

Reversion studies with *exrB* in *Escherichia coli*

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SUMMARY

exrB derivatives of H/r30 and WP2 are sensitive to ultraviolet radiation (UV), methylmethane sulphonate (MMS) and will not form colonies at 42 °C. Revertants to wild type were selected by plating on either nutrient plates containing MMS or nutrient plates incubated at 42 °C. Revertants selected for MMS resistance were temperature- and UV-resistant, and all revertants selected for temperature resistance were MMS and UV resistant. The frequency with which revertants appeared on MMS plates was about eight times as high as it was at 42 °C, and the variance in frequency of revertants among cultures started with small inocula was higher on selection at 42 °C than on MMS. The spontaneous frequency was estimated to be 10^{-6} to 10^{-7} , sufficiently low to suggest a single mutational event. Among wild-type revertants selected at 42 °C there was a stable and an unstable class. *exrB*⁺ could be co-transduced with *malB*⁺ from stable revertants to an *exrB* strain, but could not from unstable revertants. None of the revertants could support the growth of amber or ochre mutants of phage T4, nor were amber and ochre suppressed *exrB* strains wild type with regards MMS and temperature resistance. The presence of a polarity suppressor in an *exrB* strain did not modify resistance to UV or temperature. Neither UV or MMS was able to induce mutations in an *exrB* strain.

1. INTRODUCTION

exrB is a mutation, discovered in *Escherichia coli* strain B, co-transducible with *malB* and conferring sensitivity to ultraviolet (UV) radiation and to monofunctional alkylating agents (Greenberg, Berends, Donch & Green, 1974). When it occurs in or is transferred to unsuppressed *lon* strains of *E. coli*, these form non-septate filaments without treatment by UV or any added chemicals, indicating a defect in the regulation of cell division. When *exrB* occurs in *lon*⁺ or suppressed (*sul*) *lon* strains, cell division is normal but at elevated temperatures synthesis of DNA is inhibited, filaments are formed, and cell death ensues (Greenberg & Donch, 1974). In this last respect the phenotype of *exrB* resembles that of temperature sensitive mutants of *dnaB*, which are also co-transducible with *malB* (Kohiyama *et al.* 1966; Wechsler & Gross, 1971; Wechsler *et al.* 1973). While no mutant of *dnaB* has been reported to be UV-sensitive, one has been found to be sensitive to X-rays (Fangman & Russel, 1971).

In most respects tested the phenotype of *exrB* strains is the same as that of

exrA (and *lex*) strains (Donch & Greenberg, 1974*a, b*). For instance, in *exrB* strains, as in *exrA* strains, the UV induction of prophage is delayed and the proportion of cells induced is reduced; the frequency of recombinations in sexual and transductional crosses in which *exrB* strains are recipient is moderately reduced; and, endogenous DNA is degraded following UV. Like *lex* (Mount, Low & Edmiston, 1972) and *exrA* (Donch & Greenberg, 1974*b*), *exrB* is dominant to the wild-type allele in heterodiploids (Greenberg, Donch & Berends, 1974).

It is possible that the phenotypic expressions of *exrB* result from two mutations in closely linked cistrons, such as a coincidental mutation in *exrA* and the nearby *dnaB* cistrons. Alternatively, the phenotype ascribed to *exrB* could result from a deletion including both of these cistrons or to a mutation in one of these cistrons having a polarity effect on the other. The demonstration that reversions to wild type occur at a frequency ascribable to one mutational event would eliminate some of these possibilities. This will be shown in this report, together with the fact that *exrB* is not a nonsense, nor likely a frame-shift mutation, precluding its possibly having a polarity effect on *dnaB*.

2. METHODS

The strains of *E. coli* used, their relevant markers and source, are given in Table 1. The media used and methods employed for growth of bacteria, for performing transductions, and for determining UV-sensitivity have been described (Donch & Greenberg, 1968*a, b*). Mutagenesis studies were performed by previously described methods (Kondo, Ichikawa, Iwo & Kato, 1970).

