Genetic Interaction Landscape Reveals Critical Requirements for Schizosaccharomyces pombe Brc1 in DNA Damage Response Mutants

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ABSTRACT Brc1, which was first identified as a high-copy, allele-specific suppressor of a mutation impairing the Smc5-Smc6 holocomplex in Schizosaccharomyces pombe, protects genome integrity during normal DNA replication and when cells are exposed to toxic compounds that stall or collapse replication forks. The C-terminal tandem BRCT (BRCA1 C-terminus) domain of fission yeast Brc1 docks with phosphorylated histone H2A (γH2A)-marked chromatin formed by ATR/Rad3 checkpoint kinase at arrested and damaged replication forks; however, how Brc1 functions in relation to other genome protection modules remains unclear. Here, an epistatic mini-array profile reveals critical requirements for Brc1 in mutants that are defective in multiple DNA damage response pathways, including checkpoint signaling by Rad3-Rad26/ATR-ATRIP kinase, DNA repair by Smc5-Smc6 holocomplex, replication fork stabilization by Mrc1/claspin and Swi1-Swi3/Timeless-Tipin, and control of ubiquitin-regulated proteolysis by the COP9 signalosome (CSN). Exogenous genotoxins enhance these negative genetic interactions. Rad52 and RPA foci are increased in CSN-defective cells, and loss of γH2A increases genotoxin sensitivity, indicating a critical role for the γH2A-Brc1 module in stabilizing replication forks in CSN-defective cells. A negative genetic interaction with the Nse6 subunit of Smc5-Smc6 holocomplex indicates that the DNA repair functions of Brc1 and Smc5-Smc6 holocomplex are at least partially independent. Rtt107, the Brc1 homolog in Saccharomyces cerevisiae, has a very different pattern of genetic interactions, indicating evolutionary divergence of functions and DNA damage responses.

KEYWORDS Schizosaccharomyces pombe Brc1 CSN/ signalosome complex DNA damage response

Genome stability is especially at risk during the DNA synthesis (S) phase of the cell cycle, when relatively innocuous DNA lesions can impede replication or be converted into lethal chromosome breaks by passage of the replisome. These DNA lesions may originate from DNA replication errors, from toxic endogenous molecules such as free radicals arising from normal cellular metabolism, or from a wide variety of exogenous sources. Ancient prokaryotes evolved many of the most critical mechanisms for protecting genome integrity, such as homology directed repair, base excision repair, and mismatch repair. Eukaryotes inherited these mechanisms and added many more, such that even single-cell eukaryotes possess a complex array of genome protection pathways.

Brc1 protein in Schizosaccharomyces pombe plays an important role in maintaining genome stability and yet its mechanism of action remains poorly understood. Brc1 was first identified as an allele-specific, high-copy suppressor of smc6-74, which impairs the function of the Smc5-Smc6 holocomplex (Verkade et al. 1999). As with other members of the evolutionarily conserved SMC (structural maintenance of chromosomes) family of proteins, the Smc5-Smc6 complex is critical for chromosome segregation and is also important for DNA repair, especially at collapsed replication forks (De Piccoli et al. 2009; Kegel and Sjogren 2010; Pebernard et al. 2006). Brc1 is not essential for cell viability, but it is required in strains with compromised functions of the Smc5–Smc6 complex (Morikawa et al. 2004; Pebernard...
et al. 2004; Verkade et al. 1999). Brc1-defective strains are sensitive to genotoxins that stall replication forks or create DNA lesions that lead to replication fork collapse or other forms of replication stress (Sheedy et al. 2005). Furthermore, brclΔ cells have increased Rad52 foci, which indicate DNA replication difficulties even in the absence of exogenous genotoxins (Bass et al. 2012; Williams et al. 2010).

