Stead et al. BMC Genetics 2012, 13:36
http://www.biomedcentral.com/1471-2156/13/36

BMC Genetics

RESEARCH ARTICLE Open Access

Mcm2 phosphorylation and the response to replicative stress

Brent E Stead, Christopher J Brandl, Matthew K Sandre and Megan J Davey*

Abstract

Background: The replicative helicase in eukaryotic cells is comprised of minichromosome maintenance (Mcm) proteins 2 through 7 (Mcm2-7) and is a key target for regulation of cell proliferation. In addition, it is regulated in response to replicative stress. One of the protein kinases that targets Mcm2-7 is the Dbf4-dependent kinase Cdc7 (DDK). In a previous study, we showed that alanine mutations of the DDK phosphorylation sites at S164 and S170 in *Saccharomyces cerevisiae* Mcm2 result in sensitivity to caffeine and methyl methanesulfonate (MMS) leading us to suggest that DDK phosphorylation of Mcm2 is required in response to replicative stress.

Results: We show here that a strain with the *mcm2* allele lacking DDK phosphorylation sites (*mcm2*$_{AA}$) is also sensitive to the ribonucleotide reductase inhibitor, hydroxyurea (HU) and to the base analogue 5-fluorouracil (5-FU) but not the radiomimetic drug, phleomycin. We screened the budding yeast non-essential deletion collection for synthetic lethal interactions with *mcm2*$_{AA}$ and isolated deletions that include genes involved in the control of genome integrity and oxidative stress. In addition, the spontaneous mutation rate, as measured by mutations in *CAN1*, was increased in the *mcm2*$_{AA}$ strain compared to wild type, whereas with a phosphomimetic allele (*mcm2*$_{EE}$) the mutation rate was decreased. These results led to the idea that the *mcm2*$_{AA}$ strain is unable to respond properly to DNA damage. We examined this by screening the deletion collection for suppressors of the caffeine sensitivity of *mcm2*$_{AA}$. Deletions that decrease spontaneous DNA damage, increase homologous recombination or slow replication forks were isolated. Many of the suppressors of caffeine sensitivity suppressed other phenotypes of *mcm2*$_{AA}$ including sensitivity to genotoxic drugs, the increased frequency of cells with RPA foci and the increased mutation rate.

Conclusions: Together these observations point to a role for DDK-mediated phosphorylation of Mcm2 in the response to replicative stress, including some forms of DNA damage. We suggest that phosphorylation of Mcm2 modulates Mcm2-7 activity resulting in the stabilization of replication forks in response to replicative stress.

Background

DNA replication is tightly regulated to ensure that genomes are copied once and only once per cell division cycle [1]. In addition, cells must respond to assaults that damage DNA and/or disrupt replication forks by preventing the initiation of DNA replication and stabilizing active replication forks [2]. One of the targets for these regulatory events is the replicative helicase that unwinds DNA at the replication fork [3-8]. The catalytic core of the replicative helicase in eukaryotic cells is a heterohexameric complex comprised of the minichromosome maintenance proteins 2 through 7 (Mcm2-7; [9,10]). Mcm2-7 activity is tightly controlled during the initiation of DNA replication and is targeted in response to replicative stress [3,6-8,11-14].

In vivo, Mcm2-7 functions within the CMG complex comprised of Cdc45, Mcm2-7 and the tetrameric GINS complex (Sld5, Psf1, Psf2 and Psf3) [11,12]. The catalytic activity for DNA unwinding resides in Mcm2-7 with Cdc45 and GINS playing roles in limiting Mcm2-7 activity to S phase and providing scaffolding functions within the replisome [11,15-17]. CMG is isolated from replicating yeast cells as part of the RPC (replisome progression complex) that includes the checkpoint protein Mrc1, the fork pausing complex Tof1-Csm3, the histone chaperone FACT and the sister chromatid cohesion factor, Ctf4 [16].

Mcm2-7 activity is also regulated by phosphorylation. A recent study from our laboratory showed that

* Correspondence: mdavey5@uwo.ca
Department of Biochemistry, Schulich School of Medicine & Dentistry, University of Western Ontario, London, ON N6A 5C1, Canada

BioMed Central

© 2012 Stead et al.; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

phosphorylation of *Saccharomyces cerevisiae* Mcm2 by the Dbf4-dependent kinase, Cdc7 (DDK) at S164 and S170 is important for a proper response to DNA damage [5]. Strains containing a non-phosphorylatable allele of *mcm2* (*mcm2*$_{AA}$) grow similarly to wild type cells in normal growth conditions but are sensitive to the DNA alkylating agent, methyl methanesulfonate (MMS) and to caffeine. Caffeine is a purine analogue with pleiotropic effects. In general, caffeine inhibits PI3K-related kinases, which in yeast include TOR (Tor1 and Tor2), Mec1 and Tel1 [18-21]. TOR controls cell growth in response to nutrients and stress whereas Mec1 and Tel1 are both checkpoint kinases that also have roles in control of replication initiation (Mec1) and telomere maintenance (Tel1) [3,22-24].

Here, we show that in addition to MMS and caffeine, the *mcm2*$_{AA}$ strain is sensitive to the ribonucleotide reductase inhibitor, hydroxyurea (HU) and the base analogue 5-fluorouracil (5-FU), but not phleomycin, a radiomimetic drug. The phosphomimetic glutamic acid substitutions at S164 and S170 suppress sensitivity to these drugs. We examined the genetic network within which *mcm2*$_{AA}$ functions and found 9 deletions that have synthetic slow growth or lethal interactions with *mcm2*$_{AA}$ and 16 deletions that suppress the caffeine sensitivity of *mcm2*$_{AA.}$ The identities of these gene deletions are consistent with a role for Mcm2 phosphorylation in the response to DNA damage and replicative stress and include two members of the RPC. A role in response to replicative stress is emphasized by the higher than wild type spontaneous mutation rate in the *mcm2*$_{AA}$ strain and a lower than wild type mutation rate with the *mcm2*$_{EE}$ phosphomimetic allele. Most of the gene deletions that suppressed the caffeine sensitivity of *mcm2*$_{AA}$ also relieved other phenotypes of *mcm2*$_{AA}$. We propose that phosphorylation of Mcm2 by DDK is required in response to replicative stress to stabilize Mcm2-7 at replication forks.

Results

Growth of *mcm2*$_{AA}$ and *mcm2*$_{EE}$ cells in the presence of replicative stress

We examined the growth of the *mcm2*$_{AA}$ strain on media containing agents that cause replicative stress (Figure 1A & B). The *mcm2*$_{AA}$ strain had reduced growth relative to *MCM2* on YPD plates containing MMS or 5-FU (Figure 1A), but not on plates containing phleomycin (Figure 1B). These agents have different effects on DNA stability in budding yeast. MMS damages DNA by methylating guanines and adenines [25]. The effects of 5-fluorouracil in yeast are twofold: it inhibits the pyrimidine biosynthesis pathway and results in misincorporation of uracil into nascent DNA [26]. Phleomycin is structurally similar to bleomycin, a radiomimetic drug that causes double stranded DNA breaks [27]. The *mcm2*$_{AA}$ strain also grows poorly upon constant exposure to the ribonucleotide reductase inhibitor, hydroxyurea (HU), which interferes with the integrity of DNA replication forks and induces an S phase checkpoint [28,29].

We noted previously that Mcm2 in which S164 and S170 are altered to glutamic acids (*mcm2*$_{EE}$) acts like a phosphomimic, allowing growth of cells in the presence of caffeine and MMS, and has the same activity *in vitro* as phospho-Mcm2 [5]. If phosphorylation of Mcm2 is required in response to 5-FU and HU, then *mcm2*$_{EE}$ should be insensitive to these agents. As predicted, the *mcm2*$_{EE}$ strain grew similarly to wild type cells in the presence of 5-FU and HU (Figure 1C). Substitution of Glu for S at position 164 or 170 also resulted in wild type growth consistent with the requirement to mutate both Ser to Ala to obtain a phenotype [5].

Synthetic lethal/slow growth interactions with *mcm2*$_{AA}$

The sensitivity of the *mcm2*$_{AA}$ strain to caffeine, MMS, 5-FU and HU suggests that phosphorylation of Mcm2 is required in response to replicative stress. Furthermore, the increased frequency of RPA foci in these cells [5] suggests disruption of replication forks or an inability to respond to replicative stress [30-32]. If our model is correct, then mutations that increase genomic instability will be synthetic lethal or show slow growth with *mcm2*$_{AA}$. After screening the *S. cerevisiae* non-essential deletion collection for synthetic lethal interactions with *mcm2*$_{AA}$ and confirming the interactions by tetrad dissection, we found 8 gene deletions that result in no or slow growth when combined with *mcm2*$_{AA}$ (Table 1 and Additional file 1). Three of the eight gene deletions that display synthetic lethal or slow growth interactions with *mcm2*$_{AA}$ affect cell stress responses or cell cycle. In particular, the synthetic interaction of *mcm2*$_{AA}$ with *chk1Δ*, a deletion in the gene encoding a checkpoint effector kinase is consistent with the idea that *mcm2*$_{AA}$ is important in response to replicative stress. In addition, *ctf4Δ*, *sod1Δ* and *img1Δ* all lead to genomic instability or increase DNA damage [33-36] and their negative synthetic interactions with *mcm2*$_{AA}$ support the idea that Mcm2 phosphorylation is important in response to DNA damage.

