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

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In addition to spellchecking during DNA replication and modulating recombination, DNA mismatch repair (MMR) promotes cytotoxic responses to certain DNA-damaging agents [1]. In mammalian cells, the best-studied response is to S_n1-type methylating agents, including N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) [1]. Notably, MMR-deficient mammalian cells are resistant to the cytotoxic effects of these agents. A recent report showed that MMR deficiency conferred resistance to MNNG in yeast cells crippled for both homologous recombination (*rad52*Δ) and the detoxifying enzyme methylguanine methyltransferase (*mgt1*Δ) [2]. To better understand the response, we searched for additional genes modulating sensitivity to MNNG in *rad52*Δ *mgt1*Δ budding yeast. In addition to alleles of known MMR genes, we isolated an allele of *DCD1* encoding the enzyme deoxycytidylate deaminase, which influences the dCTP:dTTP nucleotide pool ratio by catalyzing the conversion of dCMP to dUMP [3]. Models of the MMR-dependent cytotoxic response to S_n1-type methylating agents have included the incorporation of dTTP opposite O^6-methyl guanine ($O^{6met}G$) in the template [1]. Our findings lend further support to this aspect of the MMR-dependent response and highlight a mechanism for 'methylation' resistance that may be of therapeutic relevance for human cancer.

To better understand the response of budding yeast to DNA methylation damage, we mutagenized a *rad52*Δ *mgt1*Δ strain to ~33% survival with ethyl methanesulfonate, and screened for mutants resistant to MNNG. After screening ~10,000 colonies, 18 colonies repeatedly tested resistant. In appropriate crosses, one-half of the *rad52*Δ *mgt1*Δ segregants were MNNG resistant, suggesting that a single gene mutation was responsible for the resistance trait and that the mutation was unlinked to either *RAD52* or *MGT1* (data not shown). Crosses to a *rad52*Δ *mgt1*Δ strain produced diploids that were each sensitive to MNNG, indicating that all 18 $MNNG^r$ mutations were recessive. Next, we performed complementation tests amongst the mutant collection and with MMR genes that, when mutated, have been found to confer resistance to MNNG, i.e. *mlh1*Δ, *msh2*Δ, *pms1*Δ, *msh6*Δ ([2] and our unpublished data). Not surprisingly, complementation tests suggested that we had isolated multiple alleles of *MLH1* (6), *MSH2* (2), *PMS1* (3) *and MSH6* (6). However, one recessive mutation defined a separate complementation group, initially designated *drm1-1* (damage response to methylation).

To identify, by complementation, the gene associated with the MNNG resistance, we transformed the *drm1-1* strain with a centromere-based yeast genomic library. Among ~20,000 transformants screened for MNNG sensitivity, two complemented colonies were identified and the library clones isolated. Sequencing revealed that these clones harbored identical genomic inserts containing seven potential open reading frames, including the *DCD1* gene. We sequenced the *DCD1* gene in the MNNG-resistant (*rad52*Δ *mgt1*Δ) strain and detected a

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mutation (G to T) predicted to cause a serine to phenylalanine change at residue 178, a residue conserved in the human deoxycytidylate deaminase gene *Dctd1* (Figure S1 in Supplemental Data).

To further substantiate that the S178F change in Dcd1 was responsible for MNNG resistance in the *drm1-1* strain, we introduced the *dcd1-S178F* mutation into the genome of the wild-type *W303* strain. Because *DCD1* is non-essential, we also constructed a *dcd1*Δ strain. The *dcd1-S178F* and *dcd1*Δ strains were each crossed with a *rad52*Δ *mgt1*Δ strain and haploid, double and triple mutant strains were generated. When compared with the *rad52*Δ *mgt1*Δ 'parental' strain, the *rad52*Δ *mgt1*Δ *dcd1-S178F* and *rad52*Δ *mgt1*Δ *dcd1*Δ strains showed an increased level of MNNG resistance that was statistically indistinguishable from both the *rad52*Δ *mgt1*Δ *pms1*Δ and *rad52*Δ *mgt1*Δ *mlh1*Δ strains (Figure 1A and Figure S2 in Supplemental Data). In addition, *dcd1* deficiency in wild-type (*RAD52 MGT1*) cells did not confer detectable resistance to MNNG. Taken together with the LD_{50} values of each strain (see Figure 1 legend), our results show that an inactivating mutation of *DCD1* in *rad52*Δ *mgt1*Δ cells confers resistance to S_n1-type DNA methylation damage.

