Elevated dNTP levels suppress hyper-recombination in Saccharomyces cerevisiae S-phase checkpoint mutants

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ABSTRACT

MEC1, the essential yeast homolog of the human ATR/ATM genes, controls the S-phase checkpoint and prevents replication fork collapse at slow zones of DNA replication. The viability of hypomorphic mec1-21 is reduced in the rad52 mutant, defective in homologous recombination, suggesting that replication generates recombinogenic lesions. We previously observed a 6-, 10- and 30-fold higher rate of spontaneous sister chromatid exchange (SCE), heteroallelic recombination and translocations, respectively, in mec1-21 mutants compared to wild-type. Here we report that the hyper-recombination phenotype correlates with lower deoxyribonucleoside triphosphate (dNTP) levels, compared to wild-type. By introducing a dun1 mutation, thus eliminating inducible expression of ribonucleotide reductase in mec1-21, rates of spontaneous SCE increased 15-fold above wild-type. All the hyper-recombination phenotypes were reduced by SML1 deletions, which increase dNTP levels. Measurements of dNTP pools indicated that, compared to wild-type, there was a significant decrease in dNTP levels in mec1-21, dun1 and mec1-21 dun1, while the dNTP levels of mec1-21 smt1, mec1-21 dun1 smt1 and smt1 mutants were ~2-fold higher. Interestingly, higher dNTP levels in mec1-21 dun1 smt1 correlate with ~2-fold higher rate of spontaneous mutagenesis, compared to mec1-21 dun1. We suggest that higher dNTP levels in specific checkpoint mutants suppress the formation of recombinogenic lesions.

INTRODUCTION

MEC1 is the essential yeast homolog (1) of the human ATR/ATM genes (for review, 2) and shares some functions with both ATM (mutated in ataxia telangiectasia) and ATR (ATM and Rad3 related). MEC1 prevents replication fork collapse (3), a function shared with ATR, but not ATM, in mammalian cells (for review, 2). Similar to ATR, MEC1 is required for viability; mec1 lethality can be suppressed by mutations in SML1, CRT1 or DIF1, or by over-expression of RNR1 or RNR3, all of which increase the activity of ribonucleotide reductase (for review, 4). SML1 mutations elevate deoxyribonucleoside triphosphate (dNTP) levels 2-fold compared to wild-type, implying that increasing dNTP levels is sufficient to suppress mec1 lethality (5).

MEC1’s function in controlling dNTP levels is mediated by RAD53, which, upon activation by DNA damage and in S phase, triggers the DUN1-mediated induction of ribonucleotide reductase (6). While both MEC1 and RAD53 are essential, DUN1 is non-essential (7). Basal dNTP levels are lower in specific mec1 and rad53 hypomorphic mutants, compared to wild-type (8). Observations that dun1 exhibits a longer S phase than wild-type and is defective in the degradation of Sml1 suggest that basal dNTP levels are also lower in dun1 (9). Thus, yeast can maintain viability at dNTP levels that are both higher and lower than in wild-type.

Lower dNTP levels may correlate with a hyper-recombination phenotype and decreased spontaneous point mutation rates while higher dNTP levels may correlate with an increase in spontaneous or DNA damage-associated mutagenesis, compared to wild-type. For example, dun1 mutants exhibit higher levels of spontaneous heteroallelic recombination (10) and lower levels of spontaneous mutagenesis that results in...
canavanine resistance (11). Over-expression of RNR1, which results in higher dNTP levels, leads to higher levels of 4-nitroquinoline-N-oxide (4NQO)-associated mutagenesis (12). The rnr1-D57N mutant, which has 2- to 3-fold higher dNTP levels, exhibits a similar increase in spontaneous mutation rates (13). One possible explanation is that replicative DNA polymerases may require higher levels of dNTPs for insertion of particular bases opposite damaged sites (12).