Table 1. *Strains of bacteria used*

Strain	Relevant markers	Source
PAM 26	<i>lon exrB malB</i>	This laboratory
PAM 27	<i>lon exrB malB⁺</i>	P1.B251 × PAM 26
PAM 28	<i>lon exrB malB⁺ (sul)</i>	Spontaneous mutant of PAM 26
H/r30	<i>lon sul malB arg</i>	E. Witkin
PAM 453	<i>lon sul exrB malB⁺ arg</i>	P1.PAM 27 × H/r30
WP2	<i>lon sul malB trp</i>	E. Witkin
PAM 442	<i>lon sul exrB malB⁺ trp</i>	P1.PAM 27 × WP2
POP901	<i>malB trp</i>	M. Hofnung
POP913	<i>malB trp⁺ suA</i>	M. Hofnung
PAM 117	<i>malB⁺ exrB trp</i>	P1.PAM 27 × POP901
PAM 118	<i>malB⁺ exrB trp⁺ suA</i>	P1.PAM 27 × POP913

Abbreviations are as recommended by Demerec *et al.* (1966).

3. RESULTS

(i) *Reversion of PAM 453 to wild type*

From each of ten cultures of PAM 453 (*exrB arg*) originating from a single clone and grown 18 h at 37 °C in JN broth, 2 × 10⁷ cells were spread on JN agar plates with and without 0.04 % MMS (K and K Chemical Co.). The former were incubated at 42 °C the latter at 33 °C. Preliminary experiments established that wild-

type H/r30 survived as well on MMS agar plates as it did on JN agar plates and that all survivors of PAM 453 on MMS agar plates were UV as well as MMS-resistant.

The number of colonies surviving each treatment, MMS and 42 °C, is shown in Table 2. Purified clones were grown in JN broth at 33 °C for 18 h and tested for resistance to MMS and UV and for growth at 42 °C. All clones which survived purification were resistant to all three stresses. Therefore, resistance to all three were inherited concomitantly and none of the 375 resistant clones examined were resistant to one but not the others.

Table 2. *Reversions to methylmethane sulphonate and temperature resistance in PAM 453*

Culture no.	No. of resistant colonies: Selection	
	MMS	42 °C
1	23	0
2	40	0
3	39	0
4	27	2
5	31	0
6	34	7
7	53	1
8	30	28
9	27	0
10	30	1
Total	336	39

There were about eight times as many survivors on MMS agar plates as on JN plates incubated at 42 °C (Table 2). It is also apparent that the variance from culture to culture in number of survivors was relatively less on MMS agar plates than plates incubated at 42 °C. The relative uniformity in numbers of survivors among cultures plated on MMS agar plates and the fact that there were eight times as many on MMS agar plates as plates incubated at 42 °C, suggests that growth at 42 °C merely selected spontaneous mutants already present in the cultures, whereas MMS might induce such mutations as well as selecting them. We have tested the mutagenic response of H/r30 and PAM 453 to MMS and were not able to demonstrate that MMS (0.04 %) was mutagenic in PAM 453, though it was mutagenic for H/r30 (data not shown). We must conclude, then, that both MMS and 42 °C select for spontaneous revertants. The frequency of spontaneous mutations to *ExrB*⁺ phenotype is estimated from the data to be 10⁻⁶ to 10⁻⁷. This frequency is sufficiently high to exclude the possibility that we were observing the resultant of two independent, simultaneous mutations.

