Conditional Genetic Interactions of RTT107, SLX4, and HRQ1 Reveal Dynamic Networks upon DNA Damage in S. cerevisiae

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ABSTRACT The DNA damage response (DDR) is a dynamic process that is crucial for protecting the cell from challenges to genome integrity. Although many genome-wide studies in Saccharomyces cerevisiae have identified genes that contribute to resistance to DNA-damaging agents, more work is needed to elucidate the changes in genetic interaction networks in response to DNA lesions. Here we used conditional epistatic miniarray profiling to analyze the genetic interaction networks of the DDR genes RTT107, SLX4, and HRQ1 under three DNA-damaging conditions: camptothecin, hydroxyurea, and methyl methanesulfonate. Rtt107 and its interaction partner Slx4 are targets of the checkpoint kinase Mec1, which is central to the DDR-signaling cascades. Hrq1 recently was identified as a novel member of the RecQ helicase family in S. cerevisiae but is still poorly characterized. The conditional genetic networks that we generated revealed functional insights into all three genes and showed that there were varied responses to different DNA damaging agents. We observed that RTT107 had more genetic interactions under camptothecin conditions than SLX4 or HRQ1, suggesting that Rtt107 has an important role in response to this type of DNA lesion. Although RTT107 and SLX4 function together, they also had many distinct genetic interactions. In particular, RTT107 and SLX4 showed contrasting genetic interactions for a few genes, which we validated with independently constructed strains. Interestingly, HRQ1 had a genetic interaction profile that correlated with that of SLX4 and both were enriched for very similar gene ontology terms, suggesting that they function together in the DDR.

KEYWORDS genetic interaction profiles DNA damage response helicase conditional interactions

Mapping of genetic interactions has been a valuable and powerful approach to reveal connections within complex biological systems (Baryshnikova et al. 2013). Much of this work has been done in Saccharomyces cerevisiae because of the tools in place to create double mutants and the availability of vast arrays of mutant libraries. Although providing great biological insights, most screens to date have been conducted under unperturbed growth conditions, whereas many networks in cells respond to environmental stimuli.

A significant type of environmental stimuli is DNA damage, which can be caused by external factors, such as exposure to genotoxins or ultraviolet light, or internal factors, such as replication fork stalling or DNA polymerase error (Lindahl 1993). Cells are constantly exposed to these insults that, if not properly repaired, may compromise genomic integrity or ultimately lead to cell death. Because of the vital importance of genomic integrity, cells have complex mechanisms to regulate the DNA damage response. DNA damage is detected by sensors, which trigger a signaling cascade, leading to the activation of the kinases Mec1 and Tel1, the yeast homologs of mammalian ATR (ATM and Rad 3-related) and ATM (ataxia-telangiectasia mutated). These kinases in turn elicit various cellular responses, including cell-cycle arrest, DNA repair, apoptosis, and/or DNA damage-induced transcriptional program (Putnam et al. 2009; Finn et al. 2012).
One of the downstream phosphorylation targets of Mec1 is Rtt107/Slx4, which is required for reinitiating replication after repair of alkylating DNA damage (Rouse 2004; Roberts et al. 2006). Deletion of the \textit{RTT107} gene results in hypersensitivity to DNA-damaging agents such as the DNA-alkylating agent methyl methane-sulfonate (MMS), the nucleotide reductase inhibitor hydroxyurea (HU), and the topoisomerase I poison camptothecin (CPT) (Chang et al. 2002; Rouse 2004; Parsons et al. 2006; Roberts et al. 2006). Rtt107 contains several BRCT (BRCA1 C-terminal) homology domains, which often serve as phospho-binding modules to recruit signaling complexes and repair factors to DNA damage-induced lesions (Rouse 2004; Mohammad and Yaffe 2009). Consistent with a role as a scaffold for protein-protein interactions during the DNA damage response, Rtt107 interacts with a number of DNA repair and recombination proteins and is recruited to sites of DNA lesions (Chin et al. 2006; Roberts et al. 2006, 2008; Ohouo et al. 2010; Leung et al. 2011; Ullal et al. 2011).

The best-characterized Rtt107-interacting partner is the replication-specific endonuclease Slx4, which interacts with the N-terminal BRCT domains of Rtt107 (Roberts et al. 2006). Slx4 is required for Mecl-dependent phosphorylation of Rtt107 and, like Rtt107, facilitates resumption of DNA replication after DNA damage (Roberts et al. 2006). However, it has become clear over the last few years that Rtt107 also has Slx4-independent functions, and vice versa. Consistent with this, the defects in DNA damage response are generally more severe in \textit{rtt107Δ} mutants than in \textit{slx4Δ} mutants, and \textit{rtt107Δ slx4Δ} double mutants are more sensitive to MMS than either of the single mutants (Roberts et al. 2006).

