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Competence Mutants

III. Responses to Radiations

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Class 3 *com*$^-$ mutants [normal in deoxyribonucleic acid (DNA) uptake but poor in ability to transform] were investigated with regard to ultraviolet (UV) and X-ray sensitivity of colony-forming ability and with regard to their ability to be transformed by UV- and X-ray-irradiated DNA. Three mutants, *com*$^-$40, 60, and 78, were highly UV-sensitive in colony-forming ability. None of the mutants was more sensitive than wild type to UV-irradiated transforming DNA; in fact, six of the mutants showed considerably greater resistance. Two of the mutants (*com*$^-$40 and 60) were slightly more sensitive to X ray in colony formation, whereas most of the mutants showed some degree of sensitivity to X-ray-irradiated transforming DNA. In addition, the physical fate of X-ray-irradiated transforming DNA has been examined, and in one case (*com*$^-$48) there was a significant drop in sedimentation value of X-ray-irradiated donor DNA after uptake by recipient cells. The *com*$^-$ mutants analyzed have been classified on the basis of their UV and X-ray sensitivities, and, where appropriate, possible biochemical lesions have been implicated.

Class 3 *com*$^-$ mutants, although normal in deoxyribonucleic acid (DNA) uptake, yield low levels of transformants (J. H. Caster, E. H. Postel, N. Stanton, and S. H. Goodgal, Bacteriol. Proc., p. 60, 1969; reference 3). In the second paper of this series (19), we have shown the variable ability of these mutants to form donor-recipient complexes, i.e., some are defective in the association step (*dad*$^-$ mutants, for donor association defective), whereas others are able to form what appear to be normal donor-recipient complexes (*dab*$^-$ mutants, for donor association biologically defective), but do not yield biologically active recombinant DNA.

Since the repair of radiation-induced damage in DNA and normal recombinational events previously have been thought to share common paths (10, 27), we decided to investigate the nature of the ultraviolet (UV) and X-ray sensitivity of class 3 mutants. While some of the UV repair mechanisms are established as separate from recombination [e.g., photoreactivation (5) and dimer excision in the dark repair system (10)], later steps in the dark repair system, i.e., widening of gaps, repair synthesis, and rejoining of strands (27), and recombination repair (10), have been shown to share common functions with normal recombination enzymes (15). Therefore, while UV sensitivity is frequently found in recombination-deficient strains (4, 27), not all UV-sensitive strains are defective in recombination (10, 15). Similarly, mutants which perform genetic recombination with low efficiency (4, 11) show X-ray sensitivity, although not all X-ray-sensitive mutants are recombination-deficient (7, 11, 26). The integration process in transformation involves, among other things, breakage of strands and final covalent insertion of the donor single strand into the recipient DNA to yield an uninterrupted bihelix (9). It is not known in detail how many and what kinds of steps are involved in this recombinational process. Because, as stated above, none of our class 3 mutants can carry out recombination of donor and recipient DNA species completely (i.e., form biologically active recombinant DNA), and because of the overwhelming evidence in favor of some commonly shared recombinational and repair enzymes, we predicted that some of our class 3 mutants should prove to be UV- or X-ray sensitive, or both. Indeed, previous studies have shown that several class 3 mutants are UV-sensitive in their colony-forming capacity (3). Therefore, we investigated both the UV and X-ray sensitivity of class 3 mutants with regard to their ability to form colonies and to act as recipients when transformed by irradiated DNA. In addi-

tion, the physical fate of X-ray-irradiated transforming DNA was examined in *com*⁻ mutants.

MATERIALS AND METHODS

The strains, competent cultures, and DNA preparations are described in the accompanying paper (19).