The presence of six BRCT (BRCA1 carboxy terminal) domains is a defining structural feature of Brc1 that is shared with the evolutionary conserved Saccharomyces cerevisiae Rtt107 and human PTIP proteins (Munoz et al. 2007; Rouse 2004). These proteins also share the ability to bind histone H2A (or H2AX in mammals) that has been phosphorylated by the ATM/ATR family of master DNA damage response checkpoint kinases (Li et al. 2012; Manke et al. 2003; Williams et al. 2010; Yan et al. 2011). This chromatin-specific interaction is mediated through the C-terminal pair of BRCT domains as also seen in DNA damage response mediator proteins such as human Mdc1 and fission yeast Crb2 (Du et al. 2006; Kilkenny et al. 2008; Stucki et al. 2005). Despite the overall structural similarities of Brc1, Rtt107, and PTIP and their importance for protecting genome integrity, it remains unclear whether they have conserved functions. Here, we investigate Brc1 by generating an epistatic miniarray profile (E-MAP) consisting of the quantitative analysis of genetic interactions between brclΔ and a S. pombe gene deletion library (Roguev et al. 2007). These E-MAP data provide novel insights into the functional relationships between Brc1 and other genome protection pathways in fission yeast.

**MATERIALS AND METHODS**

**Strains and genetic methods**

The strains used in this study are listed in Supporting Information, Table S1. Standard fission yeast methods were used as described previously (Forsburg and Rhind 2006). New null alleles of csn1, csn5, ddb1, spd1, ptk1, sde2, raf1, and snt1 were constructed using targeting constructs in which the entire open reading frames were replaced by KanMX6 as described previously (Bahler et al. 1998). Successful deletion of these genes was verified by polymerase chain reaction. Tetrad analysis was performed to construct double mutants and verified by polymerase chain reaction.

**Epistatic miniarray profile (E-MAP)**

E-MAP screens were performed and normalized as described previously (Roguev et al. 2008). Complete E-MAP profiles can be found in File S1.

**Gene Ontology (GO) analysis**

GO enrichment analysis used the Princeton implementation of GO term finder (http://go.princeton.edu/cgi-bin/GOTermFinder) (Boyle et al. 2004). Analysis used a p-value cut off of 0.01. For the fission yeast brclΔ E-MAP, the 56 SSL genes were compared with the background population of 2026 genes that produced E-MAP values (File S2). For the budding yeast rtt107Δ E-MAP, the 33 SSL genes (Collins et al. 2007) were compared with a background population consisting of all genes in budding yeast (File S3).

**Survival assay**

DNA damage sensitivity assays were performed by spotting 10-fold serial dilutions of exponentially growing cells onto yeast extract with glucose and supplements plates, and treated with indicated amounts of hydroxyurea (HU), camptothecin (CPT), and methyl methanesulfonate (MMS). For ultraviolet (UV) treatment, cells were serial diluted onto yeast extract with glucose and supplements plates and irradiated using a Stratagene Stratagene Stratalinker UV source. Cell survival was determined after 3–4 d at 30°C.

**Microscopy**

Cells were photographed using a Nikon Eclipse E800 microscope equipped with a Photometrics Quantix charge-coupled device camera and IPLab Spectrum software. All fusion proteins were expressed at their own genomic locus. Rad52-yellow fluorescent protein (YFP)—and RPA (Rad1)-green fluorescence protein—expressing strains were grown in Edinburgh minimal medium until mid-log phase for focus quantification assays. Quantification was performed by scoring 500 or more nuclei from three independent experiments.

**RESULTS**

**Quantitative genetic interaction analysis of Brc1**

To gain new functional insights into Brc1 we carried out an E-MAP analysis to quantify the genetic interactions between brclΔ and a S. pombe gene deletion library of nonessential genes (Kim et al. 2010; Roguev et al. 2007). E-MAP values were determined with a simple growth phenotype that measures negative (aggravating) interactions, such as synthetic sick/lethal (SSL) interactions, as well as positive (alleviating) interactions in which the double mutant is healthier than would be expected based on the growth of the two single mutants. An SSL interaction often identifies proteins that function in distinct but parallel pathways, whereas a positive interaction score may indicate either suppression or masking effects, in which loss of one gene masks the effect of losing another, as seen when two proteins act together in a common complex or pathway (Collins et al. 2007; Roguev et al. 2007).

The resulting Brc1 E-MAP consists of 2026 interaction scores (Table S2). Of these, 56 genes displayed a significant negative genetic interaction with brclΔ (interaction score < −2.5) and 23 displayed positive genetic interactions (interaction score >2). Most genes have genetic interactions scores close to zero. The results of this screen are summarized in Table S2.