Although Slx4 has been studied in the context of its interaction with Rtt107, the SLX4 gene was first identified in a synthetic lethal screen with \textit{SGS1}, which encodes for a RecQ helicase (Mullen et al. 2001). DNA helicases represent an important class of enzymes involved in the DNA damage response and have roles in recognition of DNA damage, DNA recombination, and stabilization of stalled replication forks (Brosh 2013). In \textit{S. cerevisiae}, Sgs1 was thought to be the only RecQ helicase family member until recently, when Hrq1 was identified as a novel RecQ helicase (Barea et al. 2008; Kwon et al. 2012). The functions of Hrq1 have only been preliminarily characterized, but based on the relationship between Slx4 and Sgs1, Hrq1 may also have linkages to Slx4 that have yet to be uncovered.

Initial genome-wide studies to characterize genetic function in response to DNA damage measured the fitness of deletion mutants exposed to a variety of genotoxic insults (Giaever et al. 2004; Parsons et al. 2006; Hillenmeyer et al. 2008). However, these studies only evaluated the requirement of single genes for resistance to DNA-damaging agents, whereas the effects on genetic networks were only studied in a small directed screen (St Onge et al. 2007). Two recent studies used genetic interaction mapping to gain new insights into the DNA damage response (Bandyopadhyay et al. 2010; Guenole et al. 2013). In the initial study, all possible double mutants of 418 genes were created and exposed to MMS to evaluate changes in the genetic interaction network (Bandyopadhyay et al. 2010). Using this approach, the authors demonstrated that differential genetic interactions are better able to reveal functions in the DNA damage response and identified new roles for several genes. A follow-up work expanded on this work and interrogated 55 query genes crossed to a library of more than 2000 genes in MMS, CPT, and zeocin conditions (Guenole et al. 2013). Analysis of the differential genetic networks revealed several genes that were hubs of genetic interactions, and additional experiments demonstrated that these genes had novel roles in the DNA damage response.

Here we use a similar approach of measuring conditional genetic interactions to study further the functions of Rtt107, Slx4, and Hrq1. We analyzed the significantly interacting gene pairs to identify those that emerged or changed in response to DNA damage. Overall, Rtt107 exhibited more genetic interactions than SLX4 or HRQ1 in CPT conditions, indicating an important role for Rtt107 in responding to CPT. Furthermore, SLX4 and RTT107 showed distinct, and sometimes even opposing, genetic interactions, even though the protein products exist at least in part as a complex in the cell. Interestingly, the interaction profile and enriched gene ontology terms for \textit{HRQ1} most closely resembled that of \textit{SLX4}, suggesting that they have overlapping functions in the DNA damage response.

**MATERIAL AND METHODS**

**Yeast strains**

All yeast strains used in this study are listed in Supporting Information, Table S1 and created using standard yeast genetic techniques (Ausubel 1987). Complete gene deletions were achieved using one-step gene integration of polymerase chain reaction-amplified modules (Longtine et al. 1998). Mutants for conditional epistatic miniarray profiling (cE-MAP) screens were constructed in the BY4742 background, whereas all other strains were constructed in the W303-1A background.

**cE-MAP**

cE-MAP screens were performed and normalized as described previously (Collins et al. 2010), with the exception that we expanded the number of plates per query strain to accommodate all drug conditions tested (see Figure 1). In brief, deletion mutants of the query genes were crossed, using a Singer robot, to a library of 1536 mutants covering a number of categories, including kinases/phosphatases and chromatin biology. We used 10 and 15 μg/mL of CPT, 50 and 100 mM of HU, and 0.0075 and 0.0125% of MMS (Sigma-Aldrich), along with two no-drug controls. All strains and conditions were screened in triplicate. Complete cE-MAP profiles can be found in File S1.

Differential S-scores were calculated by subtracting the S-score in yeast extract peptone dextrose from the S-score in each drug condition for each gene pair, and converted to Z-scores. Corresponding p-values were corrected for multiple testing using the fdrtool R package (Strimmer 2008) and conditional genetic interactions were considered significant for q-values < 0.05. cE-MAP networks were visualized with Cytoscape (Cline et al. 2007).