UV irradiation of cells. Cultures were grown in 3.7% Brain Heart Infusion (Difco Laboratories, Detroit, Mich.) supplemented with nicotinamide adenine dinucleotide (2 μg/ml) and hemin (10 μg/ml) until they reached a turbidity of 10^9 cells/ml. They were then chilled and diluted 30-fold in iced saline containing 0.37% Brain Heart Infusion. Seven milliliters of each diluted culture was placed on a plastic petri dish (100 by 15 mm) and irradiated in the dark with a General Electric germicidal lamp. The incident doses were 0, 25, 50, 100, 200, 300, and 400 ergs per mm^2. Samples (0.1 ml) were removed at each dose, diluted in ice-cold Brain Heart Infusion, and plated on agar for formation of viable colonies. The handling procedures were carried out in the absence of white light to avoid photodynamic inactivation of the cells, which can occur in 0.37% Brain Heart Infusion.

UV irradiation of DNA and its assay in transformation. *Nov*^{r} DNA (resistant to 25 μg of novobiocin per ml) and *ery*^{r} DNA (resistant to 15 μg of erythromycin per ml) were irradiated at a concentration of 30 μg/ml in 0.015 M sodium citrate-saline-0.37% Brain Heart Infusion. The incident doses were 0, 100, 200, 300, 500, and 1,000 ergs per mm^2. The irradiated DNA samples were mixed with each recipient competent culture to give a final ratio of 1 μg of DNA:5 × 10^8 cells, incubated at 34 C for 30 min, and plated for transformants and viable centers.

X-ray-irradiation of cells. The cultures were prepared as described above for UV irradiation of cells. The samples were placed 12.5 cm from the target of an OEG-60 Machlett beryllium-window tube operated at 50 kv (peak) and 20 ma with four layers of aluminum foil as a filter. The dose rate was 10 krads/min. The incident doses were 0, 2.5, 5, 7.5, 10, 15, 20, 25, and 30 krads.

X-ray-irradiation of DNA and its assay in transformation. The *nov*^{r} DNA was prepared as described for the UV irradiation, and the assay also was the same. The incident X-ray doses were 0, 5, 10, 20, 30, and 50 krads. For the physical experiments, donor DNA was labeled with ^{32}P, and the recipient cells were ^3H-labeled. The preparation of lysates and their sucrose density gradient centrifugation are described in the accompanying paper (19).

DNA uptake. The uptake of DNA was measured by a procedure previously described (3).

RESULTS

Colony-forming ability after UV irradiation. In a preliminary screening of 70 *com*⁻ mutants for colony-forming ability after UV irradiation, we found that three of the four highly sensitive mutants—*com*⁻40, 60, and 78—bind DNA normally (i.e., class 3; reference 3).

Figure 1 shows the colony survival rates of the pertinent class 3 mutants as a function of UV dose. As was found previously, *com*⁻40, 60, and 78 show high sensitivity, whereas the rest of the mutants exhibit the sensitivity range of the Rd parent. The 37% survival doses (Fig. 1) are listed in Table 1. *Com*⁻40 and 60 are 11 times, *com*⁻78 is 18 times, and *com*⁻13 is 1.2 times more sensitive than the parental strain. The rest of the mutants show somewhat higher resistance to UV than does the Rd strain; these differences are small but consistent in repeated experiments.

