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Genetics

Differences in mutagenic and recombinational DNA repair in enterobacteria

(*recA* inducibility/*umuCD*/induced mutagenesis/DNA dot-blot analysis)

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ABSTRACT **The incidence of recombinational DNA repair and inducible mutagenic DNA repair has been examined in *Escherichia coli* and 11 related species of enterobacteria. Recombinational repair was found to be a common feature of the DNA repair repertoire of at least 6 genera of enterobacteria. This conclusion is based on observations of (*i*) damage-induced synthesis of RecA-like proteins, (*ii*) nucleotide hybridization between *E. coli recA* sequences and some chromosomal DNAs, and (*iii*) *recA*-negative complementation by plasmids showing SOS-inducible expression of truncated *E. coli recA* genes. The mechanism of DNA damage-induced gene expression is therefore sufficiently conserved to allow non-*E. coli* regulatory elements to govern expression of these cloned truncated *E. coli recA* genes. In contrast, the process of mutagenic repair, which uses *umuC*$^+$ *umuD*$^+$ gene products in *E. coli*, appeared less widespread. Little ultraviolet light-induced mutagenesis to rifampicin resistance was detected outside the genus *Escherichia*, and even within the genus induced mutagenesis was detected in only 3 out of 6 species. Nucleotide hybridization showed that sequences like the *E. coli umuCD*$^+$ gene are not found in these poorly mutable organisms. Evolutionary questions raised by the sporadic incidence of inducible mutagenic repair are discussed.**

The SOS system of *Escherichia coli* is a sophisticated cellular response to DNA damage and involves induced synthesis of several DNA repair enzymes and changes in the normal cycles of cell division and replication. The key to the integration of these activities is a common transcriptional control mechanism in which expression of at least 17 genes is repressed by LexA protein repressor (reviewed in refs. 1 and 2). After DNA-damaging treatments RecA protein causes proteolysis of the LexA repressor (3), and SOS gene expression ensues (4, 5). Expression of the *recA* gene is regulated in this way, leading to increased levels of RecA protein in SOS-induced cells.

A second role of RecA protein is in recombination. This activity is essential for both homologous recombination (6) and the major pathway of postreplication repair (7), which reconstitutes gapped daughter DNA strands by recombinational exchange (8, 9). The large effect of this repair on survival is shown by the UV resistance of *tsl-1* and *recA281 lexA* mutants, which are repair proficient but do not induce expression of many SOS genes (10–13). Conversely, inhibition of recombination repair by *recA*-negative complementation causes radiosensitization without inhibiting induction of the SOS genes (14). *recA*-negative complementation can be caused by cloned truncated *recA* genes whose products are thought to impair the recombinational activity of chromosomally encoded *recA*$^+$ protein by subunit mixing.

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The distinction between recombination repair and mutagenesis is best emphasized by the properties of *umuCD* mutants (15, 16). These mutants are deficient in mutagenesis induced by agents such as UV and are moderately radiosensitive. However, physical assays show that they are proficient in postreplication repair (17), which, as previously mentioned, is primarily recombinational. Genetic and nucleotide sequence analyses of mutated genes indicate that *umuC*$^+$ and *umuD*$^+$ gene products may act in a tolerance mechanism that permits synthesis of DNA on lesion-containing templates (18–22). Although the altered DNA synthesis might affect semiconservative replication, there is other evidence that it influences repair replication (23–26). The sites of this activity might be a small fraction of gaps produced, either by excision repair or by chromosome replication as a prelude to postreplication repair. Because survival is enhanced with an intrinsic probability of mutagenesis, the process has been called mutagenic or error-prone repair. Mutations in the *umuCD* operon can be complemented by plasmid genes from at least eight unrelated groups of plasmids (27–29), even though the genes have little nucleotide homology with each other or with the *umuCD* operon (refs. 2 and 29; P. Oliver, personal communication). Two genes, *imp* of TP110 (29) and *mucAB* of R46 and pKM101 (30), also show SOS-inducible expression like that of the *umuCD* operon (31, 32). Thus, these plasmid gene products are analogous to the *umuCD* gene products in both function and regulation.

