BACTERIOPHAGE T4 GENE *32* PARTICIPATES IN EXCISION REPAIR AS WELL AS RECOMBINATIONAL REPAIR OF UV DAMAGES

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ABSTRACT

Gene *32* of phage T4 has been shown previously to be involved in recombinational repair of UV damages but, based on a mutant study, was thought not to be required for excision repair. However, a comparison of UV-inactivation curves of several gene *32* mutants grown under conditions permissive for progeny production in wild-type or *polA*$^-$ hosts demonstrates that gene *32* participates in both kinds of repair. Different gene *32* mutations differentially inactivate these repair functions. Under conditions permissive for DNA replication and progeny production, all gene *32* mutants investigated here are partially defective in recombinational repair, whereas only two of them, *P7* and *P401*, are also defective in excision repair. *P401* is the only mutant whose final slope of the inactivation curve is significantly steeper than that of wild-type T4. These results are discussed in terms of interactions of gp32, a single-stranded DNA-binding protein, with DNA and with other proteins.

REPAIR of radiation damages in phage T4 can be accomplished by several pathways (for reviews see BERNSTEIN and WALLACE 1983; DRAKE and RIPLEY 1983; CONKLING and DRAKE 1984a,b). By analogy with its host, T4 is thought to have at least three major repair pathways: photoreactivation, excision repair and recombinational repair. Photoreactivation depends largely on host functions (DULBECCO 1950) but may be stimulated directly by T4 gene *32* protein (HÉLÈNE *et al.* 1976). It can be eliminated by growing the irradiated phages in the dark. Here, I will only consider the other pathways: excision repair and recombinational repair.

The T4 repair pathways probably correspond to the three large UV targets ("vulnerable centers") deduced by BARRICELLI (1956) from multiplicity reactivation data (BARRICELLI and METCALFE 1968). HARM (1961) has suggested that these vulnerable centers might correspond to three sets of T4 genes that have to function prior to repair. The fact that there are more than three T4 genes involved in radiation repair is rationalized by the common credo of pathway analysis of radiation-sensitive mutants: if the effects on survival of two lesions are additive, the two corresponding genes are believed to act in different pathways. The effects of lesions in the same pathway are not additive, even if they occur in different genes (HARM 1963; EBISUZAKI 1966).

Using this rationale, many investigators have attempted to classify the dif-

ferent pathways according to the participating gene products (for review see BERNSTEIN and WALLACE 1983). Specifically, excision repair requires the T4 *denV* (endonuclease V) gene and, in addition, T4 genes *1, 30, 42* and *45* and the host's *polA* gene, but not T4 DNA polymerase (gene *43*) (MAYNARD-SMITH and SYMONDS 1973; SCHNITZLEIN, ALBRECHT and DRAKE 1974). It was believed not to require T4 gene *32* (MAYNARD-SMITH, SYMONDS and WHITE 1970; WALLACE and MELAMEDE 1972). Recombinational repair requires T4 gene *32* and at least 15 other T4 genes (BERNSTEIN and WALLACE 1983). It can be error-prone or error-free and has a cold-sensitive and a cold-insensitive component (CONKLING and DRAKE 1984a,b). Results of pathway analyses using certain recombination- and repair-deficient mutants were, however, ambiguous. This led to the conclusion that pathways can overlap, with respect to both participating gene products and the damages they can repair (HAMLETT and BERGER 1975; EBISUZAKI, DEWEY and BEHME 1975; CUNNINGHAM and BERGER 1977). One possible interpretation of these complex results is that repair functions of different pathways are performed by enzyme complexes and that certain proteins can participate in different complexes, thus appearing to perform different functions.

