DIFFERENTIAL INDUCTION AND REPAIR OF ULTRAVIOLET DAMAGE LEADING TO TRUE REVERSIONS AND EXTERNAL SUPPRESSOR MUTATIONS OF AN OCHRE CODON IN *ESCHERICHIA COLI* B/r WP2

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MUCH of the published work on the mechanisms by which radiation-induced lesions result in genetic mutations in bacteria has involved the reversion to prototrophy of amino-acid requiring strains, in particular the tryptophan auxotroph *Escherichia coli* B/r WP2 isolated by WITKIN. It has been realised for some time that mutant prototrophs are not all true reversions to wild type. HILL (1963), for example, found that some spontaneous and ultraviolet-induced prototrophs of B/r WP2 grew more slowly and were unstable. She suggested that they might be suppressor mutants. WITKIN (1963) also gave evidence that suppressor mutations, capable of overcoming at a single step auxotrophies at several loci, were induced by ultraviolet light (UV) under certain conditions. Unfortunately the difficulty in performing genetic analysis in the B family of *E. coli* has hitherto precluded a study of the involvement of true reversion and suppressor mutation in the several phenomena associated with radiation mutagenesis. Recently BRIDGES, DENNIS and MUNSON (1967) showed that many prototrophs derived from B/r WP2 contained suppressors capable of suppressing chain terminating codons in T4 phage, and a simple way of distinguishing suppressor mutants from true revertants was described. (Chain terminating codons UGA, UAG ("amber") and UAA ("ochre") appear to code for no amino acid and thus prevent the assembly of the polypeptide chain beyond the point at which they are situated in the messenger RNA.)

In this paper we show that the suppressors in B/r WP2 are all ochre suppressors from which we infer that the parent B/r WP2 has an ochre (UAA) codon at a tryptophan locus. A similar conclusion has been drawn by OSBORN and PERSON (1967). We also investigate the induction of true and suppressor mutations by UV under different conditions, and compare their relative susceptibility to various processes which have been described for the removal (or bypassing) of genetic damage.

MATERIALS AND METHODS

Organisms: Escherichia coli B/r WP2 Hcr+ tryptophan-requiring, originally isolated by DR. EVELYN WITKIN, was obtained from DR. C. O. DOUDNEY in 1959. A derived strain deficient in excision repair, *E. coli* B/r WP2 Hcr−, was kindly supplied by DR. RUTH HILL. Chain terminating

TABLE 1

Mutants of phage T4 used for testing suppressors

Number	Type	Locus
B22	amber	DNA polymerase
B17	amber	coat protein
B272	amber	coat protein
N24	ochre	*rII*
AP53	ochre	*rII*
360	ochre	*rII*

mutants of phage T4 were obtained from DR. LESLIE BARNETT and DR. C. CLARKE and are listed in Table 1. Bacteria designated RRU were isolated in our laboratory, other strains of *E. coli* were obtained from DR. EVELYN WITKIN.

Microbiological techniques: Bacteria were grown to mid-logarithmic phase ($\sim 2 \times 10^8$/ml) in "M" glucose-salts medium of HAAS and DOUDNEY (1957) with 10 μg/ml tryptophan. They were normally plated on "M" agar plates supplemented with 0.75 μg/ml tryptophan or (where stated) with 5% v/v Oxoid nutrient broth. When about 5×10^7 bacteria were spread on to these plates, prototrophic mutants appeared after 48 hours as distinct colonies in a lawn of residual growth of the auxotrophs. At lower plating densities ($<10^3$ bacteria per plate) the auxotrophs formed small individual colonies.

Initially, the ability of strains to support phage growth was tested using the standard layer plate technique. Subsequently a more rapid routine method was adopted with phage B22. About 10^7 phage were spread on to the surface of a dried "M" plate. By means of a loop a small amount of growth was removed from colonies to be tested, emulsified in a drop of "M" medium and streaked on to a marked area of the plate. Bacteria unable to support the phage formed a thick streak of growth similar to that formed by *E. coli* B/r. Ability to support the phage was revealed by the nonappearance of growth or by a weak, obviously partly lysed streak.

Mutagenesis: UV was from a low pressure mercury vapour lamp at a dose rate of either 1.4 or 3.3 erg mm^{-2} sec^{-1} depending on the distance between source and sample.

Methods for the induction of mutants by UV in frozen bacteria have been described elsewhere (ASHWOOD-SMITH and BRIDGES 1966).