The purpose of this report is to document the relative contributions made to the UV resistance of *E. coli* and related enterobacteria by the two processes of recombination and mutagenic DNA repair. The approach involved introducing either radioprotective R46 or pKM101 plasmids containing analogues of the *umuC*$^+$ and *umuD*$^+$ genes or plasmids that, through *recA*-negative complementation, selectively inhibit recombinational repair in *E. coli*. This approach and subsequent analyses of SOS-inducible proteins and nucleotide homology indicate that the nonmutagenic process of recombination repair is widespread. Certain species of *Escherichia* appear to be the exception, rather than the rule in possessing an additional mutagenic repair system.

MATERIALS AND METHODS

The types and origins of the bacteria used are listed in Table 1. Plasmids used were pKM101 (39), R46 (40), pMH21 (11), pBR322 (41), pCS68 (42), pPE13 (43), and pSK100 (32). Most methods used have been described earlier (44). Additional protocols were as follows. Interspecies conjugal transfers of pKM101 or R46 employed auxotrophic *E. coli* AB1157-(pKM101) or DT17 or *Salmonella typhimurium* TA1535 as donors. Cells to be conjugated were concentrated 10-fold and spread onto a dry, warmed Luria agar plate and incubated 45 min at 37°C. After resuspension in 10 mM $MgSO_4$, females

Abbreviation: bp, base pair(s).

Table 1. Bacteria used and their origins

Bacterium	Strain	Relevant genotype	Source, ref., or donor
Escherichia coli	TK701	*umuC*$^+$	T. Kato (19)
	TK702	*umuC*	T. Kato (19)
	DT17	*trp R46*	D. Tweats
	AB1157	*umuC*$^+$ *argE3 thr-4 leu-6 thi-1 proA2 his-4*	Mill Hill collection (33)
	MH1		Human
Escherichia aurescens	NCTC29855		NCTC (34)
Escherichia blattae	NCTC10965		NCTC (35)
Escherichia alkalescens	NCTC1601		
Escherichia decarboxylata	NCTC10599		NCTC
Escherichia dispar	NCTC4168		NCTC (36)
Salmonella typhimurium	LT22		C. H. Clarke
	TA1535	*hisG4 bio* pKM101	B. Sedgwick (37)
Klebsiella aerogenes	NCTC418		G. T. Yarranton
Shigella sonnei	S81		P. van der Putte
Citrobacter intermedius	MH23		Soil sample, H. Rogers
Proteus mirabilis	MH29		Monkey feces, A. Leach
	PG1300		J. Hofemeister (38)
Proteus rettgeri	MH10		Tamarin feces, A. Leach

NCTC, National Collection of Type Cultures, Public Health Laboratory, Colindale, England.

receiving R46 or pKM101 were selected on minimal plates containing ampicillin or tetracycline, respectively, each at 15 μg/ml. *Klebsiella* and *Citrobacter*, however, required ampicillin at 500 μg/ml for effective selection.

Rifampicin-resistant mutants were assayed by using a triple overlay technique. Aliquots containing approximately 10^7 cells were poured with 3 ml of 0.6% Luria agar onto plates containing 25 ml of Luria agar. After the first layer solidified, a second 3-ml layer was poured and the plate was incubated immediately at 37°C. Total numbers of viable cells were scored after appropriate dilution and plating in the same way. After 3 hr, mutagenesis plates received a third layer of 3 ml of 0.6% Luria agar containing sufficient rifampicin to give a final concentration throughout the plate of 100 μg/ml. Plates were scored after 3 days' growth.