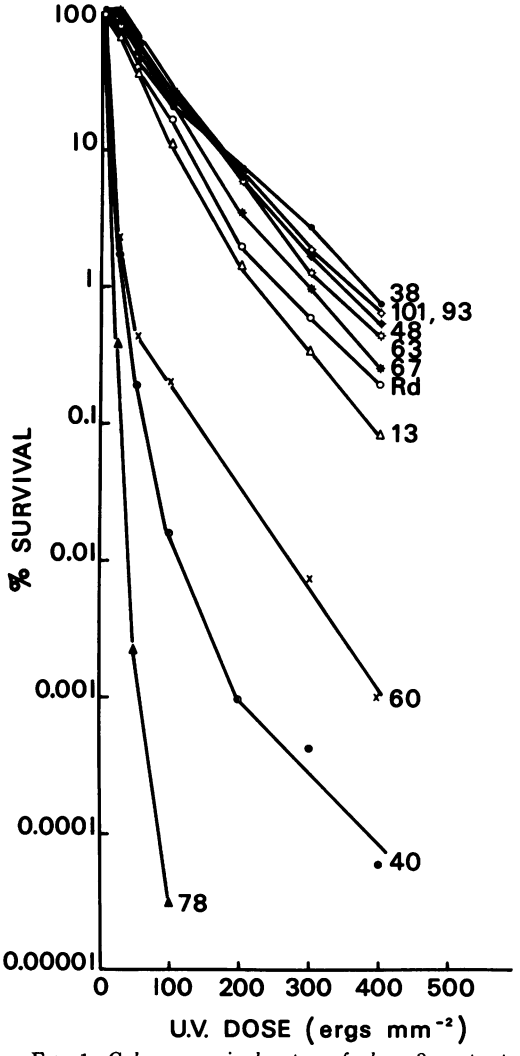

FIG. 1. *Colony survival rates of class 3 mutants after UV irradiation.*

TABLE 1. *37% Survival doses for colony-forming ability after ultraviolet irradiation of class 3 com⁻ mutants*

Strain	37% Dose (ergs/mm²)	37% Survival dose ratio (Rd:*com*⁻)
Rd (parent)	180	1.0
com⁻ 4	260	0.69
com⁻ 13	150	1.2
com⁻ 38	260	0.69
com⁻ 40	20	11
com⁻ 48	250	0.72
com⁻ 60	20	11
com⁻ 63	230	0.78
com⁻ 67	200	0.90
com⁻ 78	10	18
com⁻ 93	240	0.75
com⁻101	240	0.75

Survival of UV-irradiated transforming DNA in class 3 com⁻ mutants. It was expected that the mutants with impaired colony formation after UV irradiation would also show a reduced ability to repair UV-irradiated transforming DNA. Setlow et al. (23) showed that three of their highly UV-sensitive ("cell sensitivity") *Haemophilus influenzae* mutants DB112, 115, and 116 also demonstrate a high degree of "transforming DNA sensitivity" of the *nov*ʳ marker (resistant to 2.5 μg of novobiocin per ml). However, DB112, 115, and 116 are normally transformable strains; for the nontransformable UV "cell sensitive" mutant DB117, there was no transformation data. Munakata and Ikeda (18) showed that, in *Bacillus subtilis*, highly UV-sensitive strains yielded considerably smaller numbers of transformants with UV-irradiated DNA than did the parental strain.

We tested the survival of transforming activity of two independent DNA preparations (*nov*ʳ and *ery*ʳ) in our *com*⁻ strains with various UV doses. The effect of 500 ergs/mm² irradiation dose is shown in Table 2. In all cases, the *nov*ʳ marker was more sensitive than the *ery*ʳ marker, although the relative transformation efficiencies of the unirradiated markers were similar in each of the strains.

In none of the strains, however, was there a significantly lower rate of marker survival compared to Rd, and in many strains the survival rate of irradiated DNA proved to be even greater than in the parent. This can be seen in Table 2, columns 3 and 5, where the per cent survival at 500 ergs/mm² irradiation dose is expressed as the increase in resistance over Rd. The largest increases in UV resistance for both markers (Table 2, experiment 2) occurred in *com*⁻48, 38, and 4 and, in experiment 3, in *com*⁻78, 101, and 93. The UV sensitivity for *com*⁻13, 60, 63, 67, and 40 varied within a two-fold range from Rd.

The slightly greater sensitivity of the *nov*ʳ marker in *com*⁻60 and the *ery*ʳ marker in *com*⁻40 does not compare with the 11-fold sensitivity of colony-forming ability in these mutants discussed above. *Com*⁻78, which was 18 times more sensitive in colony-forming ability, proved to be four times more resistant in marker survival. One possible explanation of these results is that there may be a sufficient amount of residual repair activity in these mutants to repair transforming DNA but an insufficient amount, either qualitatively or quantitatively, to repair chromosomal damage.

