the Trim Galore script (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/), and reads were mapped to the S. cerevisiae genome using bowtie2 on local mode (Langmead and Salzberg 2012). Reads were counted, and differential expression analysis were performed using DESeq2 (Love et al. 2014). Two or three independent replicates of each strain were analyzed.

**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, RNA-seq data, and other experimental reagents are available upon request. File S1 contains detailed descriptions of all supplemental files, as well as Table S1 and Figure S1. File S2 contains the significant SGA hits. File S3 contains the full SAINT analysis results. File S4 contains the transcriptomic changes identified by RNA-seq. Supplemental material available at figshare: https://doi.org/10.25387/g3.13154291.

**RESULTS AND DISCUSSION**

The genetic interactome of HRQ1

In a companion study (Sanders, Nguyen, et al.; manuscript #401709), we crossed the hrq1Δ and hrq1-K318A alleles to the single-gene deletion and TS allele collections to generate all possible double mutants and assessed the growth of the resulting spore clones to identify negative and positive genetic interactions. A discussion of the significant positive and negative genetic interactions can be found therein. Here, we used GO Term mapping to identify cellular processes enriched for hrq1 genetic interactors in those data. These processes were similar when considering the hrq1Δ and hrq1-K318A datasets alone or together, so we present the combined results for simplicity. For all of the negative genetic interactions with hrq1Δ and hrq1-K318A, the top 10 GO terms were transcription by RNA polymerase II, regulation of organelle organization, DNA repair, chromatin organization, mitotic cell cycle, peptidyl-amino acid modification, cytoskeleton organization, mitochondrion organization, organelle fission, and response to chemical.

### Table 2 Gene Ontology (GO) Term enrichment of negative genetic interactors with hrq1

| GO Term (GO ID) | Genes Annotated to the GO Term | GO Term Usage | Genome Frequency of Use |
|-----------------|--------------------------------|---------------|-------------------------|
| transcription by RNA polymerase II (GO:0006366) | ABF1, CDC28, CDC73, CEG1, CSE2, EAF7, ESS1, GIM3, HMO1, HTZ1, MED11, NAB3, NUT1, RAD4, SDS3, SFG73, SIN3, SPT15, SPT3, SPT8, SRB2, SRB6, STH1, SUA7, SWI4, TAF11, TAF2, YJR084W | 28 of 191 genes, 14.66% | 536 of 6436 annotated genes, 8.33% |
| regulation of organelle organization (GO:0033043) | APC4, BDF2, CDC15, CDC20, CDC28, CDC73, CT16, DAM1, EB18, ESS1, GIC1, LTI1, MOB1, PEF1, PSE1, SDS3, SIN3, SPO16, TGS1, UTH1, VPS41 | 21 of 191 genes, 10.99% | 326 of 6436 annotated genes, 5.07% |
| DNA repair (GO:0006281) | ABF1, ACT1, BDF2, CDC28, CDC73, CST9, EAF7, NSE4, NSE5, POL1, PRP19, RAD14, RAD33, RAD4, RAD52, RAD54, RAD59, RNH201, RTT107, SIN3, STH1 | 21 of 191 genes, 10.99% | 300 of 6436 annotated genes, 4.66% |
| chromatin organization (GO:0006325) | ABF1, BDF2, CDC28, CLP1, CT16, EAF7, ESS1, GIC1, HTZ1, LGE1, RAD54, SDS3, SFG73, SIN3, SRT1, SPT3, SPT8, STH1, SWC5, UTH1, YCS4 | 21 of 191 genes, 10.99% | 310 of 6436 annotated genes, 4.82% |
| mitotic cell cycle (GO:0000278) | ACT1, APC4, CDC10, CDC15, CDC20, CDC28, CDC34, DAM1, GIC1, LTI1, MOB1, PEF1, POL1, PSE1, SIC1, SIN3, SWI4, TUB2, YCS4 | 20 of 191 genes, 10.47% | 206 of 6436 annotated genes, 5.07% |
| peptidyl-amino acid modification (GO:0018193) | ACT1, APJ1, CDC15, CDC28, CDC73, CST9, DBF2, EAF7, ESS1, LIP5, NSE4, NSE5, PSE1, SFG73, SMT3, SPO16, SPT3, SPT8, SWF1, TDA1 | 20 of 191 genes, 10.47% | 244 of 6436 annotated genes, 3.79% |
| cytoskeleton organization (GO:0007010) | ACT1, BBP1, CDC10, CDC15, CDC28, CDC31, CMD1, CTF13, DAM1, EB18, ENT1, ENT3, GIC1, NDC1, SPC29, STH1, SWF1, TUB2 | 18 of 191 genes, 9.42% | 272 of 6436 annotated genes, 4.23% |
| mitochondrion organization (GO:0007005) | ACT1, ATG1, ATG3, COA4, FCJ1, MDM35, PAM16, PAM17, PHB2, PTC1, QCR2, RCF2, SAM37, TIM18, TOM70, UTH1, YJR120W, YME1 | 18 of 191 genes, 9.42% | 279 of 6436 annotated genes, 4.33% |
| organelle fission (GO:0048285) | APC4, CDC10, CDC15, CDC20, CDC28, CST9, DAM1, DBF2, EPB2, GIC1, LTI1, MOB1, PSE1, RAD52, SPO16, TUB2, YCS4 | 17 of 191 genes, 8.90% | 268 of 6436 annotated genes, 4.16% |
| response to chemical (GO:0042221) | ACT1, ASK10, GIM3, GPR1, IRA2, MUP3, PTC1, SRB2, TDA1, TIM18, TMA19, TUB2, VPS27, YJR084W, YLR225C, YOS9 | 16 of 191 genes, 8.38% | 567 of 6436 annotated genes, 8.81% |
II, regulation of organelle organization, DNA repair, chromatin organization, mitotic cell cycle, peptidyl-amino acid modification, cytoskeleton organization, mitochondrion organization, organelle fission, and response to chemical (Table 2). Similarly, for all of the positive genetic interactions with *hrq1Δ* and *hrq1-K318A*, the top 10 GO terms were mitotic cell cycle, cytoskeleton organization, regulation of organelle organization, lipid metabolic process, DNA repair, transcription by RNA polymerase II, chromatin organization, chromosome segregation, organelle fission, and rRNA processing (Table 3). The DNA repair-related genes were not enriched for known or putative ICL repair genes as one might expect considering the ICL sensitivity phenotypes of *hrq1Δ* and *hrq1-K318A* cells. However, a chemogenomics screen exposing all of known or putative ICL repair genes as one might expect for known or putative ICL repair genes as one might expect considering the ICL sensitivity phenotypes of *hrq1Δ* and *hrq1-K318A*. Cells. However, a chemogenomics screen exposing all of known or putative ICL repair genes as one might expect for known or putative ICL repair genes as one might expect considering the ICL sensitivity phenotypes of *hrq1Δ* and *hrq1-K318A*。