**Growth and DNA damage sensitivity assays**

Overnight cultures grown in yeast extract peptone dextrose were diluted to 0.5 OD600. The cells were tenfold serially diluted and spotted onto solid yeast extract peptone dextrose plates or plates with MMS, CPT, or HU (Sigma-Aldrich) at the indicated concentrations. The plates were then incubated at 30° for 2 d and subsequently photographed.

**Gene Ontology (GO) analysis**

The Database for Annotation, Visualization, and Integrated Discovery was used for GO term enrichment analysis (Dennis et al. 2003). For each query gene, a list of significantly interacting genes was compiled that included all drug conditions and both positively and negatively interacting genes. Multiple testing correction was done using the Benjamini method, and enriched GO terms were considered significant for q-values < 0.05.

**RESULTS**

Genetic interaction profiles were considerably altered when exposed to DNA-damaging agents

To gain a global understanding of the functions of Rtt107, Slx4, and Hrq1, we measured genetic interactions with representative genes.
across the genome under three DNA damaging conditions (MMS, HU, and CPT). To achieve this, we used a version of the synthetic genetic array technology, the epistatic miniarray profile (E-MAP), to map genetic interactions for RTT107, SLX4, and HRQ1 under different DNA-damaging conditions, which we termed cE-MAP (Tong et al. 2001; Baryshnikova et al. 2010; Collins et al. 2010). During the mutant selection process, the plates were expanded to accommodate the four different conditions tested and to measure each condition with three technical replicates (see Figure 1 for workflow).

The analysis pipeline for E-MAP calculates S-scores, which reflect both the strength of the genetic interaction and the statistical confidence (Collins et al. 2010). For a broad assessment of how DNA damage affected the genetic networks, we first calculated the Pearson’s correlations of the query genes’ S-score profiles under the three drug conditions (Figure 2A). Supporting the idea that genetic networks respond significantly to external stimuli, the genetic interaction profiles generated under unperturbed growth conditions clustered away from the profiles generated under DNA-damaging conditions. Strikingly, the profiles for RTT107 in DNA-damaging conditions were closely correlated, regardless of the type of DNA insult. In contrast, the profiles for SLX4 and HRQ1 clustered together by the type of DNA-damaging agent. In addition to these major patterns, the genetic interaction profiles of all query genes under the same type of DNA-damaging agent also were positively correlated to one another (for example, see the positive correlations between the RTT107, SLX4, and HRQ1 profiles in MMS). Finally, we observed that the two concentrations of drug used produced very similar profiles; thus, we averaged the scores of the two concentrations. This improved the confidence of our S-scores since they were represented by six replicates instead of three; these sets of average S-scores were used for all further analyses.

According to previously published thresholds, S-scores lower than -2.4 or greater than 2.0 are considered significant genetic interactions (Collins et al. 2010). Consistent with the dynamic nature of the DNA damage response, the distribution of the S-scores for each query gene changed significantly between the unperturbed growth conditions and the DNA-damaging conditions (Figure 2B and Figure S1). For example, under HU conditions, the S-score distribution for each query gene became broader, indicating that the number and strength of the genetic interactions increased (Kolmogorov-Smirnov test, two-sided, p-value < 1.5 × 10^{-7} for all comparisons). Strikingly, rt107Δ mutants had the broadest distribution of S-scores of the three query mutants, i.e., the most significant genetic interactions, followed by slx4Δ mutants, then hrq1Δ mutants (Figure 2B). We note that this was consistent with the relative DNA damage sensitivity of these mutants (Figure 2C). The distribution of the S-scores of all conditions for all three query genes were significantly different from one another, although the distributions for SLX4 and HRQ1 looked more similar to one another than that of RTT107 (Kolmogorov-Smirnov test, two-sided, p-value < 7.8 × 10^{-16} for all comparisons, Figure S1D).

**RTT107 exhibited more conditional genetic interactions than SLX4 or HRQ1**

To identify genetic interactions under DNA damage conditions that were significantly different from unperturbed growth conditions, we adapted a published method (Bandypadhyay et al. 2010). Specifically, the differences between the S-score in the DNA damage condition and the S-score in unperturbed growth conditions for each gene pair were subtracted from the average of all the differential scores and divided by the standard error to calculate a Z score. Genes with significant Z scores after correcting for multiple testing are listed in Table 1 (q-value < 0.05). In total there were 569 gene pairs found to have significant differential interaction in the drug conditions tested. Of these, 378 were negative interactions (DNA damage-induced sickness or lethality), and 191 were positive interactions (DNA damage-induced epistasis or suppression). To test the reliability of this approach, we looked for known condition-specific genetic interactions. Consistent with our previously published results, deletion of DOT1 and BRE1 suppressed the DNA damage sensitivity of rt107Δ in MMS (Levesque et al. 2010). Furthermore, the suppression by dot1Δ was additionally observed in HU but not in CPT, whereas the suppression by bre1Δ was limited to MMS, in both the cE-MAP data and the independently constructed mutants (Figure 3).