Dot-blot hybridization was done as described elsewhere (42, 45). Probes were a 3-kilobase-pair (kbp) *Bam*HI fragment of pPE13 containing the *recA*$^+$ and flanking regions or a 700-bp *Eco*RI/*Pst* I internal fragment of the *recA*$^+$ structural gene, a 1.2-kbp *Eco*RI/*Hin*dIII fragment of pCS68 containing 1.1 kbp of *ada*$^+$ gene sequence, and a 2.9-kbp *Hpa* I fragment of pSK100 containing *umuC*$^+$*D*$^+$ and flanking DNA. Fragments were separated by electrophoresis through acetate-buffered agarose gels and purified by their affinity with ground glass (46).

RESULTS

The UV dose response for cell survival shows that *E. coli* was the most resistant of the organisms tested, followed by *Salmonella typhimurium*, *Proteus mirabilis* and *Citrobacter intermedius*, and the more sensitive *Klebsiella aerogenes* and *Shigella sonnei* (Fig. 1).

The ability of plasmids encoding a truncated RecA protein to negatively complement chromosomal *recA*$^+$ activity was used to diagnose the use of recombinational repair in non-*E. coli* species (14). In all cases transformation with pMH21 caused UV radiosensitization (Fig. 1), thus indicating the activity of recombination repair in the untransformed host organism. Recombination activity in these species was also indicated by SOS induction of RecA-like proteins by nalidixic acid treatment (Fig. 2) (12, 47). The pMH21 transformants,

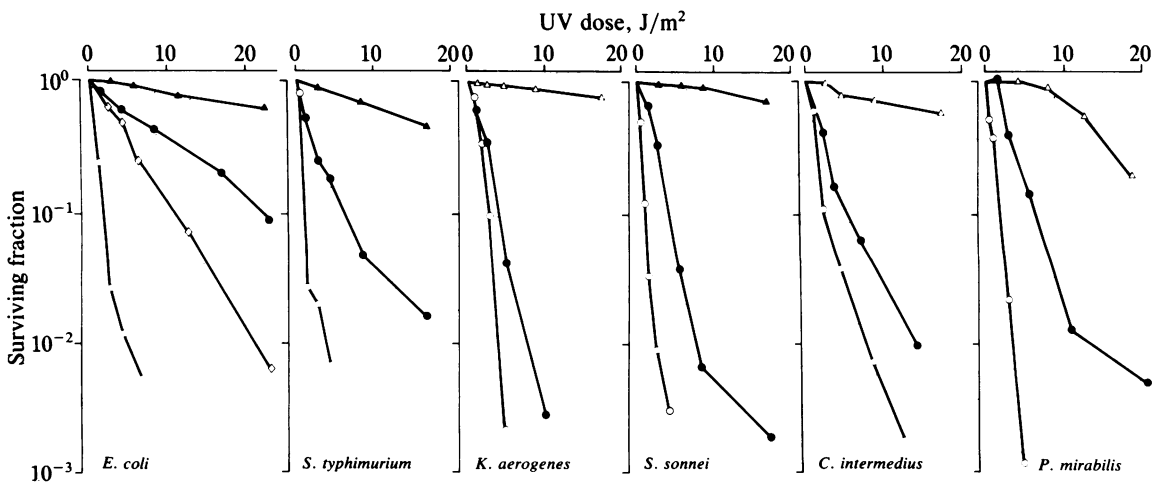

FIG. 1. Survival of six species of enterobacteria after UV irradiation. Wild types of each species (●) became more UV sensitive when carrying pMH21 (○) and more resistant with either pKM101 (▲) or R46 (△). *E. coli* TK701 and its *umuC* derivative, TK702 (◇), were used. *P. mirabilis* is strain PG1300.

