Genetic regulation of Dna2 localization during the DNA damage response

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ABSTRACT

DNA damage response pathways are crucial for protecting genome stability in all eukaryotes. *Saccharomyces cerevisiae* Dna2 has both helicase and nuclease activities that are essential for Okazaki fragment maturation, and Dna2 is involved in long-range DNA end resection at double-strand breaks. Dna2 forms nuclear foci in response to DNA replication stress and to double-strand breaks. We find that Dna2-GFP focus formation occurs mainly during S phase in unperturbed cells. Dna2 co-localizes in nuclear foci with 25 DNA repair proteins that define recombination repair centers, in response to phleomycin induced DNA damage. To systematically identify genes that affect Dna2 focus formation, we crossed Dna2-GFP into 4293 non-essential gene deletion mutants, and assessed Dna2-GFP nuclear focus formation after phleomycin treatment. We identified 37 gene deletions that affect Dna2-GFP focus formation, 12 with fewer foci and 25 with increased foci. Together these data comprise a useful resource for understanding Dna2 regulation in response to DNA damage.
INTRODUCTION

The maintenance of the genome stability is an essential process in living cells. DNA lesions perturb cellular DNA replication and transcription processes, and failure to repair damaged DNA can lead to mutagenesis, tumorigenesis and lethality. In order to combat DNA damage cells activate DNA damage response mechanisms to arrest cell cycle progression, detect DNA lesions, amplify the DNA damage signal, and execute DNA repair (Rouse and Jackson, 2002; Harrison and Haber, 2006).

Dna2 is a conserved DNA-specific ATPase present in organisms from yeast to humans. Dna2 has both helicase and nuclease activities that are essential for Okazaki fragment maturation (Budd and Campbell 1997) and it plays a crucial role in repairing DNA double strand breaks (Zhu et al. 2008) and in telomere and mitochondrial DNA maintenance (Choe et al. 2002; Duxin et al. 2009; Budd and Campbell 2013; Ronchi et al. 2013). Depletion of Dna2 causes incomplete DNA replication and genomic instability (Budd and Campbell 1995; Liu et al. 2000), and the ATPase and nuclease activities of Dna2 are essential for cell survival (Lee et al. 2000; Budd et al. 2000; Budd et al. 1995; Formosa and Nittis 1999). Overexpression of Dna2 has been detected in a variety of cancers, and is associated with poor patient outcome (Strauss et al. 2014; Peng et al. 2012).

DNA double strand breaks occur upon exposure to exogenous agents, such as ionizing radiation, or indirectly through replication fork collapse at DNA damage sites. If left unrepaired double strand breaks can cause genomic instability, cell death and tumorigenesis (Mehta and Haber 2014; Jackson and Bartek 2009). Dna2 participates in DNA repair by homologous recombination (HR). In HR, Sae2 and the MRX (Mre11-
Rad50-Xrs2) complex initiate DNA resection at the double strand break, whereas long range DNA resection is catalyzed either by Exo1 or Dna2 in collaboration with the Sgs1/Top3/Rmi1 helicase-topoisomerase complex (Mimitou and Symington 2008; Zhu et al. 2008). The resulting 3′ single stranded DNA is coated by Replication Protein A (RPA), which serves as a substrate for Rad51 filament formation (mediated by Rad52, Rad55-Rad57 and Rad54), and as a primer for subsequent DNA synthesis following strand invasion at a homologous DNA sequence that serves as a template for repair (Sugiyama and Kowalczykowski 2002; Sung 1997; Chen et al. 2013).

