Spontaneous mutagenesis in exponentially growing and stationary-phase, *umuDC*-proficient and -deficient, *Escherichia coli dnaQ49*

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Spontaneous mutations occur not only in exponentially growing bacteria but also in non-dividing or slowly dividing stationary-phase cells. In the latter case mutations are called adaptive or stationary-phase mutations. High spontaneous mutability has been observed in temperature sensitive *Escherichia coli dnaQ49* strain deficient in 3′→5′ proofreading activity assured by the ε subunit of the main replicative polymerase, Pol III. The aim of this study was to evaluate the effects of the *dnaQ49* mutation and deletion of the *umuDC* operon encoding polymerase V (Pol V) on spontaneous mutagenesis in growing and stationary-phase *E. coli* cells. Using the *argE3* revertion system in the AB1157 strain, we found that the level of growth-dependent and stationary-phase Arg+ revertants was significantly increased in the *dnaQ49* mutant at the non-permissive temperature of 37°C. At this temperature, in contrast to cultures grown at 28°C, SOS functions were dramatically increased. Deletion of the *umuDC* operon in the *dnaQ49* strain led to a 10-fold decrease in the level of Arg+ revertants in cultures grown at 37°C and only to a 2-fold decrease in cultures grown at 28°C. Furthermore, in stationary-phase cultures Pol V influenced spontaneous mutagenesis to a much lesser extent than in growing cultures. Our results indicate that the level of Pol III desintegration, dependent on the temperature of incubation, is more critical for spontaneous mutagenesis in stationary-phase *dnaQ49* cells than the presence or absence of Pol V.

Spontaneous mutations occur in exponentially growing bacteria, but also in non-growing or slowly growing cells as a result of exposure to non-lethal selection. The latter mutations, called adaptive, starvation-associated or stationary-phase mutations, differ from...
spontaneous growth-dependent mutations, which appear in the genome randomly before exposure to selection (Cairns et al., 1988; Cairns & Foster, 1991; Hall, 1990; Foster, 1993; Foster, 1999; Bull et al., 2000).

It has been found that mutations in the dnaE gene encoding the α polymerizing subunit of Escherichia coli DNA polymerase III (Pol III), the enzyme responsible for duplication of the bacterial chromosome, lead to changes in spontaneous mutagenesis in replicating bacteria. It has also been shown that mutations in dnaE modify the level of spontaneous mutations in stationary-phase cultures. Foster and coworkers (1995) have shown that dnaE915, an antimutator allele of dnaE, causes a 3-fold decrease in adaptive Lac+ reversion in the FC40 strain. Results obtained by others also support a direct role of the α subunit in adaptive mutagenesis (Harris et al., 1997).

The polymerase activity and processivity of the α subunit are highly stimulated by the ε subunit (and vice versa). The ε subunit, the product of the dnaQ gene, contains a 3’→5’ exonuclease that functions in proofreading of replication errors but also plays an important structural role within the Pol III core. In dnaQ<sup>TS</sup> mutants (such as dnaQ49 investigated in this work), at temperatures above 30°C, the ability of ε to interact physically with the α subunit is disrupted which leads to instability of the replication complex (Takano et al., 1986; Jonczyk et al., 1998; Taft-Benz & Schaaper, 1998). In dnaQ49 bacteria replicating at a non-permissive temperature damage to DNA induces the SOS response that in turn switches on several DNA repair error-free and error-prone pathways (Friedberg et al., 1995). In E. coli the SOS response involves over 40 unlinked genes (Courcelle et al., 2001; Crowley & Courcelle, 2002) creating the SOS regulon. Expression of these genes is coordinately regulated by the LexA and RecA proteins. Generation of single stranded DNA (ssDNA) fragments by failed attempts to replicate past lesions leads to the formation of RecA-ssDNA filaments that facilitate proteolytic self-cleavage of LexA. Autodigestion of LexA, that acts as a transcriptional repressor, leads to expression of LexA-regulated genes. Among them are the umuDC genes encoding DNA polymerase V which participates in translesion DNA synthesis (TSL) (Walker, 1998; Woodgate, 1999; Friedberg & Gerlach, 1999; Bridges, 1999; Janion, 2001). Replication by Pol V across DNA damage comes at the cost of reduced fidelity leading to formation of mutations even during replication of an undamaged template. Therefore, to limit mutations, expression of the umuDC operon is tightly regulated and proceeds at a late stage during the SOS response.