A discussion of the strongest negative synthetic genetic interactions with *hrq1Δ* and *hrq1-K318A* is included in our companion manuscript (Sanders, Nguyen, et al.; manuscript #401709). Briefly, this included synthetic interactions with genes encoding genome integrity factors (e.g., RAD14 and *CBC2*) and mitochondrial proteins (e.g., *MRM2* and *TOM70*), consistent with the known roles of *Hrq1* and human RECQL4 in genome maintenance (Ghosh et al. 2011; Singh et al. 2012; Choi et al. 2013; Bochman et al. 2014; Choi et al. 2014; Leung et al. 2014; Rogers et al. 2017; Nickens et al. 2018; Rogers et al. 2020a) and their nuclear and mitochondrial localization (Croteau et al. 2012; Koh et al. 2015; Kumari et al. 2016).

Deletion alleles of *ARP8* and *SHE1* and *TS* alleles of *ACT1*, *ARP3*, *CSE2*, *MPS1*, and *MPS3* are among the strongest positive synthetic genetic interactors with *hrq1Δ* and/or *hrq1-K318A* (Tables 3 and S5). *Arp8* is a chromatin remodeling factor (Shen et al. 2000), and *Cse2* is a Mediator complex subunit required for RNA polymerase II regulation (Gustafsson et al. 1998), consistent with the GO Term enrichment described above. This may suggest that like human RECQL5 (Aygün et al. 2008; Izumikawa et al. 2008; Saponaro et al. 2014), *Hrq1* plays a role in transcription.