Dna2 has recently been shown to be a target of different post-translation regulation pathways. In fission yeast, Dna2 is phosphorylated by the checkpoint effector kinase Cds1 during replication stress, and phosphorylation is essential to stabilize stalled replication forks and to prevent reversal of arrested forks (Hu et al. 2012). In budding yeast, Dna2 is direct target of Cdk1 and Mec1 kinases, and Dna2 itself directly stimulates Mec1 kinase activity (Chen et al. 2011; Kumar and Burgers 2013; Kosugi et al. 2009). Dna2 is also regulated by the SUMO pathway (Makhnevych et al. 2009). Previous studies indicate that Dna2 forms nuclear foci during DNA damage and DNA replication stress (Lisby and Rothstein 2009; Makhnevych et al. 2009; Tkach et al. 2012). Here we characterize the formation of Dna2 foci in response to double strand DNA breaks, and apply a genome-wide screen to systematically identify gene deletion mutants that change Dna2 focus formation levels.

MATERIALS AND METHODS

Strains and Media

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Strains used in this study are listed in Table S1, and are derivatives of BY4741 (Brachmann et al. 1998). Low-fluorescence media (yeast nitrogen base supplemented with 5 g/l ammonium sulphate, 2% (w/v) glucose, 150 mg/l methionine, 20 mg/l histidine, 100 mg/l leucine and 20 mg/l uracil) was used for high-throughput screening.

The DNAS-yEmCherry strain was constructed by transforming JTY5 with a PCR product containing yEmCherry::CaURA3 and targeted to DNA2 (Dna2-mOrr-fw and Dna2-mOrr-rv primers; Table S2). The template for the PCR, pKT-yEmCherry-CaURA3, was constructed by replacing mCherry sequences in the plasmid pKT-mCherry-CaURA3 with yEmCherry sequences amplified from pNEB31 (Silva et al., 2012) with primers yEmRFP_F and yEmRFP_R (Table S2). The plasmid pKT-mCherry-CaURA3 was constructed by replacing GFP in the plasmid pKT209 (Sheff and Thorn 2004) with mCherry, and was a kind gift from Mike Cox in Brenda Andrews’ lab.

Microscopy and Image Analysis

For analysis of Dna2-GFP nuclear foci, GFP fusion proteins that co-localized with Dna2-yEmCherry, and Dna2-GFP foci in gene deletion backgrounds, cultures were grown to saturation in YPD, diluted into fresh YPD to OD600 = 0.1, and grown for 2 h at 30°C before treating with 5 µg/ml phleomycin for 2h. Eleven z slices with a 0.4 µm step size were acquired using Volocity imaging software (PerkinElmer) controlling a Leica DMI6000 microscope with the fluorescein isothiocyanate, Texas Red and differential interference contrast filter sets (Quorum Technologies). Dna2-yEmCherry foci, ORF-GFP foci, and co-localizing foci were counted in at least 100 cells. Functions of the proteins tested for co-localization with Dna2 were annotated with GO-Slim terms.
downloaded from the Saccharomyces Genome Database (www.yeastgenome.org; accessed on 4/4/15) and GO functions from GeneMANIA (www.genemania.org; accessed on 4/3/15) (Montoyo et al. 2014). Protein interactions for the proteins tested for Dna2 co-localization were downloaded from GeneMANIA, using data from BioGRID (www.thebiogrid.org) small-scale studies.