Rad53 is phosphorylated in the *mcm2*$_{AA}$ strain

One potential role of Mcm2 phosphorylation in response to replicative stress is in the induction of a checkpoint signal leading to phosphorylation of Rad53, detected by decreased migration through SDS-PAGE. We examined Rad53 by Western blotting in the *MCM2* and *mcm2*$_{AA}$ strains before and after treatment with 0.02% MMS, which triggers the S phase checkpoint. As seen in Figure 2, the migration of Rad53 is slower in the presence of MMS in both strains, suggesting that signalling in response to DNA

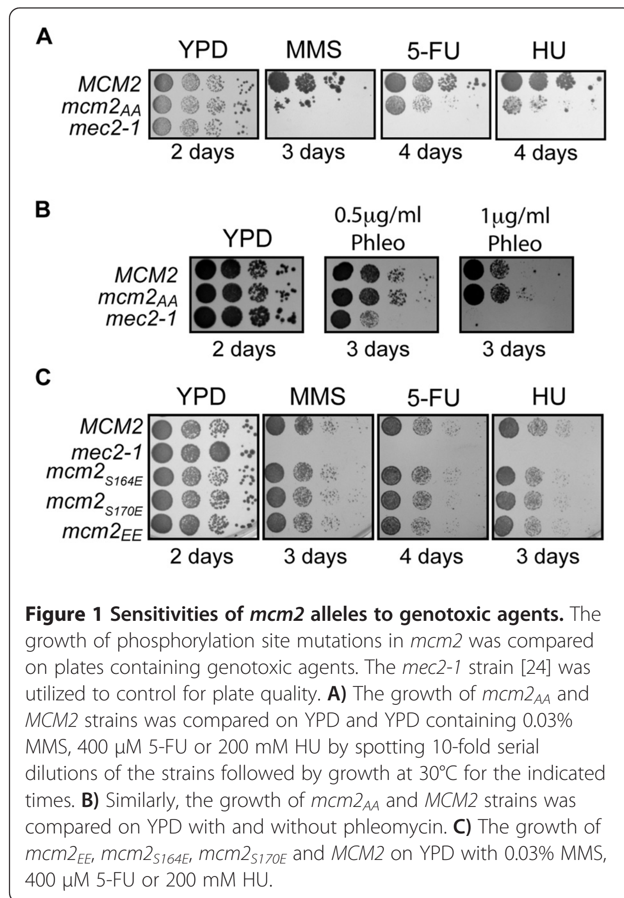

Figure 1 Sensitivities of *mcm2* alleles to genotoxic agents. The growth of phosphorylation site mutations in *mcm2* was compared on plates containing genotoxic agents. The *mec2-1* strain [24] was utilized to control for plate quality. **A)** The growth of $mcm2_{AA}$ and *MCM2* strains was compared on YPD and YPD containing 0.03% MMS, 400 μM 5-FU or 200 mM HU by spotting 10-fold serial dilutions of the strains followed by growth at 30°C for the indicated times. **B)** Similarly, the growth of $mcm2_{AA}$ and *MCM2* strains was compared on YPD with and without phleomycin. **C)** The growth of $mcm2_{EE}$, $mcm2_{S164E}$, $mcm2_{S170E}$ and *MCM2* on YPD with 0.03% MMS, 400 μM 5-FU or 200 mM HU.

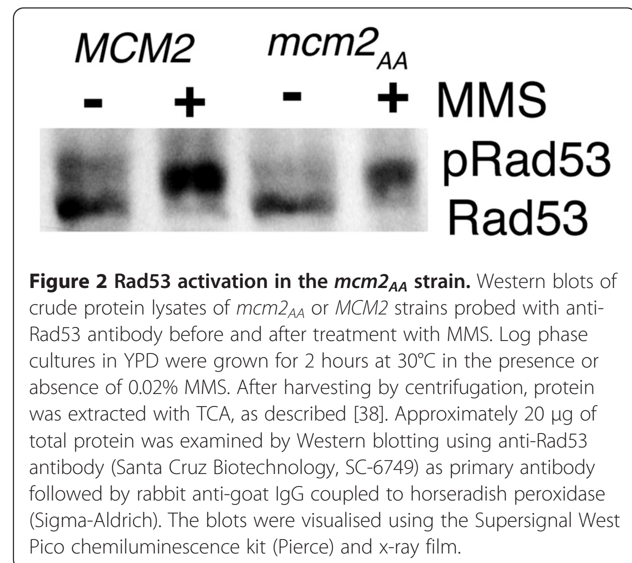

Figure 2 Rad53 activation in the $mcm2_{AA}$ strain. Western blots of crude protein lysates of $mcm2_{AA}$ or *MCM2* strains probed with anti-Rad53 antibody before and after treatment with MMS. Log phase cultures in YPD were grown for 2 hours at 30°C in the presence or absence of 0.02% MMS. After harvesting by centrifugation, protein was extracted with TCA, as described [38]. Approximately 20 μg of total protein was examined by Western blotting using anti-Rad53 antibody (Santa Cruz Biotechnology, SC-6749) as primary antibody followed by rabbit anti-goat IgG coupled to horseradish peroxidase (Sigma-Aldrich). The blots were visualised using the Supersignal West Pico chemiluminescence kit (Pierce) and x-ray film.

damage is intact and that phosphorylation of Mcm2 is not required to activate checkpoint. We also tested for a genetic interaction between $mcm2_{AA}$ and a checkpoint deficient allele of *RAD53* (*mec2-1* [24]). After mating the strains and generating spore progeny by tetrad dissection, none of the spore colonies contained both mutations indicating a synthetic lethal interaction, consistent with Mcm2 phosphorylation functioning in a parallel pathway to Rad53 (Additional file 1).

Table 1 Synthetic lethal or slow growth interactions with $mcm2_{AA}$

Gene	ORF	Function	GO	growth
*chk1*Δ	YBR274W	Checkpoint Kinase	1,2,5	lethal
*ctf4*Δ	YPR135W	Sister Chromatid Cohesion	1,2	lethal
*sod1*Δ	YJR104C	Response to oxygen radicals	1,6,8	slow
*bud23*Δ	YCR047C	Bud site selection	2,3,4	lethal
*pep3*Δ	YLR148W	Vesicular docking/Vacuolar biogenesis	3,8	lethal
*skn1*Δ	YGR143W	Sphingolipid biosynthesis	7,8	slow
*img1*Δ	YCR046C	Mitochondrial genome maintenance	9	lethal
*vma13*Δ	YPR036W	Subunit of Vacuolar ATPase	10	lethal

Genes are grouped by their GO terms as annotated in the *Saccharomyces cerevisiae* database [37]. Gene ontology: (1) Response to cell stress/chemical stimuli (2) cell cycle, (3) transport, (4) RNA metabolic process, (5) signalling process/protein modification process, (6) transcription, (7) carbohydrate metabolic process, (8) cell wall, membrane, & vesicle mediated transport, (9) mitochondrial organization, and (10) other.

Increased mutation rate in $mcm2_{AA}$ cells

If phosphorylation of Mcm2 is important in response to DNA damage and/or replicative stress, cells containing the non-phosphorylatable allele of *mcm2* ($mcm2_{AA}$) would be predicted to accumulate mutations at a higher rate than cells with *MCM2* or $mcm2_{EE}$. To test this, we utilized the *CAN1* forward mutation assay in which a mutation rate is determined from the number of canavanine resistant colonies that arise. *CAN1* encodes a transporter that enables the toxic compound canavanine to enter cells. If *CAN1* function is lost, then the cell is rendered resistant to canavanine. We grew *CAN1* strains containing different alleles of *mcm2* for several generations in liquid media without selection before determining the number of canavanine resistant colonies and the mutation rate using the method of the median [39,40]. The mutation rate was nearly two-fold higher in the $mcm2_{AA}$ strain than with *MCM2* (5.5×10^{-7} v. 3.2×10^{-7}), consistent with an inability to respond properly to spontaneous DNA damage in the absence of Mcm2 phosphorylation. Significantly, the mutation rate in the $mcm2_{EE}$ strain was half that of the *MCM2* strain (1.6×10^{-7}).

Suppressors of the caffeine sensitivity of $mcm2_{AA}$

As $mcm2_{AA}$ is predicted to interfere in the response to replicative stress, second site mutations that decrease DNA damage or increase the capacity for DNA repair would be expected to act as suppressors. We therefore screened the haploid deletion strain collection for gene deletions that suppress the caffeine sensitivity of $mcm2_{AA}$.