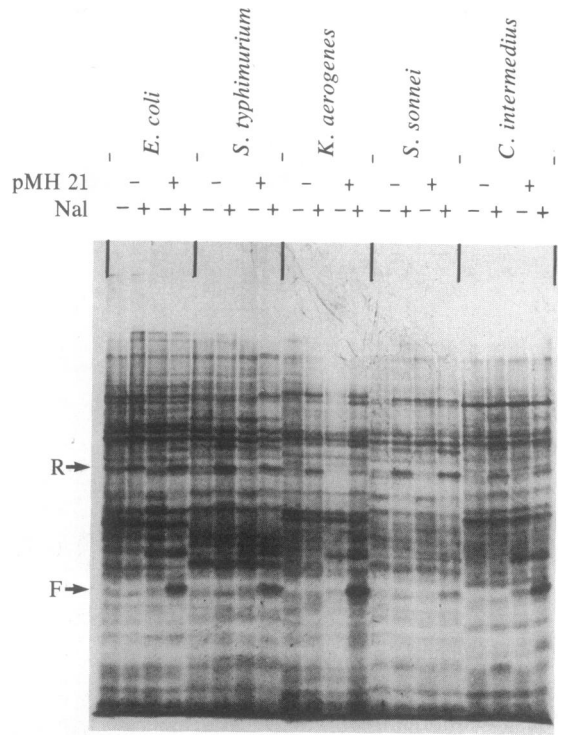

FIG. 2. Induced synthesis of RecA-like proteins and expression of a truncated *E. coli recA* gene in different species. Arrows R and F indicate full-size RecA protein and an amino-terminal fragment of RecA protein encoded by pMH21. Nal, treatment with nalidixic acid at 40 μg/ml 30 min prior to and during 30 min of incubation with [^{35}S]methionine. Proteins were separated by electrophoresis through a NaDodSO$_4$/10% polyacrylamide gel and visualized by autoradiography.

which undergo *recA*-negative complementation, also induce synthesis of plasmid-encoded truncated *E. coli* RecA protein (Fig. 2). The control mechanism of *recA* gene expression in these enterobacteria is therefore sufficiently conserved to show normal induction after interspecies transfer. Similar induced proteins were found in *P. mirabilis* PG1300 (data not shown).

The activity of mutagenic repair can be manifested by induced mutability after UV irradiation. UV irradiation of *E. coli* AB1157 caused the increase in mutagenesis to rifampicin resistance expected for this *umuC*$^+$*D*$^+$ organism (Figs. 3*A* and 4*A*). Induced mutagenesis was not reduced by inhibiting recombination repair by *recA*-negative complementation with pMH21 (Fig. 4*A*), again emphasizing the separate nature of these two inducible processes.

In contrast, little increase in frequency of rifampicin-resistant mutants was detected in UV-irradiated *Salmonella typhimurium, Shigella sonnei, K. aerogenes, C. intermedius, P. mirabilis* (Fig. 3*A*), *E. coli umuC*, or *P. rettgeri* (data not shown). Poor induced mutability was a feature of both laboratory *P. mirabilis* strain PG1300 (data not shown) and fecal isolate MH29 (Fig. 3*A*). Even within the genus *Escherichia* only three out of six species exhibited UV-induced mutability to rifampicin resistance (Fig. 3*B*). The failure to detect induced mutants in genera other than *Escherichia* was not due to some inherent inability to tolerate the changes in metabolism needed for expression of rifampicin resistance. This was shown by the mutability of *Salmonella typhimurium, Shigella sonnei, K. aerogenes, C. intermedius*, and *P. mirabilis* after receiving either pKM101 or R46 (Fig. 4*B*). As previously found with some of these organisms (38–40, 48), the enhancement of mutability was accompanied by increased cellular survival (Fig. 1). The radiation resistance conferred by pKM101 or R46 therefore reflects the potential