GO analysis of the SSL mutants identified in this screen revealed significant enrichment of genes involved in key cellular processes, including cellular response to DNA damage stimulus, DNA repair, DNA damage checkpoint, chromatin modification, and cell denudation (Table 1). The strongest SSL score was obtained with csn1 (SSL = −15.1), which encodes a subunit of the COP9/Signalosome (CSN) complex that has important functions in the protection of genome integrity (Mundt et al. 1999). Genome protection was also highlighted by other genes with the greatest SSL scores, such as apn2 (SSL = −14.6), which encodes an apurinic/apyrimidinic endonuclease required for base excision repair (Fraser et al. 2003), hrg1 (SSL = −14.1), which encodes a RecQ type DNA helicase that plays an important role in DNA interstrand cross-link repair (Grocooch et al. 2012), and rad26 (SSL = −12.5), which encodes an ATRIP ortholog required for the activity of Rad3/ATR checkpoint kinase (Edwards et al. 1999). For comparison, the recently analyzed brc1 SSL interaction with dcd1, which encodes a deoxyctydylate deaminase required to maintain a proper balance of dNTPs, was −7.8 in this screen (Sanchez et al. 2012). All of these data are consistent with Brc1 playing an important role in genome protection. GO analysis of the 23 genes that displayed positive genetic interactions with brclΔ failed to yield specific process enrichment terms.

**Comparison of Brc1 and Rtt107 E-MAPs**

Fission yeast Brc1 and budding yeast Rtt107 are 6-BRCT domain proteins that bind γH2A and are important for survival of DNA lesions formed in S-phase (Bogliolo et al. 2007; Cobb et al. 2005; Fernandez-Capetillo et al. 2004; Marti et al. 2006; Papamichos-Chronakis
and density of double mutants compared with single mutants in serial dilution assays, we detected strong negative interactions with csn1, ddb1, and pnk1; moderate negative genetic interactions with csn5, rad26, rad17, srs2, swi3, and sde2; and only weak genetic interactions with raf1 and snt1. In most cases these negative genetic interactions were strikingly enhanced when cells were exposed to low or moderate doses of genotoxins such as UV light, HU, CPT, or MMS (Figure 1, Figure 2, Figure S1, and Figure S2). Notably, the negative genetic interactions with raf1 and snt1 became obvious in the presence of these genotoxins. Of the 13 SSL interactions that were retested, only set1 failed to confirm the results of the large-scale E-MAP screen (Figure S2C).

We also retested the positive genetic interactions between brc1 and csi1 (centromere clustering protein), msh2 (mismatch DNA repair MutS homolog), and chp1 (centromeric DNA binding protein CENP-B homolog). None of these interactions were confirmed in dilution assays performed in the absence of genotoxins; however, the csi1Δ allele clearly suppressed brc1Δ genotoxic sensitivity (Figure S3A). In contrast, double mutants involving brc1Δ and msh2Δ or chp1Δ grew more poorly than single mutants when tested in the presence of genotoxins (Figure S3, B and C).

Deneddylase-independent activities of the CSN are especially critical in the absence of Brc1

Brc1 displays negative genetic interactions with Csn1 and Csn5, which are members of the CSN. The hallmark activity of CSN consists of the deneddylation of the cullin subunit of cullin-RING E3 ligases (CRLs), which favors CRL disassembly to maintain cycles of CRL assembly and disassembly that are needed protect CRL components from self-destruction (Cope et al. 2002). As mentioned previously, we confirmed the strong negative genetic interaction between brc1 and csn1 by creating and testing a new csn1Δ null allele, which caused a modest growth defect that was substantially enhanced when combined with brc1Δ (Figure 1A, untreated). As previously described, the csn1Δ cells were mildly sensitive to the topoisomerase I inhibitor CPT, the DNA alkylating agent MMS, UV light, and the ribonucleotide reductase (RNR) inhibitor HU, which slows DNA replication (Hayles et al. 2013; Mundt et al. 1999). In comparison with brc1Δ or csn1Δ strains, the double mutant brc1Δ csn1Δ cells displayed very poor growth in the presence of these genotoxins (Figure 1A). We also confirmed the negative genetic interaction between brc1 and csn5Δ mutants did not share the genotoxicity phenotype of csn5Δ cells (Mundt et al. 2002), in our assays csn5Δ cells were sensitive to