Since mec1 null mutants require sml1 for viability, mec1 hypomorphic mutants that retain essential function are useful in determining which genetic instability phenotypes correlate to lower dNTP levels. Such mutants include mec1-21, which is defective in the S-phase checkpoint (6,14) and results from a G to A substitution (G882S) at position 2644 outside of the kinase domain (15). RAD52 is required for double-strand break repair (for review, 16), and compared to mec1-21, viability in the mec1-21 rad52 mutant is significantly reduced, suggesting that double-strand breaks spontaneously arise in mec1-21 (17). Our previous studies have indicated that compared to wild-type, mec1-21 exhibits significantly higher rates of spontaneous homologous recombination between sister chromatids, homologs and non-homologous chromosomes (18). The hyper-recombination phenotype is dependent on RAD52 and G2 checkpoint genes (18), suggesting that spontaneous DNA breaks generated by DNA replication are repaired by homologous recombination.

We demonstrate that, compared to wild-type, mec1-21 hyper-recombination phenotype is SML1-dependent and correlates with lower basal levels of dNTPs. Deleting SML1 in mec1-21 dun1 mutant also correlates to a modestly higher rate of spontaneous mutagenesis. These studies suggest that higher dNTP levels may suppress hyper-recombination in hypomorphic mec1 mutants. Thus, an adequate supply of dNTPs is an important MEC1 function in maintaining genomic stability.

MATERIALS AND METHODS

Media and yeast strains

Standard media for the culture of yeast, SC (synthetic complete, dextrose), SC-HIS (SC lacking histidine), SC-TRP (SC lacking tryptophan), SC-URA (SC lacking uracil), YP (yeast extract, peptone) and YPD (YP, dextrose), are described by Burke et al. (19). YP(A)D contains YPD with 80 mg/l adenine. CAN plates contain SC medium and 60 mg/l of canavanine.

Relevant yeast strains are listed in Table 1. The mec1-21 strain YA197 (Y620), and the YA184 and YA185 strains used to PCR amplify sml1::URA3 and mec1::TRP1, respectively, are derived from W303; all other strains are of the S288c background. Strains used to measure SCE contain two overlapping his3 fragments, positioned in tandem at trp1, and were derived from YB163 (20,21). Diploid strains were used to measure translocations that were derived from a cross of one haploid (YB109) that contains the his3 fragments on one copy of chromosomes II and IV, and another which did not contain the his3 fragments (YA102) (22). To measure heteroallelic recombination, we replaced the ade2-101 alleles in YB109 and YA102 with ade2-n (YB318) and ade2-a (YB315), respectively, by two-step gene replacement using the plasmid pKH9 (23). Heteroallelic recombination was measured by selecting for Ade1 revertants.

We used the mec1-21 missense mutant to measure spontaneous recombination. The original MATa mec1-21 strain (Y620) (6) was backcrossed 10 times with strains in the S288c background [YB163, YA166 (21) and YB315] to generate meiotic segregants that either do (YB312) or do not (YB316, YB314) contain his3 recombinational substrates to measure SCE. We introduced the sml1::KanMX and sml1::URA3 allele in yeast strains by PCR-mediated gene replacement; the primers used for amplifying sml1::KanMX or sml1::URA3 knockout fragments were the same: 5'-CAT ATCGTTACTGTTTGGAAATCCG-3' and 5'-TAAA GGGAAGGAAATGCGACG-3'. The construction of mec1-1 sml1::KanMX (YB327) was described earlier (17).

To measure translocations and heteroallelic recombination in mec1 strains, mutations were introduced into two haploids by either genetic crosses or by one-step gene replacement (24); one haploid contains the his3 replacement substrates and ade2-n (YB318), while another (YB315) contained ade2-a but no recombinational substrates. YB318 was crossed with YB313 to generate the MATa-ade2-n mec1-21 meiotic segregant (YB319) that contains the GAL::his3-Δ5 and trp1::his3-Δ3. YB325 is a homozygous mec1-21 diploid that was then used to measure translocations and heteroallelic recombination.

Additional checkpoint mutants were made by either one-step gene disruption (24) or by genetic crosses and screening the phenotype of the appropriate meiotic segregant. The primer pairs used to amplify dun1::KanMX fragments were, 5’-AGAAGGCCCTGAA TACCATAAATA3 and 5’-CATGTCAGAGATTTAG AGGAAA3, respectively. We made the mec1-21 dun1::KanMX sml1::URA3 haploid (YB380) by introducing sml1::URA3 into mec1-21 dun1::KanMX (YB369) by one-step gene disruption (24). All gene disruptions were confirmed by PCR.

