Divergent adaptation of *Escherichia coli* to cyclic ultraviolet light exposures

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The genetic changes taking place during adaptive evolution are particularly interesting in evolutionary biology. As a consequence of adaptive evolution, natural populations of an organism under selective conditions change genetically and phenotypically after a number of generations in order to survive in that particular environment. When a DNA-damaging and mutagenic agent like UV light is experimentally used as a selective factor, natural resistance of bacteria to this agent is normally increased through processes of mutation and selection. Since UV-induced mutagenesis is not restricted to particular chromosomal regions, different UV resistance mechanisms will equally probably evolve as a consequence of cyclic UV irradiation. However, it is also possible that as a consequence of the selective process, one UV resistance mechanism is preferentially selected, causing adaptive convergence of different bacterial cultures. This may occur if the most abundant or lethal kind of DNA lesion is preferentially managed by a particular DNA repair pathway and even by a specific repair enzyme or if resistance mechanisms that decrease bacterial fitness tend to be eliminated from the populations. To examine which of these two alternatives actually takes place, five cultures of *Escherichia coli* were treated in parallel for 80 successive UV irradiation cycles. At the end, these five cultures gave rise to different grades of UV resistance and after a preliminary characterization we found that adaptation to cyclic UV irradiation was a consequence of selection of advantageous mutations arising in different genes related to repair and replication of DNA.

### Introduction

An interesting problem of basic knowledge in biology is the nature and reproducibility of genetic changes associated with adaptive evolution of parallel populations of an organism. When these populations undergo strong environmental stress it is expected that they will develop similar phenotypes to enable individuals to survive under such conditions; however, although these populations may be phenotypically identical, they may be genetically diverse if there are different adaptive mechanisms.

Several studies on the extent of reproducibility of adaptive evolution have been carried out in microbial systems. In these studies, diverse bacterial populations growing in particular environments for thousands of generations have been followed and their phenotypic and genetic changes studied (Lenski and Travisano, 1994; Bennett and Lenski, 1997; Lenski et al., 1998, Nakatsu et al., 1998). From these studies it has been established that selection not only promotes genetic divergence of populations living in different environments, but that even in identical environments parallel populations may diverge if they find alternative adaptive solutions.

UV light is thought to have had a major impact on the early evolution of life because it is absorbed by nucleic acids producing several types of damage that interfere with replication and transcription of DNA. If UV-induced damage is not repaired or eliminated from DNA, it may lead to mutagenesis and cell death. To counteract the lethal effects of this and other DNA-damaging agents, different repair mechanisms have developed through evolutionary history; *in Escherichia coli*, for example, several DNA repair mechanisms have been described that enable cells to eliminate or tolerate damage to its genetic material.

When UV light is experimentally used as a selective factor in *E. coli*, natural resistance to this agent is commonly increased, making this response an excellent model to study the kind and recurrence of genetic changes associated with the emergence of a particular phenotype. The induction in *E. coli* of resistance to UV or X-rays by successive irradiation cycles (Wright and Hill, 1968; Mouton et al., 1970; Ewing, 1995, 1997; Rames et al., 1997) can be envisaged as adaptive evolution of DNA repair systems driven by processes of mutation and selection.

Since UV-induced mutagenesis is aleatory and spreads over the whole chromosome, theoretically different UV resistance mechanisms are equally prone to evolve after cyclic exposures to radiation, propitiating adaptive diversification of bacterial populations. However, in cells periodically facing massive DNA damage, it is conceivable that as a result of the selective process, adaptation of different populations will converge to one UV resistance mechanism. Although adaptive convergence is controversial, a few cases of functional, mechanistic and even structural convergence have been observed (Wichman et al., 2000; Timsit, 2001; Zakon, 2002), however, a true case of convergence in genetic sequence is still to be found. In UV resistance, convergence could result if: (i) a specific pathway or even a single repair step is essential to manage the most abundant or lethal kind of DNA lesion; (ii) mutants with lower fitness are counter-selected despite their high efficiency of DNA repair.

In the present work some characteristics of five UV-resistant derivatives of *E. coli* PQ30, obtained from a single progenitor by parallel cyclic UV irradiation (80 growth–irradiation cycles), are described. The results support divergent adaptation of different populations because neither UV resistance mechanism nor similar high UV resistance levels were attained.