**Identification of Dna2 Focus Regulators**

*DNA2-GFP* (AYY3) was crossed with an array of 4293 strains (Costanzo et al. 2010) from the haploid non-essential yeast gene deletion collection (Giaever et al. 2002) using synthetic genetic array methodology (Baryshnikova et al. 2010). The resulting strains, expressing Dna2-GFP in the context of deletion of individual non-essential genes, were grown and imaged after treatment with phleomycin or with vehicle as a control, as described previously (Tkach et al., 2012). Briefly, the haploid strains were grown to saturation overnight in minimal media and further sub-cultured to mid-log phase (~16 h growth time) in low fluorescence media. Cells were transferred to 384-well slide plates to a final density of 0.045 OD<sub>600</sub> ml<sup>-1</sup> and incubated at 30°C for 2 h in low fluorescence medium (control), or low fluorescence medium plus 5 µg/ml phleomycin. Images from four fields per well were acquired in the green (405/488/640 primary dichroic, 540/75 emission band-pass filter, 800 ms exposure) and red channels (405/561/640 primary dichroic, 600/40 emission band-pass filter, 2,000 ms exposure) on an EVOTEC Opera confocal microscope system (PerkinElmer). The complete set of images from the high-throughput screen is available from the Yeast Resource Center Public Image Repository (Riffle and Davis 2010) at the URL http://images.yeastrc.org/yimit-2015. The images
were scored by visual inspection for strains that exhibited decreases in Dna2-GFP foci in phleomycin, or increases in Dna2-GFP foci in the untreated samples, relative to control. Positives were examined in low throughput as indicated above. The number of Dna2-GFP foci per cell was quantified by visual analysis of at least 100 cells, in duplicate. We assessed whether the mean number of Dna2-GFP foci per cell in each mutant was detectably different from wild type by applying a two-tailed t-test, assuming equal variance. The network of genes that affect Dna2 focus formation was drawn in Cytoscape (www.cytoscape.org), and overlaps with other data sets were assessed using a hypergeometric test in R. GO term enrichment was analyzed with the GO Term Finder (go.princeton.edu) using the deletion collection screened as the ‘universe’, and p-values corrected for multiple testing are reported.

For gene deletions with a decreased fraction of cells with Dna2-GFP foci, the total GFP fluorescence and nuclear GFP fluorescence was measured after segmenting 10 cells and nuclei for each mutant in ImageJ (http://imagej.nih.gov/ij/). Nuclear focus intensity was measured by segmenting 15-20 individual foci and measuring the GFP fluorescence in ImageJ. Unbudded (G1) cells were excluded from the analysis. We assessed whether the mean GFP fluorescence intensity per cell, the mean nuclear GFP fluorescence intensity per cell, and the mean nuclear focus GFP fluorescence intensity in each mutant was detectably different from wild type by applying a two-tailed t-test, assuming equal variance.

**Drug sensitivity:** To assay phleomycin sensitivity, cultures were grown overnight at 30°C in YPD. Cultures were diluted to an OD<sub>600</sub> of 1, serially diluted tenfold, spotted on
YPD medium with or without 2.5 or 5 µg/ml phleomycin, and grown for 2-3 days at
30°C before imaging.

Results and Discussion

Dna2 forms nuclear foci in S and G2 phases

Dna2, like many DNA damage response proteins, forms nuclear foci in response
to double strand breaks and DNA replication stress (Lisby and Rothstein 2009;
Makhnevych et al. 2009; Tkach et al. 2012; Chen et al. 2011). In addition to being
regulated by DNA damage, the intracellular localization of Dna2 is connected to cell
cycle phase via CDK phosphorylation. In G1 arrested cells Dna2 is mainly cytoplasmic,
whereas during S, G2, and M phases Dna2 displays a nuclear localization (Kosugi et al.
2009). To investigate the cell cycle distribution of Dna2 foci in unperturbed cells and
cells with double strand DNA breaks, we quantified Dna2-GFP foci in unbudded (G1),
small budded (S), and large budded (G2) cells, in both asynchronous cultures and
cultures treated with phleomycin. Phleomycin, an antibiotic of the bleomycin family,
causes free radical mediated DNA damage, including double strand breaks (Moore 1988;
Sleigh 1976). In unperturbed cells, Dna2 foci were mainly found in S phase, in 24% of
small budded cells (Table 1), suggesting that Dna2 foci can arise during DNA replication.
Following 2 hours of treatment with phleomycin, Dna2 foci were found in small budded
and large budded cells, but rarely in unbudded (G1) cells (Table 1). We arrested cells in
G1 with mating pheromone, and treated the arrested cells with phleomycin (Figure 1A
and 1B), confirming that Dna2 foci do not form efficiently during G1 phase. These
results are in agreement with the established roles of Dna2 in Okazaki fragment
maturation (in S phase), and roles in double strand break repair (DNA resection during G2/M phase).