As reported previously, *DCD1* deficiency in yeast resulted in elevation of dCTP pools and a slight decrease in dTTP pools [4] and reduced the frequency of spontaneous [4] and MNNG-induced [5] G/C to A/T mutations. To address possible yeast strain background effects in our study, we measured dNTP pool levels in the most relevant strains used, as described [6]. Consistent with a previous study [4], *dcd1*Δ in our strain background also resulted in elevated levels of dCTP and slightly diminished dTTP pools (see Table 1). Notably, MMR deficiency (*mlh1*Δ) had no significant effect on dNTP pools. The MMR-dependent cytotoxic response to S_n1-type methylating agents generally is considered to be dependent on the formation of $O^{6met}G$/T mispairs during replication [1]. Because of the increased dCTP: dTTP ratio due to *dcd1*Δ, the likelihood of incorporation of T opposite $O^{6met}G$ by polymerase is predicted to be greatly diminished. Therefore, we reasoned that MNNG treatment of *dcd1*Δ cells resulted in fewer $O^{6met}G$/T mispairs, less involvement by MMR, and cytotoxic resistance/tolerance, assuming that *DCD1* and MMR act in the same methylation damage response pathway. To test this notion, we compared the MNNG responses of *rad52*Δ *mgt1*Δ *dcd1*Δ and *rad52*Δ *mgt1*Δ *pms1*Δ *dcd1*Δ strains: the quadruple mutant appeared no more resistant to MNNG than the triple mutant (Figure 1A,B; see legend for LD_{50} values). These results support the idea that in yeast the formation of $O^{6met}G$/T mispairs during replication is required for the observed MMR-dependent cytotoxicity of MNNG. Shown in Figure 1B is a schematic incorporating the *DCD1*-related findings reported here and those from a previous report [2].

The MMR-dependent response to S_n1-type methylating agents most likely involves additional proteins besides those normally associated with MMR-dependent spellchecking (for a review, see [1]). Our study has uncovered one such protein, DCD1, which modulates dCTP:dTTP pool levels and therefore influences sensitivity to agents that induce formation of $O^{6met}G$. Several studies with cultured rodent cells suggested that Dctd deficiency increased the dCTP:dTTP pool ratio [7-9]. However, we are not aware of any isogenic pairs of proficient/deficient cell lines to test rigorously the role of Dctd in the response to methylation damage. Of interest, however, Meuth [7] showed that elevated dTTP pools increased sensitivity to MNNG in Chinese hamster ovary cells and speculated that misincorporation of thymine opposite $O^{6met}G$ was the basis for the observed toxicity.

Finally, our findings may also have relevance to cancer chemotherapy. For example, reduced DCTD levels in a tumor might compromise the clinical response to the S_n1-type methylation agent temozolomide or the purine analog 6-mercatopurine, used in the treatment of glioblastoma multiforme [10] and certain hematological malignancies [11], respectively.

Supplemental data

Refer to Web version on PubMed Central for supplementary material.