With the exception of *com*⁻48, all the "resistant" recipients (*com*⁻78, 4, 38, 93, and 101) were *dad*⁻ mutants, i.e., did not associate

TABLE 2. *Survival of ultraviolet-irradiated transforming DNA in class 3 com⁻ mutants*

Recipient strain	Donor marker irradiated			
	*nov*ʳ		*ery*ʳ	
	% Survival[a]	% Survival ratio (*com* :Rd)	% Survival	% Survival ratio (*com* : Rd)
Expt 1				
Rd	6.8	1.0	32	1.0
Expt 2				
Rd	6.8	1.0	54	1.0
com⁻13	7.7	1.1	76	1.4
com⁻48	35	5.2	101	1.9
com⁻38	53	7.8	72	1.3
com⁻ 4	85	12.3	325	6.0
Expt 3				
Rd	12	1.0	37	1.0
com⁻ 60	6.3	0.52	42	1.1
com⁻ 63	14	1.2	40	1.1
com⁻ 67	15	1.3	64	1.7
com⁻ 78	42	3.5	154	4.2
com⁻101	61	5.1	370	10
com⁻ 93	68	5.7	348	9.4
Expt 4				
Rd	13	1.0	37	1.0
com⁻40	23	1.8	26	0.7

[a] The per cent survival for each marker designates the number of transformants produced at 500 ergs/mm² compared to the unirradiated control. The frequency of transformants with the unirradiated DNA compared to Rd were: 10^{-5} for *com*⁻40; 10^{-4} for *com*⁻4, 13, 93, and 101; 10^{-3} for *com*⁻38, 48, 60, 67, and 78; and 10^{-2} for *com*⁻63.

donor DNA with the recipient chromosome. It is possible that the small amount of DNA which becomes integrated in these mutants contains fewer photolesions than the average population of irradiated DNA which is taken up by the cell. This could be the result of an enhancement of a recognition and selection mechanism already present in the wild type. Beattie and Setlow (1) and H. Harm (8) have demonstrated the lethality to the recipient of damaged DNA which becomes integrated. This could not explain our results, since all but one of the "resistant" recipients belong to the *dad*$^-$ category. To rule out cell death attributable to irradiated DNA, we tested all the transformation mixtures for total viable centers and found no significant drop with doses as high as 1,000 ergs/mm^2.

The resistance of *com*$^-$48 cannot be explained in terms of reduced killing, since it is a *dab*$^-$ mutant. If, however, *com*$^-$48 is indeed similar to *com*$^-$56 (J. M. Caster and S. H. Goodgal, *manuscript in preparation*), the DNA pieces which it integrates are smaller than normal. These small pieces of integrated donor DNA may be less lethal to the recipient, perhaps because these pieces are less likely to contain a photolesion or perhaps because of a more efficient dimer excision system.

One other possible event which could enhance transformation with UV-irradiated DNA is an increase in recombination frequency. Moderate UV doses are known to stimulate recombination (6, 11, 12) and have been used on recipient cells to enhance genetic recombination and the rescue of genetic markers during transformation in *B. subtilis* (2). However, it should be noted that there is no evidence for UV stimulation of recombination in the *H. influenzae* transformation system (Goodgal, *unpublished observations*).