A technique was developed for inducing mutations with hydroxylamine without appreciable lethality. Logarithmic phase bacteria were suspended in 5 ml phosphate buffer (pH6) which was then added to 95 ml of the same buffer at room temperature containing hydroxylamine to a final concentration of 2×10^{-2} M and which had been bubbled with oxygen-free nitrogen for 10 min. Bubbling was continued for a further 20 min and then enough acetone was added (as a concentrated aqueous solution) to make a final concentration of 5%. The whole suspension was passed through a membrane filter and the bacteria washed with "M" medium and resuspended at a concentration of about 5×10^8/ml. The mutant frequency after this treatment was about 1 in 10^7.

Photoreversal: A 250 watt photoflood lamp was used with a filter cutting out light below 3,900 Å thus largely eliminating wave-lengths causing "indirect" photoreversal, see e.g. WITKIN (1966a). The dose rate was approximately 2,000 ergs mm^{-2} sec^{-1}.

RESULTS

The nature of the mutation: (i) *E. coli* B/r WP2. The existence of suppressors of chain terminating codons in prototrophic mutants was determined by observing whether or not growth of T4 phage bearing amber mutations could occur. Among 50 prototrophic mutants initially tested (both spontaneous and induced)

33 supported phage B22 which is suppressible by weak suppressors (amber or ochre). None supported phages B17 or B272 which require strong suppressors (i.e. probably amber). It was therefore clear that many of the prototrophic mutants contained weak (probably ochre) suppressors of chain-terminating codons. All 33 were slower growing than *E. coli* B/r to a greater or lesser degree when tryptophan was not supplied, although in the presence of tryptophan the growth rate was close to that of the parent auxotroph.

Sixteen of the remaining 17 prototrophs not supporting B22 grew as fast as the parent whether or not tryptophan was supplied. We have no reason to believe that these are not true revertants at a chain terminating codon in WP2.

Supporting evidence that WP2 contains a chain-terminating codon came from the use of hydroxylamine as a mutagen. At pH 6, hydroxylamine appears to cause only G-C→A-T transition in phage (CHAMPE and BENZER, 1962). Although this has not been fully established in bacteria, if the same principle were to hold it would follow that the ochre codon UAA (which at the DNA level is exclusively A-T base pairs) would not be mutated, and codons UAG and UGA would only mutate to UAA, also chain-terminating. There should therefore be no true hydroxylamine-induced revertants at a chain termining codon. Of 24 prototrophic mutants of WP2 induced by hydroxylamine, all were lysed by phage B22. The level of spontaneous mutants was such that only one spontaneous true revertant would be expected on the average in 24 tested colonies. The result is consistent with our hypothesis and also indicated that, at least under some conditions, hydroxylamine may induce G-C→A-T transitions in bacteria as well as phage. To determine whether the suppressors were ochre or amber it was necessary to test with an ochre T4 mutant. All the ochre mutants available to us were at the *rII* locus and as this locus is only expressed in the presence of λ prophage, *E. coli* WP2 was made lysogenic for λ and prototrophic mutants were induced by UV. Of mutants tested all supported the growth of ochre phages N24, AP53 and 360 and thus contained ochre suppressors. In the absence of any amber suppressors among revertants it is a reasonable inference that WP2 contains an ochre codon at a tryptophan locus.

(ii) Other strains of *E. coli*. Prototrophic revertants of several other auxotrophic strains of *E. coli* have been examined for ability to support growth of amber phages B17 and B22 (Table 2). All strains gave rise to some prototrophic suppressor mutants capable of growing phage containing chain-terminating codons.

Mutagenesis by UV in unfrozen bacteria: The majority of mutants induced by UV at room temperature in *E. coli* B/r WP2 are suppressor mutants (Figure 1), in agreement with the supposition of HILL (1963) and WITKIN (1963). Nevertheless on tryptophan-supplemented plates some true revertants (10 to 15%) were observed. Examination of the dose response curve reveals the interesting fact that true revertants are induced linearly with the UV dose, whereas the suppressor mutants are induced according to the square of the dose. Thus at high doses the *proportion* of true revertants is negligible and the overall response curve for all mutants is close to a dose-squared proportionality. As with ionizing radia-

TABLE 2

Strains of B/r whose prototrophic mutants were tested for the presence of chain-terminating suppressors