*SHE1* is a microtubule-associated protein (Bergman et al. 2012), as is human RECQL4 (Yokoyama et al. 2019). Likewise, *Mps1* and *Mps3* are linked to the microtubule cytoskeleton as proteins necessary for spindle pole body function (Friederichs et al. 2011; Meyer et al. 2013). We attempted to determine if *Hrq1* also binds to microtubules using an *in vitro* microtubule co-sedimentation assay (Walker et al. 2019), but found that *Hrq1* alone pellets during ultracentrifugation (data not shown). We hypothesize that this is due to the natively disordered N-terminus of *Hrq1* (Rogers et al. 2017; Rogers et al. 2020a), which may mediate liquid-liquid phase separation (LLPS) of recombinant *Hrq1* in solution. Ongoing experiments are addressing the LLPS of *Hrq1* alone and in combination with its ICL repair cofactor *Pso2* (Rogers et al. 2020a).

*ACT1* encodes the *S. cerevisiae* actin protein (Gallwitz and Seidel 1980), and *Arp3* is a subunit of the Arp2/3 complex that acts as an actin nucleation center (Machesky and Gould 1999). It is unclear why mutation of these cytoskeletal factors yields increased growth in combination with *hrq1* mutations. However, *arp3* mutation also decreases telomere length (Ungar et al. 2009). Thus, this synthetic

### Table 3 Gene Ontology (GO) Term enrichment of positive genetic interactors with *hrq1*

| GO Term (GO ID)                          | Genes Annotated to the GO Term | GO Term Usage in Gene List | Genome Frequency of Use |
|-----------------------------------------|--------------------------------|---------------------------|-------------------------|
| mitotic cell cycle (GO:0000278)          | ACT1, APC11, BRN1, CDC48, CDC6, CLB3, CSM1, DBP11, IFL1, MCD1, MPS1, MPS3, MYO2, PDSS, PYY1, PSE1, PSF1, SMC4, SP76, VRP1 | 20 of 151 genes, 13.25% | 373 of 6436 annotated genes, 5.80% |
| cytoskeleton organization (GO:0007010)   | ACT1, AIM14, ARP3, CDC48, CLB3, ICE2, IFL1, LAS17, MPS1, MPS2, MPS3, MYO2, NUM1, PYY1, RSP5, SPC29, STH1, TSC11, VRP1 | 19 of 151 genes, 12.58% | 272 of 6436 annotated genes, 4.23% |
| regulation of organelle organization (GO:0033043) | AIM14, APC11, ARP3, CDC48, CDC6, CLB3, IFL1, LAS17, MPS1, PCP1, PYY1, PSE1, RSP5, SEC23, SGV1, SP76, TSC11, VRP1 | 18 of 151 genes, 11.92% | 326 of 6436 annotated genes, 5.07% |
| lipid metabolic process (GO:0006629)     | ALG14, CDC1, CHO2, DGA1, GAA1, GPI10, GPI2, GWT1, LC1B, MGA2, OPI3, PHS5, RSP5, SAC1, SUR1, TSC11, VPS4 | 18 of 151 genes, 11.92% | 347 of 6436 annotated genes, 5.39% |
| DNA repair (GO:0006281)                  | ACT1, ARP8, CDC1, DBP11, IXR1, MCD1, NHP10, PDSS, POB3, POL3, PSF1, RAD3, RHN201, RSC2, SLX5, SLX8, STH1, TEL1 | 18 of 151 genes, 11.92% | 300 of 6436 annotated genes, 4.66% |
| transcription by RNA polymerase II (GO:0006366) | CAM1, CSE2, IXR1, MGA2, MOT1, NHP10, PDC2, POB3, RAD3, RGR1, RSC2, RSP5, SVG1, SP76, STH1 | 15 of 151 genes, 9.93% | 536 of 6436 annotated genes, 8.33% |
| chromatin organization (GO:0006325)     | ARP8, CAC2, CDC6, IES1, MGA2, MPS3, NHP10, ORC6, POB3, RSC2, RSP5, SP76, STH1, TEL1 | 14 of 151 genes, 9.27% | 310 of 6436 annotated genes, 4.82% |
| chromosome segregation (GO:0007059)      | APC11, BRN1, CDC48, CSV1, IFL1, MCD1, MPS1, MPS3, PDSS, RSC2, SMC4, SP24C, STH1 | 13 of 151 genes, 8.61% | 210 of 6436 annotated genes, 3.26% |
| organelle fission (GO:0048285)           | APC11, BRN1, CLB3, CSV1, IFL1, MCD1, MPS1, MPS3, NUM1, PDSS, PSE1, SMC4 | 12 of 151 genes, 7.95% | 268 of 6436 annotated genes, 4.16% |
| rRNA processing (GO:0006364)             | BMS1, FAL1, MAK5, MOT1, MRM2, POP4, RPF2, RPS23A, RPS6B, RPS9B, RPS5, SLX9 | 12 of 151 genes, 7.95% | 352 of 6436 annotated genes, 5.47% |
The genetic effect may be related to the role of Hrq1 in telomere maintenance (Bochman et al. 2014; Nickens et al. 2018).