Colony-forming ability after X-ray-irradiation. X-ray-irradiation is believed to induce single-stranded cuts in the DNA directly by radiation chemical processes (17). Irradiation of *com*$^-$ mutants with various doses of X-ray-irradiation and measurement of the survival of colony-forming ability should provide an estimate of the relative ability of each strain to rejoin strand breaks in their chromosomes. An examination of 11 class 3 *com*$^-$ mutants showed that only two were more sensitive to X-ray-radiation than the parent Rd strain. These two strains were *com*$^-$40 and 60 (Fig. 2). The 37% survival doses for all the strains are shown in Table 3. The sensitivity values (increase in sensitivity over Rd; Table 3, column 3) varied from experiment to experiment, averaging 2.2 for *com*$^-$40 and 1.7 for *com*$^-$60. In addition to X-ray sensitivity, *com*$^-$40 and 60 were also shown above to be UV-sensitive in colony-forming ability. This suggests that, in these two strains, there is a unique block which is common to transformation, UV repair, and X-ray repair. A similar mutant, DB117, which was selected for its UV sensitivity and which is nontransformable and X-ray-sensitive, has been described by Setlow et al. (22).

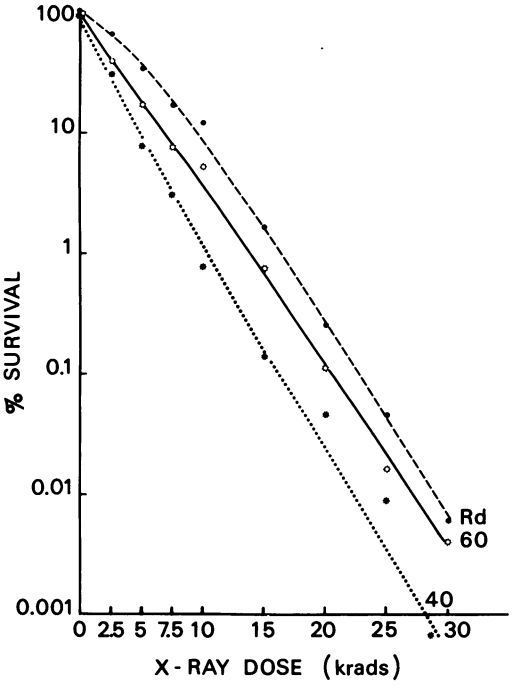

FIG. 2. *Colony survival rates of Rd, com*$^-$*40, and com*$^-$*60 after X-ray-irradiation.*

Survival of X-ray-irradiated transforming DNA in class 3 com mutants. In these experiments, *nov*^{r} DNA was exposed to a range of X-ray-irradiation, and the mutant strains were tested for their ability, relative to the Rd control, to produce transformants with the irradiated DNA.

The results of a number of experiments showed that, except for Rd and *com*$^-$38, the number of transformants induced by irradiated donor DNA species was reduced in all strains. Table 4 gives the 37% survival doses from these experiments. The sensitivity values (increase in sensitivity over Rd; Table 4, column 3) range from 1.3 to 5.3 times the Rd value. Of the three most sensitive recipients, *com*$^-$40 (4.8-fold), *com*$^-$60 (4.5-fold), and *com*$^-$48 (5.3-fold), the first two were also sensitive to X-

TABLE 3. *37% Survival doses for colony-forming ability after X-ray-irradiation of class 3 com⁻ mutants*

Strain	Sensitivity	
	37% Dose (krads)	37% Survival dose ratio (Rd:*com*⁻)
Expt 1[a]		
Rd	5.0	1.0
com⁻40	1.8	2.9
com⁻60	2.8	1.8
Expt 2		
Rd	5.0	1.0
com⁻ 63	5.0	1.0
com⁻ 67	5.0	1.0
com⁻ 13	5.0	1.0
com⁻101	5.0	1.0
com⁻ 40	2.5	2.0
com⁻ 60	2.5	2.0
Rd	5.0	1.0
com⁻ 4	5.0	1.0
com⁻13	5.0	1.0
com⁻63	5.0	1.0
com⁻67	5.0	1.0
com⁻93	5.0	1.0
Rd	5.0	1.0
com⁻48	5.0	1.0
com⁻38	5.0	1.0
com⁻78	5.0	1.0
com⁻40	3.0	1.7
com⁻60	3.5	1.4

[a] Same experiment as in Fig. 1.

ray-irradiation in colony-forming ability (*see above*), although to a lesser degree (2- to 3-fold). From these results we conclude that *com*⁻40 and 60 do have reduced ability to repair X-ray-irradiated DNA, whether it is chromosomal or exogenous transforming DNA.