Sensitivity to caffeine was chosen because of the strong phenotype it elicits with *mcm2AA*. Candidates were re-mated, isolated by tetrad dissection and re-tested on YPD with caffeine. Sixteen gene deletions were identified (Figure 3). We classified these genes by biological functions based on gene ontology annotations in the Saccharomyces Genome Database [37] as well as their reported functions in the literature (Table 2). These classifications yielded four groups of genes: cell stress, cell cycle, protein folding and "other" functions. Interestingly, half of the deletions, when independent of *mcm2AA*, were sensitive to caffeine indicating roles for these genes in response to caffeine. Four of these, *tof1Δ*, *mbp1Δ*, *ume6Δ* and *sip18Δ* were as sensitive to caffeine as *mcm2AA*. Others, such as *rad9Δ*, *rad2Δ*, *pdr15Δ* and *hrd1Δ*, displayed an intermediate sensitivity. In addition, three showed decreased sensitivity to caffeine compared to wild type *MCM2* (*yhp1Δ*, *ssm4Δ* and *rpl8bΔ*). We also note that some of the deletions, such as *tof1Δ*, *rad9Δ* and *pac10Δ*, resulted in only partial suppression (Figure 3).

Our hypothesis predicts that deletions that suppress the caffeine sensitivity of *mcm2AA* will also decrease the mutation rate in the *mcm2AA* strain. Therefore, we repeated the *CAN1* forward mutation assay on a subset of the deletion strains. As shown in Figure 4, seven of the 11 deletions tested decreased the mutation rate, both with the deletion alone and in the presence of *mcm2AA*. The exceptions were *tof1Δ*, *rad2Δ* and *ume6Δ*. Deletion of *tof1* causes genomic instability [41-43] and *rad2Δ* is deficient in nucleotide excision repair [44]. Ume6 is involved in the expression of several genes and deletion of *ume6* increases homologous recombination [45-48].

To further evaluate the mechanisms by which the gene deletions suppress *mcm2AA*, we spotted strains containing the suppressor deletions and either *MCM2* or *mcm2AA* onto YPD plates containing MMS, 5-FU and HU (Figure 5, Table 3 and Additional file 1). All of the deletions that suppressed the caffeine sensitivity of *mcm2AA* also suppressed at least one other drug sensitivity of *mcm2AA*, exemplified by *rad2Δ* and *ssm4Δ* (Figure 5). Many of the deletions in the cell stress group also lead to sensitivity to these drugs in the *MCM2* background, thus likely accounting for the complex phenotypic patterns. For example, the *srs2* and *rad9* deletions do not suppress the sensitivity of *mcm2AA* to MMS or 5-FU and only partially suppress on HU (Figure 5). Some of the deletions decrease sensitivity to the drugs in an otherwise wild type background. For example, the *yhp1Δ* strain grows faster than the wild type strain on plates containing MMS or 5-FU (Figure 5). This increased growth is also noted in the *mcm2AA yhp1Δ* strain. Therefore, the *yhp1* deletion likely functions non-specifically to suppress *mcm2AA*. Of note, Yhp1 is a transcriptional repressor that along with Yox1 is involved in the cyclic transcription of a set of genes that includes *MCM2-7* [49].

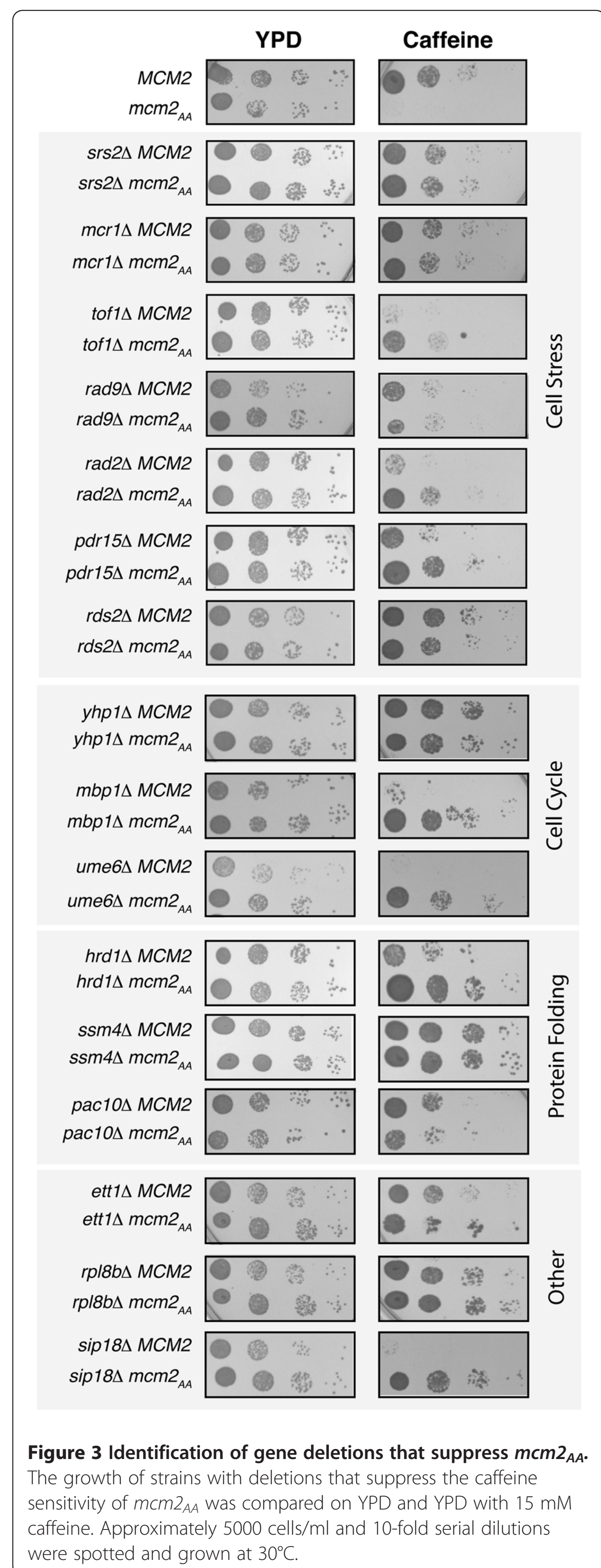

Figure 3 Identification of gene deletions that suppress *mcm2AA*. The growth of strains with deletions that suppress the caffeine sensitivity of *mcm2AA* was compared on YPD and YPD with 15 mM caffeine. Approximately 5000 cells/ml and 10-fold serial dilutions were spotted and grown at 30°C.

Table 2 Suppressors of the caffeine sensitivity of *mcm2*$_{AA}$

Gene	ORF	Function	GO
*srs2*Δ	YJL092C	DNA repair, helicase	1
*mcr1*Δ	YKL150W	Oxidative stress response	1
*tof1*Δ	YNL273W	Subunit of fork pausing complex	1,2,5
*rad9*Δ	YDR217C	Transmission of checkpoint signal	1,2,4,56
*rad2*Δ	YGR258C	Nucleotide excision repair	1,4,6
*pdr15*Δ	YDR406W	Cellular detoxification	1,3
*rds2*Δ	YPL133C	Transcription factor	1,6,7
*yhp1*Δ	YDR451C	Transcription factor/cell cycle	2,4,6
*mbp1*Δ	YDL056W	Transcription factor/cell cycle	2,4,6
*ume6*Δ	YDR207C	Transcription factor	2,4,5,6
*hrd1*Δ	YOL013C	Ubiquitin ligase/ER assoc. decay	8
*ssm4*Δ	YIL030C	Ubiquitin ligase/ER assoc. decay	8
*pac10*Δ	YGR078C	Protein folding	8
*sip18*Δ	YMR175W	Osmotic stress	9
*rpl8b*Δ	YLL045C	Ribosomal protein	9
*ett1*Δ	YOR051C	Translation termination	9

Genes are grouped by their GO terms as annotated in the *Saccharomyces cerevisiae* database [37]. Horizontal lines separate different classes. Gene ontology: (1) Response to cell stress/chemical stimuli (2) cell cycle, (3) transport, (4) RNA metabolic process, (5) signalling process/protein modification process, (6) transcription, (7) carbohydrate metabolic process, (8) ER-mediated degradation & protein-folding and (9) other.

However, deletion of *yhp1* alone did not affect expression of *MCM3-lacZ* and had little or no effect on cell growth [49]. The *ett1Δ* deletion on MMS or HU and *sip18Δ* on HU similarly increase growth of cells containing the wild type and mutated *mcm2* alleles (Table 3 and Additional file 1).