Strain	Phenotype	Some prototrophs lysed by		Presumed mutant codon
		B22	B17	
RRU 35	Leu⁻	+	+	amber
RRU 14	Leu⁻	+	+	amber
RRU 24	His⁻	+	+	amber
B 19/r	Pro⁻	+	+	amber
RRU 5	Met⁻	+	+	amber
H/r 30	Arg⁻	+	+	amber
WP2	Try⁻	+	—	ochre
WU36	Tyr⁻	+	—	ochre

tion (BRIDGES, LAW and MUNSON, in preparation), true revertants induced by UV in this strain appear to owe their origin to a single energy-absorption event, whereas two independent events appear to be involved in the formation of suppressor mutants by UV.

E. coli WP2 Hcr⁻ is a strain sensitive to UV isolated by RUTH HILL (1965). It cannot perform host cell reactivation of UV-irradiated phage and is able to excise thymine dimers from its DNA at less than 1/100th the rate of the Hcr⁺ strain (R. B. SETLOW, personal communication). It is more mutable than the Hcr⁺ strain (HILL 1965; ASHWOOD-SMITH and BRIDGES 1966) and this increased

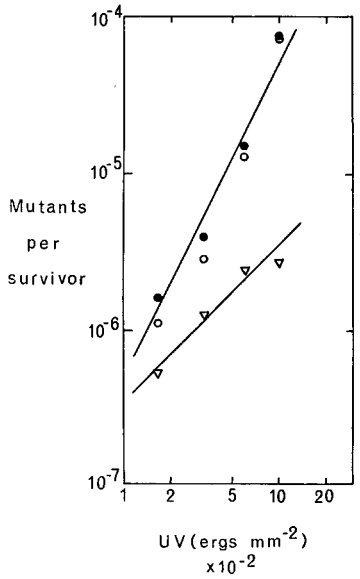

FIGURE 1.—Induction of prototrophic mutants (total, ●; suppressor, ○; true, ▽) of *E. coli* WP2 Hcr⁺ by UV at room temperature.

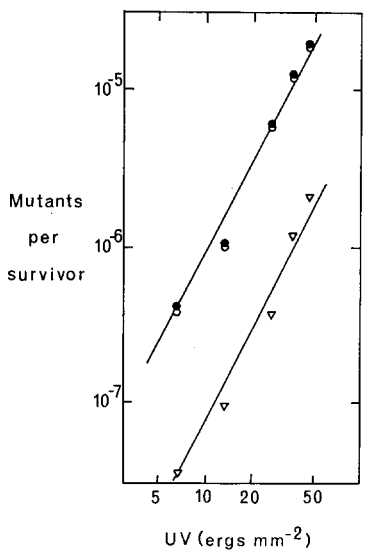

FIGURE 2.—Induction of prototrophic mutants of *E. coli* WP2 Hcr⁻ by UV at room temperature. (Symbols as for Figure 1.)

sensitivity appears to apply to damage leading to both true and suppressor mutations (Figure 2). The UV damage which leads to both true and suppressor mutations in the Hcr⁻ strain appears therefore to be excisable. An interesting difference from *E. coli* B/r WP2 is the dose-squared response curve for true as well as suppressor mutations in *E. coli* WP2 Hcr⁻.

UV mutagenesis of frozen bacteria: When UV is given to *E. coli* frozen at −79°C, much greater mutability is observed (ASHWOOD-SMITH and BRIDGES 1966). Examination of the dose response curve for *E. coli* WP2 Hcr⁺ (Figure 3) exposed at −79°C revealed marked differences from that with unfrozen bacteria (Figure 1). The proportion of true revertants was higher (as much as 45% in some experiments) and suppressor mutants as well as true revertants followed a dose response curve that was more nearly linear than dose-squared.

Quite unlike WP2 Hcr⁺, the repair-deficient WP2 Hcr⁻ when frozen demonstrated a response curve for UV which resembled that for unfrozen Hcr⁻ bacteria in having a low proportion of true revertants which increased in proportion to the square of the dose. The induction of both true and suppressor mutants was two to three times more sensitive to UV at −79°C than at 22°C.