Some *com*⁻ strains which exhibited normal sensitivity (Rd level) to X-ray-irradiation in colony-forming ability were transformed more poorly than was Rd by X-ray-irradiated transforming DNA. There are a number of possible explanations for this increased sensitivity.

(i) As mentioned above, the repair rate has a greater effect on transforming DNA than on chromosomal DNA.

(ii) In the *com*⁻ mutants tested, X-ray-irradiated DNA may not be taken up with the same efficiency as unirradiated DNA.

(iii) After uptake of irradiated DNA, the *com*⁻ mutants tested may recognize that the DNA is damaged and may not integrate it. *Com*⁻48, which after treatment with transforming DNA may associate smaller than usual segments of donor DNA with its chromosome (J. H. Caster and S. H. Goodgal, *manuscript in preparation*), and the *dad*⁻ mutants *com*⁻67, 63, 78, and 93, which associate little if any donor DNA (19), are reasonable candidates for imposing additional damage on X-ray-irradiated DNA.

(iv) Alternatively, the X-ray-irradiated DNA may be lethal in these *com*⁻ mutants, as in the case of the UV-irradiated DNA (1, 8). Although there was no change in total viable centers when each of the transformation mixtures was tested, the occurrence of a small fraction of killed cells cannot be excluded.

(v) One explanation of those strains in which chromosomal damage is repaired normally but integration or recombination is defective for transforming DNA is that separate repair and recombination enzyme systems are involved.

(vi) The X-irradiated DNA sensitivity of *com*⁻48 and 56 may be related to the fact that the donor DNA integrated in *com*⁻56 is smaller than that in Rd (J. M. Caster et al., Bacteriol Proc., p. 34, 1971). In this case, additional single-strand breaks, induced in the

TABLE 4. *37% Survival doses of X-ray irradiated transforming DNA in class 3 com⁻ mutants*

Strain	Sensitivity	
	37% Survival dose (krads)	37% Survival dose ratio (Rd:*com*⁻)
Expt 1		
Rd	47	1.0
com⁻ 93	26	1.8
com⁻ 4	37	1.3
com⁻ 63	24	2.0
com⁻ 13	31	1.5
com⁻101	25	1.9
com⁻ 67	28	1.7
Expt 2		
Rd	58	1.0
com⁻38	60	1.0
com⁻ 4	26	2.2
com⁻67	24	2.4
com⁻78	21	2.8
com⁻60	13	4.5
com⁻40	12	4.8
com⁻48	11	5.3
Expt 3		
Rd	50	1.0
com⁻38	57	0.88
com⁻78	31	1.6
com⁻56	21	2.4
com⁻48	18	2.8

DNA by X ray, may provide a better substrate for an enzyme which fragments unirradiated transforming DNA into small, inactive pieces.

Our findings are not without precedent. In a normally transformable, UV-sensitive *H. influenzae* mutant (DB116), transforming DNA was also X-ray sensitive (23), although this mutant itself was resistant to X-ray inactivation. It was shown that, in this mutant, the rejoining rate for single-stranded breaks, after a dose of 20 krads, was slower than in the wild type. The interpretation for these observations was that the rejoining rate is more critical for the survival of the X-ray-irradiated transforming DNA than it is for the survival of the cell. Except for the fact that DB116 is normally transformable and shows transforming DNA UV sensitivity, it is similar to our *com*$^-$78 mutant. Furthermore, Kato and Kondo (15) described an *Escherichia coli* mutant (R15) which was sensitive to both UV and X rays and which showed a reduced capacity to reactivate X-ray-irradiated phage but a normal capacity to reactivate UV-irradiated phage. Their interpretation was that excision repair is independent of the recombination system in this mutant. R15 also lacks DNA polymerase (14). It would be interesting if our *com*$^-$40 and 60 strains turned out to be similarly *pol*$^-$, since in many other respects they are like the *E. coli* R15 mutant.