**Dna2 co-localizes with proteins that form Rad52 repair centers at double strand breaks**

In response to double strand breaks, a number of DNA repair and checkpoint proteins re-localize from diffuse nuclear distribution to distinct sub-nuclear foci. The recombination repair protein Rad52 forms foci that co-localize with double strand breaks (Lisby et al. 2003; Lisby et al. 2001), and some repair proteins are known to co-localize in foci with Rad52 (Lisby et al. 2004). Not all proteins that form nuclear foci co-localize with Rad52, however (Tkach et al. 2012; Gallina et al. 2015). To systematically analyze proteins that co-localize with Dna2 in response to phleomycin, we fused Dna2 to yeast enhanced monomeric Cherry (yEmCherry) (Keppler-Ross et al. 2008; Silva et al. 2012), and crossed it to 55 GFP-tagged ORF strains, including 27 proteins that we found form nuclear foci in DNA replication stress (Tkach et al. 2012), and an additional 28 proteins reported to form nuclear foci in DNA damage (Gasior et al. 1998; Melo et al. 2001; Frei and Gasser 2000; Lisby et al. 2004; Srikumar et al. 2013; Denervaud et al. 2013). Among the 55 proteins, 25 co-localize with Dna2 detectably, with the extent of co-localization ranging from 55% (Ygr042w) to 2% (Mre11) (Table 2, Figure 2, and Table S3).

We plotted a network of the proteins that co-localize detectably with Dna2 foci (Figure 2C), with edge thickness corresponding to the percent of foci that co-localize with Dna2 foci, and with Gene Ontology process term indicated for each. Consistent with the roles of Dna2 in DNA replication and double strand break repair, we found that most of the 25 proteins that co-localize with Dna2 in phleomycin have connections to repair of
double strand breaks. All the representatives of the RAD52 epistasis group that were tested co-localized with Dna2, with Rad52 found with Dna2 at the highest frequency (54% of Dna2 foci contain Rad52). Of particular interest, Ygr042w showed a similarly high frequency of co-localization with Dna2, consistent with a recent report that the fission yeast homologue, Dbl2, co-localizes with recombination repair foci (Yu et al. 2013), and suggesting that Ygr042w could play a role in recombination repair. Dna2 functions in the resection step of double strand break repair in concert with Sgs1/Top3/Rmi1 (Zhu et al. 2008) and co-localized frequently with each of the members of the complex (Figure 2C). Dna2 also co-localized frequently with the ssDNA binding protein RPA, a regulator of resection (Niu et al. 2010; Cejka et al. 2010; Chen et al. 2013). Dna2 showed only a weak co-localization with the MRX complex (Mre11, Rad50, Xrs2), consistent with 2-step resection models where ends resected initially by MRX/Sae2 are handed off to Dna2 (Mimitou and Symington 2011). As previously suggested for MRX and Rad52 (Lisby et al. 2004), the weak co-localization of Dna2 and MRX detected could reflect proteins that are in the same repair center but that are not associated with the same DNA end, since multiple DNA ends can associate with a single Rad52 focus (Lisby et al. 2003). DNA damage checkpoint proteins co-localize with Dna2 robustly, including the Mec1 activators Dpb11, Ddc1, and Ddc2, and the effector kinase Rad53 (Figure 2C). We found that complex members tended to show similar frequencies of co-localization (Rfa1/Rfa2, Sgs1/Top3/Rmi1, MRX, RFC, Rtt107/Slx4, Rad55/57; Figure 2C and Table S3).

We analyzed the extent of protein-protein interactions among the 25 proteins that co-localize with Dna2 foci, and among the 28 proteins that form nuclear foci but do not co-localize detectably with Dna2 foci following phleomycin treatment (Figure 3). We
noted that the proteins that co-localized with Dna2 form a dense network of protein-protein interactions (5.04 interactions on average), whereas the protein-protein interactions among the proteins that did not co-localize with Dna2 are sparser (1.75 interactions on average). Additionally, proteins that co-localize with Dna2 foci for the most part are annotated on GO processes involved in DNA repair, DNA replication, and DNA damage response. The proteins that did not co-localize with Dna2 foci are involved in some distinct processes, notably RNA catabolism, suggesting that some of these proteins form nuclear foci with functions that are distinct from Rad52 repair centers.