Previously, we observed that the *mcm2*$_{AA}$ strain has a higher frequency of cells with RPA foci [5]. RPA is the single-stranded DNA binding protein and thus foci represent generation of single stranded DNA. In wild type cells, RPA is diffuse in the nucleus (Figure 6A). In a low percentage of wild type cells, RPA foci will appear. Since a higher frequency of cells contain foci when treated with DNA damaging agents, the foci are thought to represent ongoing repair processes or disruption of the replication fork, both of which generate stretches of single stranded DNA [30-32]. Interestingly, in cells containing *mcm2*$_{AA}$, RPA foci appear in a much higher frequency of cells; ~20 percent (Figure 6A and B; [5]). As a means of determining the mechanisms by which the gene deletions suppress *mcm2*$_{AA}$, we tested whether they also suppress the increased frequency of cells with RPA foci. We transformed a plasmid encoding GFP-tagged Rpa1 into a subset of the suppressor strains and scored each for cells with RPA foci (Figure 6B). In isolation, the gene deletions had a higher ratio of cells with RPA foci than wild type (Figure 6B) with the *tof1, rad9, mbp1, hrd1, ssm4, sip18* and *rpl8b* deletions having p values less than 0.05, reflecting the effect of these deletions on genomic stability. Despite this increase, deletion of most of the genes tested suppressed the increased frequency of RPA foci in the *mcm2*$_{AA}$ strain; *srs2Δ* did not ($p \geq 0.05$). Deletion of *tof1* also did not suppress *mcm2*$_{AA}$. Indeed, *tof1Δ* in the

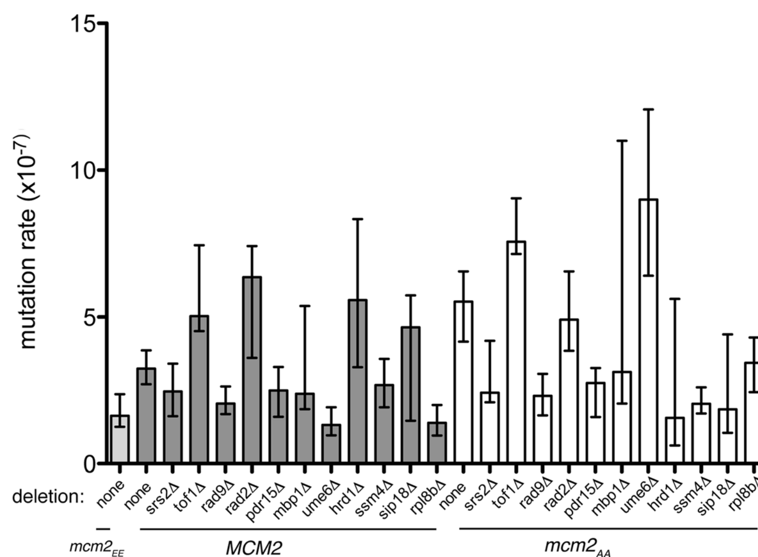

Figure 4 Mutation rates of *mcm2* alleles and strains with gene deletions that suppress *mcm2*$_{AA}$. The mutation rates of *MCM2* and *mcm2*$_{AA}$ strains with and without gene deletions that suppress *mcm2*$_{AA}$ were calculated as described [39,40] using the *CAN1* forward mutation assay. Dark grey bars are the deletions in the *MCM2* background; white bars are with *mcm2*$_{AA}$ and the light grey bar (far left) is the *mcm2*$_{EE}$ strain. The error bars represent the upper and lower confidence limits (95%) of the mutation rates and were calculated from the 95% confidence limits of the median determined from the binomial distribution [40].

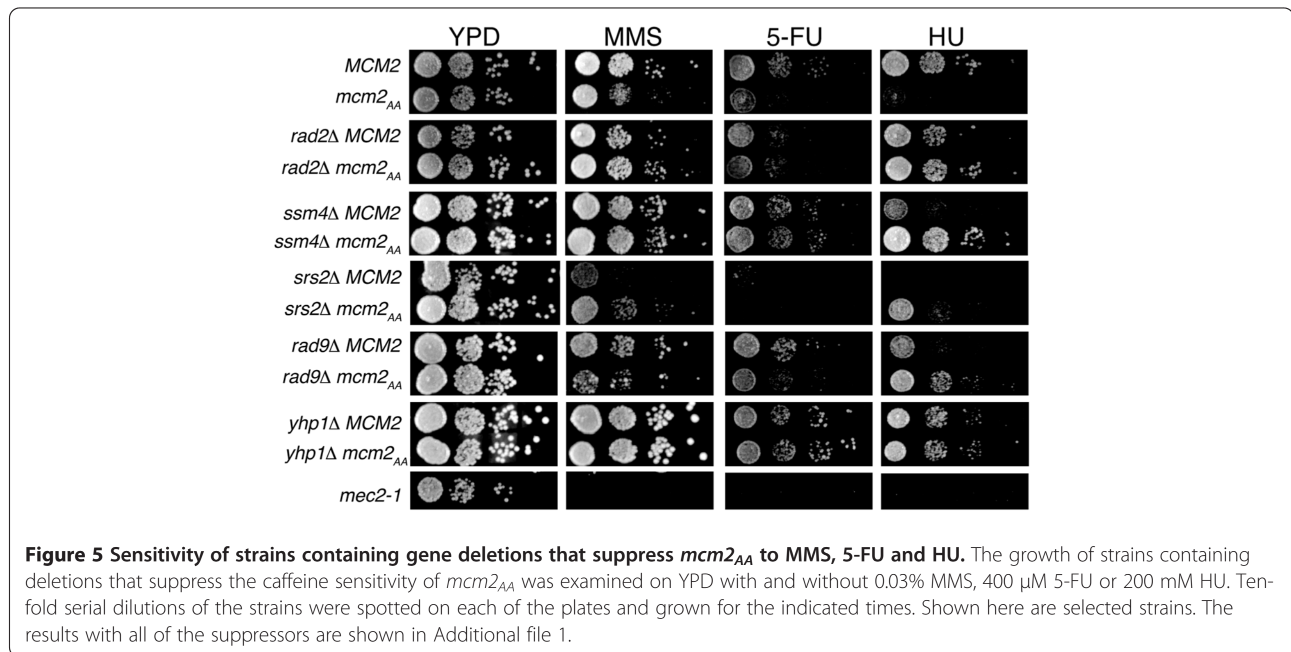

Figure 5 Sensitivity of strains containing gene deletions that suppress *mcm2$_{AA}$* to MMS, 5-FU and HU. The growth of strains containing deletions that suppress the caffeine sensitivity of *mcm2$_{AA}$* was examined on YPD with and without 0.03% MMS, 400 μM 5-FU or 200 mM HU. Ten-fold serial dilutions of the strains were spotted on each of the plates and grown for the indicated times. Shown here are selected strains. The results with all of the suppressors are shown in Additional file 1.

wild type background greatly increases the frequency of cells with RPA foci. This increased frequency might be explained by the observation that disruption of the *tof1* ortholog in *S. pombe* (*swi1*) decouples polymerases, generating excess single stranded DNA [50-52].

Table 3 Phenotypes of deletions that suppress the caffeine sensitivity of *mcm2$_{AA}$*

	Sensitivities				Suppression of *mcm2$_{AA}$*				
mutation	caff	MMS	5-FU	HU	MMS	5-FU	HU	RFA foci	mutation rate
*srs2*Δ	R	S	S	S	-	-	+	+	+
*mcr1*Δ	R	R	S	S	-	+	+	ND	ND
*tof1*Δ	S	S	R	S	+	+	+/−	-	-
*rad9*Δ	S	S	R	S	-	-	+	+	+
*rad2*Δ	S	S	S	S	+	+	+	+	-
*pdr15*Δ	S	R	S	R	+	+	+	+	+
*rds2*Δ	R	S	S	S	+	+/−	+	ND	ND
*yhp1*Δ	R+	R+	R+	R	+	+	+	ND	ND
*mbp1*Δ	S	R	R	S	+	+	+	+	-
*ume6*Δ	S	R	R	R	+	+	-	+	-
*hrd1*Δ	S	S	R	S	+	+	+	+	+
*ssm4*Δ	R+	R	R	S	+	+	+	+	+
*pac10*Δ	R	S	S	S	+	+	+	ND	ND
*sip18*Δ	S	S	R	R+	+	+	+	+	+
*rpl8b*Δ	R+	S	S	S	+	+	+	+	+
*ett1*Δ	R	R+	R	R+	+	+	+	ND	ND

The sensitivities of the deletion strains (with wild type *MCM2*) to each drug is indicated by "R" for growth similar to wild type, "S" for growth slower than wild type or "R+" for better than wild type growth. For suppression of the phenotypes associated with *mcm2$_{AA}$*, "-"indicates no suppression, "+" indicates suppression, "E" indicates epistasis and "ND" indicates that the test was not performed on that strain.

Discussion

Our findings suggest a role for phosphorylation of Mcm2 by DDK in response to replicative stress. Specifically, we demonstrate that the *mcm2$_{AA}$* strain is sensitive to drugs that cause replicative stress, has an increased mutation rate and that *mcm2$_{AA}$* interacts with genes involved in the response to replicative stress. Along with our previous study showing that phosphorylation of Mcm2 at S164 and S170 slows DNA unwinding and results in enhanced DNA binding by Mcm2-7 *in vitro* [5], our results lead to a model in which phosphorylation of Mcm2 slows DNA unwinding by Mcm2-7 and/or stabilizes the replication fork as part of the proper response to replicative stress.