Determining rates of spontaneous recombination and mutations

The rates (events per cell division) of spontaneous SCE, heteroallelic recombination, translocations and mutations in CAN1 were determined by the method of the median (25), as previously performed (21). Rates of spontaneous heteroallelic recombination were determined on cells inoculated on YP(A)D, on which there is no growth advantage for Ade1 revertants. Rates of mutations in CAN1 were determined by selecting for resistance to canavanine. We determined the statistical significance by the Mann–Whitney U-test (26).

Determining dNTP levels

The methods of measuring dNTPs in yeast are as described in Chabes et al., (13). At a density between 0.5 × 10^7 and 1.5 × 10^7 cells/ml, ~1 × 10^6 cells were
Table 1. Yeast strains

| Strain  | Genotype | Source (syonym) |
|---------|----------|-----------------|
| YA102   | MATα-inc ura3-52 his3-A20 lys2-801 trp1-A1 ade2-101 | This lab |
| YA165   | MATα ura3-52 his3-A20 trp1-A1 leu2-1 | F. Winston (FY250) |
| YA166   | MATα ura3-52 his3-A20 trp1-A1 leu2-1 | F. Winston (FY251) |
| YA184   | MATα trp1-A1 leu2-3,112 his3-11,15 ura3-1 can1-100 sm1::URA3 rad53::HIS3 RAD5 | R. Rothstein (W2105-17B) |
| YA185   | MATα trp1-A1 leu2-3,112 his3-11,15 ura3-1 can1-100 mcm1-A::TRP1 sm1::HIS3 RAD5 | R. Rothstein (U963-61A) |
| YA195   | MATα his3-A1 leu2-300 mit150-A sm1::KanMX | ResGene (512) |
| YA196   | MATα:MATα his3-A1/− leu2-300 ura3-A0/− mit150-A/− lys2-A0/− mcm1-A::KanMX/− | ResGene (23275) |
| YA197   | MATα ade2-A1 trp1-A1 leu2-3,112 his3-A11,15 ura3-1 can1-100 mcm1-A1 | S. Elledge (Y620) |
| YA224   | MATα his3-A1 leu2-300 mit150-A sm1::KanMX | ResGen (3798) |
| YB313   | MATα-inc ura3-52 his3-A20 lys2-801 trp1-A1 gal3-A1 mec1-21 | Derived from cross of YB311×YA165 |
| YB314   | MATα ura3-52 his3-A20 lys2-801 trp1-A1 gal3-A1 mec1-21 | Derived from cross of YB312×YA166 |
| YB315   | MATα ura3-52 his3-A20 ade2-A1 lys2-801 trp1-A1 gal3-A1 | Derived from YA102 |
| YB316   | MATα ura3-52 his3-A20 ade2-A1 lys2-801 trp1-A1 gal3-A1 mec1-21 | Derived from cross of YB315×YB314 |
| YB317   | MATα ura3-52 his3-A20 ade2-A1 lys2-801 trp1-A1 gal3-A1 sm1::KanMX | sml1::KanMX disruption in YB315 |