Gp32 is an example of such proteins. It is a single-stranded DNA-binding protein that is essential in replication and recombination and that participates in repair (ALBERTS and FREY 1970; BALDY 1970; WU and YEH 1973) and packaging (MOSIG, GHOSAL and BOCK 1981). In T4-infected bacteria, gp32 covers most single-stranded DNA regions and regulates its own synthesis at the translational level by specifically binding to a leader segment of the gene *32* mRNA (KRISCH and ALLET 1982; VON HIPPEL *et al.* 1983; FULFORD and MODEL 1984). The binding of gp32 to DNA is thought to be partially responsible for its role in repair (HÉLÈNE *et al.* 1976). In addition, there is genetic (MOSIG, BERQUIST and BOCK 1977; MOSIG *et al.* 1979) and biochemical (FORMOSA, BURKE and ALBERTS 1983) evidence that gp32 interacts with many other T4 DNA replication and recombination proteins. These interactions are thought to play important roles in the assembly of these proteins into functional replication and recombination complexes (BRESCHKIN and MOSIG 1977a,b; ALBERTS *et al.* 1983; KARAM, TROJANOWSKA and BAUCOM 1983; MOSIG 1983; NOSSAL and ALBERTS 1983).

From our earlier results (MOSIG, BERQUIST and BOCK 1977; MOSIG *et al.* 1979; MOSIG, SHAW and GARCIA 1984), we suspected that T4 gene *32* protein might participate in excision repair as well as recombinational repair. Since different gene *32* mutations under conditions restrictive (BRESCHKIN and MOSIG 1977a,b) as well as permissive for progeny particle production (MOSIG and BRESCHKIN 1975; MOSIG, BERQUIST and BOCK 1977) differentially affect interactions with other proteins, and consequently affect different steps in DNA replication and recombination, we suspected that the failure to detect involvement of gene *32* in excision repair may have been a consequence of the specific mutations used in earlier studies. Our present results confirm that, under conditions that are permissive for progeny production by undamaged phage, most gene *32* mutants are specifically defective in recombinational repair. In

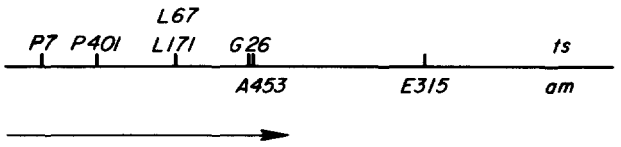

FIGURE 1.—A map of the gene *32* mutations used here. Order and distances of the mutations were determined by MOSIG, BERQUIST and BOCK (1977). The order of *amA453* and *tsG26* could not be determined unambiguously. The arrow indicates the direction of transcription.

addition, at least two gene *32* mutants (*P7* and *P401*) are also defective in excision repair.

MATERIALS AND METHODS

Phage: T4D wild type and the gene *32* mutants shown in Figure 1, as well as the *L171-rII* double mutants (Figure 4), have been used previously (MOSIG, BERQUIST and BOCK 1977). *r71* maps in *rIIA*, *rb42* in *rIIB* and *r1272* deletes the entire *rII* region.

Bacteria: *Escherichia coli* strains B and S/6 (restrictive for *am* mutants, permissive at 30° for the *ts* mutants used here) and CR63 (permissive for *am* mutants) have been used before (MOSIG, BERQUIST and BOCK 1977). The *polA1* strain p3478 (DE LUCIA and CAIRNS 1969) was obtained from J. CAIRNS.

UV-irradiation: Phage particles grown from single young plaques were freed from bacterial debris by low-speed centrifugation and filtering through 0.6 M nitrocellulose (Schleicher and Schuell). They were pelleted by centrifugation for 1 hr at 20,000 rpm and resuspended in "washing fluid" (10 mM Tris, pH 7.4, 1 mM $MgCl_2$, 0.1% NaCl, 0.01% gelatin) to a titer of approximately 5×10^8/ml. Ten milliliters of the phage suspension were UV irradiated with continuous agitation in a Petri dish at a distance of 130 cm from a 15-watt General Electric 15T8 germicidal lamp. At the intervals shown in the figures, aliquots were withdrawn, appropriately diluted and plated in dim yellow light on metal-top plates under permissive conditions (*ts* mutants at 30° on B, *am* mutants at 30° on CR63 bacteria) using exponential phase plating bacteria. Media and plating conditions were as described by ADAMS (1959) except that the soft-agar overlay contained only 0.4% agar, to enhance plaque sizes and efficiencies of plating.