The physical interactome of Hrq1
To complement our genetic analysis of hrq1 alleles, we also sought to identify the proteins that physically interact with Hrq1 in vivo. To do this, we cloned the sequence for a 3xFLAG tag in frame to the 3’ end of the HRQ1 gene, replacing its native stop codon. The tag does not disrupt any known activities of Hrq1, as demonstrated by the DNA ICL resistance of the Hrq1-3xFLAG strain (Fig. S1 and data not shown). Next, we snap-froze and cryo-lysed cells to preserve macromolecular complexes in near-native states (Mosley et al. 2011), immunoprecipitated Hrq1-3xFLAG and its associated proteins from the lysates, and analyzed them using a quantitative proteomics approach.

Overall, 290 interacting proteins were identified (Table S6), 77 of which had a SAINT score > 0.75 and were thus considered significant (Figure 1A). These 77 proteins are enriched for GO Term processes such as rRNA processing, ribosomal small subunit biogenesis, ribosomal large subunit biogenesis, cytoplasmic translation, transcription by RNA polymerase I, transcription by RNA polymerase II, RNA modification, DNA repair, chromatin organization, and peptidyl-amino acid modification (Table 4). Further, these categories are representative of the entire set of 290 proteins.

To demonstrate the robustness of these data, we identified Hrq1-interacting proteins that are subunits of larger macromolecular complexes involved in several of the GO Term processes listed above. For instance, among the rRNA processing and ribosomal small subunit biogenesis proteins (Figure 1B), several members of the small ribosomal subunit processome (https://www.yeastgenome.org/complex/CPX-1604) are significant Hrq1 interactors. Many more such proteins had SAINT scores < 0.75, suggesting that they may be secondary interactors (i.e., they physically interact with a significant Hrq1 interactor rather than Hrq1 directly) and/or more weakly associated subunits of the processome. Similarly, the transcription by RNA polymerase I (Figure 1C) and transcription by RNA polymerase II (Figure 1D) proteins contain members of multiple macromolecular complexes, including the RNA polymerase I (https://www.yeastgenome.org/complex/CPX-1664) and RNA polymerase II (https://www.yeastgenome.org/complex/CPX-2662) complexes themselves. As with the hrq1 SGA data in our companion manuscript (Sanders, Nguyen, et al.; manuscript #401709), these links to transcription are intriguing and reminiscent of the links of human RECQL5 to transcription (Aygun et al. 2008; Izumikawa et al. 2008; Saponaro et al. 2014).

Despite knowing some mechanistic details of how Hrq1 functions in ICL repair and telomere maintenance (Bochman et al. 2014; Rogers et al. 2017; Nickens et al. 2018; Nickens et al. 2019; Rogers et al. 2019), transcription by RNA polymerase I (C), and transcription by RNA polymerase II (D) factors are also shown. Members of macromolecular complexes associated with these processes are labeled and color coded: small ribosomal subunit processome (https://www.yeastgenome.org/complex/CPX-1604), green; RNA polymerase I (https://www.yeastgenome.org/complex/CPX-1664), II (https://www.yeastgenome.org/complex/CPX-2662), and III (https://www.yeastgenome.org/go/GO:0005666), red; PAF1 complex (https://www.yeastgenome.org/complex/CPX-1664) and RNA polymerase II (https://www.yeastgenome.org/complex/CPX-2662) complexes themselves. As with the hrq1 SGA data in our companion manuscript (Sanders, Nguyen, et al.; manuscript #401709), these links to transcription are intriguing and reminiscent of the links of human RECQL5 to transcription (Aygun et al. 2008; Izumikawa et al. 2008; Saponaro et al. 2014).