Susceptibility of premutational damage to mutation frequency decline: One of the more puzzling features of UV mutagenesis is "mutation frequency decline" which itself is a manifestation of the "broth effect." These phenomena may be briefly described as follows. If UV irradiated bacteria are exposed to conditions tending to decrease RNA and protein synthesis (e.g. amino acid starvation, chloramphenicol treatment) fewer mutations are induced than under conditions

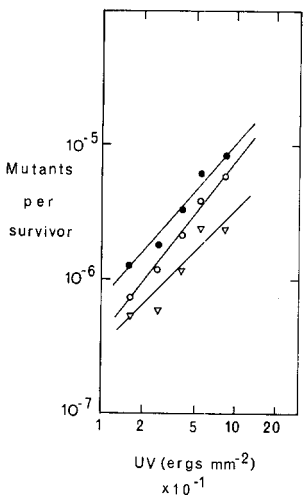

FIGURE 3.—Induction of prototrophic mutants by UV irradiation of *E. coli* WP2 Hcr⁺ at —79°C. (Symbols as for Figure 1.)

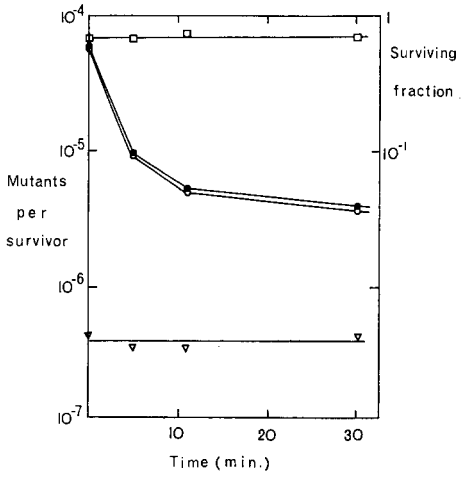

FIGURE 4.—Decline in mutation frequency as determined on broth-enriched plates during incubation of *E. coli* WP2 Hcr⁺ at 37° without tryptophan after UV irradiation; viability, □; total mutants, ●; suppressor mutants, ○; true revertants, ▽.

tending to encourage RNA and protein synthesis (e.g. supply of amino acids, nutrient broth). If auxotrophic bacteria are incubated in a minimal medium lacking the required amino acid and plated at intervals on plates enriched with a low level of nutrient broth, the number of mutant colonies subsequently appearing declines within 15 minutes or so of incubation to a plateau value one fifth or less of the initial value (depending on the degree of broth enrichment). The same low level is found for bacteria plated immediately after irradiation on minimal plates with a low level of tryptophan but no other amino acids (MUNSON and BRIDGES 1966).

The essentials of the phenomenon were described by WITKIN (1956) for mutation to prototrophy in *Salmonella typhimurium* and *E. coli* B/r (including WP2). The broth effect and mutation frequency decline (mfd) have also been demonstrated for mutation from Lac$^-$ to Lac$^+$ (WITKIN and THEIL 1960) but there is no appreciable effect with mutation to streptomycin resistance or streptomycin independence (WITKIN and THEIL 1960).

WITKIN (1963, 1966a, b) has postulated that mfd is a characteristic of mutations at suppressor loci which appear to be concerned with the translation of the genetic message into polypeptide chains. It is obviously of fundamental importance to know whether the broth effect is lesion-specific or locus-specific. This knowledge can only come from an exhaustive study of mfd in mutation systems known to give rise to either true or suppressor mutations. We can make a start by comparing true and suppressor mutations to prototrophy in B/r WP2, where both types are subject to the same environmental conditions for expression and selection.

Figure 4 shows very clearly that it is only suppressor mutants which respond to the stimulating effect of nutrient broth on RNA and protein synthesis.

It is noteworthy that the proportion of true revertants is highest (over 50%) where UV-irradiated bacteria are plated on unsupplemented minimal agar. Although this result cannot be compared quantitatively with those for enriched media, it provides confirmatory evidence that suppressor mutations are more susceptible than true reversions to conditions tending to inhibit protein synthesis.

Susceptibility of premutational damage to photoreversal: The photoreversal of the mutagenic action of UV on WP2 by treatment with light of longer wavelength immediately after irradiation has been studied intensively in recent years by WITKIN (1966a). She has shown that in WP2 Hcr$^+$ premutational UV damage susceptible to broth enrichment (i.e. that leading to suppressor mutants) is removed under the influence of light *indirectly by a dark repair system.* The splitting of pyrimidine dimers by the photoreactivation enzyme (SETLOW 1965) is not directly involved; removal of dimers in this direct way affects only the rate and not the extent of indirect photoreversal.