Another member of the SOS regulon, the sfiA (sulA) gene, is also induced as one of the latest (Kuzminov, 1999). The gene encodes a protein that inhibits cell division which subsequently leads to filamentous growth of cells. Thus, the massive appearance of filaments proves sfiA expression, and may indicate the induction of the SOS response (Janion et al., 2002).

The aim of this work was to test the effect of the dnaQ49 mutation in the ε subunit of Pol III, and of the deletion of the umuDC operon on spontaneous mutagenesis in exponentially growing as well as stationary-phase bacteria. We examined the frequencies and specificity of spontaneous mutations in E. coli AB1157 strain bearing an ochre mutation in chromosomal argE3 gene. Only argE3→Arg<sup>+</sup> revertants showed the ability to grow into colonies on minimal plates without arginine. In comparison to a dnaQ<sup>+</sup> strain, AB1157<sup>dnaQ49</sup> growing at the non-permissive temperature of 37°C exhibited a high rate of spontaneous Arg<sup>+</sup> revertants accompanied by SOS induction. The same strain additionally deprived of Pol V (AB1157<sup>dnaQ49ΔumuDC</sup>) showed a 10-fold decrease in the level of growth-dependent Arg<sup>+</sup> revertants, while the level of stationary-phase revertants decreased only twice. The above observation, as well as the differences in the SOS induction levels and in
specificities of the Arg+ revertants in growing and stationary-phase cultures, indicate distinct mutagenic pathways in growing and resting bacterial cells.

**MATERIALS AND METHODS**

**Bacterial strains.** The bacteria used in this study were *E. coli* K12 strain AB1157 (genotype: F- thr-1 leuB6 proA2 his4 thi1 argE3 lacY1 galK2 rpsL supE44 ara-14 xyl-15 mtl-1, ttx-33 (Bachmann, 1987)) and its dnaQ49 and ΔumuDC derivatives. In the present work AB1157 dnaQ49 was obtained by P1 transduction of dnaQ49zae-502::Tn10 from the NR9695 into the AB1157 strain (Schaaper & Cornacchio, 1992), whereas AB1157 dnaQ49 ΔumuDC by transduction of ΔumuDC595::cat from RW82 into AB1157 dnaQ49 (Woodgate, 1992). All dnaQ49 strains were stored on minimal medium to limit the appearance of suppressors. For each constructed strain three independently isolated clones were tested. Experiments were repeated 4–6 times, each in duplicate, and standard deviations (S.D.) were calculated.

**Media and plates.** LB (Luria–Bertani) medium consisted of 1% Bacto-tryptone, 0.5% yeast extract, and 0.5% NaCl. E medium consisted of C-salts (Vogel & Bonner, 1956) supplemented with glucose (0.5%), casamino acids (0.2%), thiamine (10 μg/ml) and Arg, His, Thr, Pro and Leu, each at 25 μg/ml. E-Arg was the E medium deprived of arginine and solidified with 1.5% Difco Agar.

**Mutation experiments.** Mutation experiments were performed as described before (Nowosielska & Grzesiuk, 2000). Bacteria were grown overnight at 28°C or 37°C with shaking in E medium, centrifuged, suspended in fresh E medium, and grown for 3 h. Aliquots of 100 μl were plated onto LB plates to estimate the number of viable cells, and onto E-Arg plates to estimate the number of Arg+ revertants. The frequency of mutation was the number of Arg+ revertants per viable cell. Colonies on LB plates were counted after one day of incubation. Arg+ colonies required 2 days to become visible on E-Arg plates. For further incubation under conditions limiting growth, E-Arg plates were sealed with parafilm and the appearing revertants were counted daily up to the 11th day. The mutation spectra of Arg+ revertants were studied as described elsewhere (Śledziewska-Gójska et al., 1992). The suppressor activities of the Arg+ revertants were examined by testing their sensitivity to a set of amber (B17) and ochre (oc427, ps292, ps205) mutants of T4 phage. From each mutation experiment 50 colonies, of growth-dependent and station-
ary-phase Arg\(^+\) revertants, were tested. The results of phage typing revealed the kind of suppressor present and the type of mutation (Table 1).