Figure 1 Identification of the Hrq1-3xFLAG interactome by IP-MS and SAINT. A) Overview of the 290 interactions identified by SAINT in anti-FLAG Hrq1 purifications. The graph compares the FC-B score against the SAINT probability score. The dashed line represents the 0.75 probability cut-off. The highest confidence hit, Hrq1, is shown in red. Subsets of the 290 interactors enriched for rRNA processing and ribosomal small subunit biogenesis (B),

| G3 Genomes | Genomes | Genetics |
|------------|---------|----------|
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with our proteomic investigation, focusing on wild-type Hrq1 rather than Hrq1-K318A, we would start with focused experiments, than Hrq1-K318A. Although we have been able to use this mutant to learn about ICL repair and telomere maintenance, its utility as an experimental tool to investigate other processes is unknown. How-

ever, if we were to perform immunopuri-
eral components or crosslinking cells, enriching for telomeric repeat DNA, for instance, treating cells with ICL damage to enrich for repair pathways (Kuranda et al. 2006). Thus, more direct assays of the transcriptional response to Hrq1 mutation were needed. To gain a transcription-wide perspective, we therefore performed RNA-seq analysis of wild-type, hrq1Δ, and hrq1-K318A cells. Compared to wild-type, 107 genes were significantly downregulated and 28 genes were significantly upregulated in hrq1Δ cells (Table S7). Similarly, 301 and 124 genes were down- and upregulated, respectively, in hrq1-K318A cells compared to wild-type. Similar to the SGA and proteo-

tic work by Bochman et al. 2006), suggesting that the Hrq1-K318A protein is still recruited to its sites of action in vivo but somehow disrupts transcription as a catalytically inert roadblock. It should also be noted that Hrq1-K318A protein levels are similar to those of wild-type Hrq1 when expressed from the Hrq1 locus (Bochman et al. 2014), so hrq1-K318A effects are not due to changes in helicase levels when the protein is mutated.

As a purine analog, caffeine does cause transcriptional stress, but it has pleiotropic effects on yeast cells, affecting multiple biological pathways (Kuranda et al. 2006). Thus, more direct assays of the transcriptional response to Hrq1 mutation were needed. To gain a transcription-wide perspective, we therefore performed RNA-seq analysis of wild-type, hrq1Δ, and hrq1-K318A cells. Compared to wild-type, 107 genes were significantly downregulated and 28 genes were significantly upregulated in hrq1Δ cells (Table S7). Similarly, 301 and 124 genes were down- and upregulated, respectively, in hrq1-K318A cells compared to wild-type. Similar to the SGA and proteomic data sets, the GO Terms of these differentially expressed genes (DEGs) were enriched for processes such as response to chemical, meiotic cell cycle, mitotic cell cycle, rRNA processing, and chromo-

Table 4 Gene Ontology (GO) Term enrichment of proteins that physically interact with Hrq1

| GO Term (GO ID) | Genes Annotated to the GO Term | GO Term Usage in Gene List | Genome Frequency of Use |
|----------------|--------------------------------|---------------------------|-------------------------|
| rRNA processing (GO:0006364) | BMS1, BUD21, CBF5, CIC1, DBP10, DBP3, DBP9, DHR2, DIP2, ECM16, ENP2, ERB1, ESF1, FUN12, GAR1, KRE33, MAK5, MOT1, MPP10, MRD1, NHP2, NOP56, NOP8, NTA2, NTA5, NUG1, RL7, ROK1, RPL1A, RPS6A, RPS8A, RPR12, RPP8, TDR1, URA1, UTP10, UTP22, UTP9 | 21 of 75 genes, 28.00% | 149 of 6436 annotated genes, 2.32% |
| ribosomal small subunit biogenesis (GO:0042274) | BMS1, BUD21, DHR2, DIP2, ECM16, ENP2, FUN12, KRE33, MPP10, MRD1, NTA5, NUG1, RL7, ROK1, RPS19A, RPS6A, RPS8A, RPR12, SGD1, TDR1, UTP10, UTP22, UTP9 | 18 of 75 genes, 24.00% | 122 of 6436 annotated genes, 1.90% |
| cytoplasmic translation (GO:0002181) | FUN12, NIP1, RPL12A, RPL1A, RPL23A, RPL43A, RPS19A, RPS25B, RPS6A, RPS8A | 10 of 75 genes, 13.33% | 201 of 6436 annotated genes, 3.12% |
| transcription by RNA polymerase I (GO:0006360) | CDC73, CTR9, DHR2, LE01, MOT1, RPA49, RBP5, UTP10, UTP9 | 9 of 75 genes, 12.00% | 69 of 6436 annotated genes, 1.07% |
| transcription by RNA polymerase II (GO:0006366) | CDC73, CTR9, HHF1, HTA1, LE01, MOT1, RBP5, RTG3 | 8 of 75 genes, 10.67% | 536 of 6436 annotated genes, 8.33% |
| RNA modification (GO:0009451) | AIR2, CBFS, GAR1, KRE33, NHP2, NOP56, PUS1, RRP8 | 8 of 75 genes, 10.67% | 177 of 6436 annotated genes, 2.75% |
| DNA repair (GO:0006281) | CDC73, CTR9, HTA1, LE01, PDS5, RFC3 | 6 of 75 genes, 8.00% | 300 of 6436 annotated genes, 4.66% |
| chromatin organization (GO:0006325) | CTR9, FPR3, FPR4, HHF1, HTA1, LE01 | 6 of 75 genes, 8.00% | 310 of 6436 annotated genes, 4.82% |
| peptidyl-amino acid modification (GO:0018193) | CDC73, CTR9, FPR3, FPR4, HHF1, HTA1, LE01 | 6 of 75 genes, 8.00% | 244 of 6436 annotated genes, 3.79% |

