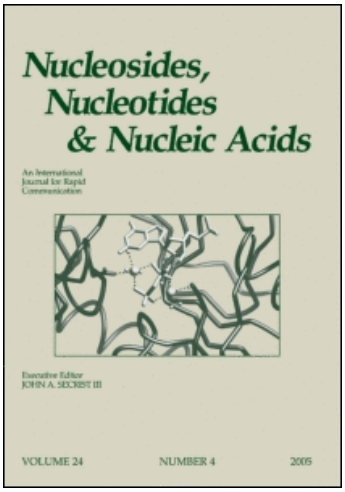

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The Role of Glycosylases in Mismatch Repair: The Use of 7-Deazapurines as Repair-Resistant Lesions in Oligonucl, Eotide-Directed Mutagenesis

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THE ROLE OF GLYCOSYLASES IN MISMATCH REPAIR: THE USE OF 7-DEAZAPURINES AS REPAIR-RESISTANT LESIONS IN OLIGONUCLEOTIDE-DIRECTED MUTAGENESIS.

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Summary: The mechanism of mismatch repair from phage DNA was studied using the techniques of oligonucleotide-directed mutagenesis. 7-Deaza-analogues of deoxy-adenosine and deoxy-inosine were found to be excised with impaired repair efficiency following transfection into E.coli JM101.

Despite the wealth of information available describing the great variety of factors involved in DNA repair, relatively little is known about the mechanisms of mismatch repair. Although a number of repair-deficient cell lines has been characterised, no enzyme responsible solely for the removal of mispaired nucleotides has, to our knowledge, been discovered to date. The aim of our work is to elucidate the individual factors involved in mismatch repair, using the techniques of chemical synthesis and molecular biology. In this report we describe our attempts to establish the role played by glycosylases in the repair of misincorporated nucleotides from duplex viral DNA, by constructing mismatches consisting of a pyrimidine in the parent strand and a non-complementary purine in the daughter strand.

2'-Deoxy-7-deazapurines differ from their parent compounds in one important aspect: their glycosidic bonds are highly resistant to acidic hydrolysis. It seems reasonable to expect that these bonds should also be resistant to enzymatic hydrolysis; their repair from a mismatch would thus be expected to be inefficient, providing that a glycosylase enzyme was involved in any part of the repair pathway.

The system used to screen the repair of the above mismatches consisted of the filamentous bacteriophage M13mp9, which served as a template for DNA polymerase I in primer extension reactions using the mismatch-carrying oligonucleotides as primers during in vitro synthesis of

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                  Pro Ser Leu Ala Ala Gly Arg Arg Ile Pro Gly
M13 mp9    5' CCA AGC TTG GCT GCA GGT CGA CGG ATC CCC GGG  3'
                                      ⇅
M13 op7       --- --- --- --- --- --- TGA --- --- --- ---

Oligonucleotides:    3'   A CGT CCA CCT GCC TAG      5'
                          - --- --- A-- --- ---
                          - --- --- I-- --- ---
                          - --- ---DI-- --- ---
                          - --- ---Tu-- --- ---
```

Figure 1: The target site of M13mp9 and the oligonucleotide sequences used in the site-directed mutagenesis experiments described below. The abbreviations I, DI and Tu stand for deoxyinosine, deoxydeazainosine, and deoxytubercidin. Oligonucleotides containing A and Tu were used as primers on the wild type M13mp9 template, while the G, I and DI carrying oligomers were used on the opal mutant template.

the fully double-circular replicative form of this phage. The selected site of mutation was an arginine 7 (CGA) codon of the polylinker sequence of M13mp9. A series of oligodeoxyribonucleotides 16 bases long was synthesized by the phosphotriester approach. When these oligomers were used as primers for the polymerase reactions, they annealed to the template in such a way as to place the central purine residue opposite a non- complementary pyrimidine. The sequences of the target site and the oligonucleotides are shown in Figure 1. The section of the M13 sequence which is of interest codes for a so-called α-peptide, which is capable of complementing with the truncated product of the β-galactosidase gene of E.coli JM101. Bacteria infected with this phage exhibit therefore a $\beta\text{-gal}^+$ phenotype. A transition mutation of the cytidine residue within the arg 7 codon generates an opal TGA nonsense codon, which stops protein synthesis at this point. Infection with the opal mutant results thus in a $\beta\text{-gal}^-$ phenotype. The repair efficiency can thus be monitored by counting the numbers of the blue and the colourless plaques following transfection and plating out on indicator plates containing a substrate for β-galactosidase.

The results of our experiments are summarised in Table 1. Deoxyinosine, which is known to be excised by the action of hypoxanthine glycosylase, was found to be removed with similar efficiency to deoxyguanosine from a I/T or a G/T mismatch. By contrast, deoxydeazainosine was removed more than 10-fold less efficiently.

Table 1: The efficiency of repair of purine/pyrimidine mismatches from the replicative form of the filamentous bacteriophage M13mp9, following transfection into E.coli JM101.

MISMATCH + strand/ - strand	(-) STRAND PHENOTYPE	%MUTANTS	TOTAL NUMBER OF PLAQUES
T/G	blue	6	2937
T/I	blue	3	4850
T/DI	blue	41	3616
C/A	white	3	3278
C/Tu	white	21	4468

Similarly, the repair of deoxyadenosine and deoxytubercidin differ also by an order of magnitude in favour of the deaza analogue.

As the presence of the 7-deazapurines in the oligonucleotides was not found to inhibit either P1 endonuclease, or snake venom phosphodiesterase, the differences in the rates of repair of these lesions from duplex DNA can only be explained by their resistance to the action of N-glycosylases.

Although DNA repair is known to be accomplished in general by a number of separate factors, our results indicate that mismatch repair, at least in the case of purine/pyrimidine mismatches, is to a great extent performed by a DNA N-glycosylase.