We visualized the conditional genetic interactions in a network in which nodes represented query or array genes and edges represented significant conditional genetic interactions. The edges were colored according to the drug condition that the genetic interaction occurred in (Figure 4A). As revealed by the network map, there was a subset of genes that interacted with all three query genes, suggesting that they play a more general role in the DNA damage response. These included the homologous recombination genes RAD52, RAD55, and RAD57 (Figure 4B). Aside from this group of genes, there were also subsets that interacted with only two out of the three query genes. HRQ1 and SLX4 shared the greatest number of interacting genes, and this represented a significant overlap between these two groups (Fisher’s exact test, greater, p-value = 2.2 × 10^{-16}, Figure 4A). Further supporting shared functions of RTT107 and SLX4, there was also a significant overlap between their interacting genes (Fisher’s exact test, greater, p-value = 1.7 × 10^{-14}). Conversely, each query gene had unique genetic interactions, and RTT107 had the greatest number of these (Figure 4A). Whereas the majority of the unique genetic interactions with
RTT107 occurred under CPT conditions, HRQ1 and SLX4 had minimal numbers of unique interactions in CPT.

When comparing the total number of genetic interactions, we found that RTT107 had many more positively interacting genes than either SLX4 or HRQ1 (Figure 4C). After these interactions were separated into each drug condition, it became clear that the biggest difference in interactions occurred during exposure to CPT for both positive and negative interactions (Figure 4, D and E). Taken together, these data suggest that Rtt107 plays an important role in responding to protein-bound nicks induced by CPT. To support this, RTT107 also had a strong positive/epistatic genetic interaction with TOP1, the molecular target of CPT, whereas this interaction was absent for SLX4 and HRQ1 (Figure 5A).

Genes with condition-specific interactions were enriched for functions in the DNA damage response