2020a), we still know very little about this protein and what it may be doing in vivo. Thus, we took the unbiased approach described above with our proteomic investigation, focusing on wild-type Hrq1 rather than Hrq1-K318A. Although we have been able to use this mutant to learn about ICL repair and telomere maintenance, its utility as an experimental tool to investigate other processes is unknown. How-

ter, if we were to perform immunopurification-mass spectrometry (IP-MS) with Hrq1-K318A, we would start with focused experiments, for instance, treating cells with ICL damage to enrich for repair complexes or crosslinking cells, enriching for telomeric repeat DNA, and identifying the associated proteins. Indeed, such experiments are ongoing.

Transcriptomic perturbations caused by mutation of Hrq1

Due to the links between the Hrq1 and transcription identified through SGA and IP-MS, we decided to determine if the S. cerevisiae transcriptome is altered by Hrq1 mutation. First, we tested the effects of the general transcription stressor caffeine (Kuranda et al. 2006) on hrq1Δ and hrq1-K318A cells. As shown in Figure 2A, the hrq1-K318A strain was much more sensitive to 10 mM caffeine than wild-type, though the hrq1Δ strain displayed little-to-no caffeine sensitivity. To obtain more quantitative data, we performed growth curve experi-

ments for wild-type, hrq1Δ, and hrq1-K318A cells in the absence and presence of increasing concentrations of caffeine. At high levels of caffeine, the hrq1Δ strain was significantly (P < 0.0001) more sensitive than wild-type, but again, the hrq1-K318A mutant displayed greater sensitivity at a wider range of concentrations (Figure 2B). These data mirror the increased sensitivity of the hrq1-K318A strain to DNA ICL damage compared to the hrq1Δ mutant (Bochman et al. 2014; Rogers et al. 2020a), suggesting that the Hrq1-K318A protein is still recruited to its sites of action in vivo but somehow disrupts transcription as a catalytically inert roadblock. It should also be noted that Hrq1-K318A protein levels are similar to those of wild-type Hrq1 when expressed from the Hrq1 locus (Bochman et al. 2014), so hrq1-K318A effects are not due to changes in helicase levels when the protein is mutated.

As a purine analog, caffeine does cause transcriptional stress, but it has pleiotropic effects on yeast cells, affecting multiple biological pathways (Kuranda et al. 2006). Thus, more direct assays of the transcriptional response to Hrq1 mutation were needed. To gain a transcription-wide perspective, we therefore performed RNA-seq analysis of wild-type, hrq1Δ, and hrq1-K318A cells. Compared to wild-type, 107 genes were significantly downregulated and 28 genes were significantly upregulated in hrq1Δ cells (Table S7). Similarly, 301 and 124 genes were down- and upregulated, respectively, in hrq1-K318A cells compared to wild-type. Similar to the SGA and proteomic data sets, the GO Terms of these differentially expressed genes (DEGs) were enriched for processes such as response to chemical, meiotic cell cycle, mitotic cell cycle, rRNA processing, and chromo-

some segregation (Table S8).