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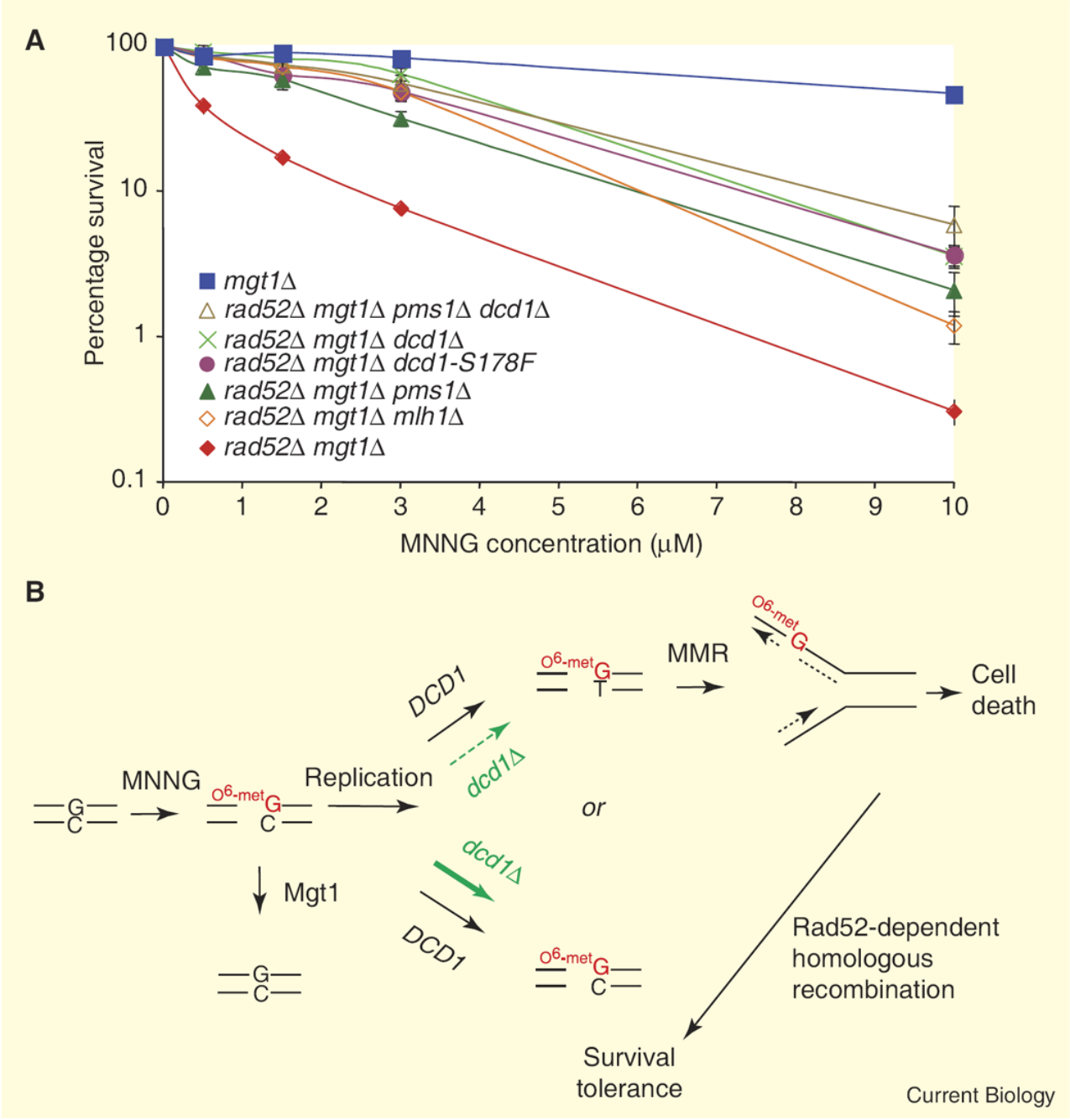

Figure 1.

(A) MNNG survival curves: strains were exposed for 45 min to increasing amounts of MNNG. Error bars represent standard error of the mean. The LD_{50} values for *mgt1*Δ, *rad52*Δ *mgt1*Δ *dcd1*Δ *pms1*Δ, *rad52*Δ *mgt1*Δ *dcd1*Δ, *rad52*Δ *mgt1*Δ *dcd1-S178F*, *rad52*Δ *mgt1*Δ *pms1*Δ, *rad52*Δ *mgt1*Δ *mlh1*Δ and *rad52*Δ *mgt1*Δ strains were 9.7 μM, 2.4 μM, 2.2 μM, 2.1 μM, 1.8 μM, 1.6 μM and 0.25 μM MNNG, respectively. (B) A model for the cellular response to MNNG treatment in budding yeast. The scheme is modified from a previous report [2] to include the role of *DCD1*, which, when absent, results in reduced $O^{6met}G/T$ mispair formation following replication, reduced MMR-dependent processing and resistance to cell death. With the exception of the 'heavy' and 'dashed' arrows signifying consequences in the *dcd1*Δ strain, the

model applies to wild-type yeast. Further, following replication, remaining $O^{6met}G/C$ residues will either be detoxified by the methyltransferase or diluted out by subsequent cell cycles. Although as shown the model infers MMR-dependent ‘futile repair’, ‘direct signaling’ must be considered (for reviews, see [1,12]). Support for the futile cycling model comes from several studies including *in vitro* results showing reiterative MMR excision cycles on $O^{6met}G/T$ mismatch-containing plasmid substrates [13]. Support for direct signaling comes from a ‘separation-of-function’ allele of mouse Msh6 that compromises spellchecking without affecting the DNA-damage response [14] and from studies showing that MutSα–MutLα complexes are required for ATR–ATRIP signaling from methylation damage sites [15].

Table 1

Measurements of deoxynucleotide pools.

Strain	dNTPs, pmol/10^6 cells			
	dATP	dTTP	dCTP	dGTP
*rad52*Δ *mgt1*Δ	1.33 ± 0.23	4.39 ± 0.96	2.02 ± 0.22	0.87 ± 0.22
*rad52*Δ *mgt1*Δ *mlh1*Δ	1.74 ± 0.24	4.92 ± 0.88	2.84 ± 0.16	1.13 ± 0.14
*rad52*Δ *mgt1*Δ *dcd1*Δ	1.68 ± 0.36	2.14 ± 0.30	7.49 ± 0.59	0.73 ± 0.17

Using mid-log phase yeast cultures grown in rich medium, dNTP pools were determined in duplicate on each of three separate extractions.