**Microscopic observations.** The experiments were performed according to Yigit and Reznikoff (1997). Briefly, bacteria were collected by centrifugation of liquid cultures or by washing off solid medium (E-Arg plates), suspended in 0.9% NaCl, spread onto glass slides, air-dried and fixed with 100% methanol. The bacteria were then stained with acridine orange (0.15 mg/ml) in 10 mM phosphate buffer (pH 7.4) and examined under a fluorescence microscope (Nikon Microphot S.A., chroma HQ-FLP 41012 filter) with 100× objective lens. All the photographs were taken under the same conditions and at the same 1800-fold magnification.

**RESULTS**

**SOS response in AB1157\(dnaQ49\)**

In early studies on the SOS system it was shown that filamentous growth is characteristic for the induction of the SOS response (Witkin, 1967). Taking advantage of this feature, Janion and coworkers demonstrated that in starved bacteria the SOS system is induced by a cAMP-dependent process but only when cells regain their growth (Janion *et al.*, 2002). Here we observed SOS induction in *E. coli* AB1157\(dnaQ49\) (Fig. 1). Microscopic preparations were made of overnight liquid \(dnaQ49\) cultures in E medium and from the suspensions obtained by washing E-Arg plates (incubated for one, six and ten days) with 0.9% NaCl. The wild type strain showed...
no filamentation when grown either in liquid or on solid medium, thus indicating no induction of the SOS response. The dnaQ49 strain incubated at the permissive 28°C showed no filamentation when grown in liquid medium, weak filamentation after one day of incubation on E-Arg plates, and almost normal cell growth after 6 and 10 days of incubation (Fig. 1).

In contrast to the data obtained at 28°C, strong filamentation was observed in dnaQ49 liquid culture grown overnight at 37°C. Unexpectedly, in preparations of one-day culture on E-Arg plates at 37°C, filaments were rare, but appeared again in 6- and 10-day cultures. However, the degree of filamentation after 10 days of incubation appeared lower than that after 6 days (Fig. 1). In the dnaQ49ΔumuDC strain, induction of the SOS response was at a similar level as in dnaQ49 (not shown).

**Growth-dependent and stationary-phase mutations in dnaQ49 strains**

In *E. coli* AB1157 reversion to prototrophy of the argE3 locus proceeded in E minimal liquid medium with the frequency of 2.7 Arg⁺ colonies/10⁸ cells (Table 2). A similar frequency was observed in AB1157 with deletion of the *umuDC* operon. Introduction of the temperature-dependent dnaQ49 mutation into AB1157 dramatically increased the frequency of growth-dependent revertants at the non-permissive temperature of 37°C (870 × 10⁻⁸ cells vs. 2.7 × 10⁻⁸ cells in wt strain). At 28°C the frequency of Arg⁺ revertants was also elevated although to a much lesser degree in comparison to the wild type strain (57 vs. 2.7 × 10⁻⁸ cells). Additional deletion of the *umuDC* operon in dnaQ49 bacteria led to a 2-fold (28.2 × 10⁻⁸ cells) and over 10-fold (80.2 × 10⁻⁸ cells) decrease in the level of the Arg⁺ revertants at 28°C and 37°C, respectively.

When AB1157 bacteria were plated on E-Arg plates and incubated for several days, Arg⁺ colonies that arose were the result of *argE3→Arg⁺* reversion. The Arg⁺ colonies that appeared between the 4th and 11th day of incubation were regarded as stationary-phase revertants. Frequency of mutations was calculated per viable cell. The number of the cells was determined by washing E-Arg plates after cutting off the colonies of growth-dependent Arg⁺ revertants formed before the 4th day (Nowosielska & Grzesiuk, 2000) Since the number of living cells did not decrease during incubation, the initial values