All the recombinants were tested for their ability to support the growth of a series of amber and ochre mutants of phage T4. None were able to do so. Furthermore, none of 28 independently isolated *arg*⁺ revertants of the PAM 453 were

resistant to UV, MMS or to inhibition of growth at 42 °C, though 25 were able to support the growth of T4 *am* 882. *exrB* is thus clearly not an amber nonsense mutation. Similar experiments were done with PAM 442 (*exrB trp*). None of 50 independently isolated revertants to UV and temperature-resistance were *trp*⁺ and, whereas, most of the *trp*⁺ revertants isolated were able to support T4 ochre

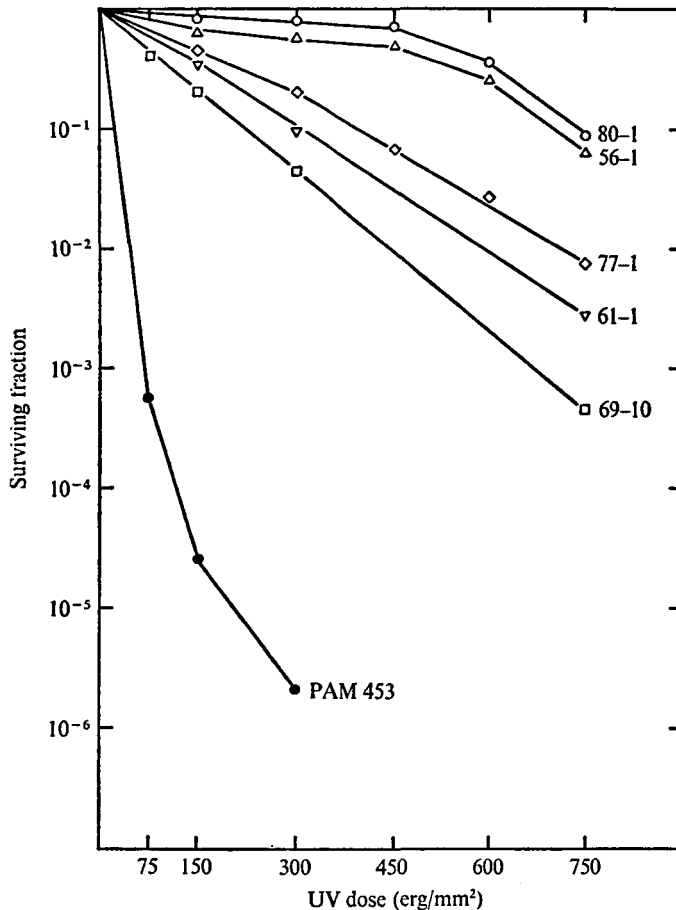


Fig. 1. Fraction of colony formers as a function of UV dose in PAM 453 and some of its mutants selected for their ability to form colonies at 42 °C.

mutants, none were MMS-, UV- or temperature-resistant. *exrB* is, therefore, not an ochre nonsense mutation and not being a nonsense mutation would not likely exert any polarity effect (Drake, 1970).

(ii) *Stability of revertants selected for temperature resistance*

In the course of isolating and purifying ten independent temperature-resistant mutants of PAM 453, it was observed that five of these were slightly less resistant

to UV than wild-type H/r30. The UV survival curves of two fully UV-resistant and three partially UV-resistant revertants are shown in Fig. 1. It was suspected that the latter isolates might result from a mutation at a suppressor locus and might be unstable. Unstable suppressor mutations have been reported for *E. coli* (Hill, 1963; Gundersen, 1963; Schwartz, 1967*a, b*; Brody & Yanofsky, 1965; Hill, Foulds, Soll & Berg, 1969) and have been attributed to heterogeneity in a suppressor mutation either because of an episome or because of a redundancy in the suppressor cistron. Ten revertants were grown in JN broth at 33 °C and 50 clones randomly picked. Revertants were examined through one to five serial passages and clones at the end of each passage examined for UV and temperature resistance. It is seen in Table 3 that five isolates appeared stable and five were unstable. Stable and unstable revertants were also observed with PAM 442.

Table 3. *Stability of temperature resistant revertants of PAM 453*

Revertant no.	% UV- and temperature-resistant:*				
	passage number†				
	1	2	3	4	5
56-1	100	100	—	—	—
68-45	100	100	—	—	—
80-1	100	100	100	—	—
73-3	100	100	100	—	—
62-3	100	100	100	—	—
63-4	76	68	—	—	—
69-10	58	86	96	90	76
61-1	70	74			
77-1	74				
67-38	72				

* Fifty clones grown 18–24 h in JN broth at 37 °C. Tested by spot test.