To further analyze the functions of RTT107, SLX4, and HRQ1 revealed by the conditional genetic interactions, we looked at the enrichment of GO terms using the Database for Annotation, Visualization, and
| Query     | Drug | Interaction | Significant Genes                                                                 |
|-----------|------|-------------|-----------------------------------------------------------------------------------|
| HRQ1      | CPT  | Negative    | ASFI, CHL1, CLB5, CTF4, DCC1, DDC1, MM11, MM52, MRE11, PBY1, RAD17, RAD24, RAD52, RAD54, RAD55, RAD57, RAD59, RTT101, RTT109, SAE2 |
| HRQ1      | CPT  | Positive    | CYC8, GMH1, PDA1                                                                  |
| HRQ1      | HU   | Negative    | ARP4, ASFI, BAS1, BMH1, BRE1, CLB5, ERG5, ERJ5, GCN1, GCN20, GET2, GNP1, HPC2, IRA2, LAT1, LGE1, LST4, MET18, MFT1, MK51, MRC1, MRE11, NPI1, PB2, PDB1, PDE2, PRM1, POL32, RAD52, RAD54, RAD55, RAD57, RIM21, RPL34B, RPS21B, RTF1, RTG3, RTT109, SDC1, SEC22, SEC66, SF37, SNX4, SPF1, SUA7, SWD1, SWD3, SW4, SYC1, UBA3, UBP15, UBP6, URE2, VPS8, YTA7 |
| HRQ1      | HU   | Positive    | AIM2, APS3, ARO1, ARP7, BTS1, BUL1, CAP1, COQ2, CUE3, CYC8, CYT1, DCR2, FEN1, HO52, MSS18, NGL2, PET130, TH16, YMR102C |
| HRQ1      | MMS  | Negative    | DAL81, FKS1, GDF1, GDF2, GNPI, MMS2, MOG1, MHP1, MRE11, MSH4, PBY1, PET18, PHOS, POL32, RAD10, RAD18, RAD27, RAD59, REV1, REV3, REV7, RVS161, SAK1, SCS7, SRO9, STP1, TRS33, UBC13, VMA21, YGL081W, YSW6 |
| HRQ1      | MMS  | Positive    | CLB5, HST3, ILM1, KAP122, PMR1, SUR4                                                |
| RTT107    | CPT  | Positive    | AGL2, BCK1, BFA1, BUB2, CHS5, CLB2, CLB5, COG5, CRN1, CSG2, DCC1, DDC1, DEP1, ECM33, ELA1, ERV14, FET3, FKS1, FUN30, GAS1, HPC2, IRA2, IRC21, KE2X, LAS2, LEM3, LGE1, LEE1, MEK3, NCL1, OPI3, OST3, PAC1, PB1Y, PEP8, PFA4, PMT1, PMT2, PPH21, PPH3, PPM1, PRE9, PPS2, RAD17, RAD24, RAD54, RAD55, RAD57, RAD61, RHDS5, RDI1, REV7, RGA1, RRD2, RTF1, RST1, RXT2, SAP30, SC57, SEC22, SEC28, SLA1, SM1, SMY1, SPF1, SRO9, STV1, SUR4, SW4, TPK3, UBP14, VPS24, VPS27, VPS29, VPS35, VPS5, VPS8, VPS9, YDR061W, YJR088C, YLR246W, YPL150W, ZDS1 |
| RTT107    | CPT  | Positive    | APQ12, ARP7, BUB1, BUB3, CDC28, CYC8, CYT1, DBF2, DPB4, ELC1, ELGI, ESS1, GAC1, GAL80, HAD1, IK3, IPT1, JHD2, LIA1, MIP1, MK22, MMS4, MRC1, MRT4, NAP1, NCS2, NF1, NOP12, OAF1, PAP2, PEF1, POL32, P54, RAD27, RPL11B, RPL8B, RPS15B, RPS4A, RSC4, SAC3, SAM37, SCL1, SFL1, SFL2, SGF29, SLCl, SME3, SPT2, SSN2, TED1, TOP1, TRM10, TUB3, TUP1, UBX4, UFO1, YCR505C, YLR287C, YNR004W |
| RTT107    | HU   | Negative    | ABG2, BAS1, BUB2, CRN1, DUG2, GCN1, IRA2, MAD2, NPR1, OXR1, PBY2, PPS2, PSCC22, SER2X, SLX9, SLY14, SPF1, SUR4, SW4, URE2, YDL089W |
| RTT107    | HU   | Positive    | AEP2, AIM26, ARO1, ARP7, BTS1, BUB3, BUD21, BUD6, BUL1, CDC48, CYC8, CYK3, CYT1, DBF1, ECM5, ERG6, HDA1, LIM1, LAG1, MET22, NAP1, NUP2, PEF1, PET130, PEX17, PMR1, POL30, PPM1, RIM101, RPS15B, RPS28B, RPS30A, SCL1, SFE1, SPE3, SPT21, SSE1, TH16, TSA1, UBX4, UFO1 |
| RTT107    | MMS  | Negative    | BCK1, BFA1, CLB2, CRN1, DCC1, DUG2, FG1, GF1, GNP1, HRT1, IRC21, LM7, LEE1, MBP1, MMS2, PAC1, POL30, PPM1, PTC2, RAD17, RAD18, RAD27, REV7, RST1, SEC75, SLX9, SRO9, SRS2, STP1, TEL1, TSA1, UBC13, VMA21, YGL081W, YPL041C |
| RTT107    | MMS  | Positive    | AIM29, APQ12, ARO1, BMH1, BTS1, CSM3, CYT1, DBF2, DOT1, ELCI, FEN1, GAL80, GSF2, HST3, ILM1, LGE1, MRC1, MSS18, NAM7, NPI1, PER1, RAD52, RMD11, ROTO2, RPL8B, RPS15B, RPS4A, RSC4, SPT2, TEPI, TMA23, TOP1, TUB3, UBO1 |
| SLX4      | CPT  | Negative    | ARP4, ASFI, CHL1, CK1, CLB5, CSM3, CTF4, DCC1, DDC1, DIA2, GAS1, LAS21, LEM3, LGE1, MM51, MMS2, PBY1, PRM1, PHIS, RAD17, RAD24, RAD52, RAD54, RAD55, RAD57, RAD59, RDI1, RTT101, RTT109, SAE2, SF37, SRS2, STV1 |
| SLX4      | HU   | Negative    | ASFI, AIM22, ARL3, ARP4, BAS1, BMH2, BRE1, CLB5, COG5, CWH41, DCC1, ECM30, ERG5, ERJ5, FKH2, GCN1, GEF20, GNP1, HCM1, HPC2, INPS3, IRA2, LGE1, LST4, MDS3, MRE11, NPI1, OXR1, PBY2, PBY1, PDB1, PDE2, PFA4, PRM1, PRE9, PPS2, OCR10, ONO1, RAD54, RAD55, RAD57, RPL41B, RPS11A, RTF1, SDC1, SEC22, SEC66, SF37, SKY3, SN1G, SPF1, STV1, SUA7, SUR4, SWD1, SWD3, SW4, SYC1, SYF2, UBP15, URE2, VPS7, YDR061W, YER064C, YPR063C, YSY6 |
| SLX4      | HU   | Positive    | APS3, BTS1, BUL1, CAP1, CDC36, COQ2, CYT1, FEN1, HO52, IMP2, MDM38, MFT1, MSS18, NGL2, PET123, PET130, RPL43A, RPN13, SCD6, SPT21, TAF9, TOP1, YDL176W, YIP3 |
| SLX4      | MMS  | Negative    | CKB1, DIA2, EN4, ERV25, GF1, GNPI, HST3, IMP2, MHP1, PAC1, PET18, POL32, PPH3, PSY3, RAD18, RAD26, RAD27, RAD59, REV3, REV7, RRD1, SAE2, SAE185, SEC75, STP1, TOM3, TRS2, UBC13, UBC13, VMA21, YGL081W |
| SLX4      | MMS  | Positive    | BUD14, DOT1, MSH4, NAM7, RPS21B |