When a replication fork encounters DNA damage such as a base lesion or a break in the DNA strand, synthesis by the replicative polymerases at that fork halts. A series of events must then occur for replication to proceed [53]. While double stranded DNA breaks, base damage or nucleotide depletion each induce the S phase checkpoint, which inhibits further initiation of DNA replication and stabilizes replication forks, the form of the response differs depending on the type of perturbation [54,55]. Given the sensitivity of the *mcm2$_{AA}$* strain to MMS and 5-FU, we propose that phosphorylation of Mcm2 by DDK is required to stabilize replication forks in response to DNA base damage. The lack of sensitivity to phleomycin with this strain suggests that Mcm2 phosphorylation may not be required in response to double strand breaks. That DDK phosphorylation would trigger Mcm2 participation in the response to

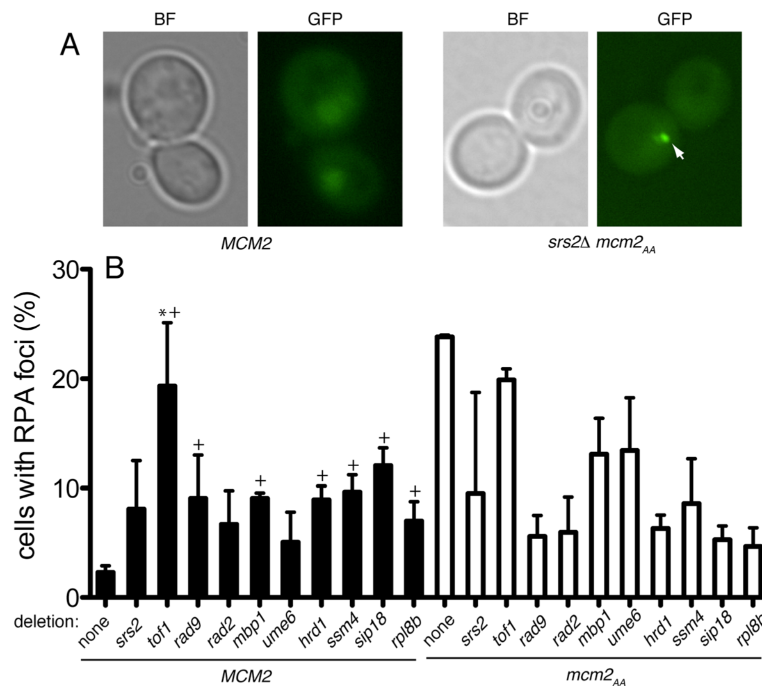

Figure 6 RPA foci in the suppressor strains. Cells with RPA foci were identified using GFP fused to Rpa1 as described [5]. **A)** An example of RPA foci is shown. Bright field (BF) and fluorescent (GFP) images are shown for *MCM2* (left panels) and *srs2*Δ $mcm2_{AA}$ (right panels). A RPA focus in the *srs2*Δ $mcm2_{AA}$ image is indicated by the white arrow. **B)** The percentage of cells containing RFA foci was measured in triplicate experiments and standard deviation was determined. The asterisks indicate strains that do not have statistically significant different frequency of RPA foci compared to $mcm2_{AA}$ ($p \geq 0.05$, Student's unpaired *t*-test) while the crosses indicate strains that are statistically different from wild type ($p < 0.05$).

replicative stress is not surprising given DDK participates in responses to DNA damage and replicative stress and is a target of Rad53 during the S phase checkpoint [56-60].

Genetic interactions with $mcm2_{AA}$

The genetic interactions with $mcm2_{AA}$ are most consistent with a requirement for Mcm2 phosphorylation in response to disruption of the replication fork. The effect of the suppressing deletions can be explained as either decreasing spontaneous DNA damage, which would otherwise disrupt replication forks or increasing the capacity for rescue of disrupted forks by recombination. In contrast, the deletions that result in synthetic lethal interactions increase spontaneous DNA damage, perturb the replication fork and/or are required for checkpoint responses. Mapping the interactions within the 25 genes connected to $mcm2_{AA}$ indicates that about 15 form a network independently of *mcm2* (Figure 7). Most of the interacting genes have roles in response to DNA damage and replicative stress. Interestingly, several of the genes interact with *CDC7* and *DBF4,* the genes encoding the two components of DDK (Figure 7).

Of the 25 genetic interactions with *mcm2* that we isolated, only one was previously identified. Deletion of *rad9* has a synthetic lethal interaction with *mcm2-1*, a temperature sensitive allele [63]. Interestingly, here *rad9Δ* suppresses some, but not all, of the phenotypes associated with $mcm2_{AA}$. The difference may result from allele specificity; *mcm2-1* is expected to affect Mcm2's essential role in Mcm2-7 function whereas the $mcm2_{AA}$ allele affects Mcm2 activity in response to replicative stress.

Deletion of three genes, *HRD1, SSM4* and *PAC10* whose products are involved in targeting misfolded proteins for degradation [64,65], suppress $mcm2_{AA}$. One possible mechanism for these suppressors is that misfolded proteins induce the unfolded protein response (UPR) which in turn provides protection against reactive oxygen species which can damage DNA [66]. However, the mechanism of suppression is more complex since $mcm2_{AA}$ is still sensitive to caffeine or MMS in the presence of tunicamycin, which induces the UPR (data not shown).

Regulating helicase progression in response to replicative stress

We propose that phosphorylation of Mcm2 by DDK is required in response to replicative stress. This role is not recognition of damage or replication fork collapse since the S phase checkpoint is intact at least up to Rad53 phosphorylation. Furthermore, $mcm2_{AA}$ has synthetic lethal

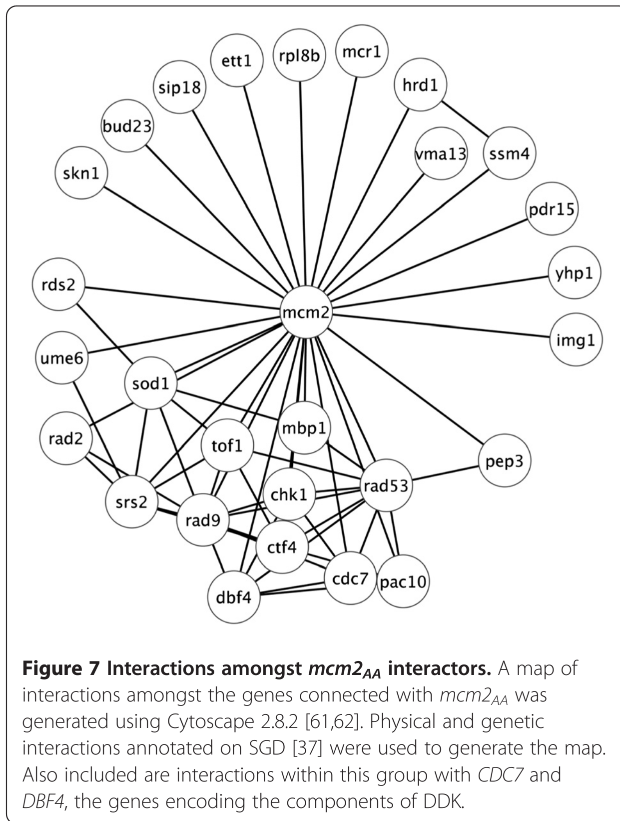

Figure 7 Interactions amongst *mcm2*$_{AA}$ interactors. A map of interactions amongst the genes connected with *mcm2*$_{AA}$ was generated using Cytoscape 2.8.2 [61,62]. Physical and genetic interactions annotated on SGD [37] were used to generate the map. Also included are interactions within this group with *CDC7* and *DBF4*, the genes encoding the components of DDK.

interactions with *chk1Δ* and *mec2-1*, mutations in the genes encoding the checkpoint effector kinases in yeast [2,67-69]. Synthetic lethal interactions often indicate function of the interacting genes in parallel pathways. Based on the previously observed biochemical activities of Mcm2-7 with DDK-phosphorylated or phosphomimetic Mcm2 [5], we surmise that phosphorylation of Mcm2 may stabilize Mcm2-7 on DNA and/or slow the helicase. DNA helicases are predicted to contact DNA through the sugar phosphate backbone, not the bases [70]. Therefore, unlike replicative polymerases that stall at sites with missing or damaged bases, the helicase will continue unwinding DNA and may decouple from the polymerase. Indeed single stranded DNA generated by decoupling of helicase from the replicative polymerases is proposed to generate a checkpoint response [71-77]. Decoupling appears to be regulated so that the helicase does not advance too far from the rest of the replisome, leading to complete disassembly of replication forks [78]. One role for Mcm2 phosphorylation may be to slow the helicase so that it does not proceed too far ahead of the replicative polymerases. In this model, the accumulation of RPA foci in the *mcm2*$_{AA}$ strain is not due to spontaneous decoupling of the helicase from polymerase but rather is due to DNA damage triggering decoupling of the helicase.