In WP2 Hcr$^-$ (which is deficient in dark repair) mutations are induced by lesions (mostly pyrimidine dimers) which in the normal strain would have been largely excised. Photoreversal in WP2 Hcr$^-$ is due to direct dimer splitting by the photoreactivation enzyme; indirect photoreversal of the dark repair type does not occur in this strain.

We have examined the proportions of true and suppressor mutants in both strains after UV irradiation with and without treatment with light of wavelengths which favour dimer-splitting rather than indirect repair. Approximately 200 colonies were picked off and tested at each point.

Figure 5 shows that both true and suppressor mutations were photoreversed to a considerable extent in *E. coli* WP2 Hcr$^-$ indicating the involvement of pyrimidine dimers in the production of both types of mutation. In *E. coli* WP2 Hcr$^+$ given the same light treatment (Figure 6) there was a much smaller reversal of both true and suppressor mutations, showing that photoreactivable dimers play a smaller role in the induction of both true and suppressor mutants in this strain.

DISCUSSION

It is clear from our results that *E. coli* B/r WP2, and all the other mutable auxotrophs examined have chain terminating codons at their respective auxotrophic loci. In the case of WP2 this would seem to be an ochre codon. The fact that no mis-sense or frame-shift mutations are found among those *E. coli* auxotrophs commonly used for mutation studies suggests the possibility that only mutants containing chain terminating codons mutate to prototrophy at an easily measurable rate. One reason for this probably lies in the fact that several suppressor loci may mutate as well as the chain terminating codon to give a prototrophic organism.

The ability of amber mutants of T4 to distinguish true from suppressor proto-

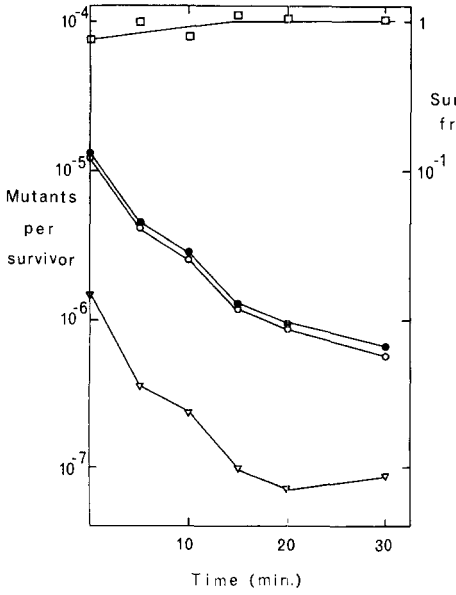

FIGURE 5.—Photoreactivation of *E. coli* WP2 Hcr$^-$ as a function of time of exposure to light. (Symbols as for Figure 4.)

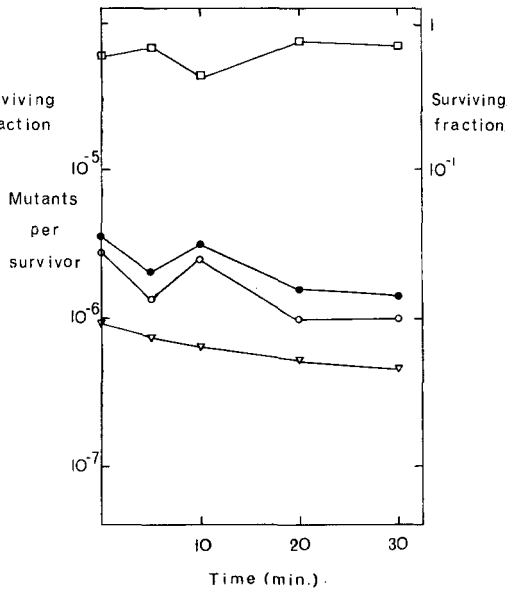

FIGURE 6.—Photoreactivation of *E. coli* WP2 Hcr+ as a function of time of exposure to light (Symbols as for Figure 4.)

trophs will undoubtedly be a useful tool in the field of mutagenesis, particularly mutagen specificity. For example, true revertants at the ochre codon in WP2 must be due to a coding change at an adenine-thymine base pair in the DNA, and if the change is a transition to guanine-cytosine it must be at the first position of the codon since transitions at the second and third positions would still yield chain terminating codons.