Strains to monitor translocations and heteroallelic events

| Strain  | Genotype | Source (syonym) |
|---------|----------|-----------------|
| YB109   | MATα ura3-52 his3-A20 ade2-A101trp1-A1 gal3-A1 trp1::his3-A5::HOcs lys2-A1 (leaky) | This laboratory |
| YB131   | MATα ura3-52 his3-A20 ade2-A2 trp1-A1 gal3-A1 leu2-3,112 GAL1::his3-A5::HOcs lys2-A1 (leaky) | Derived from cross of YB313×YB318 |
| YB191   | MATα ura3-52 his3-A20 ade2-A2 trp1-A1 gal3-A1 leu2-3,112 GAL1::his3-A5::HOcs lys2-A1 (leaky) mec1-21 | sml1::KanMX disruption in YB318 |
| YB192   | MATα ura3-52 his3-A20 ade2-A2 trp1-A1 gal3-A1 leu2-3,112 GAL1::his3-A5::HOcs lys2-A1 (leaky) sm1::KanMX | sml1::KanMX disruption in YB319 |
| YB323   | YB316×YB320 | This laboratory |
| YB325   | YB316×YB319 | This laboratory |
| YB348   | YB315×YB318 | This laboratory |
| YB373   | MATα ura3-52 his3-A20 ade2-A2 trp1-A1 gal3-A1 leu2-3,112 mec1-21 sml1::KanMX | sml1::KanMX disruption in YB316 |
| YB374   | MATα ura3-52 his3-A20 ade2-A2 trp1-A1 gal3-A1 leu2-3,112 GAL1::his3-A5::HOcs lys2-A1 (leaky) mec1-21 sm1::KanMX | sml1::KanMX disruption in YB319 |
| YB375   | YB373×YB374 | This laboratory |
| YB376   | MATα inc ura3-52 his3-A20 ade2-A101trp1-A1 gal3-A1 trp1::his3-A5::HOcs, his3-A5::HOcs, his3-A5::HOcs | This laboratory |
| YB377   | MATα inc mec1-21 | This laboratory |
| YB378   | MATα inc mec1-21 | Tenth backcross of Y620 with YB163 |
| YB379   | MATα inc mec1-21 | Tenth backcross of Y620 with YB163 |
| YB380   | MATα inc mec1-21 | sml1::KanMX disruption in YB316 |
| YB381   | MATα inc mec1-21 | sml1::KanMX disruption in YB316 |
| YB382   | MATα inc mec1-21 | sml1::KanMX disruption in YB316 |
| YB383   | MATα inc mec1-21 | sml1::KanMX disruption in YB316 |
| YB384   | MATα inc mec1-21 | sml1::KanMX disruption in YB316 |
| YB385   | MATα inc mec1-21 | sml1::KanMX disruption in YB316 |
| YB386   | MATα inc mec1-21 | sml1::KanMX disruption in YB316 |
| YB387   | MATα inc mec1-21 | sml1::KanMX disruption in YB316 |
| YB388   | MATα inc mec1-21 | sml1::KanMX disruption in YB316 |

*All strains listed below have the same genotype as YB163 unless indicated. Mating type is added for clarity. YB333 and YB334 may contain either ura3-52 or ura3-A0 and lys2-A0 or lys2-801.

harvested by filtration through 25mm White AAWP nitrocellulose filters (0.8 μm, Millipore AB, Solna, Sweden). The filters were immersed in 500 μl of ice-cold extraction solution (12% w/v trichloroacetic acid, 15mM nitrocellulose filters (0.8 μm, Millipore AB, Solna, Sweden, 99%) added to 800 μl of ice-cold Freon-trioctylamine mixture. The mixture was vortexed and centrifuged for 1 min at 20000 g. The aqueous phase was collected and added to 800 μl of ice-cold Freon-trioctylamine mixture. The mixture was vortexed and centrifuged as described earlier. A 475 μl aliquot of the aqueous phase was pH-adjusted with 25 μl of 1M NH₄HCO₃, pH 8.9, the deoxyribonucleotides were separated from the ribonucleotides by boronate affinity chromatography (Affi-Gel 601, Bio-Rad) and quantified by HPLC. Another 47.5 μl aliquot of the aqueous phase was mixed with 152.5 μl of water and used for the HPLC quantification of NTP pools. Separation of nucleotides was done on a Partisil 5 SAX column (PolyLC Inc., Columbia, MD, USA) using a UV-2075 Plus detector (Jasco, Mölndal, Sweden). Nucleotides were isotopically.
Key points in the text:

- Western blots
  - To detect Rad53 phosphorylation after HU and MMS exposure, protein samples were obtained from log phase cells (A600 = 0.5–1) exposed to 200 mM HU or 0.01% MMS for 2 h. Proteins (10 μg) were separated on 10% acrylamide/0.266% bis-acrylamide gels, transferred to nitrocellulose membrane and exposed to a Rad53 antibody (yC-19, Santa Cruz). The secondary antibody was peroxidase conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratory, Inc.). The signals were developed using ECL western lightning kit (NEL102, PerkinElmer Life Science, Inc.).