The idea that replicative stress triggers a requirement for Mcm2 phosphorylation by DDK is supported by the synthetic lethal interaction of *mcm2*$_{AA}$ with deletions that lead to genomic instability. Examples include *ctf4Δ* and *img1Δ*. Ctf4 is involved in sister chromatid cohesion and is integral to the RPC [16,36,79-81]. Deletion of *img1* leads to loss of functional mitochondria which in turn leads to genomic instability [34,35]. Increased DNA damage is also noted in strains with increased levels of ROS, such as *sod1Δ* [33] and *vma13Δ* [82,83], both of which are synthetically lethal with *mcm2*$_{AA}$. Rad9 is important for transmission of checkpoint signalling with deletion of *rad9* resulting in cells that fail to arrest in response to DNA damage [84,85]. The increased homologous recombination noted with *srs2Δ*, *rad2Δ* and *ume6Δ* may suppress *mcm2*$_{AA}$ by providing a means to resolve stalled replication forks [45,86-88]. Finally, the model that Mcm2 phosphorylation may be required to slow replication forks is supported by the ability of *tof1Δ* to suppress defects of *mcm2*$_{AA}$ only in the presence of genotoxic agents (*tof1Δ* does not suppress the increased mutation rate or increased frequency of RPA foci). Lack of Tof1, a member of the RPC, slows replication forks [43], however this occurs only in the presence of replicative stress, such as seen in the presence of genotoxic agents [42].

Conclusions

Phosphorylation of Mcm2 by DDK is required for the proper response to replicative stress, but not to induce a checkpoint. This phosphorylation event likely slows the Mcm2-7 helicase and/or stabilizes replication forks. In the absence of Mcm2 phosphorylation, the mutation rate is increased.

Methods

Materials

Caffeine, HU and MMS were purchased from Sigma Aldrich (99% purity); geneticin (G418) from either United States Biological or Santa Cruz Biotechnology; phleomycin from Santa Cruz Biotechnology; 5-FU from Nutritional Biochemicals Corp. YPD is 1% yeast extract, 2% peptone and 2% *D*-glucose. The yeast strains MDY167 (*MCM2-URA3*), MDY169 (*mcm2*$_{AA}$*-URA3*) and MDY191 (*mcm2*$_{EE}$*-URA3*) are described in Stead et al. [5] and the *mec2-1* strain in [24].

Synthetic lethal screen

The genetic screens were a modification of SGA analysis [89]. Manipulation of the gene arrays was performed manually using a 3.18-mm 48-pinner tool (V&P Scientific, San Diego, CA). Screens were performed using MDY169 (*MAT***α** *mcm2*$_{AA}$*-URA3*) or MDY167 (*MAT***α** *MCM2-URA3* [5]) mated with the haploid yeast Magic Marker deletion collection generated from the diploid strain collection (Open Biosystems; [90]). Diploids were selected and then sporulated before selecting haploid cells that

contained a gene deletion (Kan^R) and $mcm2_{AA}$ or *MCM2* (Ura^+). Plates were scanned on a HP Scanjet 3970 and colony size was compared using ImageQuant TL (GE Healthcare). A synthetic lethal or slow growth (SSL) phenotype was assigned if the colony size was smaller in the $mcm2_{AA}$ cross than in the *MCM2* cross. A false positive rate (growth when the gene deletion is reported as lethal in SGD [37]) was roughly 5% and is similar to false positive rates reported previously [89]. From this initial screen, we compiled a list of 234 genes that had SSL interactions with $mcm2_{AA}$. Next, the candidates were retested from the mating step in triplicate. Forty-one SSL interactions were identified in this step. The deletions were then re-tested for genetic interactions with $mcm2_{AA}$ by re-mating followed by tetrad dissection. SSL interactions were verified if Ura + and $G418^R$ spore progeny were never recovered or grew slowly relative to the corresponding single mutations.

Screen for suppressors of caffeine sensitivity

A screen to isolate deletions that suppress the caffeine sensitivity of $mcm2_{AA}$ was performed by pinning the haploid $mcm2_{AA}$ strains that also contained a gene deletion (generated as described above) to YPD containing 15 mM caffeine. The plates were incubated at 30°C for 4 days, imaged and then quantified. A spot was determined to contain a deletion that suppresses $mcm2_{AA}$ when its size was larger than the spot size of the $mcm2_{AA}$ strain. The deletion of 369 genes suppressed the growth defect of $mcm2_{AA}$ in caffeine in the initial screen. These candidate deletions were re-mated in triplicate, haploids generated and retested for sensitivity to caffeine, resulting in 86 strains containing a deletion that potentially suppresses $mcm2_{AA}$. The 86 were strains re-mated, sporulated and tetrads dissected. A G418 resistant, Ura + colony was identified, grown to saturation and 10-fold serial dilutions were spotted onto YPD with and without 15 mM caffeine.

Mutation rate assay

The forward mutation assay was performed as described in [91]. Briefly, at least 20 colonies were inoculated into 10 x 1 ml YPD and the cultures grown overnight at 30°C to 1–2 x 10^8 cells/ml. Each culture was diluted to approximately 200 cells/1 ml YPD and grown to 1–2 x 10^8 cells/ml. Cells were then plated (~ 10^7) on CM-Arg plates containing 25 μg canavanine/ml and appropriate dilutions were made before plating on YPD. Colonies on each plate were counted to determine the number of canavanine resistant cells per 10^7 cells. The mutation rate was calculated using the method of the median [39,40].

Additional file

Additional file 1: Stead et al., Supplemental Data.

Acknowledgements
This work was supported by a Canadian Institutes of Health Research Operating Grant (MOP 68926) to MJD and by funding from Schulich School of Medicine & Dentistry. BES was supported by a Natural Science and Engineering Research Council PGS-D fellowship.

Authors' contributions
BES carried out the screens and drug assays with assistance from CJB and MKS and wrote a draft of the manuscript. MJD did the microscopy, mutation assays, Rad53 blot and edited the manuscript with CJB. All authors read and approved the final manuscript.

Received: 29 January 2012 Accepted: 7 May 2012
Published: 7 May 2012

References
1. Bell SP, Dutta A: **DNA replication in eukaryotic cells.** *Annu Rev Biochem* 2002, **71:**333–374.
2. Segurado M, Tercero JA: **The S-phase checkpoint: targeting the replication fork.** *Biol Cell* 2009, **101:**617–627.
3. Randell JC, Fan A, Chan C, Francis LI, Heller RC, Galani K, Bell SP: **Mec1 is one of multiple kinases that prime the Mcm2-7 helicase for phosphorylation by Cdc7.** *Mol Cell* 2010, **40:**353–363.
4. Sheu YJ, Stillman B: **Cdc7-Dbf4 phosphorylates MCM proteins via a docking site-mediated mechanism to promote S phase progression.** *Mol Cell* 2006, **24:**101–113.
5. Stead BE, Brandl CJ, Davey MJ: **Phosphorylation of Mcm2 modulates Mcm2-7 activity and affects the cell's response to DNA damage.** *Nucleic Acids Res* 2011, **39:**6998–7008.
6. Cortez D, Glick G, Elledge SJ: **Minichromosome maintenance proteins are direct targets of the ATM and ATR checkpoint kinases.** *Proc Natl Acad Sci U S A* 2004, **101:**10078–10083.
7. Montagnoli A, Valsasina B, Brotherton D, Troiani S, Rainoldi S, Tenca P, Molinari A, Santocanale C: **Identification of Mcm2 phosphorylation sites by S-phase-regulating kinases.** *J Biol Chem* 2006, **281:**10281–10290.
8. Yoo HY, Shevchenko A, Dunphy WG: **Mcm2 is a direct substrate of ATM and ATR during DNA damage and DNA replication checkpoint responses.** *J Biol Chem* 2004, **279:**53353–53364.
9. Bochman ML, Schwacha A: **The Mcm complex: unwinding the mechanism of a replicative helicase.** *Microbiol Mol Biol Rev* 2009, **73:**652–683.
10. Forsburg SL: **Eukaryotic MCM proteins: beyond replication initiation.** *Microbiol Mol Biol Rev* 2004, **68:**109–131.
11. Moyer SE, Lewis PW, Botchan MR: **Isolation of the Cdc45/Mcm2-7/GINS (CMG) complex, a candidate for the eukaryotic DNA replication fork helicase.** *Proc Natl Acad Sci U S A* 2006, **103:**10236–10241.
12. Pacek M, Tutter AV, Kubota Y, Takisawa H, Walter JC: **Localization of MCM2-7, Cdc45, and GINS to the site of DNA unwinding during eukaryotic DNA replication.** *Mol Cell* 2006, **21:**581–587.
13. Sheu YJ, Stillman B: **The Dbf4-Cdc7 kinase promotes S phase by alleviating an inhibitory activity in Mcm4.** *Nature* 2010, **463:**113–117.
14. Ishimi Y, Komamura-Kohno Y: **Phosphorylation of Mcm4 at specific sites by cyclin-dependent kinase leads to loss of Mcm4,6,7 helicase activity.** *J Biol Chem* 2001, **276:**34428–34433.
15. Costa A, Ilves I, Tamberg N, Petojevic T, Nogales E, Botchan MR, Berger JM: **The structural basis for MCM2-7 helicase activation by GINS and Cdc45.** *Nat Struct Mol Biol* 2011, **18:**471–477.
16. Gambus A, Jones RC, Sanchez-Diaz A, Kanemaki M, van Deursen F, Edmondson RD, Labib K: **GINS maintains association of Cdc45 with MCM in replisome progression complexes at eukaryotic DNA replication forks.** *Nat Cell Biol* 2006, **8:**358–366.
17. Ilves I, Petojevic T, Pesavento JJ, Botchan MR: **Activation of the MCM2-7 helicase by association with Cdc45 and GINS proteins.** *Mol Cell* 2010, **37:**247–258.
18. Hall-Jackson CA, Cross DA, Morrice N, Smythe C: **ATR is a caffeine-sensitive, DNA-activated protein kinase with a substrate specificity distinct from DNA-PK.** *Oncogene* 1999, **18:**6707–6713.
19. Kuranda K, Leberre V, Sokol S, Palamarczyk G, Francois J: **Investigating the caffeine effects in the yeast Saccharomyces cerevisiae brings new insights into the connection between TOR, PKC and Ras/cAMP signalling pathways.** *Mol Microbiol* 2006, **61:**1147–1166.
20. Reinke A, Chen JC, Aronova S, Powers T: **Caffeine targets TOR complex I and provides evidence for a regulatory link between the FRB and kinase domains of Tor1p.** *J Biol Chem* 2006, **281:**31616–31626.