† Each passage from a culture which tested as UV-resistant.

Phage P1 was grown on a stable revertant of PAM 453 and was used to transduce PAM 28 (*malB exrB sul*) to Mal⁺. Thirty-seven per cent of these were as resistant to UV as strain B/r. This would happen if *exrB*⁺ had been co-transduced with *malB*⁺ from the revertant to PAM 28. The cotransductional frequency of *exrB*⁺ and *malB*⁺ was similar to that achieved in transductions in which known *exrB*⁺ strains, e.g. B/r, were used as donors. It is clear that stable revertants to wild-type phenotype of PAM 453 likely resulted from the mutation *exrB* to *exrB*⁺. Similar results were obtained with a stable revertant of PAM 442.

Attempts to transduce UV resistance together with *malB*⁺ from the unstable revertant 69-10 to PAM 28 were unsuccessful, none of 100 Mal⁺ transductants examined being more resistant to UV than PAM 28. When strain B was used as a recipient, 37% of the transductants were UV-sensitive. An interpretation of these results is that *exrB* was not mutated in unstable strains, the resistant phenotype resulting from a mutation at a suppressor locus not yet mapped and not co-transducible with *malB*.

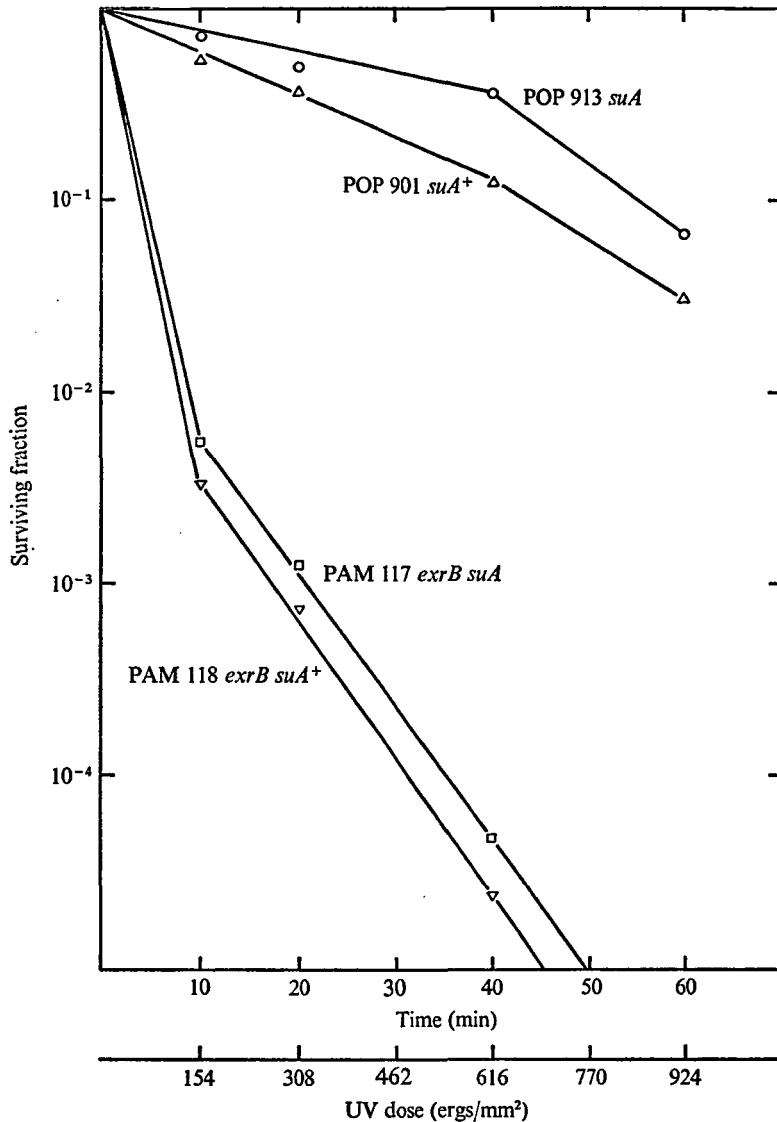


Fig. 2. Fraction of colony formers as a function of UV dose in *exrB* derivatives of POP901 *trp* and POP913 *trp*⁺ *suA*.

