Supplemental Data:
Involvement of deoxycytidylate deaminase in the response to S_n1-type methylation DNA damage in budding yeast

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Supplemental Experimental Procedures

Media and growth conditions

All media were prepared as described [S1] except that synthetic media contained increased leucine (60 mg/liter). Growth and sporulation were at 30°C. Sporulation of diploid cells and tetrad dissections were performed as described [S2].

Strain constructions

All yeast strains are derivatives of a *RAD5 CAN1* W303-1B unless otherwise noted (see Table 1, [S3,S4]. W303 derivatives (*pms1Δ* and *rad52Δ*) were described previously [S2,S5].

MGT1 and *DCD1* disruption was constructed in haploid strains using a PCR-based gene disruption method [S6] utilizing *TRP1* as a selective marker and the following primers, with uppercase letter sequences complementary to *MGT1 or DCD1* sequences and lowercase denoting selective marker sequences: *MGT1*-H1 (5'-AAAAAAATTG AAAACGGTCG CATTTTTGAT CTAAATGGACCAACG gagcagattgtactgag -3'), *MGT1*-H2 (5'-ACATAACTATTTCTTATGTTTATTTTCCT AAAATCCTTTATCCAAtgtgcggtatttcacacc -3') and *DCD1*-H1 (5'-GCACATTAAA GGTGTGAATG CTACACTAAT CAATATAACA CGATTgagcagattgtactgag -3'), *DCD1*-H2 (5'-TTATATATATGTTACATTAGTTCGAACTGAAAAGGAACTTATGAA tgtgcggtatttcacacc -3'). Each disruption was confirmed by PCR.

MNNG sensitivity by cytotoxicity and "spotting" assays

Sensitivity to a pulse of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG; Sigma Chemical Co., St. Louis, MO) was determined by exposing exponentially growing

yeast cells to varying amounts of MNNG for 45 minutes followed by plating of appropriate dilutions on YPD. Surviving colonies were counted after 3 days of incubation at 30°C. For all strains, a plot of ln(survival %) vs. dose yielded a straight line and an LD_{50} was calculated as $-\ln(2)/\alpha$, where α is the slope of the straight line.

For spotting assays, relevant yeast strains were grown overnight to saturation in YPD, serially diluted (1:5) and spotted with a 48-prong replicator onto synthetic complete medium (CSM) and CSM containing 0.35 μM MNNG plates and grown at 30°C for 2 days.

MNNG-resistant colonies were then screened by replica plating onto CSM containing 0.19μM MNNG.

Measurement of dNTP pools

Analysis of dNTP pools was measured in duplicate on each of 3 separate extractions of 100 mls each of mid-log phase yeast cultures grown in YPD medium (between 2 and 4 x 10^9 cells). The dNTPs were extracted from the cultures as described Muller [S7]. The dNTP levels were measured in duplicate from diluted extracts using the protocol described previously [S8]. Each value represents the average, +/– standard deviation, derived from duplicate analyses of three different extracts.

Supplemental References

S1. Sherman, F. (1991). Getting started with yeast, Volume 194 (Academic press Inc.).

S2. Erdeniz, N., Dudley, S., Gealy, R., Jinks-Robertson, S., and Liskay, R.M. (2005). Novel *PMS1* alleles preferentially affect the repair of primer strand loops during DNA replication. Mol. Cell. Biol. *25*, 9221-9231.

S3. Thomas, B.J., and Rothstein, R. (1989). Elevated recombination rates in transcriptionally active DNA. Cell *56*, 619-630.

S4. Zou, H., and Rothstein, R. (1997). Holliday junctions accumulate in replication mutants via a RecA homolog-independent mechanism. Cell *90*, 87-96.

S5. Smith, J., and Rothstein, R. (1999). An allele of *RFA1* suppresses *RAD52*-dependent double-strand break repair in *Saccharomyces cerevisiae*. Genetics *151*, 447-458.

S6. Baudin, A., Ozier-Kalogeropoulos, O., Denouel, A., Lacroute, F., and Cullin, C. (1993). A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*. Nucleic Acids Res. *21*, 3329-3330.
S7. Muller, E.G. (1994). Deoxyribonucleotides are maintained at normal levels in a yeast thioredoxin mutant defective in DNA synthesis. J. Biol. Chem. *269*, 24466-24471.
S8. Sherman, P.A., and Fyfe, J.A. (1989). Enzymatic assay for deoxyribonucleoside triphosphates using synthetic oligonucleotides as template primers. Anal. Biochem. *180*, 222-226.