21. Wanke V, Cameroni E, Uotila A, Piccolis M, Urban J, Loewith R, De Virgilio C: **Caffeine extends yeast lifespan by targeting TORC1.** *Mol Microbiol* 2008, **69:**277–285.
22. Saiardi A, Resnick AC, Snowman AM, Wendland B, Snyder SH: **Inositol pyrophosphates regulate cell death and telomere length through phosphoinositide 3-kinase-related protein kinases.** *Proc Natl Acad Sci U S A* 2005, **102:**1911–1914.
23. Tercero JA, Diffley JF: **Regulation of DNA replication fork progression through damaged DNA by the Mec1/Rad53 checkpoint.** *Nature* 2001, **412:**553–557.
24. Weinert TA, Kiser GL, Hartwell LH: **Mitotic checkpoint genes in budding yeast and the dependence of mitosis on DNA replication and repair.** *Genes Dev* 1994, **8:**652–665.
25. Beranek DT: **Distribution of methyl and ethyl adducts following alkylation with monofunctional alkylating agents.** *Mutat Res* 1990, **231:**11–30.
26. Seiple L, Jaruga P, Dizdaroglu M, Stivers JT: **Linking uracil base excision repair and 5-fluorouracil toxicity in yeast.** *Nucleic Acids Res* 2006, **34:**140–151.
27. Moore CW: **Cleavage of cellular and extracellular *Saccharomyces cerevisiae* DNA by bleomycin and phleomycin.** *Cancer Res* 1989, **49:**6935–6940.
28. Cobb JA, Bjergbaek L, Shimada K, Frei C, Gasser SM: **DNA polymerase stabilization at stalled replication forks requires Mec1 and the RecQ helicase Sgs1.** *EMBO J* 2003, **22:**4325–4336.
29. Santocanale C, Diffley JF: **A Mec1- and Rad53-dependent checkpoint controls late-firing origins of DNA replication.** *Nature* 1998, **395:**615–618.
30. Bernstein KA, Shor E, Sunjevaric I, Fumasoni M, Burgess RC, Foiani M, Branzei D, Rothstein R: **Sgs1 function in the repair of DNA replication intermediates is separable from its role in homologous recombinational repair.** *EMBO J* 2009, **28:**915–925.
31. Clausing E, Mayer A, Chanarat S, Muller B, Germann SM, Cramer P, Lisby M, Strasser K: **The transcription elongation factor Bur1-Bur2 interacts with replication protein A and maintains genome stability during replication stress.** *J Biol Chem* 2010, **285:**41665–41674.
32. Wu X, Shell SM, Zou Y: **Interaction and colocalization of Rad9/Rad1/Hus1 checkpoint complex with replication protein A in human cells.** *Oncogene* 2005, **24:**4728–4735.
33. Gralla EB, Valentine JS: **Null mutants of Saccharomyces cerevisiae Cu, Zn superoxide dismutase: characterization and spontaneous mutation rates.** *J Bacteriol* 1991, **173:**5918–5920.
34. Coppee JY, Rieger KJ, Kaniak A, di Rago JP, Groudinsky O, Slonimski PP: **PetCR46, a gene which is essential for respiration and integrity of the mitochondrial genome.** *Yeast* 1996, **12:**577–582.
35. Rasmussen AK, Chatterjee A, Rasmussen LJ, Singh KK: **Mitochondria-mediated nuclear mutator phenotype in *Saccharomyces cerevisiae*.** *Nucleic Acids Res* 2003, **31:**3909–3917.
36. Kouprina N, Kroll E, Bannikov V, Bliskovsky V, Gizatullin R, Kirillov A, Shestopalov B, Zakharyev V, Hieter P, Spencer F, *et al*: **CTF4 (CHL15) mutants exhibit defective DNA metabolism in the yeast Saccharomyces cerevisiae.** *Mol Cell Biol* 1992, **12:**5736–5747.
37. **Saccharomyces Genome Database.**www.yeastgenome.org
38. Foiani M, Liberi G, Piatti S, Plevani P: **Saccharomyces cerevisiae as a model system to study DNA replication**. In *Practical Approach Edited by Cotterill S.* Edited by Eukaryotic DNA, Replication A. Oxford, UK: Oxford University Press; 1999:185–200.
39. Lea D, Coulson C: **The distribution of the numbers of mutants in bacterial populations.** *J Genet* 1949, **49:**264–285.
40. Foster PL: **Methods for determining spontaneous mutation rates.** *Methods Enzymol* 2006, **409:**195–213.
41. Bairwa NK, Mohanty BK, Stamenova R, Curcio MJ, Bastia D: **The intra-S phase checkpoint protein Tof1 collaborates with the helicase Rrm3 and the F-box protein Dia2 to maintain genome stability in *Saccharomyces cerevisiae*.** *J Biol Chem* 2011, **286:**2445–2454.
42. Hodgson B, Calzada A, Labib K: **Mrc1 and Tof1 regulate DNA replication forks in different ways during normal S phase.** *Mol Biol Cell* 2007, **18:**3894–3902.
43. Tourriere H, Versini G, Cordon-Preciado V, Alabert C, Pasero P: **Mrc1 and Tof1 promote replication fork progression and recovery independently of Rad53.** *Mol Cell* 2005, **19:**699–706.
44. Habraken Y, Sung P, Prakash S, Prakash L: **Transcription factor TFIIH and DNA endonuclease Rad2 constitute yeast nucleotide excision repair factor 3: implications for nucleotide excision repair and Cockayne syndrome.** *Proc Natl Acad Sci U S A* 1996, **93:**10718–10722.
45. Sweet DH, Jang YK, Sancar GB: **Role of *UME6* in transcriptional regulation of a DNA repair gene in *Saccharomyces cerevisiae*.** *Mol Cell Biol* 1997, **17:**6223–6235.
46. Jani NM, Lopes JM: **Transcription regulation of the Saccharomyces cerevisiae PIS1 gene by inositol and the pleiotropic regulator, Ume6p.** *Mol Microbiol* 2008, **70:**1529–1539.
47. Steinfeld I, Shamir R, Kupiec M: **A genome-wide analysis in Saccharomyces cerevisiae demonstrates the influence of chromatin modifiers on transcription.** *Nat Genet* 2007, **39:**303–309.
48. Williams RM, Primig M, Washburn BK, Winzeler EA, Bellis M, Sarrauste de Menthiere C, Davis RW, Esposito RE: **The Ume6 regulon coordinates metabolic and meiotic gene expression in yeast.** *Proc Natl Acad Sci U S A* 2002, **99:**13431–13436.
49. Pramila T, Miles S, GuhaThakurta D, Jemiolo D, Breeden LL: **Conserved homeodomain proteins interact with MADS box protein Mcm1 to restrict ECB-dependent transcription to the M/G1 phase of the cell cycle.** *Genes Dev* 2002, **16:**3034–3045.
50. Sommariva E, Pellny TK, Karahan N, Kumar S, Huberman JA, Dalgaard JZ: ***Schizosaccharomyces pombe* Swi1, Swi3, and Hsk1 are components of a novel S-phase response pathway to alkylation damage.** *Mol Cell Biol* 2005, **25:**2770–2784.
51. Vengrova S, Dalgaard JZ: **RNase-sensitive DNA modification(s) initiates *S. pombe* mating-type switching.** *Genes Dev* 2004, **18:**794–804.
52. Noguchi E, Noguchi C, McDonald WH, Yates JR 3rd, Russell P: **Swi1 and Swi3 are components of a replication fork protection complex in fission yeast.** *Mol Cell Biol* 2004, **24:**8342–8355.
53. McGlynn P, Lloyd RG: **Recombinational repair and restart of damaged replication forks.** *Nat Rev Mol Cell Biol* 2002, **3:**859–870.
54. Chang M, Bellaoui M, Boone C, Brown GW: **A genome-wide screen for methyl methanesulfonate-sensitive mutants reveals genes required for S phase progression in the presence of DNA damage.** *Proc Natl Acad Sci U S A* 2002, **99:**16934–16939.
55. Putnam CD, Jaehnig EJ, Kolodner RD: **Perspectives on the DNA damage and replication checkpoint responses in Saccharomyces cerevisiae.** *DNA Repair* 2009, **8:**974–982.
56. Duncker BP, Brown GW: **Cdc7 kinases (DDKs) and checkpoint responses: lessons from two yeasts.** *Mutat Res* 2003, **532:**21–27.
57. Gabrielse C, Miller CT, McConnell KH, DeWard A, Fox CA, Weinreich M: **A Dbf4p BRCA1 C-terminal-like domain required for the response to replication fork arrest in budding yeast.** *Genetics* 2006, **173:**541–555.
58. Ogi H, Wang CZ, Nakai W, Kawasaki Y, Masumoto H: **The role of the Saccharomyces cerevisiae Cdc7-Dbf4 complex in the replication checkpoint.** *Gene* 2008, **414:**32–40.
59. Pessoa-Brandao L, Sclafani RA: **CDC7/DBF4 functions in the translesion synthesis branch of the RAD6 epistasis group in Saccharomyces cerevisiae.** *Genetics* 2004, **167:**1597–1610.
60. Weinreich M, Stillman B: **Cdc7p-Dbf4p kinase binds to chromatin during S phase and is regulated by both the APC and the RAD53 checkpoint pathway.** *EMBO J* 1999, **18:**5334–5346.
61. Cline MS, Smoot M, Cerami E, Kuchinsky A, Landys N, Workman C, Christmas R, Avila-Campilo I, Creech M, Gross B, *et al*: **Integration of biological networks and gene expression data using Cytoscape.** *Nat Protoc* 2007, **2:**2366–2382.
62. Smoot ME, Ono K, Ruscheinski J, Wang PL, Ideker T: **Cytoscape 2.8: new features for data integration and network visualization.** *Bioinformatics* 2011, **27:**431–432.
63. Costanzo M, Baryshnikova A, Bellay J, Kim Y, Spear ED, Sevier CS, Ding H, Koh JL, Toufighi K, Mostafavi S, *et al*: **The genetic landscape of a cell.** *Science* 2010, **327:**425–431.
64. Alvarez P, Smith A, Fleming J, Solomon F: **Modulation of tubulin polypeptide ratios by the yeast protein Pac10p.** *Genetics* 1998, **149:**857–864.
65. Ismail N, Ng DTW: **Have you HRD? Understanding ERAD Is DOAble!** *Cell* 2006, **126:**237–239.
66. Gregersen N, Bross P: **Protein misfolding and cellular stress: an overview.** *Meth Mol Biol* 2010, **648:**3–23.
67. Chen Y, Sanchez Y: **Chk1 in the DNA damage response: conserved roles from yeasts to mammals.** *DNA Repair (Amst)* 2004, **3:**1025–1032.