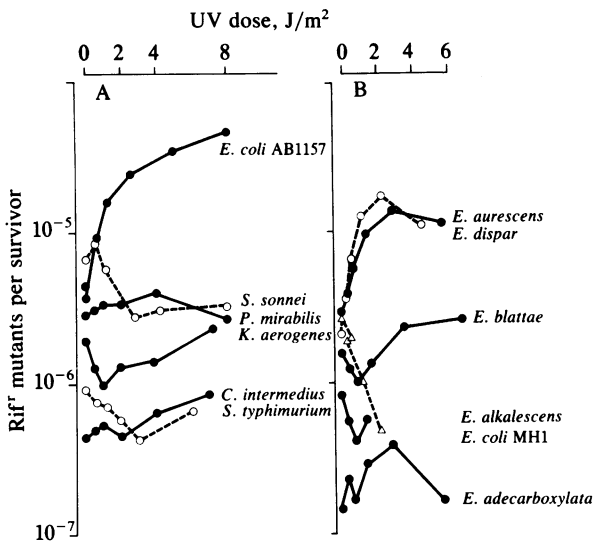

FIG. 3. Frequency of rifampicin-resistant mutants in different genera of enterobacteria (*A*) and different species of *Escherichia* (*B*). Values expressed are for the total incidence of mutant colonies and consist of both spontaneous and UV-induced mutants.

contribution that mutagenic DNA repair could make to cellular survival.

DNA homology tests were done to determine whether the incidence of recombinational and mutagenic repair systems correlated with the presence of *recA*-like and *umuCD*-like chromosomal sequences (Fig. 5). An additional control probed the same DNAs with the *ada* sequence, whose gene product acts in the unrelated adaptive repair system for alkylation damage. Compared with *recA* or *ada*, the incidence of *umuCD*-like sequences in these species was limited. Even though the *umuC*$^+$*D*$^+$ probe contained flanking regions and was hybridized at low stringency, only four species, all within the genus *Escherichia*, showed hybridization with the

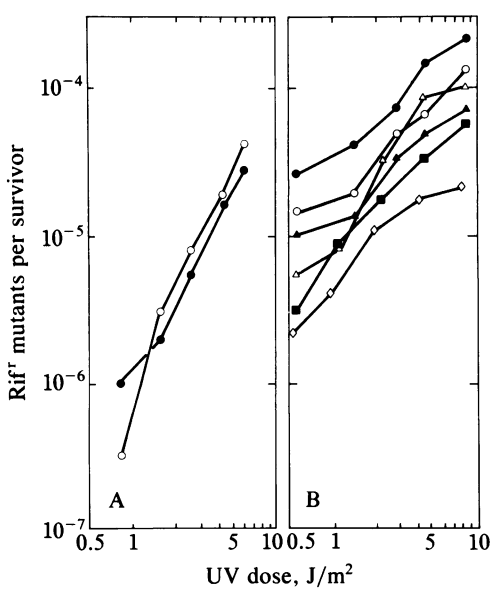

FIG. 4. (*A*) *recA*-negative complementation by pMH21 does not inhibit UV-induced mutagenesis to rifampicin resistance in *E. coli* AB1157. Transformed (○) and untransformed cells (●) displayed UV-sensitive and -resistant phenotypes similar to those shown in Fig. 1 and elsewhere (11). (*B*) Plasmids R46 and pKM101 confer high levels of UV-induced mutagenesis on *E. coli* AB1157 (●), *Salmonella typhimurium* (▲), *K. aerogenes* (△), *Shigella sonnei* (○), *C. intermedius* (■), and *P. mirabilis* PG1300 (◇). The effects of these plasmids on UV survival are shown in Fig. 1.

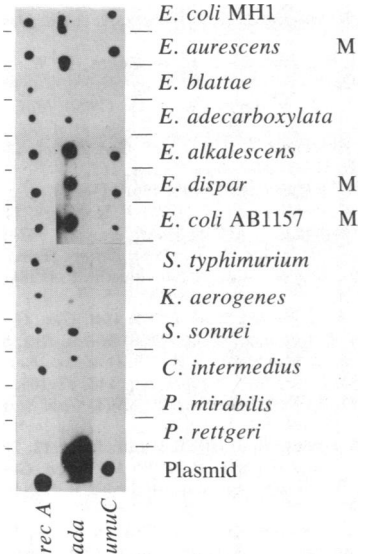

FIG. 5. Dot-blot hybridization between genomic DNAs from enterobacteria and ^{32}P-labeled probes encoding *E. coli recA, ada,* and *umuC* regions of the chromosome. M, UV-induced mutagenesis to rifampicin resistance shown in Fig. 3. Hybridization and washing were at 65°C in 0.75 M NaCl/0.075 M sodium citrate/0.5 mM EDTA/0.1 M NaH_2PO_4/0.2% $NaDodSO_4$.