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth-dependent mutations</th>
<th>Adaptive mutations</th>
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<tbody>
<tr>
<td></td>
<td>c.f.u.* at time of plating</td>
<td>Arg⁺ revertants/plate</td>
</tr>
<tr>
<td></td>
<td>×10⁸</td>
<td>Arg⁺ revertants/10⁸ cells</td>
</tr>
<tr>
<td>wt [AB1157]</td>
<td>1.68 ± 0.5</td>
<td>4.5 ± 2.0</td>
</tr>
<tr>
<td>ΔumuDC</td>
<td>2.4 ± 0.7</td>
<td>6.0 ± 2.7</td>
</tr>
<tr>
<td>dnaQ49 28°C</td>
<td>2.0 ± 0.6</td>
<td>114.0 ± 20.5</td>
</tr>
<tr>
<td>dnaQ49 37°C</td>
<td>0.3 ± 0.2</td>
<td>261.0 ± 42.5</td>
</tr>
<tr>
<td>dnaQ49ΔumuDC 28°C</td>
<td>2.0 ± 0.6</td>
<td>56.4 ± 18.0</td>
</tr>
<tr>
<td>dnaQ49ΔumuDC 37°C</td>
<td>1.2 ± 0.4</td>
<td>96.2 ± 21.0</td>
</tr>
</tbody>
</table>

*colony forming units.
were taken for calculation. In the AB1157 and AB1157ΔumuDC strains the rate of stationary-phase Arg⁺ revertants was very low (1.3 and 0.125 × 10⁻⁸ cells, respectively). The presence of the dnaQ49 allele led to an over 3-fold increase in the rate of stationary-phase Arg⁺ revertants at 28°C (4.7 × 10⁻⁸ cells) and a 30-fold increase at 37°C (39.1 × 10⁻⁸ cells) (Table 2, last column). The absence of Pol V (umuDC deletion) in the dnaQ49 strain was almost without effect on the level of Arg⁺ stationary-phase revertants incubated at 28°C (4.7 × 10⁻⁸ cells for dnaQ49 and 3.7 × 10⁻⁸ cells for dnaQ49ΔumuDC), but led to a 2-fold decrease in the rate of mutations at 37°C (39.1 × 10⁻⁸ cells for dnaQ49 and 19.3 × 10⁻⁸ cells for dnaQ49ΔumuDC).

**Specificity of Arg⁺ spontaneous revertants**

To determine the presence of suppressors in growth-dependent and stationary-phase Arg⁺ revertants, we used a previously described method (Śledziewska-Gójska et al., 1992) based upon estimation of the susceptibility of these revertants to a set of amber and ochre T4 phage mutants (see Methods for details). Two groups of Arg⁺ colonies were tested: the ones that appeared on E-Arg plates after 2 days of incubation (growth-dependent revertants), and those which arose between the 4th and the 10th day (stationary-phase revertants). The results of these tests are summarized in Table 3.

Generally, in AB1157 strains Arg⁺ growth-dependent revertants were the result of supB and supL suppressor formation due to GC→AT transitions and AT→TA transitions, respectively. Some of these reversions arose by formation of an unknown suppressor or by other unidentified pathway(s). The different specificity of the reversions observed at the permissive and the non-permissive temperature in the dnaQ49 strain is probably the result of disturbed α-ε interaction within the Pol III core at 37°C. At this temperature, in comparison to 28°C, a 2-fold decrease in GC→AT transitions, and an additional number of back mutations were observed. Interestingly, the introduction of umuDC deletion into dnaQ49 led to similar proportions of supB and supL suppressors in dnaQ49ΔumuDC and in wt strains at 37°C.

<table>
<thead>
<tr>
<th>Strain/temp. of incubation</th>
<th>Growth dependent Arg⁺ revertants (%)</th>
<th>Stationary-phase Arg⁺ revertants (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>back</td>
<td>supB</td>
</tr>
<tr>
<td>wt</td>
<td>0</td>
<td>44</td>
</tr>
<tr>
<td>dnaQ49 28°C</td>
<td>0</td>
<td>53</td>
</tr>
<tr>
<td>dnaQ49 37°C</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>dnaQ49ΔumuDC 28°C</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>dnaQ49ΔumuDC 37°C</td>
<td>0</td>
<td>45</td>
</tr>
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</table>

*Date are means of several experiments (for details see Materials and Methods).
DISCUSSION

In this work we investigated spontaneous mutagenesis in exponentially growing and in growth limited *E. coli* strains deficient in the 3′→5′ proofreading activity of Pol III, and additionally proficient or deficient in the SOS-inducible repair polymerase Pol V. The mutagenic pathways were tested in the AB1157 strain with the chromosomal argE3 ochre mutation. Reversion of the argE3 mutation to the Arg+ phenotype enabled growth on minimal medium deprived of arginine. The Arg+ revertants could arise by back mutations at the argE3 ochre site or by suppressor formation.

