Characterization of the ungI mutation of *Escherichia coli*

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Uracil DNA glycosylase (ung) excises uracil residues from DNA which arise as a result of either deamination of cytosine or misincorporation of dUMP residues by DNA polymerase. Ung mutants of *E. coli* e.g. BW310 (1) have a high rate of GC→AT mutations. An *E. coli* ung strain also deficient in dUTPase (dut), RZ1032 (2) can have as much as 14% uracil substituted for thymine and is used in site directed mutagenesis (3) where a deaminated cytosine residue is to be preserved or the uracil containing DNA template is to be destroyed (1,2,3). Since the complete nucleotide sequence of ung (w.t.) has recently been described (4), it was of interest to define the mutations in the ung gene of these strains and explain the molecular mechanisms that render them ung.

Approximately 1.0 μg of chromosomal DNA from different strains of *E. coli* were mixed with 0.1 n mole of 5'- sense (GCACCTAAAGGTACCCGATTG) and 3'- antisense (TGCCATGCAGATTCCCTCCC) primers in 100μl in the presence of 50mM KCl, 10mM Tris (pH 8.3), 1.5mM MgCl₂, 200 μM dNTPs, 2 units Taq DNA polymerase and subjected to 23 cycles of incubations as follows: 1 min. at 92°C, 2 min at 37°C and 4 min at 65°C(5). Following this polymerase chain reaction, agarose gel electrophoresis of 2.0μl aliquots showed amplification of a 0.75 kbp fragment encompassing the whole ung gene (Fig. 1, marked with an arrow head). This fragment was gel purified and subjected to sequence analysis from both ends using the above mentioned primers and AMV reverse transcriptase. The sequence of the ung gene from strain HB101 (ung) was identical to the wild type ung sequence (4). Sequences of the ung genes form RZ1032 and BW310 were identical to each other as they both possessed the same allele (ungI) (1,3) but showed a single base (G) (marked with an arrow, Fig. 2) deletion in the 43rd codon when compared to the wild type sequence. This deletion leads to a shift in the reading frame and a premature termination 16 amino acids downstream from it (Fig. 2). The reading frame of the wild type gene codes for a protein of 229 amino acids before encountering its natural termination codon (4).

REFERENCES