Zeocin resistance suppresses mutation in hypermutable *Escherichia coli*

The ability to introduce random mutations into genes cloned in bacterial vectors is particularly useful for bioengineering and molecular biological studies. The increasing importance of hypermutable bacteria for these purposes is emphasized by the commercial availability of the *E. coli* strain XL1-Red (Stratagene), which contains mutS, mutT and mutD mutations. Defects in mutS confer a hypermutable phenotype because this gene is part of the methyl directed mismatch repair (MMR) system, a post-replicative DNA repair pathway that identifies and corrects mismatched DNA duplexes (LeClerc et al., 1996). MutT mutants strains are unable to hydrolyse 8-oxodGTP and display an increase in transversions (Fowlera & Schaaper, 1997). Defects in mutD also contribute to the hypermutability of strain XL1-Red because the frequency of transitions and transversions is enhanced by the loss of the DNA polymerase proofreading function (Selifonova et al., 2001).

During preliminary experiments designed to select mutant SHV-1 β-lactamases with altered substrate specificities, we constructed a recombinant, designated pJMBlle+, which contained the gene for SHV-1 (Mercier & Levesque, 1990) in the common cloning vector pCR-Blunt (Invitrogen). Introduction of pJMBlle+ into *E. coli* hypermutators in regular use in our laboratory (Miller et al., 2002) and strain XL1-Red appeared to suppress mutation rates for β-lactam resistance. Plasmid pCR-Blunt and several other commercially available cloning and expression vectors (Invitrogen) contain the Zeocin-resistance gene ShBle. Expression of ShBle in prokaryotic and eukaryotic hosts confers resistance to the broad-spectrum antibiotic Zeocin. To investigate a possible role for the ShBle gene product in the suppression of mutation frequencies in this system, we excised the ShBle gene from pJMBlle+ using the restriction enzyme PmlI (New England Biolabs), resulting in the plasmid pJMBlle−. We then compared the effects of pJMBlle+ and pJMBlle− on endogenous antibiotic resistance mutation frequencies in a number of *E. coli* mutator strains, including XL1-Red (Table 1).

The minimum inhibitory concentration (MIC) of rifampicin (Sigma-Aldrich), a commonly used mutational marker, was determined for several *E. coli* hypermutators containing single mutator alleles (e.g. wvrD, mutS, mutH) (Table 1) and for strain XL1-Red (mutD, mutT, mutS). The MIC for rifampicin for all strains was 8 μg ml⁻¹. Mutation frequencies for rifampicin resistance, using selections at × 4 MIC, were determined as described previously (Miller et al., 2002). Hypermutator strains containing constructs expressing the ShBle gene showed 10- to 100-fold reductions in mutation frequencies for rifampicin resistance (Table 1). The presence of plasmid pJMBlle− had no influence on mutation frequencies, with rates comparable to those observed in the untransformed controls (Table 1). It therefore appears that ShBle is responsible for the decreased mutation frequencies observed in hypermutators. Interestingly, expression of ShBle did not suppress rifampicin-resistance frequencies in the non-mutator strain 1411 (Table 1).

The mechanism of action of Zeocin is unknown, but may be similar to that of the structurally related group of bleomycin/phleomycin antibiotics (Berdy, 1980). The bleomycin–Fe(II) complex, in conjunction with reducing agent and oxygen, causes nucleotide-specific DNA cleavage. The ShBle gene, isolated from *Streptoalloteichus hindustanus* (Gatignol et al., 1988), encodes a protein that binds stoichiometrically to bleomycin, inhibiting its DNA strand cleavage activity (Dumas et al., 1994).

In transposon Tn5, the bleomycin-resistance phenotype encoded by the ble gene is associated with improved host fitness, even in the absence of the drug (Blot et al., 1991). Improved fitness may be mediated by enhancement of the DNA repair system, although the exact role of ble has not been determined. Nevertheless, bleomycin resistance mediated by ble appears to be independent of its ability to confer a fitness advantage on the host bacterium (Kumagai et al., 1999). The ShBle and Ble proteins share 25 % amino acid identity. Both proteins are acidic and exhibit low isoelectric points and low molecular masses. These features suggest that their structural genes are derived from a common ancestor (Kumagai et al., 1999). Therefore, the ShBle protein may enhance DNA repair via the same mechanism as Ble. This enhancement could be sufficient to compensate for the defective mismatch repair and proofreading activities in the hypermutators that lack one or more of these error-correcting systems. However, in cells with a fully proficient set of DNA repair genes no further enhancement of repair is conferred by the ShBle protein.

Mutation analysis is an invaluable tool to investigate relationships between genotype and phenotype. The introduction of

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**Chris Thomas, Editor-in-Chief**
random mutations into cloned genes by hypermutable hosts is an easy and efficient approach to investigate this relationship. However, we would advise close monitoring of mutation frequencies to avoid inadvertent suppression of mutation by markers that may be present in cloning vectors to assist selection of recombinants. This will also apply to in vivo mutagenesis with alkylating agents since the Ble protein interferes with the mutagenic potential of such compounds (Blot et al., 1991).

**Table 1. Mutation frequencies for rifampicin resistance in** *E. coli* **Strain Genotype Reference/source**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Mutation frequency*</th>
</tr>
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<tbody>
<tr>
<td>pJMB2</td>
<td>lacI3 lacZ18 proB trp nalA rpsL</td>
<td>Miller et al. (2002) 1.7 ± (±0.39) x 10−5</td>
</tr>
<tr>
<td></td>
<td>lacI3 lacZ18 proB trp nalA rpsL</td>
<td>Miller (2003) 2.37 ± (±0.49) x 10−6</td>
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<tr>
<td></td>
<td>lacI3 lacZ18 proB trp nalA rpsL</td>
<td>Miller et al. (2003) 2.41 ± (±0.85) x 10−6</td>
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<tr>
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<td>lacI3 lacZ18 proB trp nalA rpsL</td>
<td>Miller et al. (2003) 6.42 ± (±0.30) x 10−6</td>
</tr>
<tr>
<td></td>
<td>lacI3 lacZ18 proB trp nalA rpsL</td>
<td>Miller et al. (2003) 3.18 ± (±0.67) x 10−6</td>
</tr>
<tr>
<td></td>
<td>lacI3 lacZ18 proB trp nalA rpsL</td>
<td>Miller et al. (2003) 4.86 ± (±0.71) x 10−6</td>
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**References:**


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