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**Table 1** Summary of significant enriched GO categories for biological function of genes with genetic interactions positively correlated with **brc1** (*P* ≤ 0.01)

| Process                                                                 | Brc1 E-MAP Functional Groups |
|------------------------------------------------------------------------|------------------------------|
| Cellular response to DNA damage stimulus                               | srs2, csn1, rad1, ddb1, rad2, sbs3, rad9, rad17, pku80, hus1, hat1, ddb1, mms22, rad26, hrq1, nse6, arp42, apn2, pnk1, swi3 |
| DNA repair                                                             | srs2, ddb1, mms22, rad1, rad2, sbs3, rad9, rad17, pku80, hus1, hrq1, arp42, pnk1, apn2, hat1 |
| DNA damage checkpoint                                                  | h us1, rad26, rad1, ddb1, rad9, rad17 |
| Chromatin modification                                                 | ubpb, ngt1, met3, nnt1, crr1, raf1, arp42, set1, crr1, hat1 |
| Cullin deneddylation                                                    | csn1, csn5, csn71 |
| Others                                                                 | cbp11, ddc1, vps60, SPBC1711.15C, SPBC1289.14, mpp6, SPAC1635.01, SPAC1071.09C, SPBP16F5.05C, nna30, nup60, SPBP16F5.08C, rga8, SPBC651.02, urn1, SPCC1442.02, ppk3, sde2, SPBC27.05, pmp3, SPBC1711.09C, hmt1, atg22, fep1 |

GO, Gene Ontology; E-MAP, epistatic minarray profile.
Table 2 Summary of significant enriched GO categories for biological function of genes with genetic interactions positively correlated with RTT107 (P ≤ 0.01)

| Process                                           | RTT107 E-MAP Functional Groups |
|---------------------------------------------------|--------------------------------|
| DNA metabolic process                             | RRM3, MND2, SG51, RM11, POL30, SPT4, TSA1, MRE11, SW6, XRS2, RTT101, SLX5, TEL1, TOP3, SRS2, MSH1, NEJ1, NUP84 |
| DNA repair                                        | RRM3, MRE11, XRS2, SG51, SLX5, TEL1, SRS2, MSH1, NEJ1, POL30, NUP84, SPT4 |
| Double-strand break repair                        | SRS2, TEL1, NEJ1, NUP84, MRE11, XRS2, SG51 |
| Double-strand break repair via nonhomologous end joining | SRS2, NEJ1, MRE11, XRS2 |
| Response to stress                                | RRM3, YOR338W, SG51, RM11, POL30, SPT4, TSA1, SW6, XRS2, MRE11, RTT101, SLX5, TEL1, SRS2, MSH1, NEJ1, NUP84 |
| Telomere maintenance and organization             | TOP3, TEL1, RRM3, XRS2, SG51, SLX5 |
| Cell cycle                                       | CLB1, TSA1, MND2, XRS2, SW6, YOR338W, SG51, RTT101, RM11, TEL1, TOP3, CDC10, POL30 |
| Chromosome organization                           | RRM3, MND2, XRS2, SG51, SLX5, RM11, TEL1, TOP3, POL30, NUP84, SPT4 |
| DNA recombination                                 | SRS2, TOP3, MND2, XRS2, SW6, SG51 |
| Others                                            | RPN6, RPA190, YNR048W, GET2, AIM4, REB1, NUT1, BEM2, RPN10, TAH1, SRP40, DST1 |

GO, Gene Ontology; E-MAP, epistatic miniarray profile.

Chronic exposure to HU, CPT and MMS, although less so than csn1Δ or brc1Δ mutants. Double mutant brc1Δ csn1Δ cells grew quite poorly in the presence of these genotoxins (Figure 1B).

Ddb1 is a core member of CLR4 (Cul4-Ddb1 RING ligase) that is target of Csn deneddylation activity. We confirmed that the modest growth defect caused by ddb1Δ was substantially enhanced when combined with brc1Δ (Figure 2 untreated). The ddb1Δ cells were mildly sensitive to UV, MMS, and HU (Zolezzi et al. 2002) and CPT (Figure 2). We found that double mutant brc1Δ ddb1Δ cells were highly sensitive to these DNA-damaging agents (Figure 2).

The negative genetic interactions between Brc1 and members of the Csn and CLR4 ubiquitin ligase imply that Brc1 and Csn independently act in genome maintenance pathways that are partially complementary. The JAMM motif within the MPN domain of Csn5 is independent act in genome maintenance pathways that are partially critical in the absence of Brc1.