Table S1. Strains used in this study.

Strain	**Genotype**	**Source**
W1558-4A	*MATα ade2-1 CAN1 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5*	[S4]
NEY915	W1558-4A *mgt1::TRP1*	This study
NEY978	W1588-4A *mgt1::TRP1 rad52::HIS5*	This study
NEY984	W1588-4A *mgt1::TRP1 rad52::HIS5 pms1::LEU2*	This study
RML1	W1588-4A *mgt1::TRP1 rad52::HIS5 dcd1-S178F*	This study
NEY1373	W1588-4A *mgt1::TRP1 rad52::HIS5 dcd1::TRP1*	This study
NEY1450	W1588-4A *mgt1::TRP1 rad52::HIS5 pms1::LEU2 dcd1::TRP1*	This study
W1558-4C	*MAT*a *ade2-1 CAN1 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5*	[S4]
NEY929	W1588-4C *mgt1::TRP1 rad52::HIS5 mlh1::LEU2*	This study
NEY981	W1588-4C *mgt1::TRP1 rad52::HIS5 pms1::LEU2*	This study
NEY967	W1588-4C *mgt1::TRP1 rad52::HIS5 msh2::LEU2*	This study
NEY975	W1588-4C *mgt1::TRP1 rad52::HIS5 msh6::TRP1*	This study

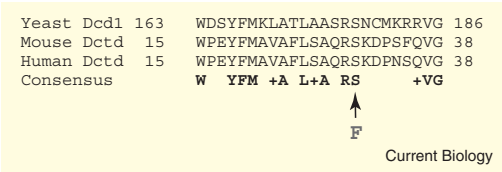

Figure S1. Alignment of yeast Dcd1 and mammalian Dctd proteins.

The 'mutated' S178 residue is shown below the consensus sequence. Numbers correspond to the amino acid position in the protein.

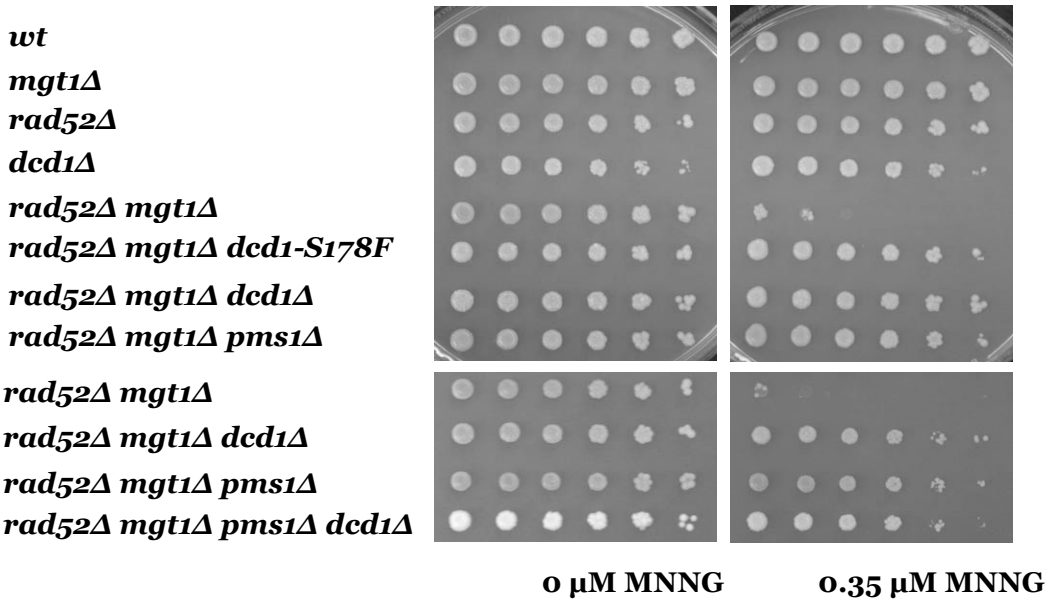

Figure S2. Comparison of MNNG responses for wild type, *mgt1Δ*, *rad52Δ*, *pms1, dcd1Δ* and *dcd1-S178F* mutations in various combinations, as measured by a fivefold serial dilution spot tests on CSM and CSM + 0.35 μM MNNG plates, as described in the Experimental Procedures.