Consistent with this possibility, Cmr1 was recently shown to form a distinct intranuclear compartment that also contains four additional proteins that fail to co-localize with Dna2 (Pph3, Apj1, Hos2, and Dus3) (Gallina et al. 2015). Together our data indicate that Dna2 foci co-localize with a subset of repair and checkpoint proteins that likely define the canonical Rad52 double-strand break repair foci (Gasior et al. 1998; Lisby et al. 2004; Lisby et al. 2001; Yu et al. 2013).

**Identification of genes affecting Dna2 focus formation**

To systematically identify the genetic requirements for Dna2 focus formation we screened a collection of 4293 haploid non-essential gene deletion mutants (Costanzo et al. 2010; Giaever et al. 2002) in the absence and presence of phleomycin. Dna2-GFP foci were visualized by high throughput confocal microscopy, and scored by visual inspection. All images from the screen are available from the Yeast Resource Center Public Image Repository (Riffle and Davis 2010) at the URL http://images.yeastrc.org/yimit-2015.

Forty-seven genes were identified that affected Dna2-GFP focus formation, either by increasing focus formation in untreated cells (32) or by decreasing focus formation in
phleomycin treated cells (15) (Table S4). These positives were re-imaged in low throughput before and after treatment with phleomycin for 2 hours, and foci in the resulting images were quantified. We confirmed that 12 mutants showed a decrease (p < 0.05) in the fraction of cells with a Dna2-GFP focus following phleomycin treatment, relative to wild type (Figure 4A), and that 25 mutants had an increased (p < 0.05) fraction of cells with Dna2 foci relative to wild type (Figure 4A). We identified three classes of mutants with increased Dna2 foci: those with increased spontaneous foci only (11), those with increase spontaneous and increased phleomycin-induced foci (7), and those with increased phleomycin-induced foci only (7) (Figure 4A). There are likely additional mutants in the deletion collection that cause increased Dna2 foci in phleomycin only, since this class was not scored in our primary screen.

The 25 gene deletions that conferred increased Dna2 foci were strongly enriched for genes involved in DNA repair and DNA damage response (Figure 4B; p=2x10^{-17} and p=5x10^{-16}). We compared these genes to those identified in a recent ‘constitutive RNR3 expression’ screen (Hendry et al. 2015), and found significant overlap (16 genes, hypergeometric p=4x10^{-21}), suggesting the presence of increased spontaneous DNA damage in these mutants, as expression of RNR3 responds specifically to DNA damage (Elledge and Davis 1990). We compared the genes that when deleted caused increased Dna2 foci to those that cause increased Rad52 foci (Alvaro et al. 2007), again finding significant overlap (10 genes, hypergeometric p=2x10^{-11}). Finally, we compared to the set of genes with negative genetic interactions with dna2-1 or dna2-2 (Budd et al. 2005), which could indicate spontaneous damage that requires Dna2 for its repair. We noted a significant overlap (10 genes, hypergeometric p=2x10^{-14}). There were only 5 genes
(CLB5, CTF8, EST3, MID1, and RIF2) in our set of 25 that were not found to have increased Rnr3 expression, increased Rad52 foci, or a negative genetic interaction with dna2. Decreased replication origin usage in clb5 mutants is proposed to cause spontaneous DNA damage (Gibson et al. 2004). Deletion of the CTF18 gene, which encodes the binding partner of Ctf8, causes increased Rad52 foci (Gellon et al. 2011). RIF2 and EST3 regulate telomere length (Wotton and Shore 1997; Hughes et al. 2000). MID1 has no clear connection to DNA repair, but it is only 403 bp from the 5’ end of the RFC3 ORF. RFC3 is essential for DNA replication and is important for DNA repair (Cullmann et al. 1995; Green et al. 2000). Thus, all 25 of the genes whose deletion causes increased Dna2 focus formation likely cause increased DNA damage when deleted, and Dna2 likely participates in the repair of that damage.