Increased RPA and Rad52 foci in csn1Δ cells

The SSL interaction between csn1Δ and brc1Δ suggested that the double mutant suffers increased rates of DNA damage or is unable to efficiently repair DNA lesions. To test this proposition we first monitored the formation of Replication Protein A (RPA) foci in csn1Δ and brc1Δ csn1Δ cells. RPA is the major single-strand DNA (ssDNA)-binding protein in eukaryotic cells (Parker et al. 1997). Formation of RPA foci in untreated cells is thought to arise predominantly from replication fork stalling or collapse and subsequent homology-directed repair that involve resection of DNA ends to generate 3’ ssDNA tails. For our assays we used strain in which Ssb1 (aka Rad11 in fission yeast), which is the largest subunit of RPA, was expressed with a YFP tag from the endogenous ssb1+ locus. In agreement with previous studies (Bass et al. 2012), we observed a significant increase in cells with RPA foci in the brc1Δ (16.0%) strain compared with wild type (7.1%). There was a much larger increase in cells with RPA foci in the

Table 3 Summary of genetic interactions involving brc1Δ

| Allele | Function | Untreated | UV | HU | CPT | MMS |
|--------|----------|-----------|----|----|-----|-----|
| csn1Δ  | Signalosome complex subunit | YES | YES | YES | YES | YES |
| ddb1Δ  | Damage DNA binding protein Part of the ubiquitin ligase complex | YES | YES | YES | YES | YES |
| csn5Δ  | Signalosome complex subunit | Yes | Yes | Yes | Yes | Yes |
| rad2Δ  | Cell cycle arrest | Yes | YES | YES | YES | YES |
| rad17Δ | RFC related checkpoint protein | – | YES | YES | YES | YES |
| srs2Δ  | ATP-dependent DNA helicase | Yes | YES | YES | YES | YES |
| pku8Δ  | Ku protein (NHEJ) | No | YES | YES | YES | YES |
| npl1Δ  | DNA kinase/phosphatase (SSBR) | YES | YES | YES | YES | YES |
| swi3Δ  | Replication fork protection complex subunit | Yes | Yes | Yes | Yes | Yes |
| sde2Δ  | Silencing defective protein | YES | YES | YES | YES | YES |
| raf1Δ  | Rik1-associated factor | No | Yes | Yes | Yes | Yes |
| set1Δ  | Histone lysine methyltransferase | No | No | R | No | No |
| snt1Δ  | Set3 complex subunit | No | No | Yes | Yes | Yes |
| cxi1Δ  | Chromosome segregation impaired protein 1 | No | R | R | R | R |
| msh2Δ  | Mut5 protein homolog 2 | No | No | No | Yes | Yes |
| cbp1Δ  | CENP-B homolog | No | No | No | Yes | Yes |

Double mutants were assessed for growth in the absence or presence of specified genotoxins. UV: ultraviolet; HU: hydroxyurea; CPT: camptothecin; MMS, methyl methanesulfonate; YES, strong negative interaction; YES, negative interaction; No, no genetic interaction; R, suppression.
There was a further small increase in the \textit{brc1Δ} \textit{csn1Δ} strain (44.2%) although the difference with \textit{csn1Δ} was not quite statistically significant (p-value = 0.08) (Figure 3A). We also monitored foci formation of Rad52, previously known as Rad22, which is essential for all forms of homology-directed repair in fission yeast (Meister et al. 2003). As observed previously (Williams et al. 2010), the frequency of Rad52-YFP foci was significantly increased in \textit{brc1Δ} cells (9.6%) as compared with the wild type. The incidence of cells with Rad52 foci was higher in the \textit{csn1Δ} strain (21.2%), and there was a further significant increase in the \textit{brc1Δ csn1Δ} strain (30.1%) (Figure 3B). These findings suggest that Brc1 prevents replication fork instability in CSN-defective cells.

**Defective relief of RNR inhibition in \textit{csn1Δ} and \textit{ddb1Δ} cells contributes to SSL interaction with \textit{brc1Δ}**

Ddb1, Cullin 4 (Pcu4), and CSN subunits, Csn1 and Csn2, are required for degradation of Spd1, which is an inhibitor of RNR (Holmberg et al. 2005). As \textit{spd1} deletion partially suppresses

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**Figure 1** Critical requirement of COP9/Signalosome (CSN) in \textit{brc1Δ} cells. Genetic interaction between Brc1 and Csn1 (A) or Csn5 (B). 10-fold serial dilutions of the indicated strains were exposed to the indicating DNA-damaging agents. Plates were incubated at 30°C for 3–4 d. CPT, camptothecin; HU, hydroxyurea; MMS, methyl methanesulfonate; WT, wild type.