### Materials and methods

**Bacteria**

Bacterial strains are listed in Table I. Most of them are derivatives of *E. coli* PQ30, a strain constructed by Quillardet and Hofnung (1985) for genotoxicity assays (Quillardet et al., 1985) and used here to quantify the SOS response in

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UV-resistant derivatives. Hfr strains of the CSH series were bought from the Cold Spring Harbor Laboratory collection.

**Growth-irradiation cycles**

A clone of *E. coli* PQ30 was grown overnight in Luria–Bertani broth (LB) and the resultant culture fractionated into five subcultures. Each subculture was then exposed to 80 growth-irradiation cycles of UV light. Each growth-irradiation cycle was carried out as follows. An aliquot of 1 ml from an early stationary culture (~5 × 10^8 cells), grown in LB broth and suspended in 0.02 M phosphate buffer, was irradiated with a 15 W Hg vapour UV germicidal lamp, calibrated at a dose rate of 1 J/m^2/s with a UVP meter model UVX-25. This type of lamp has a UV emission peak of 254 nm, which is the wavelength preferentially absorbed by nucleic acids. Following irradiation, 0.1 ml of this suspension was inoculated into 1 ml of fresh LB broth and incubated at 37°C for ~6 h to early stationary phase; incubation was in darkness to avoid photoreactivation. Starting with a dose of 10 J/m^2 for each cycle, dose was increased 2-fold every 10 growth-irradiation cycles, except in the interval between cycles 51 and 60, because of poor bacterial growth.

**UV survival**

Quick UV sensitivity tests were done by streaking LB agar plates with samples of liquid cultures and exposure to UV light from a 15 W germicidal lamp. A gradient of UV doses was used by achieving successive sections of the bacterial streaks. Quantitative dose-response curves were obtained by exposing exponentially growing cells, suspended in 0.02 M phosphate buffer, to several UV doses (bacterial suspensions were gently stirred during irradiation). Immediately after irradiation, cells were diluted in 0.02 M phosphate buffer and spread on LB agar. In both the qualitative and quantitative tests plates were incubated in the dark for 18–24 h at 37°C. Survival curves are the mean of at least three experiments.

**SOS Chromotest**

Induction of the SOS response by UV light was measured by the SOS Chromotest assay described by Quillardet and Hofnung (1985). The assay in strain PQ30 comprises determination of two enzymatic activities: inducible β-galactosidase (under control of the SOS operon) and constitutive alkaline phosphatase, denoting genotoxicity and general protein synthesis, respectively. However, the five UV-resistant derivatives lost constitutive synthesis of alkaline phosphatase. Consequently, the enzyme was undetectable in four of those UV-resistant strains while an intermediate level of alkaline phosphatase was observed in IN804 (Figure 3).

**Reversion frequency**

In order to measure the frequency of reversion from *leu^-* to *leu^+*, stationary cultures resuspended in 10^8 M MgSO_4, were irradiated with 30 J/m^2 UV light, diluted and spread over minimal agar supplemented with the necessary nutritional requirements to allow *leu^-* revertants to grow.

**Results**

In order to investigate the changes occurring during adaptive evolution to a strong selective agent, some characteristics of five UV-resistant strains, as well as chromosome location of the mutations causing their UV resistance, were determined. Characterization was done in individual clones of each derivative and the reproducibility of results in several clones was good evidence of genetic homogeneity after 80 cycles.

The results show that although some phenotypic characteristics are common to the five derivatives, UV resistance followed different courses. Interestingly, mutations causing the UV-resistant phenotype are concentrated within a short chromosomal region extending clockwise from 87 to 6 min.

**UV survival**

Figures 1 and 2 show dose-response curves after 50 and 80 growth-irradiation cycles, respectively. As can be seen, different survival percentages after UV irradiation are observed among the five resistant derivatives, suggesting different UV resistance mechanisms. Comparison of survival at 100 J/m^2 between 50 and 80 cycles shows three types of populations: those in which UV resistance increased (IN803 and IN804), those in which resistance remained the same (IN801) and those in which resistance decreased (IN802 and IN805). This decrease in resistance was not expected because the irradiation process was supposed to select increasingly efficient survival strategies.

**SOS induction**

The SOS response is induced after DNA damage or a delay in DNA replication and once activated it promotes targeted and untargeted error-free and error-prone DNA repair. Since SOS constitutive mutants (*recA730* or *lexA51*) are more resistant to killing by UV radiation and other DNA-damaging agents (Witkin et al., 1982; Krueger et al., 1983), it was quite possible that UV resistance in some of our strains was due to a partially or completely constitutive SOS system. For this reason, the SOS response of each UV-resistant derivative was determined.