Spontaneous mutagenesis in exponentially growing AB1157dnaQ49

Errors that occur during normal DNA replication can be a major source of spontaneous mutations in bacteria. In *E. coli* high fidelity of replication is ensured by the α and ε subunits of Pol III. The dnaE-encoded α subunit matches the nucleotides in the newly synthesized DNA strand to the template DNA. The dnaQ-encoded ε subunit shows 3′→5′ exonuclease activity that removes the incorrect nucleotide from the growing end of nascent DNA. This feature of ε is disturbed in the dnaQ49 strain. The mutator phenotype (a high level of spontaneous mutations) is fully expressed at the non-permissive temperature of 37°C and to a much lesser degree at the permissive 28°C. In the dnaQ49 strain mutations can arise as a result of: (i) lack of 3′→5′ exonuclease function; (ii) induction of the SOS-directed error-prone Pol V, and (iii) disturbed ε−α interaction within Pol III at a non-permissive temperature, enabling a better access of SOS-inducible polymerases to the newly synthesized fragment of DNA. In dnaQ49 growing at 37°C the high level of growth-dependent Arg+ revertants (870 vs. 2.7 in wt strain) results from the loss of the proofreading activity of the ε subunit and probably saturation of the mismatch repair system (MMR) (Mellon et al., 1996; Schaaper, 1993), as well as from the disturbance in the α−ε interaction within the Pol III core, which leads to Pol III destabilization. Filamentous growth of dnaQ49 in liquid medium at 37°C indicates strong induction of the SOS response. Elimination of *umuDC* from the dnaQ49 strain led to a 10-fold decrease in the number of growth-dependent Arg+ revertants. These results indicate that a large fraction of the growth-dependent mutations in the dnaQ49 strain at 37°C is due to spontaneous mutagenesis involving the SOS-induced repair polymerase Pol V (UmuD’2C proteins). On the other hand, since in dnaQ49ΔumuDC we still observed an elevated level of Arg+ growth-dependent revertants, and the same level of SOS induction as in dnaQ49, we assume that, in the absence of Pol V, defective polymerases III as well as pol IV or pol II may contribute to DNA mutagenesis (Nowosielska et al., 2004).

Spontaneous mutagenesis in stationary-phase AB1157dnaQ49

In spite of extensive studies on the phenomenon of adaptive mutagenesis, the mechanism of spontaneous mutation formation in the absence of DNA replication is still not clear. Studies in the FC40 system have indicated that in adaptive mutagenesis such processes as recombination (Harris et al., 1997), hypermutability (Rosche & Foster, 1999), and amplification (Slechta et al., 2003) of sequences under selection are involved. In contrast, in the argE3 → Arg+ system studied here the main source of stationary-phase mutations in dnaQ49 cells are DNA lesions left by Pol III defective in correction activity. These Arg+ reversions were mostly back mu-
tations in the reporter gene, whereas, the growth-dependent reversions resulted from suppressor formation.

Besides impaired Pol III, Pol V also contributes to the increase of Arg\(^+\) revertants. However, the action of Pol V seems to have a lesser effect on the level of Arg\(^+\) revertants in starving than in growing dnaQ49 cells. The frequency of mutations at 37°C was 2-fold lower in dnaQ49\(\Delta\)umuDC than in dnaQ49 while in growing cells this rate was about ten times decreased (Table 2).

Additionally, it seems that in the case of the dnaQ49 mutant, the temperature of incubation, permissive or non-permissive, is more important for the formation of stationary-phase Arg\(^+\) revertants than the presence or absence of Pol V. Since in the dnaQ49 strain at 37°C the level of growth-dependent as well as stationary-phase Arg\(^+\) revertants is much higher than at 28°C, we assume that the destabilization of Pol III core at 37°C is more critical for the occurrence of Arg\(^+\) revertants than the absence of properly operating 3′ → 5′ proofreading activity of the mutated ε subunit. It is possible that partial disconnection of Pol III at the replication fork facilitates the access of Pol V, Pol IV and Pol II up-regulated within the SOS system. It has been reported that Pol IV is required for adaptive mutations in the lac operon of FC40 strain and, together with Pol III, can account for all the adaptive point mutations at lac (Tomkins et al., 2003). In our argE3 → Arg\(^+\) reversion system Pol IV may play a similar role and to some extent replace Pol V (Nowosielska et al., 2004).
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