(iii) *Effect of a polarity suppressor on exrB*

To further test the possibility that the temperature sensitivity of *exrB* was the result of a polarity effect on an adjacent cistron (*dnaB*), we examined the phenotype of *exrB* in POP901 and POP913. Both of these strains were *malB*, POP901 being *trp* and its mutant POP913 *trp*⁺ as a result of a mutation in the polarity suppressors *suA*⁺ to *suA* (Morse & Primakoff, 1970; Hofnung, 1974). *exrB* was introduced into each of these strains by co-transduction with *malB*⁺ with P1 grown on PAM 27 (*exrB malB*⁺). *exrB* (UV^s) was observed in about 5% of each

of the strains. On purification a representative of each transductional class was designated PAM 117 (*exrB suA*) and PAM 118 (*exrB suA*⁺). As seen in Fig. 2, there was no significant difference between the UV survival curves of either the *su*⁺ or *su* derivatives of the *exrB*⁺ or *exrB* strains. The significant observation was, however, that both PAM 117 and PAM 118 were temperature-sensitive. From this we conclude that the temperature-sensitive manifestation of *exrB* was not modified by the polarity suppressor *suA*.

Table 4. *arg*⁺ revertants of H/r30 and PAM 453 resulting from UV irradiation of cells plated on semi-enriched medium

Organism	UV dose (ergs/mm ²)	No. of organisms treated	No. of organisms surviving treatment	No. <i>arg</i> ⁺ revertants	Revertant frequency
H/r30	0	—	1 × 10 ¹¹	122	1.2 × 10 ⁻⁹
H/r30	5	7.2 × 10 ⁹	7.2 × 10 ⁹	690*	9.5 × 10 ⁻⁸
H/r30	20	2.4 × 10 ⁹	2.2 × 10 ⁹	5700*	2.6 × 10 ⁻⁶
PAM 453	0	—	4.0 × 10 ¹⁰	120	3.0 × 10 ⁻⁹
PAM 453	2	8.6 × 10 ⁹	1.7 × 10 ⁹	10*	5.8 × 10 ⁻⁹
PAM 453	5	8.6 × 10 ⁹	4.3 × 10 ⁸	<1*	<2.3 × 10 ⁻⁹

* Corrected for spontaneous reversions.

(iv) *Effect of exrB on UV-induced reversions to arg*⁺

One of the phenotypic properties of *exrA* and *recA* strains is the preclusion in them of UV-induced mutagenesis (Witkin, 1969). That this is also a property of PAM 453 can be seen from the data in Table 4. The frequency of the mutation *arg* to *arg*⁺ was enhanced by UV in parental H/r30, in the experiment shown, by a factor of about 80-fold the spontaneous frequency at a UV dose of 5 ergs/mm² and enhanced 2000-fold at a UV dose of 20 ergs/mm². With PAM 453 exposed to UV doses of 2 and 5 ergs/mm², no significant increase in reversion to *arg*⁺ above spontaneous was observed. We conclude that in *exrB* strains as in *exrA* strains there is no enhancement of mutagenesis following treatment with UV.