- Results
  - Viability of particular mec1 hypomorphs is reduced in the rad52 mutant, deficient in homologous recombination (27,17). While ~60% of either mec1-21 or rad52 log phase cells form colonies, only ~20% of mec1-21 rad52 cells form colonies (17). Deleting SML1 in mec1-21 rad52 increased the plating efficiency to ~50% and increased the growth rate to wild-type levels (Supplementary Table S1).
  - These results suggest that deleting SML1 in mec1-21 rad52 decreases the number of lethal DNA double-strand breaks. We therefore determined whether hyper-recombination phenotypes of mec1-21 would be suppressed by SML1 deletions and correlate with dNTP levels. Unequal SCE was measured by selecting for His+ recombinants in haploid strains containing two truncated his3 gene fragments (20,21, Figure 1). Diploid strains were used to measure heteroallelic recombination between ade2-a and ade2-n (28) and ectopic recombination between GAL1::his3-A5' and trp1::his3-A3' (22, Figure 1).

- Higher rates of spontaneous, homologous recombination in mec1-21 are SML1-dependent
  - We reasoned that if lower dNTP levels correlate with higher recombination rates in mec1 mutants, then SML1 mutations would decrease homologous recombination in mec1-21 mutants. We previously observed a 6-fold increase in the rate of spontaneous SCE in the mec1-21 mutant (YB312) compared to wild-type (18). We observed no difference in the rates of spontaneous recombination between sml1 (YB326) and wild-type (YB163); however the 6-fold increase in rates of SCE was reduced to wild-type levels in mec1-21 sml1 (YB329, Table 2).

- Rates of heteroallelic recombination and translocations in mec1-21 were 10-fold higher and 23-fold higher than wild-type, respectively (Table 3). We determined whether mec1-21 hyper-recombination between homologs and non-homologs also required SML1. Heteroallelic recombination between ade2 alleles can be visualized using a colony pigment assay, where Ade+ recombinants appear as white colony sectors (Figure 2). Heavily white sectored colonies indicated that more heteroallelic recombination occurred in mec1-21 (YB325) than in wild-type (YB348), while the mec1-21 sml1 mutant (YB375) exhibited less visible sectoring than mec1-21 cells (Figure 2).

- We then measured rates of translocations and heteroallelic recombination in cells grown on medium containing excess adenine (YPAD) so that there is no growth advantage for Ade+ cells. While the rates of heteroallelic recombination and translocations in the sml1 (YB323) and wild-type (YB348) were similar (Table 3), mec1-21 sml1 diploid mutant (YB375) exhibited 8- and 4-fold lower rates of heteroallelic recombination and translocations, respectively, compared to mec1-21. However the rates of recombination in mec1-21 sml1 were still between 2- and 5-fold higher than those observed in wild-type (Table 3). Thus, SML1 deletion in mec1-21 diploid partially suppresses spontaneous heteroallelic recombination and translocations.

- Rate of spontaneous SCE in mec1-21 are further increased by dun1
  - The MEC1-mediated pathway for regulating dNTP levels involves Rad53 activation, which in turn leads to Dun1 activation (7,29). By western blots, we observed that Rad53 is partially activated to P-Rad53 in the mec1-21 hypomorph, compared to wild-type, after HU and MMS exposure (Supplementary Figure S1). We observed ~40% of the Rad53 activation (P-Rad53/Rad53) in mec1-21, compared to wild-type, while in mec1-a, the level of activation was <30% (n = 2). These data are consistent with observations that mec1-21 exhibits partial checkpoint activation after exposure to agents that cause DNA replication stress (30).

- Considering that DUN1 is required for transcriptional induction of the RNR genes (7) and is suggested to maintain basal dNTP levels (9), we asked whether mec1-21 dun1 mutants would exhibit higher levels of spontaneous recombination, compared to mec1-21. Although there is no difference between spontaneous rates of SCE in dun1 (YB370) and wild-type, we observed a 15-fold increase in the rate of SCE in the mec1-21 dun1 double mutant, compared to wild-type (Table 2). SML1 mutations conferred significantly lower rates of SCE in mec1-21 dun1 (YB369), but were still 3-fold higher than wild-type. These data indicate that DUN1 suppresses recombination in mec1-21, but a SML1 deletion only partially suppresses the hyper-recombination phenotype of the mec1-21 dun1 double mutant.