**Figure 2** Genetic interactions among Brc1, Ddb1, and Spd1. 10-fold serial dilutions of the indicated strains were exposed to the indicating DNA damaging agents. Plates were incubated at 30°C for 3–4 d. CPT, camptothecin; HU, hydroxyurea; MMS, methyl methanesulfonate; WT, wild type.
genotoxin sensitivity in \textit{ddb1}Δ and \textit{csn1}Δ cells, we investigated whether the defect in degrading Spd1 contributed to the SSL interaction between \textit{brc1}Δ and \textit{ddb1}Δ or \textit{csn1}Δ. Our genetic analyses revealed that \textit{spd1} deletion substantially suppressed the growth defects in \textit{brc1}Δ \textit{ddb1}Δ and \textit{brc1}Δ \textit{csn1}Δ backgrounds (Figure 2 and Figure 4, untreated). This suppression was also evident to varying degrees in cells treated with a panel of genotoxins (UV, HU, MMS, and CPT) (Figure 2 and Figure 4). Taken together, these data indicate that the defect in relieving Spd1-mediated inhibition of RNR in \textit{ddb1}Δ and \textit{csn1}Δ cells is a major factor in the SSL interactions with \textit{brc1}Δ, although other pathways involving Csn1 and Ddb1 must also contribute to these negative genetic interactions.

Requirement for \textit{γH2A} in \textit{csn1}Δ cells

Rad3 checkpoint kinase, the fission yeast ortholog of mammalian ATR and budding yeast \textit{Mec1}, plays a central role in replication stress response triggered by stalled and collapsed replication forks (Boddy \textit{et al.} 1998; Lindsay \textit{et al.} 1998). Rad3 has a number of important substrates, including the serine in the SQE motif in the C-terminal tail of histone H2A (Nakamura \textit{et al.} 2004). Phospho-H2A, also known as \textit{γH2A}, serves as a chromatin recruitment platform for Brc1, Crb2, and Mdb1, which all bind \textit{γH2A} through their C-terminal BRCT domains. To assess whether \textit{γH2A} is important in the absence of CSN complex, we constructed a \textit{csn1}Δ strain in which both histone H2A genes contained a mutation that changed the C-terminal SQE phosphorylation motif to AQE (\textit{hta1-S129A hta2-S128A}), which is the so-called \textit{htaAQ} genotype. In comparison with the parental strains, the \textit{csn1}Δ \textit{htaAQ} strain displayed a reduced growth phenotype that was particularly evident in the presence of a panel of genotoxins (UV, HU, CPT, MMS) (Figure 5A).

Both Brc1 and Crb2 have well-established roles in DNA damage responses that are required for survival of genotoxic stress. Crb2 is required for activation of the checkpoint kinase Chk1 in response to DNA damage. As Chk1 was reported to have a synthetic growth defect with \textit{csn1}Δ, we expected that Crb2 would be important in \textit{csn1}Δ cells. Indeed, our studies revealed that \textit{csn1}Δ \textit{crb2}Δ cells grew poorly compared to the parental strains and this defect was accentuated in the presence of the panel of genotoxins (Figure 5B). These data suggest that \textit{γH2A} interactions with both Brc1 and Crb2 are important in \textit{csn1}Δ cells.