umuCD probe. All species lacking *umuCD* hybridization showed little UV-induced mutability to rifampicin resistance. Similarly, the UV-mutable species, *E. dispar, E. aurescens,* and *E. coli* AB1157, showed hybridization. Two exceptions were a hospital isolate of *E. coli*, MH1, and *E. alkalescens*, which showed *umuCD* hybridization (Fig. 5) but poor UV-induced mutability (Fig. 3*B*). Restriction enzyme digestion and Southern hybridization from these and other isolates of *E. coli* reveal frequent DNA rearrangements within the region covered by the *umuCD* probe used and will be described in detail elsewhere. Hybridization occurred with an *E. coli recA*$^+$ probe that included flanking DNA in all species except *P. mirabilis* and *P. rettgeri* (Fig. 5). A smaller probe consisting of approximately 700 bp of the internal sequence of the *recA* gene gave the same result except that it also did not hybridize detectably with genomic DNA from *Shigella sonnei.*

DISCUSSION

The analyses of proteins and DNA homologies presented here show the presence of RecA-like proteins and genes in six genera of enterobacteria. All organisms were also similar in showing nalidixic acid-induced synthesis of these proteins. The results indicate conservation in RecA protein activities, structure, and regulation.

In *E. coli* the consequences of RecA protein's recombinational and regulatory activities were previously separated by *recA*-negative complementation (14, 44). Recombination and postreplication repair were inhibited, causing radiosensitivity. In contrast, SOS-induced gene expression was less affected, and SOS-induced gene products, including RecA protein, were synthesized. In this work similar features of negative complementation were seen when plasmids encoding an amino-terminal fragment of *E. coli* RecA protein were transferred to foreign hosts. The cells became radiosensitive but continued to show normal induction of SOS gene expression, as judged by induction of their own RecA-like proteins. It is therefore proposed that these other species use recombinational repair as *E. coli* does as part of their response to DNA damage. In *E. coli* and *P. mirabilis* this repair system is most efficient when damage-induced protein synthesis occurs (49, 50). Given RecA protein's well characterized recombinogenic properties (51) and its inducibility, it is further proposed that inducible UV resistance be considered to be primarily due to an inducible recombination repair system.

recA-negative complementation by pMH21 probably occurs by the combination of short plasmid-encoded RecA polypeptides with full-size chromosome-encoded molecules to form inactive multimeric units (14). Interspecies-negative complementation may therefore indicate a functional conservation in enterobacterial RecA protein structure permitting interspecies subunit mixing. Conservation of structure is also indicated by earlier reports of cross-reactivity of antibody to *E. coli* RecA with the equivalent *S. typhimurium* and *P. mirabilis* proteins (49, 52).

Normal SOS-induced expression of pMH21 containing a truncated *E. coli recA* gene was also seen after interspecies transfer. Thus, the control mechanism of SOS-induced gene expression within these species is also conserved enough to allow their equivalent *recA*$^+$ and *lexA*$^+$ activities to permit normal regulation of an *E. coli recA* control sequence. Conversely, introduction of plasmid-encoded *E. coli lexA* repressor into some of these species reduced synthesis of RecA-like proteins (unpublished observations), showing that *E. coli* SOS regulatory elements can also govern the SOS response of other organisms. Furthermore, reciprocal transfer of cloned *recA*$^+$ genes between *P. mirabilis* and *E. coli* complemented both recombination and regulatory defects in *recA* mutants of both species (53). Such interspecies complementation has since been related to the functional conservation of *in vitro* properties of *E. coli, P. mirabilis*, and *S. typhimurium recA*$^+$ proteins in recombination reactions, and in cleaving λ repressor and *E. coli lexA*$^+$ protein (47, 54). Collectively, these results indicate conservation of a common mechanism of inducible recombination repair and its regulation in enterobacteria. Other evidence gained with *Hemophilus influenzae* (55) and *Ustilago maydis* (56) also points to the existence of inducible recombination repair and strengthens the conclusion that this is a widespread strategy for surviving DNA damage.