RESULTS

Representative UV-inactivation curves of the different gene *32* mutants and of wild-type T4 (all plated at 30°) are shown in Figure 2. Greater UV sensitivity of the gene *32* mutants *L67* and *P7* as compared with wild-type T4 has been reported previously by BALDY (1970) and WU and YEH (1973), respectively; since *L67* and *L171* map at the same position and are of identical phenotype (MOSIG, BERQUIST and BOCK 1977), I assume that they are identical mutations. My results also show that, under these conditions permissive for progeny production, different gene *32* mutants have different UV sensitivities which follow two or three basic patterns according to the following criteria. The majority of the gene *32* mutations affect mainly the shoulders of the survival curves and have little effect on the final slopes. Whereas the terminal slope of the survival curve of wild-type T4 extrapolates to approximately 3, the terminal slope of a majority class of gene *32* mutants extrapolates to approximately 2 and the *P7* and *P401* curves have little or no shoulders. The gene *32* mutant *P401*, which is partially defective for growth at any tempera-

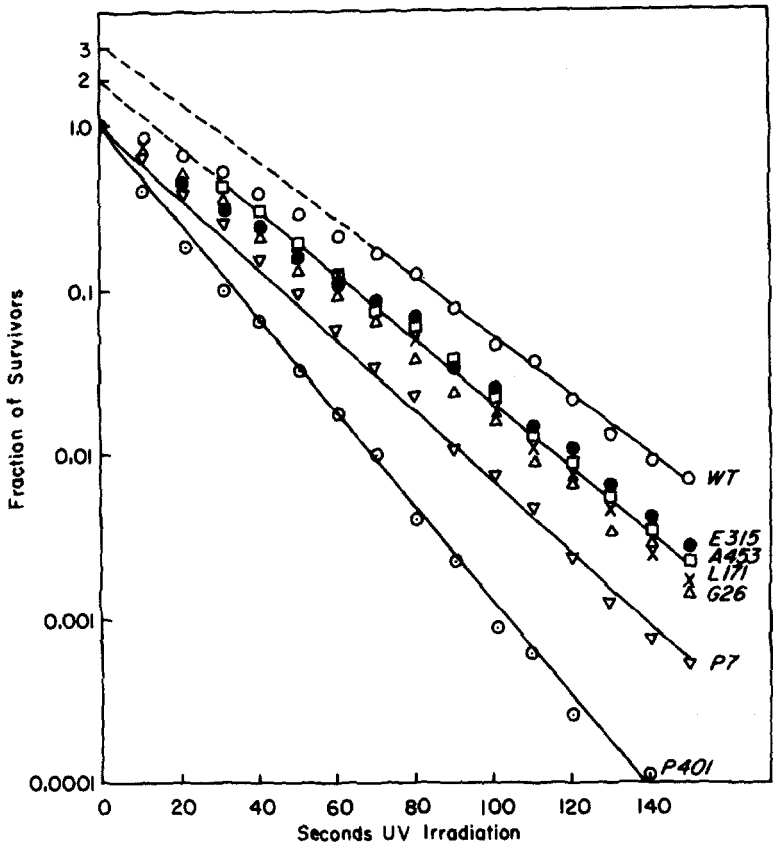

FIGURE 2.—UV-inactivation of wild-type and different gene *32* mutants in *polA*$^+$ bacteria. Free particles were irradiated as described in MATERIALS AND METHODS and plated at 30° on *E. coli* B (*ts* mutants) or CR63 (*am* mutants). Wild type, ○; *E315*, ●; *A453*, □; *G26*, △; *L171*, ×; *P7*, ▽; *P401*, ⊙.

ture at which phage T4 can grow (BRESCHKIN and MOSIG 1977a; MOSIG, BERQUIST and BOCK 1977), is the only mutant that shows a significantly steeper terminal slope than the wild-type or the other gene *32* mutants. Three repeats of these experiments, including using CR63 as plating bacteria for the *ts* mutants, gave indistinguishable results (data not shown). The steeper inactivation curve of *P401* might indicate that, in this mutant, repair is slower or fewer kinds of lesions can be repaired than in the other gene *32* mutants because its gp32 binds poorly to DNA.