DISCUSSION

In this study we used E-MAP to explore the genetic interactions of \textit{S. pombe} Brc1, a protein with six BRCT domains that binds \textit{γH2A} and is important for survival of replication stress. Brc1 was identified as an allele-specific high-copy suppressor of \textit{smc6-74} (Verkade \textit{et al.} 1999), it becomes essential in strains with compromised Smc6 or Nse4 functions, and \textit{brc1}Δ is also has strong negative genetic interactions with conditional alleles of \textit{rad60} and \textit{top2} (Morikawa \textit{et al.} 2004; Pebernard \textit{et al.} 2006; Verkade \textit{et al.} 1999). Among the 56 SSL interactions
revealed in our E-MAP analysis, only four were previously detected through classical genetic analyses: *apn2* (E-MAP score = −14.58), encoding an apurinic/apyrimidinic endonuclease and *rad2* (E-MAP score = −2.98), encoding a FEN1 endonuclease, both of which are involved in base excision repair (Alseth et al. 2004; Alseth et al. 2005); *mms22* (E-MAP score = −2.44), encoding a DNA repair protein that forms a complex with Mms1 (Dovey and Russell 2007); and *ssb3* (E-MAP score = −10.97), encoding the nonessential small subunit of the replication protein A (Cavero et al. 2010). Among the CSN subunits identified in our screen, we found that negative genetic interaction was strongest with *csn1*. This observation suggests that loss of the deoxyribonuclease activity dependent on the Csn5 subunit is not fully responsible of the SSL interaction between Brc1 and Csn1, nor are the SSL interactions totally explained by the role of Ddb1/Csn1 in controlling RNR activation through Spd1 degradation. These results suggest that Csn1 and Brc1 function in parallel in response to DNA damage and contribute to genome stability through multiple pathways. Supporting this idea, our studies revealed that *csn1Δ* mutants have increased numbers of RPA and Rad52 foci. Thus, our studies also reveal the importance of γH2A in the absence of Csn1, with our data indicating that binding of both Brc1 and Cbr2 to γH2A is important in response to replication stress. Interestingly, deregulation of CSN and its interactions are related to multiple cancers, making CSN an interesting target for cancer therapy (Fuzesi-Levi et al. 2014; Lee et al. 2011; Richardson and Zundel 2005).

The SSL interaction of *brc1* with *nse6* provides clues about the functional relationships between Brc1 and the Smc5–Smc6 complex. As mentioned previously, Brc1 was initially discovered as an allele-specific, high-copy suppressor of *smc6*-74 (Verkade et al. 1999). This type of genetic interaction often indicates a physical association; for example, the missense mutation in *smc6*-74 might impair binding to Brc1, which is a defect that might be suppressed by increasing the total cellular concentration of Brc1. It is unknown if Brc1 associates with the Smc5–Smc6 holocomplex; however, the Brc1 homolog in *S. cerevisiae* coprecipitates with multiple subunits of the Smc5–Smc6 holocomplex (Ohouo et al. 2010). Nse5 and Nse6 form a heterodimer that is part of the Smc5–Smc6 holocomplex and is required for many or all of its DNA repair functions, but unlike other subunits of the holocomplex Nse5 and Nse6 are not essential for cell viability (Pebernard et al. 2006). The SSL interaction of *brc1* with *nse6* detected in our screen strongly indicates that the DNA repair functions of Brc1 and Smc5–Smc6 holocomplex are at least partially independent.

The SSL interaction of *brc1* with *mrc1* is novel and provides insights about the requirement for Brc1 in the response to replication stress. Mrc1 (mediator of replication checkpoint) was discovered by screening for mutations that cause hydroxyurea sensitivity and are rescued by overproduction of the replication checkpoint kinase Cds1/Chk2 (Tanaka and Russell 2001). Mrc1 is conserved in budding yeast and mammals in which it is known as Mrc1 and claspin, respectively (Alcasabas et al. 2001; Kumagai and Dunphy 2000). The

![Figure 4](image-url) Genetic interactions among Brc1, Csn1, and Spd1. 10-fold serial dilutions of the indicated strains were exposed to the indicating DNA damaging agents. Plates were incubated at 30°C for 3–4 d. CPT, camptothecin; HU, hydroxyurea; MMS, methyl methanesulfonate; WT, wild type.
mrcl* gene in fission yeast is periodically transcribed during S-phase in the cell cycle and recruiting Cds1 to stalled replication forks by Mrcl is required for its efficient activation of Cds1. Mrcl appears to have both Rad53-dependent and -independent functions that stabilize replication forks in S. cerevisiae (Katou et al. 2003; Osborn and Elledge 2003), but it is unclear whether Mrcl has Cds1-independent activities in fission yeast (Nitani et al. 2006). In our brc1Δ E-MAP we uncovered a significant SSL interaction with mrclΔ (E-MAP = −2.52) but not with cds1Δ (E-MAP = −0.60), even though cds1Δ mutants are more severely sensitive to HU (Tanaka and Russell 2001). These data indicate that the absence of Brcl enhances the requirement for a Cds1-independent function of Mrcl in stabilizing replication forks.