4. DISCUSSION

Three genes linked to *malB* (*exrA*, *wvrA*, and *dnaB*) have phenotypic properties shared in part by *ExrB* strains. Revertants of *lex* (*exrA*) have been isolated which extends the similarity between *exrA* and *exrB* mutations (Mount, Walker & Kosel, 1973). When UV-resistant revertants of *lex* were isolated, two types of revertants were obtained in approximately equal proportions. One class of UV-resistant revertants appears to be true *lex*⁺ revertants and the other class carries the additional property of temperature sensitivity. The mutation of the latter class, *tsl*, cannot be separated from *lex* by genetic means and most likely represents a second mutational event in the *lex* gene. Like *tsl*, *exrB* strains are temperature-sensitive, forming filaments at the restrictive temperature. However, *exrB* strains are clearly distinct from *tsl* strains for they are UV-sensitive and possess many of the

properties associated with the *lex* mutation. Furthermore, in crosses between *exrA* and *exrB* strains the frequency of wild-type recombinants is high, indicating separate positions on the chromosome (Greenberg, Berends, Donch & Green, 1974). We have reported in this paper that *exrB* strains revert to wild type at a frequency of 10^{-6} to 10^{-7} . Therefore the possibility that the ExrB phenotype is the result of multiple mutations in genes of the *malB* region (e.g. *exrA* and *dnaB*) is nil.

A significant property of *exrB* is that strains into which it is transduced (WP2, H/r30, K12) are temperature-sensitive (Greenberg & Donch, 1974). The failure to form colonies at 42 °C is associated with an almost immediate cessation of DNA synthesis, though growth continues, leading to non-dividing filaments. The immediate inhibition of DNA synthesis suggests a failure to elongate DNA rather than a block in initiation of synthesis. In these respects the *exrB* phenotype is identical with that of *dnaB* (Wechsler & Gross, 1971). Since *dnaB*, too, is closely linked to *malB*, there were the possibilities that *exrB* is an allele of *dnaB* or that a mutation in *exrB* exerted a polarity effect on *dnaB*. The latter possibility can be excluded by two lines of evidence reported in this paper. First, polarity effects are usually exerted by nonsense mutations or possibly frameshift mutations (Drake, 1970). *exrB* is not an amber or ochre nonsense mutation because it is suppressible by neither amber nor ochre suppressors. Secondly, in a strain carrying a polarity suppressor *suA* the *exrB* phenotype was still temperature-sensitive.

As to whether *exrB* is an allele of *dnaB* – this seems unlikely because we have found *exrB* and *dnaB* form wild-type recombinants at a high frequency in crosses between strains bearing each of them (unpublished). The fact that no *dnaB* mutant has been reported to be UV-sensitive only suggests that *exrB* is not an allele of *dnaB*, since we have little information on how intensively, if at all, this pleotropic effect has been investigated. That it has been investigated we know from the report of Fangman & Russel (1971), who found X-ray but not UV sensitivity in one *dnaB* strain. It is also reported that the substantial number of *dnaB* mutants isolated form only one complementation group (Wechsler *et al.* 1973). Furthermore, in *in vitro* assays the *dnaB*⁺ product will not restore the capacity for DNA synthesis in extracts of *exrB* strains (R. Schekman, personal communication).

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REFERENCES

- BRODY, S. & YANOFSKY, C. (1965). Mechanism studies of suppressor-gene action. *Journal of Bacteriology* **90**, 687-695.
- DEMEREK, M., ADELBERG, E. A., CLARK, A. J. & HARTMAN, P. E. (1966). A proposal for a uniform nomenclature in bacterial genetics. *Genetics* **54**, 61-76.
- DONCH, J. J. & GREENBERG, J. (1968*a*). Loci of radiation sensitivity in Bs strains of *Escherichia coli*. *Genetical Research, Cambridge* **11**, 183-191.
- DONCH, J. & GREENBERG, J. (1968*b*). Ultraviolet sensitivity gene of *Escherichia coli* B. *Journal of Bacteriology* **95**, 1555-1559.