One simple interpretation of the different shoulders in different mutants is that, under these growth conditions, gp32 of the *P7* and *P401* mutants is defective in interacting with components of one additional repair pathway (or complex) in which the majority of the mutationally altered gene *32* proteins are not defective. We suspected this to be the excision repair pathway (MOSIG, BERQUIST and BOCK 1977). The *polA* (DNA polymerase I) gene of *E. coli* is known to function in the T4 *denV*-dependent excision repair pathway but not in T4 recombinational repair (MAYNARD-SMITH, SYMONDS and WHITE 1970;

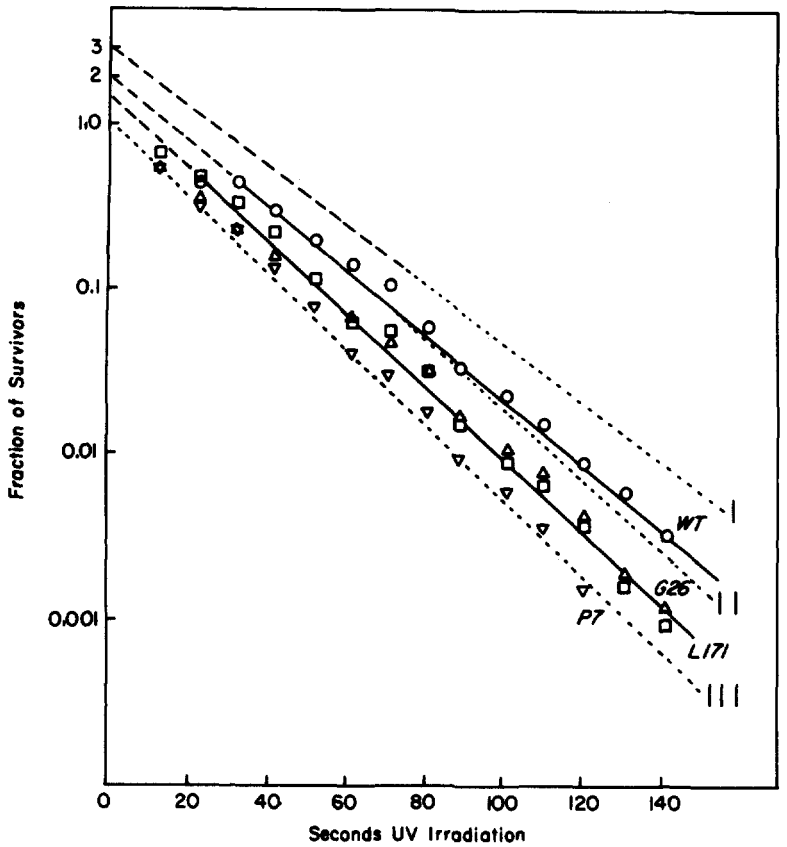

FIGURE 3.—UV-inactivation curves of wild-type and different gene *32* mutants in *polA1* bacteria at 30°. Wild type, ○; *L171*, □; *G26*, △; *P7*, ▽. For comparison, the extrapolated UV-inactivation curves of these phage in *polA*$^+$ bacteria (see Figure 2) are drawn as dotted lines (extrapolations as dashed lines): I, wild type; II, *G26* and *L171*; III, *P7*.