Despite these similarities, *E. coli* differs from many other species of *Escherichia* and enterobacteria in one important aspect of the SOS response—namely, induced mutability. In many species UV-induced mutagenesis to rifampicin resistance could not be detected. Previous reports also showed low or undetectable UV-induced mutagenesis to rifampicin resistance and amino acid prototrophy in *P. mirabilis* (38, 53) and *S. typhimurium* (57, 58). However, these species are not intrinsically immutable because introduction of plasmids R46 or pKM101 renders them mutable. R46 and pKM101 have a similar effect on nonmutable *umuCD* strains of *E. coli* (59). Therefore, the above nonmutable species can be viewed as naturally occurring *umuCD* mutants. This view is reinforced by the hybridization analyses presented here, showing that many species lack sequences hybridizing with the *E. coli umuCD* gene but retain similarities at two other repair gene regions, *recA* and *ada*.

The limited incidence of *umuCD*-like sequences and poor UV-induced mutability in enterobacteria raises a number of questions concerning the evolution and selective advantages of genes that enhance cellular mutability and resistance to DNA-damaging treatments. Firstly, the limited incidence of *umuCD*-like sequences supports earlier proposals that the gene is, or once was, part of a transposon (27, 29, 60, 61), which has recently invaded the genomes of some species of *Escherichia*. Many genes analogous to *umuC*$^+$*D*$^+$ in their involvement in mutagenic repair (27, 30) are components of multiple-transposon derived plasmids. They may, therefore, be classified with other transposon genes giving cellular

resistance to a particular type of environmental hazard. However, preliminary experiments to detect transposition by co-integrate formation with the cloned *umuC*$^+$*D*$^+$ gene of pSK100 have proved unsuccessful (unpublished observation).

In the evolution of *umuCD* and related sequences, the additional resistance to DNA damage by lesion tolerance would be expected to be advantageous. The fact that this mechanism is different from other DNA repair processes would be expected to increase this selective advantage. What then is the evolutionary role of the induced mutagenesis ensuing from this process?

Two views of mutagenesis have been forwarded. One envisages an optimal mutation frequency determined by a balance between potential gains in fitness arising through the generation of variation and loss of fitness due to deleterious mutagenesis (62). Since an inducible mutagenesis system is involved, a mechanism of "inducible evolution" has been invoked (63). The mutations produced have been envisaged either to increase the immediate fitness of the organism to withstand the treatment inducing them or to increase fitness later in changed environmental conditions. However, the notion of optimal evolutionary rates of induced mutagenesis is difficult to reconcile with observations of large differences in induced mutability in closely related species enjoying similar life styles. For example, *E. coli* with normal UV-induced mutability was isolated from the same sample of tamarin feces as poorly mutable *Proteus rettgeri* MH10.

The second view of mutagenesis is that it is an incidental product of a repair system whose primary selective advantage is enhanced survival. In terms of the tolerance mechanism proposed for inducible mutagenic repair, the selective advantage gained by survival enhancement would be increased by providing a repair activity different from other systems and would be determined by two opposing factors: the energy cost of DNA replication on damaged template DNA and the production of deleterious mutations. At its extreme this model envisages mutagenesis as an incidental by- product of a larger and more immediate effect of survival enhancement by damage tolerance. However, neither view of mutagenesis excludes the other. Experiments different from those yet described are needed to determine the relative selective merits of immediate survival enhancement by *umuCD* activity and the longer term benefits of genetic variation by induced mutagenesis.

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