CPT, camptothecin; HU, hydroxyurea; MMS, methyl methylsulfonate.
repair was one of the most overrepresented terms (5.45- and 6.42-fold enrichment, respectively). Notably, the lists of enriched GO terms for SLX4 and HRQ1 were almost identical, further suggesting that these two genes have similar functions in the DNA damage response. In contrast, RTT107 was significantly enriched only for one GO term, cell cycle checkpoint (2.95 fold enrichment, q < 0.03), although many of the GO terms that did not meet the significance cutoff were also related to the cell cycle (data not shown).

Deletion of HST3 and MRC1 suppressed the DNA damage sensitivity of \( \text{rtt107}\Delta \) but not \( \text{slx4}\Delta \) mutants

Hierarchical clustering of the significantly interacting genes revealed several patterns of genetic interaction profiles. There were sets of genes that were specific for the query gene, regardless of the DNA-damaging agent (Figure 5A). Intriguingly, there were certain genes that showed strong positive interactions with RTT107 but negative interactions with SLX4, such as HST3 and MRC1, further supporting the idea that Rtt107 and Slx4 have unique functions.

Conversely, other sets of genes were specific for the DNA-damaging agent and interacted with all three query genes under that condition (Figure 5B). For example, the CPT-specific genes included RAD24, RAD17, and DDC1, which encode for components of the 9-1-1 checkpoint clamp and RFC loader complex (Majka and Burgers, 2007). The MMS-specific genes included multiple components of the translesion synthesis pathway, such as REV3, REV7, and RAD18 (Sharma et al., 2013). Unexpectedly, the HU-specific genes included several transcription-related genes such as SWD1, SWD3, and SDC1.

Positive S-scores indicate that the double mutant exhibits better fitness than expected (multiplicative product of single mutants’ fitness), but it does not differentiate between suppression and epistasis. To further investigate the nature of the genetic interaction between HST3 and RTT107 and SLX4, we independently constructed deletion mutants and extended the analysis to include HST4, which was not on the E-MAP library. Hst3 and Hst4 are protein deacetylases that are both responsible for removing histone H3 K56 acetylation, thereby affecting replicative lifespan and response to DNA damage (Miller et al., 2006). Deletion of HST3 clearly suppressed the DNA damage sensitivity of \( \text{rtt107}\Delta \) mutants in all three drugs tested, albeit to a lesser extent in HU (Figure 6A). Confirming the striking opposite interactions of RTT107 and SLX4 observed in the E-MAP, HST3 and SLX4 showed a synergistic interaction in CPT and MMS, but not HU. In general, HST4 showed the same genetic interaction profile as HST3. However, the hst3\Delta hst4\Delta rtt107\Delta triple mutant showed a variable phenotype depending on the DNA-damaging agent, and this differed from the hst3\Delta rtt107\Delta or hst4\Delta rtt107\Delta double mutants, portraying a complex relationship between HST3 and HST4 in this genetic interaction.