The 12 gene deletions with fewer Dna2-GFP foci do not represent a coherent functional group (Figure 4B), and were not enriched for any GO term. Of the genes identified, only RAD27 has a clear connection to Dna2, in that Rad27 and Dna2 function in concert in Okazaki fragment maturation (Ayyagari et al. 2003). Several of the genes we identify are involved in translation capacity (RPP1A, RPS10A, DEG1), nutrient sensing (TCO89), and G1 transit (SWI4), and so could reflect cell cycle delays in G1, where Dna2 foci typically do not form (Table 1). It is also possible that reduced Dna2-GFP foci per cell could be caused by decreased total abundance of Dna2-GFP, or decreased nuclear abundance of Dna2-GFP. We tested these latter possibilities by quantifying total GFP fluorescence per cell, nuclear GFP fluorescence per cell, and Dna2-GFP focus intensity (Figure 4C, 4D, and Table S4). Two mutants, brp1Δ and ckb1Δ, had statistically apparent decreases in nuclear Dna2-GFP mean fluorescence intensity (Figure
4C). In both cases, the effect size was small (0.67x wild type for \( brp1\Delta \) and 0.70x wild type for \( ckb1\Delta \)), and the decrease in nuclear Dna2-GFP signal was paralleled by a similar decrease in total cellular Dna2-GFP signal. Deletion of \( YNL198C \) caused a decrease in total cellular Dna2-GFP fluorescence, but the decrease in nuclear fluorescence in this mutant could not be statistically distinguished from wild type. A similar analysis of Dna2-GFP focus intensity in the 12 mutants with decreased Dna2-GFP foci per cell revealed four strains with decreased focus intensity (Figure 4D; \( brp1\Delta \), \( ckb1\Delta \), \( deg1\Delta \), and \( ynl198c\Delta \)). Interestingly, two mutants, \( rad27\Delta \) and \( ubp3\Delta \), had increased focus intensity despite having fewer foci per cell. We conclude that none of the mutants causes a substantial decrease in Dna2-GFP expression or in nuclear abundance of Dna2-GFP. However, in \( brp1\Delta \) and \( ckb1\Delta \), decreased nuclear localization could indirectly cause a decrease in Dna2-GFP focus intensity.

To further assess the functional relationship between \( DNA2 \) and gene deletions that decrease Dna2-GFP foci, single and double mutants of \( dna2-1 \) (Budd et al., 2000) and \( ubp3\Delta \), \( ecm32\Delta \), \( swi4\Delta \), \( ckb1\Delta \), \( ynl198C\Delta \), \( deg1\Delta \), \( rad27\Delta \), \( rpp1A\Delta \), \( tos9\Delta \), \( bdp1\Delta \), \( tco89\Delta \) and \( rps10A\Delta \) were tested for phleomycin sensitivity. Double mutants of \( dna2-1 \) with \( tos9\Delta \), \( swi4\Delta \), \( ckb1\Delta \), \( rps10A\Delta \), or \( rad27\Delta \), showed increased phleomycin sensitivity relative to the relevant single mutants (Figure 5), indicating that deletion of any of these five genes exacerbates the phleomycin sensitivity of \( dna2-1 \) mutants.

In summary, we find that Dna2 nuclear foci induced by phleomycin co-localize with a group of proteins that form double-strand break repair centers with Rad52. We identified 25 genes that cause an increase in Dna2 foci when mutant, likely by promoting spontaneous DNA damage. We identified a functionally diverse group of 12 genes that
are important for robust Dna2 focus formation in phleomycin, 5 of which contribute to phleomycin resistance. Together these data will be a useful resource for understanding Dna2 compartmentalization in response to DNA damage.