- DONCH, J. J. & GREENBERG, J. (1974*a*). The effect of *lex* on UV sensitivity, filament formation and λ -induction in *lon* mutants of *Escherichia coli*. *Molecular and General Genetics* **128**, 277–281.
- DONCH, J. & GREENBERG, J. (1974*b*). The dominance of *exrA* in *Escherichia coli*. *Molecular and General Genetics* (in the Press).
- DRAKE, J. W. (1970). *The Molecular Basis of Mutation*. San Francisco, London, Cambridge, Amsterdam: Holden-Day.
- FANGMAN, W. L. & RUSSEL, M. (1971). X-irradiation sensitivity in *Escherichia coli* defective in DNA replication. *Molecular and General Genetics* **110**, 332–347.
- GREENBERG, J., BERENDS, L. J., DONCH, J. & GREEN, M. H. L. (1974). *exrB*: A *malB*-linked gene in *Escherichia coli* B involved in sensitivity to radiation and filament formation. *Genetical Research, Cambridge* **23**, 175–184.
- GREENBERG, J. & DONCH, J. (1974). Sensitivity to elevated temperatures in *exrB* strains of *Escherichia coli*. *Mutation Research* **25**, 403–405.
- GREENBERG, J., DONCH, J. J. & BERENDS, L. (1975). The dominance of *exrB* over *exrB*⁺ in heterodiploids of *Escherichia coli*. *Genetical Research, Cambridge* **25**, 39–44.
- GUNDERSEN, W. B. (1963). New type of streptomycin resistance resulting from action of the episome-like mutator factor in *Escherichia coli*. *Journal of Bacteriology* **86**, 510–516.
- HILL, R. F. (1963). The stability of spontaneous and ultraviolet-induced reversions from auxotrophs in *Escherichia coli*. *Journal of General Microbiology* **30**, 289–297.
- HILL, R. F., FOULDS, J., SOLL, L. & BERG, P. (1969). Instability of a missense suppressor resulting from a duplication of genetic material. *Journal of Molecular Biology* **39**, 563–581.
- HOFNUNG, M. (1974). Divergent operons and the genetic structure of the maltose B region in *Escherichia coli* K12. *Genetics* **76**, 169–184.
- KOHIYAMA, M., COUSIN, D., RYTER, A. & JACOB, F. (1966). Mutants thermosensibles d'*Escherichia coli* K12. 1. Isolement et caractérisation rapide. *Annals Institute Pasteur* **110**, 465–496.
- KONDO, S., ICHIKAWA, H., IWO, K. & KATO, T. (1970). Base-change mutagenesis and prophage induction in strains of *Escherichia coli* with different DNA repair capacities. *Genetics* **66**, 187–217.
- MORSE, D. E. & PRIMAKOFF, P. (1970). Relief of polarity in *E. coli* by '*suA*'. *Nature* **226**, 28–31.
- MOUNT, D. W., LOW, K. B. & EDMINSTON, S. J. (1972). Dominant mutations (*lex*) in *Escherichia coli* K-12 which affect radiation sensitivity and frequency of ultraviolet light-induced mutations. *Journal of Bacteriology* **112**, 886–893.
- MOUNT, D. W., WALKER, A. C. & KOSEL, C. (1973). Suppression of *lex* mutations affecting deoxyribonucleic acid repair in *Escherichia coli* K-12 by closely linked thermosensitive mutations. *Journal of Bacteriology* **116**, 950–956.
- SCHWARTZ, N. M. (1967*a*). Revertant instability in *Escherichia coli*. I. Mutagen and allele specificity. *Genetics* **57**, 495–503.
- SCHWARTZ, N. M. (1967*b*). Revertant instability in *Escherichia coli*. II. Genetic studies. *Genetics* **57**, 505–512.
- WECHSLER, J. A. & GROSS, J. D. (1971). *Escherichia coli* mutants temperature-sensitive for DNA synthesis. *Molecular and General Genetics* **113**, 273–284.
- WECHSLER, J. A., NÜSSLEIN, V., OTTO, B., KLEIN, A., BONHOEFFER, F., HERRMANN, R., GLOGER, L., & SCHALLER, H. (1973). Isolation and characterization of thermosensitive *Escherichia coli* mutants defective in deoxyribonucleic acid replication. *Journal of Bacteriology* **113**, 1381–1388.
- WITKIN, E. M. (1969). Ultraviolet-induced mutation and DNA repair. *Annual Review of Microbiology* **23**, 487–514.