WALLACE and MELAMEDE 1972). MAYNARD-SMITH and SYMONDS (1973) have shown that the T4 gene *32* amber mutation *A453* and a *denV* mutation have additive affects on T4 UV-inactivation. Since they believed the *A453* mutation to be a null allele, they concluded that gene *32* is required for recombinational repair but not for excision repair. We have subsequently shown, however, that gp32 of the *A453* mutant is partially active in its own DNA replication; furthermore, under restrictive conditions, it helps in *trans* to replicate the infecting DNA of a replication-defective mutant (*P7*) (BRESCHKIN and MOSIG 1977a,b). These results suggested that the *A453* peptide could also have retained its capacity for excision repair but that other mutated gene *32* proteins might have lost this function. Therefore, we tested how a *polA1* host mutation affected the survival curves of several different gene *32* mutants.

Figure 3 shows clearly that the *P7* mutant (under conditions permissive for growth) was not more sensitive to UV when plated on a *polA1* mutant than when plated on wild-type bacteria. By this criterion, then, gene *32* acts in the same repair pathway as *polA*, namely, in excision repair. On the other hand,

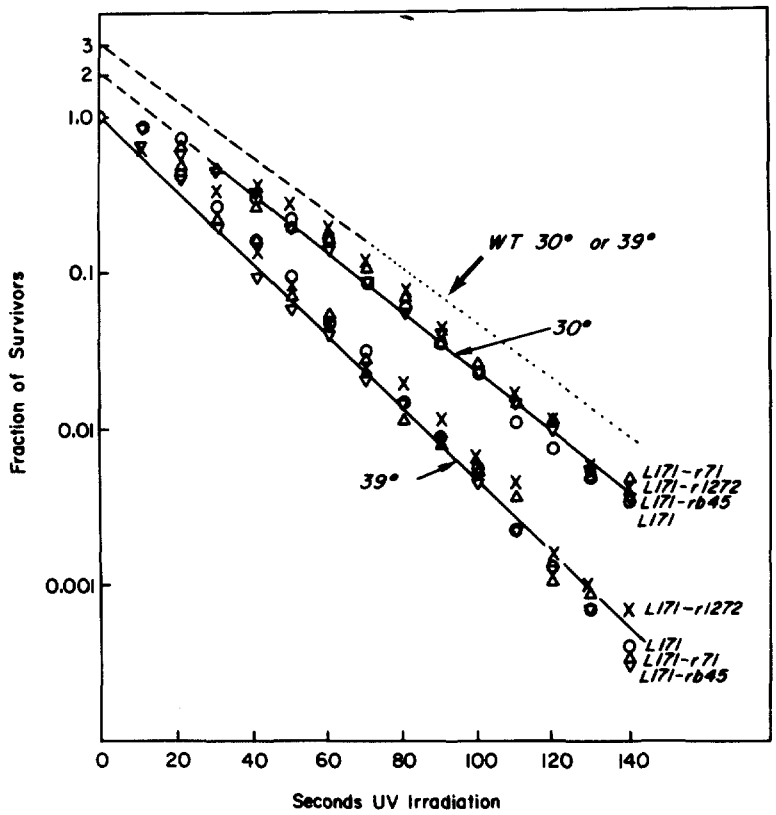

FIGURE 4.—UV-inactivation curves of *L171* and *L171-rII* double mutants in *E. coli* B at 30° and at 39°. For comparison, the wild-type curve is shown as a dotted line. *L171*, O; *L171-r71*, Δ; *L171-rb42*, ∇; *L171-r1272*, ×.

wild-type T4 and the class (II) of less UV-sensitive gene *32* mutants became more sensitive in *polA1* than in *polA*$^+$ bacteria.

The following results indicate that, at 39°, when the burst size of the *L171* mutant is small (~10) (MOSIG, BERQUIST and BOCK 1977), the *L171* peptide too is defective in both excision and recombinational repair. When plated at 39°, *L171* showed single-hit kinetics and an inactivation slope similar to that of *P7* at 30° (Figure 4). The *L171* inactivation curves at 39° in *polA*$^+$, *polA1* and *lig*$^-$ (ligase defective) hosts were identical (data not shown). Since additional *rII* mutations enhance the burst size of *L171* at 39° (MOSIG, BERQUIST and BOCK 1977), we also tested the UV sensitivities of several *L171-rII* double mutants. The results in Figure 4 show that the additional *rII* mutations have no effect on the repair potential. This result was expected, since the *rII* mutations mainly help the ligation of *L171* DNA to packageable DNA but do not affect DNA synthesis (MOSIG and BRESCHKIN 1975).