In the case of amber suppressor mutants it is already possible to make a tentative correlation between the specificity of certain mutagens and specific changes at the anticodons of various transfer RNA molecules (OSBORN, PERSON, PHILLIPS and FUNK 1967). Our knowledge of ochre suppressors is more rudimentary, only two groups being so far distinguishable in WP2 (OSBORN and PERSON 1967). It is likely that several different loci may be involved, some possibly coding ribosomal and not transfer RNA.

In our studies we have used the T4 amber mutant B22 to distinguish true revertants from ochre suppressors. This amber is suppressible even by very weak suppressors and probably detects all but a very few percent of the suppressor mutants of WP2 (authors' unpublished observations; PERSON and OSBORN, personal communication).

One of us has elsewhere (BRIDGES 1966) postulated that the dose-squared response obtained with UV mutagenesis at room temperature is due to the linear production with dose of two types of damage, A and B. Type A (pyrimidine dimer-type damage) was said to interfere with the repair of type B (the true premutational lesion) by the mutation frequency decline system. Under certain conditions a dose-squared response would be expected. The present experiments support this model for suppressor mutagenesis in the Hcr^+ strain. Only suppressor mutations show a dose-squared response and mfd. True mutations show neither.

The more nearly linear response, low proportion of suppressor mutants, and smaller broth effect observed when UV is given to frozen Hcr^+ bacteria may also be readily interpreted on this model. We may assume that type B damage, leading to both true and suppressor mutations, is greatly increased in the frozen state, whereas we know that type A damage (pyrimidine dimers) is reduced (ASHWOOD-SMITH, BRIDGES and MUNSON 1965; SMITH 1966; RAHN 1966; BRIDGES, ASHWOOD-SMITH and MUNSON 1967). The smaller amount of type A damage results in less inhibition of the mfd of type B damage with the consequence that fewer suppressor mutants appear and the dose-squared component of the response is small. Furthermore, if WITKIN is correct in her inverse correlation of dimer excision and mfd (WITKIN 1966b), it seems likely that mfd is prevented when the excision enzyme is engaged in excising dimers elsewhere than at suppressor loci (as, for example, in the presence of amino acids). Where dimer-formation is much reduceed, as after UV at −79°C, there will accordingly be little competition and therefore a smaller effectiveness on repair of premutational lesions of those conditions which alter the rate of excision of dimers.

Our results indicate that the broth effect is locus-specific (i.e. confined to suppressor loci). Other results (BRIDGES, LAW and MUNSON, in preparation)

show that it is also lesion-specific (premutational lesions induced at suppressor loci by low doses of ionizing radiation are not excisable nor do they show a broth effect).

The above model which explains certain phenomena of UV mutagenesis in the Hcr^+ strain is not, however, applicable to the Hcr^- strain where there is no excision-repair and mfd is small (MUNSON and BRIDGES 1966) or nonexistent (WITKIN 1966a). In WP2 Hcr^- both true and suppressor mutations show a dose-squared response and this is independent of whether UV is given to bacteria at 22°C or −79°C. It would seem that some other cause must be sought for the dose-squared response in this case.

Our present data with the Hcr^- strain may be used to obtain an estimate of the probability with which a potentially premutational lesion may give rise to a mutated gene at the time of or after replication, on the assumption that no dimers are excised and that some may pass through the DNA replication complex (c.f. RUPP and HOWARD-FLANDERS 1967). From the experiment shown in Figure 2 it may be seen that after 30 ergs mm^{-2} given to WP2 Hcr^- at room temperature (just before the curve begins to flatten at higher doses not shown in the figure) approximately 10^{-6} true reversions at the ochre codon are obtained. Assuming the yield for 1 erg mm^{-2} of 2537 Å light to be 2.5×10^{-6} dimers per thymine residue (BOYCE and HOWARD-FLANDERS 1964; SETLOW, CARRIER and BOLLUM 1965; SETLOW, SWENSON and CARRIER 1963; WULFF 1963), a dose of 30 ergs mm^{-2} will produce an approximate average of 2×10^{-4} dimers in the WP2 ochre codon. Depending on which base pairs in the codon can give rise to mutations (see above) the probability of a dimer giving rise to a mutation is roughly 1 to 2%.