As mentioned in Materials and methods, all UV-resistant strains lost constitutive synthesis of alkaline phosphatase. Consequently, turbidity at 600 nm, measured 2 h after UV irradiation, was instead used as an index of cell viability.

To confirm that all of the UV-resistant derivatives had actually lost constitutive synthesis of alkaline phosphatase, the enzyme was quantified by the method of Echols et al. (1961). The results showed that the enzyme was undetectable in four of the UV-resistant strains while an intermediate level of alkaline phosphatase was observed in IN804 (Figure 3).

Figure 4a shows SOS induction by UV in the parental strain and UV-resistant derivatives. For comparison, SOS induction in PQ30 was determined by titration of alkaline phosphatase.
and culture turbidity and no significant difference was found between these two methods. Although the SOS responses of the UV-resistant strains can be induced up to levels comparative to that observed in PQ30, it occurs only at higher UV doses. Interestingly, the most UV-resistant derivative, IN803, showed no SOS induction at all. As shown in Figure 4b, the lower SOS induction in all UV-resistant strains is not caused by high basal levels of β-galactosidase enzyme due to a partially constitutive SOS system.

### Genetic mapping

UV resistance was mapped by transmission to the F− UV-resistant strains of the corresponding wild-type alleles from Hfr strains CSH119 and CSH120, which differ in the origin and direction of chromosome transfer as well as the site of Tn10 insertion (Miller, 1992). As detailed in Materials and methods, genetic markers with known locations on the bacterial chromosome were used in turn to build transmission gradients based on the frequency at which each marker appears in the offspring after a specified mating time. This frequency depends on the distance of the marker from the origin of chromosome transfer.

During chromosome transmission from Hfr wild-type strains into F− UV-resistant strains, a fragment is eventually transmitted that upon recombination with its homologue in the receptor strain substitutes for that portion where the mutation(s) causing the UV-resistant phenotype is located, reverting it to wild-type. These wild-type clones were easily detected among the UV-resistant offspring and their frequency could be
correlated with the frequency of clones bearing the other genetic markers selected. From the frequencies of transmission of Tn10, metB and cisG, linear gradients of transmission were obtained and UV resistance mutations located by interpolating the frequency of clones that lost the UV-resistant phenotype. Genetic mapping by gradients of transmission allows the location of mutations with a resolution of ~1–2 min and the genes affected are then identified on the genetic map of E.coli by proximity to the mapped site. Table II shows the location of these mutations as obtained from typical gradients of transmission.

**Mutation frequencies**

It has been observed that under some environmental stresses mutator strains are favoured over non-mutator strains (Blázquez, 2003; Chopra et al., 2003). Although mutator genes may induce deleterious mutations and thus represent an indirect selective disadvantage, at the same time bacteria bearing mutators can increase their adaptive ability by generating beneficial mutations. As our UV-resistant strains were the result of an adaptation to the stressful conditions produced by cyclic irradiation, it was of interest to see if some of them had high spontaneous or induced mutation frequencies, particularly IN803 and IN805, which have mutations in genes involved in DNA synthesis. Genetic mapping in IN803 indicated that its UV resistance was due to mutations in dnaQ and dnaE, coding for, respectively, the ε and α subunits of DNA polymerase III, which are the proof-reading 3′→5′ exonuclease (Scheuermann and Echols, 1984) and the α polymerising subunits of the holoenzyme. In IN805 there is a mutation in polA, whose product is DNA polymerase I, which controls Okazaki fragment processing during normal DNA replication and repolymerization during nucleotide excision repair (Moolenaar et al., 2000).

Table III shows that both spontaneous and UV-induced reversion frequencies to leu+ vary from one strain to another. In one extreme are IN802 and IN801, with almost no induction of mutations, and on the other is IN803, with an increase in reversion frequency of >800 times after a dose of 30 J/m² UV light. Finally, in IN805 induction is similar to that in the parental strain.

**Discussion**

In addition to transient responses that alleviate the effects of physical and chemical DNA-damaging agents (adaptive and SOS responses), bacteria may adapt genetically to environmental stresses by Darwinian mutation and selection. Development of radiation resistance induced by cyclic irradiation is a clear example of this kind of adaptation and an adequate experimental model to study the genetic basis of a particular phenotype in parallel populations.

The results reported here indicate that adaptation (i.e. increased UV resistance) of *E.coli* to cyclic exposures to UV...