DISCUSSION

Gene *32* of phage T4 has been shown previously to be involved in recombinational repair of UV damages but was thought not to be required for *denV*-

dependent excision repair (for review see BERNSTEIN and WALLACE 1983). However, I conclude from results reported here that gene *32* participates in both kinds of repair. I suggest that gp32 interacts in a different manner with DNA and proteins of recombination complexes than with DNA and proteins of excision repair complexes. This conclusion is derived from three kinds of results.

(1) Under conditions that are permissive for progeny production, different gene *32* mutants show different UV sensitivities. According to this criterion, most mutants can be assigned to one of two basic inactivation curves which differ mainly in their shoulders but little in their final slopes (Figure 2). Only the mutant *P401* showed a significantly steeper final slope.

(2) At least one of the gene *32* mutants that belongs to the less sensitive class when plated at 30° changes to the more sensitive class when plated at 39° (Figure 4).

(3) A *polA1* mutation in the host renders the less sensitive T4 gene *32* mutants more UV sensitive, whereas this *polA1* mutation has no additional effect on the more sensitive gene *32* mutants (Figure 3 and data not shown).

Although there is a certain ambiguity in fitting UV-inactivation data to curves of certain shapes, my results fit best with the idea that the final slopes of UV-inactivation curves of wild-type T4 extrapolate to approximately 3, final slopes of the less sensitive gene *32* mutants extrapolate to approximately 2 and inactivation curves of the more sensitive mutants show little or no shoulder. The *polA1* host mutation mainly reduces the shoulders of the curves of wild-type T4 and the less sensitive gene *32* mutants. Thus, by classical pathway analysis, some (but not all) gene *32* mutants appear defective in excision repair at 30°. One mutant (*L171*) becomes defective in excision repair only at 39°. Since all assays, on $polA^+$ or $polA^-$ hosts or at different temperatures, were done from the same irradiated phage suspensions, I conclude that the multihit inactivation curves (Figure 2) were not caused by clumping of T4 particles.

The residual repair capacity of the less sensitive gene *32* mutants (class II) in *polA1* hosts (as compared with *P7*, which is defective in both pathways) is probably largely due to the leakiness of the p3478 mutant (GROSS and GROSS 1969). MORTELMANS and FRIEDBERG (1972) have postulated that another undefined mutation in p3478, not *polA1*, is responsible for the greater UV sensitivity of T4 phage in this host. This appears unlikely, since another *polA* mutation has similar effects as p3478 on T4 survival (WALLACE and MELAMEDE 1972). Furthermore, GROSS and GROSS (1969) have shown that the *polA1* mutation in different genetic backgrounds results in different residual polymerase activities (1% in p3478 and up to 8% in transductants derived from it which were used by MORTELMANS and FRIEDBERG 1972). In any case, the conclusion that *P7* is defective in excision repair is unaffected by this caveat, since p3478 had been shown to be defective in excision repair.

From the difference in the ability of UV-irradiated am^+-$denV^+$ and am^+-$denV^-$ to complement two *am* mutations in gene *32* (*A456* and *E315*), MAYNARD-SMITH and SYMONDS (1973) concluded that gene *32* is not involved in excision repair. I interpret their results to mean that the truncated *A453* and *E315* peptides (which correspond to only one-third or one-half, respectively,

of the normal gene *32* protein) retain their capacity to participate in excision repair even under conditions restrictive for growth. Note that these amber peptides also retain their capacity to participate in primary DNA replication and, by inference, to interact with DNA (BRESCHKIN and MOSIG 1977a,b; DANNENBERG and MOSIG 1981).