Similar conclusions are suggested by the SSL interaction of brc1Δ with swi3Δ (E-MAP = −4.6). Swi3 binds Swi1 to form the fork protection complex that stabilizes stalled replication forks (Noguchi et al. 2003; Noguchi et al. 2004). This activity is required for robust activation of Cds1 in response to HU treatment and other forms of replication stress. However, the absence of an SSL interaction between brc1Δ and cds1Δ mutations suggests that Cds1-independent activity of Swi1-Swi3 fork protection complex is especially critical in the absence of Brcl.

Although we focused on the SSL interactions identified in our brc1Δ E-MAP, we did confirm the alleviating (positive) interaction with csi1 (E-MAP = +2.14). Csi1 is implicated in centromere clustering during interphase through its interaction with Sad1 in the spindle pole body and it also has a role in tethering spindle-stabilizing factors to the spindle pole body for promoting bipolar spindle assembly (Hou et al. 2012; Zheng et al. 2014). The involvement of Csi1 in these processes is interesting in light of our evidence that recruiting Brcl to γH2A in pericentromeric heterochromatin during S-phase contributes to maintaining the heterochromatic state, which is required for efficient chromosome segregation during nuclear division (Lee et al. 2013). Indeed, genetic assays indicate that Brcl is required for mitotic chromosome stability, which suggests a role for Brcl in chromosome segregation (Verkade et al. 1999). Furthermore, we found that brc1Δ cells are moderately sensitive to the microtubule-destabilizing drug thiomamide and display increased rates of chromosome missegregation in the presence of thiabendazole (Lee et al. 2013). These effects of Brcl correlate with the genetic data linking Brcl to the Smc5-Smc6 complex (Verkade et al. 1999) and data showing that the holocomplex localizes around centromeres during S-phase and defects in the complex increase the frequency of lagging chromosomes during nuclear division (Pebernard et al. 2008). However, despite these striking correlations, it is unclear why a defect in Csi1 function should alleviate the requirement for Brcl as suggested by our genetic suppression data. In this regard it is interesting that csi1Δ cells are sensitive to the DNA-damaging agent 4-nitroquinoline 1-oxide, which causes replication stress by producing bulky adducts in DNA (Deshpande et al. 2009). Our studies further indicated that csi1Δ cells are mildly sensitive to UV light, HU, and CPT (Figure S3). Most strikingly, the csi1Δ mutation effectively suppresses sensitivity of brc1Δ cells to these genotoxins and MMS. Again, it is unobvious how this suppression happens, although we note that there are some well-known examples of mutations in different DNA repair pathways having suppressive effects; for example, eliminating the Ku complex required for nonhomologous end joining (NHEJ) suppresses defects in the Mre11-Rad50-Nbs1 complex and Ctp1 that are required for homologous recombination repair (Langerak et al. 2011), and the rad51Δ mutations suppresses UV sensitivity of nse6Δ mutants (Pebernard et al. 2006).

Finally, the list of SSL interactions derived from the E-MAP studies for Brcl (56 SSL interactions) and Rtt107 (33 SSL interactions) reveal remarkably little overlap, with only one gene, the ATP-dependent DNA helicase srs2/SRS2, being found in both screens. In S. pombe, deletion of srs2 causes elevated rates of spontaneous recombination (Doe and Whitby 2004). Furthermore, deletion of brc1 suppressed the hyper-recombination phenotype of an srs2Δ strain (Bass et al. 2012). This small degree of overlap suggests major functional differences between Brcl and Rtt107 despite their similarities in domain organization and a shared mechanism of localizing to DNA lesions through C-terminal BRCT domains binding γH2A (Li et al. 2012; Williams et al. 2010). However, comparing all genetic interactions identified by classical genetic analyses and E-MAP suggests an additional degree of overlap for Brcl and Rtt107. For example, classical genetic interactions uncovered strong negative interactions with Rqh1 and Sgs1, which are orthologous DNA helicases of the RecQ family. Nevertheless, the unexpectedly low overlap for both E-MAP lists and GO process terms suggests significant functional differences between Brcl and Rtt107, reflecting the large evolutionary divergence between S. pombe and S. cerevisiae.
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