Acknowledgements

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References


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Table 1. Frequencies of Dna2-GFP focus formation in G₁, S and G₂/M cells

<table>
<thead>
<tr>
<th></th>
<th>unbudded (G₁)</th>
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<th>large budded (G₂/M)</th>
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<tr>
<td></td>
<td>Control</td>
<td>Phleomycin</td>
<td>Control</td>
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<td>1.7</td>
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The percent of cells in each morphological class that contain a Dna2-GFP focus is indicated.
Table 2. Proteins that co-localize with Dna2-yEmCherry during treatment with phleomycin.

<table>
<thead>
<tr>
<th>ORF-GFP</th>
<th>Protein</th>
<th>Protein-GFP co-localized with Dna2-yEmCherry (%)</th>
<th>Dna2-yEmCherry co-localized with Protein-GFP (%)</th>
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<tr>
<td>YGR042W</td>
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</table>
Figure 1. Dna2-GFP focus formation in G1 and asynchronous cells. (A) Logarithmic phase asynchronous cells or cells arrested in G1 were exposed to phleomycin (5μg/ml) and imaged by confocal microscopy to detect Dna2-GFP foci. (B) The number of Dna2-GFP foci per cell was quantified for the G1-arrested and asynchronous cells. At least 100 cells were analysed in 3 independent experiments, and the percent of cells with at least 1 Dna2-GFP focus is plotted for each.
Figure 2. Co-localization of Dna2 with DNA repair and DNA damage response proteins. (A) Co-localization of the indicated ORF-GFP with Dna2-yEmCherry was measured by confocal fluorescence microscopy after phleomycin treatment. The percent of cells with co-localizing foci is plotted. (B) Representative images of proteins that co-localize with Dna2. Cells expressing Dna2-yEmCherry and the indicated ORF-GFPs were imaged after phleomycin treatment. Scale bar is 5 μm. (C) Network of the 25 proteins that co-localize with Dna2 after phleomycin treatment. Thickness of the edges corresponds to the fraction of cells displaying co-localization. Gene function is indicated by node colour.
**Figure 3. Dna2 focus protein-protein interaction network.** The proteins that were tested for Dna2 focus co-localization are represented as nodes coloured according to function, with the 25 proteins detected at the Dna2 focus on the left and the 28 proteins not found in Dna2 foci on the right. Edges correspond to protein-protein interactions curated by BioGRID, with interactions between Dna2 focus proteins in red, interactions between non-Dna2 focus proteins in blue, and interactions that bridge the two groups in green.
Figure 4. Identification of genes that affect Dna2-GFP focus formation. (A) The fraction of cells with Dna2-GFP foci in 12 gene deletions with fewer Dna2 foci after treatment with phleomycin, and in 25 gene deletions with increased Dna2 foci either in untreated cells or after treatment with phleomycin is plotted for two replicates. (B) The 37 confirmed genes that affect Dna2 foci abundance are organized in a network, with nodes coloured according to function. Edges are in red for gene deletions with fewer foci and in green for gene deletions with increased foci. (C) Dna2-GFP fluorescence intensity after treatment with phleomycin is plotted for the 12 gene deletions with fewer Dna2 foci, for whole cells (dark blue; n=10) and nuclei (light blue; n=10). Horizontal bars indicate the median Dna2-GFP fluorescence in each compartment for each mutant. Horizontal lines mark the median Dna2-GFP fluorescence in each compartment for wild type. (D) Dna2-GFP nuclear focus intensity after treatment with phleomycin is plotted for each mutant (n=12 to 20) and wild type. Horizontal bars indicate the median focus intensity for each mutant, and the horizontal line marks the median focus intensity for wild type.
Figure 5. Phleomycin sensitivity of dna2-1 double mutants. Tenfold serial dilutions of the indicated strains were spotted onto YPD or YPD containing phleomycin at the concentrations shown. Plates were imaged after 2-3 days.