BERNSTEIN and WALLACE (1983) have discussed current recombination models that can lead to repair of radiation damages in T4 DNA. These models, like those for recombinational DNA repair in *E. coli* by HOWARD-FLANDERS (1982) and LIVNEH and LEHMAN (1982), assume basic break-join recombination with some associated DNA repair synthesis. In phage T4, however, recombinational intermediates initiate new replication forks, and this mechanism is required for overall T4 DNA replication since initiation at the primary origins is turned off during T4's transcriptional program (LUDER and MOSIG 1982; DANNENBERG and MOSIG 1983; MOSIG 1983). Since, under the conditions permissive for growth used here, the gene *32* mutants (with the exception of *P401*) are not defective in DNA replication (MOSIG and BRESCHKIN 1975; BRESCHKIN and MOSIG 1977a,b), their repair deficiencies cannot be due to an overall defect in recombination. Instead, the mutants must be deficient in some recombinational steps that are not essential for initiating new replication forks. MOSIG, BERQUIST and BOCK (1977) have shown that, under the same permissive conditions, these gene *32* mutants are not defective in "splice" recombination (as defined by STAHL 1979) but are partially defective in "patch" recombination, branch migration and/or heteroduplex repair. Therefore, defects in these latter steps probably account for their recombinational repair defects. Specifically, defects in pairing of complementary DNA strands (of different parentage) across UV damages would reduce potential repair of damaged DNA by recombination without necessarily preventing initiation of DNA replication from the 3′ end of an invading strand (see Figure 5).

The defect in the recombinational steps of the gene *32* mutants discussed above can be readily explained by defective interactions of the altered gp32 with the *WXY* recombination system (CONKLING and DRAKE 1984a,b). In fact, the inactivation curves of those gene *32* mutants that are defective only in recombinational repair resemble the inactivation curves of *uvsY* (BOYLE and SYMONDS 1969) or *uvsW* mutants (WU, YEH and EBISUZAKI 1984). The products of genes *uvsX* and *uvsY* have been shown to interact with gp32 *in vitro* (FORMOSA, BURKE and ALBERTS 1983). Construction and analysis of appropriate double mutants are needed to confirm this suggestion.

The initiation of replication forks from recombinational intermediates (LUDER and MOSIG 1982) provides an additional pathway to circumvent UV damages in T4 DNA (Figure 5) and, perhaps, generally (SZOSTAK *et al.* 1983). It is, therefore, not surprising that recombinational repair pathway(s) requires all T4 replication functions (BERNSTEIN and WALLACE 1983), including the T4 topoisomerase (MISKIMINIS *et al.* 1982). HYMAN (1983) has concluded that T4 gp49 (endonuclease VII) is not involved in recombinational repair. This does not argue against a role of this protein in recombination (MIYAZAKI, RYO and MINAGAWA 1983) since gp49 is not required to initiate recombination, but to