As a second example of characterizing positive interactions, we focused on MRC1, a gene encoding for an S-phase checkpoint adaptor (Tanaka, 2010), which also showed this pattern of opposite genetic interactions with RTT107 and SLX4. Using independently constructed deletion mutants, we observed that deletion of MRC1 suppressed the DNA damage sensitivity of \( \text{rtt107}\Delta \) mutants to CPT and MMS (Figure 6B). Interestingly, the converse was observed in the case of HU, in that deletion of RTT107 mildly suppressed the sensitivity of \( \text{mrc1}\Delta \) mutants. In contrast, the slx4\Delta mrc1\Delta double mutant was clearly more sensitive to CPT and MMS than the slx4\Delta single mutant, although the deletion of SLX4 mildly suppressed the HU sensitivity of the \( \text{mrc1}\Delta \) single mutant.

DISCUSSION

In this study we used cE-MAP to generate conditional genetic interaction profiles for RTT107, SLX4, and HRQ1, to further investigate their functions in the DNA damage response. We tested CPT, HU, and MMS, which elicited specific genetic interactions with each of the query genes, and the network of interactions observed provides insight into the mechanisms of both the DNA-damaging agent and the query genes. Furthermore, we validated two specific examples of genetic interactions emerging from the cE-MAP in direct genetic tests and identified a novel genetic suppression in each case.

A critical component of the cE-MAP approach was the use of DNA-damaging agents. Because we are interested in interrogating the DNA damage response functions of the query genes, it was crucial to evaluate the genetic interactions in conditions when those functions are active. Testing three different DNA-damaging agents also provided an opportunity to compare between the responses of the genetic interaction network to each type of DNA insult.

We observed sets of genes that showed significant interactions under each specific drug condition. For each drug condition there was different DNA damage response pathways represented within the sets of genes, supporting the idea that the cell responds specifically to different types of DNA lesions. The CPT-specific genes included components of the 9-1-1 checkpoint clamp and RFC loader complex, suggesting that the DNA damage response to protein adducts involves this component of the checkpoint response (Majka and Burgers, 2007). The MMS-specific genes included multiple components of the translesion synthesis pathway, which is one of the pathways in postreplication repair that allows cells to replicate past damaged bases or bulky adducts (Sharma et al., 2013). These data suggest that it also plays a role in bypassing alkylated bases. The HU-specific genes included several transcription-related genes, which was unexpected, but could be explained by an indirect effect of the mechanism of HU, which depletes the deoxyribonucleotide triphosphate pool. Interestingly, one of the HU-specific genes, YER064C, is relatively uncharacterized but was recently shown to change its cellular localization upon exposure to HU, suggesting a role for this gene in response to replication stress (Tkach et al., 2012). Consistent with our study, the previously published E-MAP analysis of the DNA damage response revealed that genes showing significant interactions in CPT are enriched for function in the DNA damage checkpoint, whereas significant genes in MMS are enriched for post-replication repair (Guenole et al., 2013).
Figure 4  RTT107 had more significant genetic interactions than SLX4 or HRQ1. (A) Visualization of all the significant genetic interactions. Nodes represent query or array genes, and edges are colored by the drug condition that the interaction occurred in. Blue represents CPT, green represents HU, and red represents MMS. The numbers of unique interactions for each query gene are labeled for each drug condition. Indicated p-values are from Fisher’s exact tests (greater) of the genes that interact only with two out of the three query genes. (B) Enlarged view of a subset of the network indicated by the black box in (A). (C) RTT107 had more positive genetic interactions than SLX4 or HRQ1. RTT107 had more (D) positive and (E) negative genetic interactions in CPT than SLX4 or HRQ1. CPT, camptothecin; HU, hydroxyurea; MMS, methyl methane-sulfonate.
In contrast to the drug-specific genes, there were also sets of genes that showed unique interactions with each specific query gene. These interactions suggested that the query genes we interrogated have distinct functions in the DNA damage response. It is of particular interest that RTT107 and SLX4 shared only a subset of genetic interactions, given that the Rtt107 and Slx4 proteins exist as a complex in the cell (Roberts et al. 2006). An attractive model is that there are different pools of Rtt107 and Slx4 protein complexes that contribute to specific functions, since Rtt107’s interaction with SMC5/6 and Slx4’s interaction with Sln1 are independent of each other (Roberts et al. 2006; Leung et al. 2011). The human homologs of Rtt107 and Slx4, PTIP and SLX4, respectively, also have many distinct functions. While PTIP is involved in the DNA damage signaling cascades and DNA repair pathway choice (Gong et al. 2009; Wu et al. 2009; Callen et al. 2013), SLX4 has roles in Holliday junction resolution and telomere length regulation (Castor et al. 2013; Garner et al. 2013; Wan et al. 2013; Wilson et al. 2013; Wyatt et al. 2013). The data from this cE-MAP provide an opportunity to further elucidate the unique functions of Rtt107 and Slx4, which may be further dissected into responses to different DNA lesions.