So far we have considered only the induction and repair of premutational lesions and not the way in which they are converted into functional mutations. True mutations in WP2 would seem of necessity to be either transitions or transversions, and the same is probably true of those suppressor mutations coding the anti-codons of transfer RNAs. Yet pyrimidine dimers (which give rise to mutations in the Hcr^- strain) should not themselves directly miscode either RNA or DNA. It has been known for some years that, at least for UV mutagenesis, a premutational lesion does not become a functional mutation until after DNA replication (WEATHERWAX and LANDMAN 1960; DOUDNEY and HAAS 1960; LIEB 1960; WITKIN 1961). How might this occur?

RUPP and HOWARD-FLANDERS (1967) have given evidence suggesting that when a dimer is replicated in an Hcr^- strain, a gap is produced, probably opposite the dimer on one of the daughter chromatids. This gap disappears on incubation, possibly by recombination with the other daughter chromatid (HOWARD-FLANDERS, THERIOT and STEDEFORD 1967). One may imagine that a mistake or error could occur during such a recombination and thus give rise to a mutation.

Alternative nonrecombinational mechanisms might be either (i) sealing of gaps after replication by an enzyme inserting random bases or (ii) a slow replication process occurring with a lower probability than gap formation in which bases are inserted randomly opposite the dimer. WITKIN (personal communication) has suggested that the *exr* gene might be responsible for the second alterna-

tive, although it could equally well be responsible for the first. This suggestion is attractive since *exr*$^-$ bacteria are not only more sensitive to UV than *exr*$^+$ bacteria but are almost completely immutable by UV (WITKIN, personal communication).

Whatever the explanation, it now seems fairly clear that the induction of a mutation by UV depends on an unexcised dimer (or presumably a type B lesion in the case of Hcr$^+$ strains) passing through the replication complex. Once replication has occurred and a wrong base or bases have been laid down opposite the dimer the way might then be clear, at least in Hcr$^+$ strains, for the dimer to be excised and replaced by bases matching the mutant strand. If a mistake in recombination were responsible for the first mutated strand this would result in only one mutant clone arising from the original irradiated gene (cf. RYAN, FRIED and SCHWARTZ 1954, discussed by BRIDGES and MUNSON 1967; HOLLIDAY 1962).

We wish to thank DRS. EVELYN WITKIN, MARY OSBORN, STANLEY PERSON, PAUL HOWARD-FLANDERS and W. DEAN RUPP for their kindness in providing one of us (B.A.B.) with opportunities to see and discuss with them their work prior to its publication.

SUMMARY

Evidence is presented that a number of *Escherichia coli* strains have chain-terminating codons at their auxotrophic loci. They may mutate to prototrophy either by true reversion at the chain-terminating codon or by mutation at suppressor loci. The two types of prototroph may be distinguished by the ability of the latter to support growth of T4 phage also carrying chain-terminating mutations. In *E. coli* B/r WP2 Try$^-$, which appears to have an ochre codon, both types of mutation arise spontaneously. Ultraviolet light (UV) at room temperature induced a preponderance of suppressor mutations whose frequency increased with the square of the dose: In an Hcr$^+$ strain (one able to perform excision-repair) the frequency of suppressor mutants was greatly enhanced by the addition of broth to the plating medium. The frequency of suppressor mutants fell considerably if bacteria were incubated in the absence of the required amino acid before plating on broth-enriched plates. This mutation frequency decline did not occur with true revertants which also differed from suppressor mutants in being induced linearly with dose. The sensitivity of an Hcr$^-$ strain was much greater than the Hcr$^+$ strain for the induction of both true and suppressor mutants although here the true revertants, like the suppressor mutants, were induced in proportion to the square of the UV dose. In the Hcr$^-$ strain the induction of both true and suppressor mutants was directly photoreversible and therefore presumably involved pyrimidine dimers. In the Hcr$^+$ strain there was little photoreactivation of either true or suppressor mutants indicating the primary involvement of a different lesion in the DNA.—When UV was given to Hcr$^+$ bacteria at $-79°$C not only was the sensitivity much greater but the proportion of true revertants was much higher (around 40%) and the frequency of both true and suppressor mutants increased in proportion to a power of the dose nearer 1 than 2. Hcr$^-$ bacteria under the same conditions were about twice as sensitive as at 22°C,

the dose-squared response was retained and the proportion of true revertants was low, as at room temperature (around 10%).—It is concluded that under any given conditions both true and suppressor mutations are derived largely from the same initial type of damage, such differences as are observed being due to the differential repair of damage at suppressor loci. It is suggested that mutations arise during the postirradiation modification of unexcised UV damage which passes through the DNA replication complex.

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