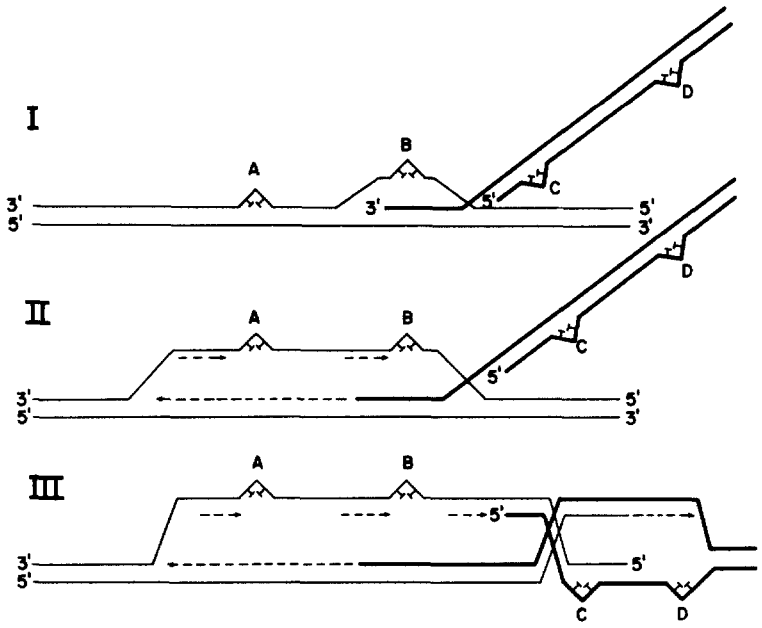

FIGURE 5.—Different ways to repair or bypass UV damages by recombinational mechanisms. For reasons of simplicity, all UV lesions are shown as thymine dimers, but the mechanism applies to other damages as well. The two parental molecules participating in these reactions are shown in thin or thick solid lines, respectively; newly synthesized DNA is shown as dashed lines. Arrowheads indicate directions of replication. I. A single-stranded 3′ terminus invades a damaged chromosome. Dimer B is displaced from its complementary strand; this step does not require pairing of another DNA strand across dimer B. II. DNA replication is initiated in the leftward direction from the recombinational intermediate shown in I (LUDER and MOSIG 1982). It uses the 3′ end of the invading single strand as primer for leading strand synthesis, which eventually displaces dimer A from its complementary strand. Lagging-strand synthesis can be initiated from priming sites for Okazaki pieces, but will stop at dimers A and B, generating gaps opposite the dimers (HOWARD-FLANDERS 1982; BERNSTEIN and WALLACE 1983). Such gaps could be repaired by the mechanisms proposed by BERNSTEIN and WALLACE (1983) (not shown). III. Branch migration in the recombinational intermediates shown in I and II in the rightward direction across dimer C displaces this dimer from its template. Branch migration in this direction can be terminated at any point by cutting with endonuclease VII (KEMPER *et al.* 1984) (not shown here). In the absence of cutting, branch migration will eventually reach the ends of the invaded (thin line) molecule. In that case, replication can be initiated from the 3′ end in the rightward direction (MOSIG, SHAW and GARCIA 1984). Such replication can displace dimer D from its template and generate gaps opposite dimer D by mechanisms similar to those shown in II for forks generated in the leftward direction.

cut recombinational intersections formed by branch migration (KEMPER *et al.* 1984; MOSIG, SHAW and GARCIA 1984).

We do not know which step of the excision repair pathway requires gp32. As discussed by BERNSTEIN and WALLACE (1983), several details of excision repair are poorly understood. It is, however, tempting to speculate that gp32 is required to maintain or stabilize the DNA strand opposite the excised lesion as a template for resynthesis of the excised DNA segment. Perhaps gp32 of *P7*, under conditions permissive for progeny production, is more defective in

excision repair resynthesis than in DNA replication, because *E. coli* DNA polymerase I, not T4 DNA polymerase, resynthesizes this DNA (MAYNARD-SMITH, SYMONDS and WHITE 1970). The earlier results had suggested that this mutant is also partially defective in repairing recombinational heteroduplexes (MOSIG, BERQUIST and BOCK 1977). In this respect, it is noteworthy that gp32 appears to interact with *E. coli* DNA polymerase I *in vivo* (BRESCHKIN and MOSIG 1977b), even though it selectively stimulates T4 DNA polymerase *in vitro* (HUBERMAN, KORNBERG and ALBERTS 1971).

Since recombination can repair or bypass UV damages by more than one mechanism (see Figure 5 and BERNSTEIN and WALLACE 1983) and since excision repair of UV damages and of recombinational heteroduplex mismatches are thought to be related (for review see WHITEHOUSE 1982), it is conceivable that recombinational and excision repair complexes operate in close vicinity and thus appear to interact. Clearly, as with genes involved in building of DNA replication and recombination complexes, it is necessary to analyze several gene *32* mutations to unravel the roles of gp32 in DNA repair.

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