- dNTP are lower in mec1-21, dun1 and mec1-21 dun1 but are increased in mec1-21 sml1 and mec1-21 dun1 sml1 mutants compared to wild-type
  - We expected that the higher and lower rates of homologous recombination exhibited by S-phase checkpoint mutants would inversely correlate with dNTP levels. We measured dNTP levels in wild-type, sml1, mec1-21, dun1, mec1-21 sml1, mec1-21 dun1 and mec1-21 dun1 sml1 strains (Figure 3). In comparison to wild-type, the S288c sml1 strains exhibited increased dNTP levels over 100%, while the S288c dun1 strains exhibited ~50–70% reduction in dNTP levels. The mec1-21 levels of dNTPs were ~50–70% of those observed in wild-type strains.
Interestingly, the dNTP levels of mec1-21 dun1 strains were similar to mec1-21. These results are consistent with observations that MEC1 is epistatic to DUN1 in controlling dNTP levels (6,8). We do not know the lowest dNTP level that maintains viability in yeast; thus, it is possible that basal dNTP levels cannot be reduced further than those observed in mec1-21 dun1.

The basal dNTP levels were similar in sml1, mec1-21 sml1 and mec1-21 dun1 sml1, and increased ~2-fold, relative to wild-type (Figure 3). The basal level of each dNTP was elevated between 2- and 4-fold in both the mec1-21 sml1 and the mec1-21 dun1 sml1 strains, relative to mec1-21 and mec1-21dun1, which exhibit lower dNTP levels than wild-type (Figure 3). These data indicate that deleting SML1 in both checkpoint mutants and wild-type leads to similar dNTP levels. These results are consistent with observations that MEC1 and DUN1 both function in a pathway to degrade Sml1 (8,9). Thus, higher dNTP levels correlate with suppression of homologous recombination in mec1-21 mutants.

Spontaneous mutagenesis rates are lower in the mec1-21 dun1 mutant but increased in mec1-21 dun1 sml1 mutants

One hypothesis is that higher dNTP levels facilitate DNA replication at stalled replication forks, preventing

Figure 1. Unequal SCE, translocation and heteroallelic recombination assays used in this study. Ovals represent centromeres and lines represent chromosomes. For simplicity, the left arms of chromosomes are not included. An arrow and feathers together denote HIS3. As indicated in the bottom left of the figure, the 5’ deletion lacks the feather and the 3’ deletion lacks the arrow. The two regions of the sequence identity shared by the his3 fragments are indicated by decorated boxes; closely-spaced diagonal-filled boxes indicate a region of ~300bp. The 117-bp HO cut site (HOcs), as indicated by an arrowhead, is located between these sequences within the his3-Δ5′:HOcs fragment. (A) The his3-truncated fragments are integrated into the trp1 locus to measure unequal SCE events. (B) Translocation events result from recombination between the same his3 fragments located each on chromosomes II and IV. Positions of the GAL1 and trp1 are shown on chromosomes II, IV and the reciprocal translocation. (C) Heteroallelic recombination between ade2-a and ade2-n generates ADE2. ADE2 and ade2 alleles are represented as boxes; ade2-a and ade2-n are separated by ~1 kb.

Table 2. Rates of spontaneous SCE in mec1 mutants

| Strain | Genotypea | Rate (×10⁶)b | Ratioc |
|--------|-----------|--------------|--------|
| YB163  | MEC1      | 1.1 ± 0.1    | 1.0    |
| YB326  | sml1      | 1.1 ± 0.2    | 1.0    |
| YB312  | mec1-21   | 6.3 ± 0.9    | 5.7    |
| YB329  | mec1-21 sml1 | 1.1 ± 0.2 | 1.0    |
| YB370  | dun1      | 1.2 ± 0.3    | 1.1    |
| YB369  | mec1-21 dun1 | 17 ± 4.0  | 15.0   |
| YB380  | mec1-21 dun1 sml1 | 3.1 ± 0.7 | 2.8    |
| YB327  | mec1-Asml1 | 1.2 ± 0.2    | 1.2    |
| aAll strains derived from S288c. For complete genotype (Table 1).\n| bRate represents the number of events per cell division; n ≥ 3.\n| cRatio represents rate of SCE in mutant/rate of SCE in wild–type.\n
Spontaneous mutagenesis rates are lower in the mec1-21 dun1 mutant but increased in mec1-21 dun1 sml1 mutants