Figure 2C shows the frequency distribution of all of the changes in expression in the hrq1Δ cells compared to wild-type, separated by down- and upregulated DEGs for each mutant. Outliers are denoted as single points, representing the transcripts whose abundances changed the most. The expression changes in most DEGs were mild decreases or increases, but several varied greatly from wild-type. As an internal control, we found that the transcription of Hrq1 in hrq1Δ cells displayed the largest decrease among all data sets relative to wild-type (Figure 2C).
The largest number of outliers were the 10 upregulated DEGs in hrq1-K318A cells. These included genes encoding two cell wall mannoproteins (TIP1 and CWPI) (Van Der Vaart et al. 1995; Fujii et al. 1999), a heat shock protein (HSP30) (Piper et al. 1997), a protein required for viability in cells lacking mitochondrial DNA (ICY1) (Dunn and Jensen 2003), a predicted transcription factor whose nuclear localization increases upon DNA replication stress (STP4) (Tkach et al. 2012), a protein of unknown function whose levels increase in response to replication stress (YER053C-A), a factor whose over-expression blocks cells in G1 phase (CP1) (Ren et al. 2016), and three proteins of unknown function that are induced by ICL damage (YLR297W, TDA6, and FMP48) (Dardalhon et al. 2007). No mutations in these 10 DEGs were significant genetic interactors with hrq1 (Sanders, Nguyen, et al.; companion manuscript #401709), but the latter are particularly tantalizing nonetheless considering the known function of Hrq1 in ICL repair (Bochman 2014; Rogers et al. 2017; Rogers et al. 2020a).

Perhaps the YLR297W, TDA6, and FMP48 gene products function in the Hrq1-Pso2 ICL repair pathway, and their levels must be elevated to compensate for the catalytically crippled Hrq1-K318A mutant. Alternatively, they may represent members of a back-up ICL repair pathway that is activated when the Hrq1-Pso2 pathway is ablated. In either case, it should be noted that the RNA-seq experiments were performed in the absence of exogenous ICL damage, but the hrq1-K318A cells appear already primed to deal with ICLs in the absence of functional Hrq1. The reasons for this are currently unknown, but our ongoing experiments are addressing this phenomenon. It should also be noted that these three genes were likewise upregulated in the hrq1Δ samples (File S4), though not to the same extent as in hrq1-K318A cells.

**CONCLUSIONS AND PERSPECTIVES**

Here, we used a multi-omics approach to comprehensively determine the *S. cerevisiae* Hrq1 interactome. The data reported here and in our companion manuscript (Sanders, Nguyen, et al.; manuscript #401709) greatly expand the known genetic and physical interaction landscape of Hrq1 in yeast, including synthetic genetic interactions with and transcriptomic changes caused by the strong hrq1-K318A allele. Various links to the known and putative roles of Hrq1 and its homologs in DNA repair, telomere maintenance, and the mitochondria were found, as well as novel connections to the cytoskeleton and transcription.

Our concurrent data also indicate that the second *S. cerevisiae* RecQ family helicase, Sgs1, is likewise involved in transcription (Sanders, Nguyen, et al.; manuscript #401709). However, it is unclear if Hrq1 and Sgs1 act together during transcription or have distinct roles, and it is unknown what these roles are. Human RECQL5 physically interacts with RNA polymerase II, controlling transcription elongation (Saponaro et al. 2014). It may also function at the interface of DNA repair and transcription by helping to resolve replication-transcription conflicts (Hamadeh and Lansdorp 2020).

It is reasonable to hypothesize that Hrq1 and/or Sgs1 function as box and whisker plots drawn using the Tukey method. The individually plotted points outside of the inner fences represent outliers (i.e., expression changes with the largest absolute values) and correspond to genes whose log2 fold change value is less than the value of the 25th quartile minus 1.5 times the inter-quartile distance (IQR) for downregulation or genes whose log2 fold change value is greater than the value of the 75th quartile plus 1.5IQR for upregulation.
similarly and, in the case of Hrq1, perhaps in the transcription-coupled repair of DNA ICL lesions. Future work should address these hypotheses, as well as the others raised throughout this manuscript, to further characterize the roles of RecQ helicases in the maintenance of genome integrity. Similar to the mechanistic identification of the roles of Hrq1 in yeast (Bochman 2014; Nickens et al. 2018; Rogers et al. 2020a), we anticipate that these data will spur additional research into exciting and unexpected functions of RecQ4 subfamily helicases.

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Note added in proof: See 4359-4368 in this issue for a related work.

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