The cE-MAP data also provided more insight into the function of the helicase Hrq1, which has only been preliminarily characterized (Kwon et al. 2012; Choi et al. 2013; Bochman et al. 2014). Interestingly, the genetic interaction profile of Hrq1 correlated more closely to SLX4 than RTT107, and the sets of genes that interacted with SLX4 or Hrq1 overlapped significantly. In addition, GO analysis of the significantly interacting genes returned almost identical GO terms for Hrq1 and SLX4. The close relationship between Hrq1 and SLX4 revealed by the cE-MAP data are supported by previous studies showing that SLX4 is synthetic lethal with SGS1, the major RecQ helicase in S. cerevisiae (Mullen et al. 2001). Moreover, there is some evidence suggesting that Hrq1 and Slx4 are both involved in interstrand crosslink repair and suppression of telomere addition (Zhang et al. 2006; Ward et al. 2012; Bochman et al. 2014). However, slx4Δ and hrq1Δ mutants display different sensitivities to DNA-damaging agents, indicating they function separately as well (Flott and Rouse. 2005; Choi et al. 2013; Bochman et al. 2014). Further experiments are needed to determine the roles of Hrq1 and Slx4 in DNA structure maintenance and the nature of their relationship in these functions. Possible routes of inquiry can be suggested by additional examination of the genetic data.

**Figure 5** Different patterns of genetic interactions were observed for genes that significantly changed their interactions in response to DNA-damaging conditions. Shown are subsets of conditional epistatic miniarray profiling (cE-MAP) data. Blue and yellow represent negative and positive genetic interactions, respectively. (A) Some genetic interactions were specific for the query genes. (B) Other genetic interactions were specific to the drug condition and were common across all query genes.
We followed up on two genes that had particularly striking conditional genetic interactions. Both HST3 and MRC1 showed a strong positive genetic interaction with RTT107 but a negative interaction with SLX4. Although Rtt107 and Slx4 form a complex, these genetic interactions suggest that not only do Rtt107 and Slx4 have independent functions, but they may have opposing functions in these contexts. Using a direct genetic test, we found that deletion of HST3, as well as HST4, suppressed the DNA damage sensitivity of rtt107Δ mutants. The known target of the Hst3 and Hst4 deacetylases is H3 K56 acetylation (H3K56ac). Whereas deletion of HST3 alone causes an increase in H3 K56ac, deletion of HST4 alone does not change the acetylation levels, and only in the double mutant are all H3 molecules completely acetylated (Celic et al. 2006). Intriguingly, the suppression of the rtt107Δ mutant phenotype in CPT was observed upon deletion of HST3 or both HST3 and HST4, but not HST4 alone. In contrast, deletion of either HST3 or HST4 alone was sufficient to suppress the DNA damage sensitivity of rtt107Δ mutants to MMS and HU. Based on this data, we speculate that the deacetylation of H3 K56ac may be important to the genetic interaction in CPT, whereas it is not relevant in MMS or HU conditions, rather there may be a different function or target of Hst3 and Hst4 involved. Similarly for the genetic interaction with SLX4, deletion of HST3 or HST4 alone exhibited the same phenotype, thus suggesting that deacetylation of H3 K56ac is not involved.

We also validated the genetic interaction with MRC1 and found that deletion of MRC1 suppressed the DNA damage sensitivity of rtt107Δ mutants in CPT and MMS but aggravated the sensitivity of slx4Δ mutants. Interestingly, the situation was different in HU, where deletion of either RTT107 or SLX4 mildly suppressed the sensitivity of mrc1Δ mutants. This finding is consistent with a model proposed by a previous study suggesting that Rtt107 and Slx4 inhibit the checkpoint adaptor protein Rad9, which is normally not important in replication stress, but becomes crucial in the absence of Mrc1 (Ohouo et al. 2013). However, this model does not explain the genetic interactions observed in CPT and MMS and reflects the distinct responses to various types of DNA lesions, as well as the multiple functions of DNA damage response proteins.

Our study contributes to the growing of body of data that has mapped genetic interactions in response to DNA damage and further validates it as a fruitful approach that reveals condition-specific functions.
and pathways in the cell. There remains much ground to be covered as we have only started to characterize the pathways specific for the multitude of environmental conditions that affect all living organisms.

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