One hypothesis is that higher dNTP levels facilitate DNA replication at stalled replication forks, preventing
replication fork regression or the formation of double-strand breaks. Higher dNTP levels can also lead to higher rates of 4NQO-associated mutagenesis (12). We therefore measured rates of spontaneous mutation at CAN1 in wild-type, mec1-21, sml1, mec1-21 sml1, mec1-21 dun1, dun1 and mec1-21 dun1 sml1 strains (Table 4). The rate of spontaneous mutation in wild-type (YB163) was $4 \times 10^{-7}$, in agreement with Datta et al. (11). In comparison to wild-type, the mutation rate is the same in sml1 and mec1-21 sml1 but lower in mec1-21 dun1 ($P < 0.05$). The rates of spontaneous mutation are increased ~2-fold in mec1-21 dun1 sml1 ($P < 0.05$), in comparison mec1-21 dun1. Thus, higher dNTP levels correlate with the higher rate of spontaneous mutagenesis in mec1-21 dun1 sml1 but not in sml1 strains.

**DISCUSSION**

The ATR (ATM) yeast homolog *MEC1* has a pivotal role in stabilizing stalled DNA replication forks and is required to maintain vital levels of deoxyribonucleotides to ensure that the genome is faithfully replicated. The viability of *mec1* hypomorphic mutants, such as *mec1-21*, is significantly reduced in *rad52* mutants (17), suggesting that double-strand breaks are spontaneously generated in *mec1* mutants. In this study, we observed that deleting *SML1* suppressed the *mec1* hyper-recombination phenotypes while increasing the rate of spontaneous mutation in *mec1-21 dun1*. We compared measurements of dNTPs in *SML1* and *sml1* deletion strains that contained combinations of the *mec1-21* and *dun1* mutations, and derived the following conclusions: (i) lower dNTP levels in *mec1-21* correlate with an increase in recombination rates, while higher dNTP levels in both *mec1-21* and *mec1-21 dun1* correlate with a decrease in recombination rates, (ii) *DUN1* can suppress homologous recombination in *mec1-21* independent of its function in maintaining dNTP levels and (iii) higher dNTP levels increase spontaneous mutagenesis in *mec1 dun1* strains. We suggest that increasing dNTP levels reduces the formation of recombinogenic DNA lesions in *mec1* hypomorphic and *dun1* mutants. This is the first study to measure dNTP levels in *mec1-21* and *dun1* mutants to demonstrate that *DUN1* is required for maintaining basal dNTP levels.

These observations may seem to contradict published reports that frequencies of gross chromosomal rearrangements (GCRs) are highest in *mec1-A*, which is deleted for *SML1* compared to *mec1-21* (31,32). However, *mec1-A* is deficient in both S-phase and G2 checkpoint functions, while the *mec1-21* hypomorph retains partial G2 checkpoint function and is less X-ray sensitive compared to the null mutant (17,18,33). We speculate that replication fork collapse is more severe in *mec1-A*, compared to *mec1-21*. Thus, the combination of replication fork collapse and the deficiency in recombinational repair of double-strand breaks in G2 likely results in the higher rate of GCRs in the *mec1-A* compared to *mec1-21*.

**Lower dNTP levels contribute to the higher rates of spontaneous recombination**

Both *mec1* and *dun1* mutants exhibit lower dNTP levels, compared to wild-type, but only *mec1-21* exhibits higher SCE recombination rates. We suggest that the higher recombination rates result from replication forks stalling due to inadequate dNTP levels. *MEC1* functions to prevent replication fork collapse, especially at slow zones of replication (3), and when ribonucleotide reductase is inhibited by HU (34). We suggest that the *MEC1*
Figure 3. dNTP levels measured in wild-type (YB163), dun1 (YB370), sml1 (YB326), mecl-21 dun1 (YB369), mecl-21 dun1 sml1 (YB380) and mecl-21 (YB312 and YB311) mutants. Two mecl-21 strains were used; YB311 MATα-inc and YB312 MATα (Table 1).