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**Table II. Map position of mutations causing UV resistance in strains derived from E.coli PQ30 by cyclic UV irradiation.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Chromosome position (min)</th>
<th>Gene</th>
</tr>
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<tbody>
<tr>
<td>IN801</td>
<td>98.5</td>
<td>radA</td>
</tr>
<tr>
<td>IN802</td>
<td>93.5</td>
<td>uvrA</td>
</tr>
<tr>
<td>IN803</td>
<td>4–6</td>
<td>dnaE, dnaQ</td>
</tr>
<tr>
<td>IN804</td>
<td>91</td>
<td>uvrA</td>
</tr>
<tr>
<td>IN805</td>
<td>87</td>
<td>polA</td>
</tr>
</tbody>
</table>

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**Fig. 4.** SOS induction in *E.coli* PQ30 and five UV-resistant derivatives irradiated with UV light. See Materials and methods for details. (A) SOS induction factor as determined by Quillardet and Hofnung (1985); viability of PQ30 was measured by culture turbidity (a) and by synthesis of alkaline phosphatase (b). In UV resistant derivatives, viability was measured only by culture turbidity. (B) SOS activity measured directly by titration of β-galactosidase.
light took place through indiscriminate selection of advantageous mutations induced by UV in DNA repair and replication genes. Although, in principle, UV resistance in each strain could be due to the accumulated effect of several mutations in various genes, our results indicate that, with one exception, it can be attributable to mutations affecting the function of only one gene product at a time, as shown in Table III. Only in strain IN803 were two types of clones obtained from the crosses; some which had reverted completely to the wild-type phenotype and others which exhibited an intermediate level of UV resistance. We interpreted this as an indication that in this case UV resistance was due to mutations in two closely related genes, which, as indicated by gene mapping, are located between 4 and 6 min on the bacterial chromosome.

During adaptive evolution high mutation rates are beneficial as they provide the variability necessary to facilitate genetic adaptation. This condition can be achieved either by activating mutagenic responses, like the SOS response, or by inhibiting antimutagenic activities, like DNA mismatch repair (Matic et al., 1995; Harris et al., 1997). Data (not shown) for a recA derivative of the parental strain PQ30, in which no UV resistance could be attained after 50 irradiation-growth cycles, indicate that development of UV resistance was very likely dependent on SOS mutagenesis. On the other hand, in a recB strain the phenotype evolved normally, indicating that the increase in UV resistance was not dependent on homologous recombination.

Despite the diversity of adaptive strategies followed by the UV-resistant derivatives, all of them lost constitutive synthesis of alkaline phosphatase, characteristic of the parental strain. For some reason there was a selective pressure against this trait during the development of UV resistance.

Alkaline phosphatase is an inducible enzyme, synthesized under phosphate limiting conditions to supply the necessary phosphate requirements by non-specifically hydrolysing organic phosphate monoesters and diesters (Wanner, 1987; Coleman, 1992; O’Brien and Herschlag, 2001). Excess alkaline phosphatase in the parental strain [up to 6% of total protein in constitutive strains according to Torrani and Rohman (1961)] has no apparent deleterious effect under normal conditions, as suggested by the stability of this trait in PQ30. However, in cells cyclically irradiated, such a condition may be disadvantageous, due either to a decrease in the availability of precursors for repair and/or replication of damaged DNA, caused by dephosphorylation of nucleotides (Garen and Levinthal, 1960; Torrani, 1960), or to deficient repair of damaged DNA caused by dephosphorylation of phosphomonoesters at nicks, gaps and ends of double-strand breaks (Weiss et al., 1968; Masamune et al., 1971), preventing further action by repair enzymes.

Another phenotypic characteristic showed by these strains is a decrease in UV induction of the SOS response. In the current model SOS is induced by gaps resulting from blocked DNA replication at sites of unrepaired DNA lesions (Sassanfar and Roberts, 1990; Sommer et al., 1991; Higashitani et al., 1995) that activate RecA protein as a co-protease to facilitate the autodigestion of LexA repressor. Therefore, it is expected that efficient DNA repair processes would generate less DNA substrate for RecA activation. Some results from strain IN801 (not shown), in which an insertion of miniTn5 caused complete loss of UV resistance and fully restored the SOS response, support the assumption that low SOS activity in our UV-resistant strains is not a defect in the SOS system but a consequence of efficient repair or replication of damaged DNA.