68. Liu Y, Vidanes G, Lin YC, Mori S, Siede W: **Characterization of a *Saccharomyces cerevisiae* homologue of *Schizosaccharomyces pombe* Chk1 involved in DNA-damage-induced M-phase arrest.** *Mol Gen Genet* 2000, **262:**1132–1146.
69. Rouse J, Jackson SP: **Interfaces between the detection, signaling, and repair of DNA damage.** *Science* 2002, **297:**547–551.
70. Enemark EJ, Joshua-Tor L: **Mechanism of DNA translocation in a replicative hexameric helicase.** *Nature* 2006, **442:**270–275.
71. Byun TS, Pacek M, Yee MC, Walter JC, Cimprich KA: **Functional uncoupling of MCM helicase and DNA polymerase activities activates the ATR-dependent checkpoint.** *Genes Dev* 2005, **19:**1040–1052.
72. Feng W, Collingwood D, Boeck ME, Fox LA, Alvino GM, Fangman WL, Raghuraman MK, Brewer BJ: **Genomic mapping of single-stranded DNA in hydroxyurea-challenged yeasts identifies origins of replication.** *Nat Cell Biol* 2006, **8:**148–155.
73. Lucca C, Vanoli F, Cotta-Ramusino C, Pellicioli A, Liberi G, Haber J, Foiani M: **Checkpoint-mediated control of replisome-fork association and signalling in response to replication pausing.** *Oncogene* 2004, **23:**1206–1213.
74. Nedelcheva MN, Roguev A, Dolapchiev LB, Shevchenko A, Taskov HB, Stewart AF, Stoynov SS: **Uncoupling of unwinding from DNA synthesis implies regulation of MCM helicase by Tof1/Mrc1/Csm3 checkpoint complex.** *J Mol Biol* 2005, **347:**509–521.
75. Sogo JM, Lopes M, Foiani M: **Fork reversal and ssDNA accumulation at stalled replication forks owing to checkpoint defects.** *Science* 2002, **297:**599–602.
76. You Z, Kong L, Newport J: **The role of single-stranded DNA and polymerase alpha in establishing the ATR, Hus1 DNA replication checkpoint.** *J Biol Chem* 2002, **277:**27088–27093.
77. Zou L, Elledge SJ: **Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes.** *Science* 2003, **300:**1542–1548.
78. Nitani N, Yadani C, Yabuuchi H, Masukata H, Nakagawa T: **Mcm4 C-terminal domain of MCM helicase prevents excessive formation of single-stranded DNA at stalled replication forks.** *Proc Natl Acad Sci* 2008, **105:**12973–12978.
79. Hanna JS, Kroll ES, Lundblad V, Spencer FA: **Saccharomyces cerevisiae CTF18 and CTF4 are required for sister chromatid cohesion.** *Mol Cell Biol* 2001, **21:**3144–3158.
80. Miles J, Formosa T: **Evidence that POB1, a Saccharomyces cerevisiae protein that binds to DNA polymerase alpha, acts in DNA metabolism in vivo.** *Mol Cell Biol* 1992, **12:**5724–5735.
81. Gambus A, van Deursen F, Polychronopoulos D, Foltman M, Jones RC, Edmondson RD, Calzada A, Labib K: **A key role for Ctf4 in coupling the MCM2-7 helicase to DNA polymerase alpha within the eukaryotic replisome.** *EMBO J* 2009, **28:**2992–3004.
82. Ando A, Nakamura T, Murata Y, Takagi H, Shima J: **Identification and classification of genes required for tolerance to freeze-thaw stress revealed by genome-wide screening of Saccharomyces cerevisiae deletion strains.** *FEMS Yeast Res* 2007, **7:**244–253.
83. Dudley AM, Janse DM, Tanay A, Shamir R, Church GM: **A global view of pleiotropy and phenotypically derived gene function in yeast.** *Mol Syst Biol* 2005, **1(2005):**0001.
84. Paulovich AG, Margulies RU, Garvik BM, Hartwell LH: **RAD9, RAD17, and RAD24 are required for S phase regulation in Saccharomyces cerevisiae in response to DNA damage.** *Genetics* 1997, **145:**45–62.
85. Toh GW, Lowndes NF: **Role of the Saccharomyces cerevisiae Rad9 protein in sensing and responding to DNA damage.** *Biochem Soc Trans* 2003, **31:**242–246.
86. Krejci L, Van Komen S, Li Y, Villemain J, Reddy MS, Klein H, Ellenberger T, Sung P: **DNA helicase Srs2 disrupts the Rad51 presynaptic filament.** *Nature* 2003, **423:**305–309.
87. Veaute X, Jeusset J, Soustelle C, Kowalczykowski SC, Le Cam E, Fabre F: **The Srs2 helicase prevents recombination by disrupting Rad51 nucleoprotein filaments.** *Nature* 2003, **423:**309–312.
88. Howlett NG, Schiestl RH: **Nucleotide excision repair deficiency causes elevated levels of chromosome gain in Saccharomyces cerevisiae.** *DNA Repair* 2004, **3:**127–134.
89. Tong A, Boone C: *Synthetic Genetic Array (SGA) Analysis in Saccharomyces cerevisiae*, Methods in Molecular Biology, Volume 313. Second Editionth edition. Totowa, NJ, U. S. A: The Humana Press, Inc; 2005:171–192. Yeast Protocols.
90. Pan X, Yuan DS, Xiang D, Wang X, Sookhai-Mahadeo S, Bader JS, Hieter P, Spencer F, Boeke JD: **A robust toolkit for functional profiling of the yeast genome.** *Mol Cell* 2004, **16:**487–496.
91. Huang ME, Rio AG, Nicolas A, Kolodner RD: **A genomewide screen in *Saccharomyces cerevisiae* for genes that suppress the accumulation of mutations.** *Proc Natl Acad Sci U S A* 2003, **100:**11529–11534.

doi:10.1186/1471-2156-13-36
Cite this article as: Stead *et al.*: Mcm2 phosphorylation and the response to replicative stress. *BMC Genetics* 2012 13:36.

Submit your next manuscript to BioMed Central and take full advantage of:

- **Convenient online submission**
- **Thorough peer review**
- **No space constraints or color figure charges**
- **Immediate publication on acceptance**
- **Inclusion in PubMed, CAS, Scopus and Google Scholar**
- **Research which is freely available for redistribution**

Submit your manuscript at
www.biomedcentral.com/submit