Table 4. Rates of spontaneous canavanine resistance in mecl mutants

| Strain   | Genotypea | Rate (×10³)b | Ratio⁡ |
|----------|-----------|--------------|--------|
| YB163    | MEC1      | 4.0 ± 0.6    | 1.0    |
| YB326    | sml1      | 4.3 ± 0.5    | 1.0    |
| YB312    | mecl-21   | 5.3 ± 1.4    | 1.0    |
| YB329    | mecl-21 sml1 | 3.8 ± 0.1 | 1.0    |
| YB370    | dun1      | 2.8 ± 1.9    | 1.0    |
| YB369    | mecl-21 dun1 | 1.8 ± 0.6 | 1.0    |
| YB380    | mecl-21 dun1 sml1 | 3.2 ± 0.5 | 0.5    |

⁡All strains derived from S288c. For complete genotype (Table 1).
³Rate represents the number of events per cell division; n ≥ 2.
³Ratio represents rate of SCE in mutant/rate of SCE in wild-type.

function in preventing replication fork collapse reduces spontaneous SCE in dun1 mutants. dun1 mutants do exhibit higher rates of spontaneous heteroallelic gene conversion between homologs (10), and it is possible that sister chromatid gene conversions are not detected in our assay (35). However, in mecl hypomorphs, stalled replication forks would lead to more replication fork collapse and recombinogenic lesions. Thus, MEC1 has two functions in suppressing recombinogenic lesions: (i) maintaining dNTP levels and (ii) preventing replication fork collapse.

When SML1 is deleted in either mecl-21 or mecl-21 dun1 strains, we speculate that higher dNTP levels reduce replication fork stalling and thus fewer replication forks collapse. However, we observed that mecl-21 sml1 mutants still exhibited some hyper-recombination between homologs or non-homologous chromosomes when dNTP levels were high. It is likely that we can detect higher levels of translocations in mecl-21 sml1 mutants because there is a lower rate of spontaneous translocations, compared to heteroallelic and SCE, in wild-type. Thus, more recombinogenic lesions still occur in mecl-21, compared to wild-type, even at high dNTP levels.

**DUN1 suppresses spontaneous homologous recombination in mecl-21**

Although dNTP levels in mecl-21, dun1 and mecl-21 dun1 are similar, the rates of spontaneous recombination are synergistically increased in the double mutant, compared to the single mutants. We suggest that there may be two different reasons for these observations. First, although the overall dNTP levels are the same, the rate of dNTP production may be lower in mecl-21 dun1, compared to dun1, leading to more replication stalling. Second, Schollaert et al. (36) reported that CHK1 is required for hydroxyurea resistance in dun1 or sml1 dun1 mutants, suggesting additional DUN1 functions in promoting replication. This possibility is supported by the observation that SML1 deletion only partially suppresses the hyper-recombination phenotype in mecl-21 dun1. We suggest that MEC1 and DUN1 may have redundant roles in suppressing recombinogenic breaks.

While MEC1 may prevent replication fork collapse, a possible function of DUN1 in preventing recombinogenic lesions is to promote translesion synthesis. DUN1 functions in promoting spontaneous mutagenesis in both wild-type and in pol3 mutants (11). We observed that the rate of spontaneous mutation was similar in dun1 and mecl-21 dun1. Thus, it is possible that failure to promote translesion synthesis in mecl-21 dun1 leads to more DNA replication stalling at sites of spontaneous damage, increasing the number of collapsed replication forks and recombinogenic lesions.

**Spontaneous mutagenesis increases and spontaneous recombination decreases in mecl-21 dun1 as dNTPs levels increase due to SML1 deletion**

4-NQO-associated mutagenesis increases with higher dNTP levels in strains deleted for genes that encode error-prone polymerases (12), suggesting that replicative polymerases function as translesion polymerases on non-bulky lesions when dNTP levels are higher (12). However, we did not observe higher levels of spontaneous mutagenesis in sml1, compared to wild-type. Considering that Sm1 is degraded in S-phase, the S-phase dNTP levels are likely similar in wild-type and in sml1 mutants (8). We speculate that higher dNTP levels may promote translesion synthesis in cells where replication forks frequently stall, as in S-phase checkpoint mutants when the DNA polymerase encounters slow zones of...
replication. Here, we speculate that higher dNTP levels in mecl-21 dun1 sml1 promote more translesion synthesis and thus reduce recombiniticogenic lesions.

CONCLUSIONS

mecl-21 hyper-recombination phenotype correlates with low dNTP levels and can be suppressed by SML1 mutations. We suggest that higher levels of dNTPs reduce recombiniticogenic lesions by promoting either replication fork progression or translesion synthesis. This is the first study to show that higher rates of recombination in a mutant deficient in wild-type dNTP levels can be suppressed by increasing dNTP concentrations.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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