All mutations conferring UV resistance mapped within a short chromosomal stretch of only 18 min extending from 87 to 5 min in a clockwise direction, where several DNA repair and replication genes are located. In some strains these mutations are easily assigned to particular genes; for example, in IN801 the UV resistance mapped at 98.5 min, where only radA is near at 99.6 min (Diver et al., 1982), and in IN805 it mapped at 87 min, very close to polA at 87.2 min (Rudd, 1998), therefore, radA and polA are very likely involved in the UV resistance of these strains. IN803, one of the two most resistant strains, seems to have mutations in two closely related genes, between 4 and 6 min on the bacterial chromosome. One is located between 1.75 and 4.75 min and the other between 5 and 6 min. We have assumed that the first corresponds to dnaE (4.42-4.5 min) and the second to dnaQ [5.1 min], coding for the α and ε subunits of DNA polymerase III, respectively (Scheuerman and Echols, 1984).

In IN802 and IN804 the mutations mapped at 93.5 and 91 min, respectively, where a set of DNA-related loci is found (Rudd, 1998): lexA (91.71 min), dinF (91.72 min), dnaB (91.87 min), uvrA (92.01 min) and ssb (92.08 min).

Since in these strains resolution of mapping by gradients of transmission does not allow the establishment of which genes are causing the resistant phenotypes, more accurate mapping is necessary. However, we consider that in the case of IN802 uvrA is very likely involved in UV resistance because it is the nearest repair gene to the mapped site. On the other hand, in IN804 UV resistance may be due to mutations in either of the other genes mentioned above and for the moment it is not possible to conclude unambiguously which one is causing the phenotype.

The above considerations indicate that during adaptation of Escherichia coli to cyclic UV irradiation, mutations induced in DNA repair or replication genes were indiscriminately selected. Included among these genes are: uvrA and polA, involved in excision repair; dnaE and dnaQ, involved in DNA replication; radA and dinF, whose functions have not yet been elucidated, though a role in processing Holiday junctions during recombination and recombinational repair has been proposed for the former (Beam et al., 2002); and finally, dnaB, which codes for a replication and recombination DNA helicase.

Interestingly, one of the most UV-resistant phenotypes was not the result of an increase in repair efficiency but was apparently a combined effect of an increase in misinsertions carried out by the polymerizing α subunit of polymerase III and a decrease in proof-reading activity of the ε subunit. Mapping results indicated that this strain has mutations affecting both subunits of the enzyme, giving rise to a mutator-like phenotype. However, this mutator activity seems to be different from

<table>
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<tr>
<th>Bacterial strain</th>
<th>UV dose (J/m²)</th>
<th>Induction factor</th>
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<tbody>
<tr>
<td>PQ30</td>
<td>7 × 10⁻¹⁰</td>
<td>2.3 × 10⁻³</td>
</tr>
<tr>
<td>IN801</td>
<td>3 × 10⁻¹⁰</td>
<td>1.9 × 10⁻⁹</td>
</tr>
<tr>
<td>IN802</td>
<td>5.3 × 10⁻¹⁰</td>
<td>4.3 × 10⁻⁹</td>
</tr>
<tr>
<td>IN803</td>
<td>2.8 × 10⁻⁹</td>
<td>2.4 × 10⁻⁶</td>
</tr>
<tr>
<td>IN804</td>
<td>1.2 × 10⁻⁹</td>
<td>5.9 × 10⁻⁸</td>
</tr>
<tr>
<td>IN805</td>
<td>8.4 × 10⁻¹⁰</td>
<td>1.7 × 10⁻⁸</td>
</tr>
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</table>
others previously known in dnaE and dnaQ (Fijalkowska et al.,
1997), because it promotes misinsertions almost exclusively
during replication of UV-damaged DNA (see Table III). In
addition, most misreplication events occurring at sites of
damage are not removed because of the mutated dnaQ, thus
allowing fast, SOS-independent DNA translation synthesis.
These events seem to be unimportant in non-irradiated cells,
as shown by the low spontaneous mutation frequencies (Table III),
thus avoiding the costs of high spontaneous dele-
terious mutation rates.

All these results indicate that not only the UV-induced
mutagenesis but also the selective process occurred in a
random-like way because no preferential UV resistance
mechanism was chosen. This adaptive divergence is clearer
in cultures IN803 and IN804, which attained similar levels of
UV resistance through different mechanisms, as evidenced by
the results of genetic mapping and the UV-induced mutation
frequencies. Future activities should include more accurate
monitoring by cleavage of LexA repressor.

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