To test some of the above-mentioned possibilities, we examined the physical fate of X-ray-irradiated DNA in these strains.

Physical fate of X-irradiated DNA in com$^-$48, 60, and 78 and Rd recipient strains. To determine whether X-ray-irradiated DNA sensitivity may be the result of physical changes (i.e., reduced uptake, reduced integration, breakdown of irradiated DNA, or reduced ability to join single-stranded regions in the DNA), we determined the physical fate of X-ray-irradiated donor DNA in *com*$^-$ mutants. The experiment described above was extended by an analysis of the DNA from donor-recipient complexes. The donor DNA was labeled with ^{32}P, and the *nov*^{r} marker and recipient strains were labeled with ^{3}H and the *str*^{r} marker. The data for DNA uptake and transformation (Table 5) show that the amount of donor radioactivity taken up by each strain did not vary with X-ray dose to the donor DNA (Table 5, column 3). In agreement with the experiment in Table 5, the X-ray-irradiated DNA was considerably more sensitive in *com*$^-$48, 60, and 78 than in Rd.

Randolph and Setlow (20) found that radioactively labeled DNA (^{3}H in their case) was more sensitive to X-ray-irradiation than was unlabeled DNA in the wild-type *H. influenzae*. They attributed this increased sensitivity to the higher molecular weight of the labeled DNA preparation.

In our experiment, it was found that transformation of the *nov*^{r} marker was lower with ^{32}P-labeled DNA than with unlabeled DNA (compare the 37% survival doses in Tables 4 and 5), and it is possible that the increased sensitivity is attributable to additional damage caused by ^{32}P decay. Nevertheless, the rate of survival as a function of X-ray-irradiation dose and the sensitivity values (increased sensi-

TABLE 5. *DNA uptake, transformation, and survival rates of X-ray-irradiated ^{32}P-labeled nov^R DNA in Rd and com$^-$48, 60, and 78 strains*

Strain	Dose (krad)	DNA uptake (counts/min/ml of transformation mixture)	Transformants per ml	% Survival	37% Survival dose (krads)	37% Survival dose ratio (Rd:*com*$^-$)
Rd	0	3.8×10^3	1.8×10^6	100	26	1.0
	20	3.8×10^3	7.9×10^5	44		
	50	3.8×10^3	3.4×10^5	18		
com$^-$48	0	6.1×10^3	1.7×10^3	100	3	8.7
	20	6.0×10^3	2.5×10^0	0.2		
	50	6.0×10^3	0	0		
com$^-$60	0	2.7×10^3	6.4×10^3	100	7	3.7
	20	2.8×10^3	4.6×10^2	7.2		
	50	2.7×10^3	3.0×10^1	0.5		
com$^-$78	0	2.8×10^3	1.2×10^3	100	9	2.9
	20	2.7×10^3	1.5×10^2	12		
	50	2.8×10^3	2.8×10^1	2.4		

tivity compared to Rd) are similar for both labeled and unlabeled DNA preparations for the strains *com*⁻60 and 78, and Rd. The sensitivity value for *com*⁻48 is two- to threefold greater for labeled than for unlabeled DNA. Additional single-strand breaks in the DNA resulting from ^{32}P decay could explain these results if we assume again that, in *com*⁻48, fragmentation after uptake is facilitated by single-strand breaks in the DNA.

Sucrose gradient sedimentation of lysates of donor-recipient complexes after uptake of X-ray-irradiated DNA. To test the effect of the recipient on the physical fate of X-ray-irradiated DNA, lysates were prepared from cells exposed to ^{32}P-labeled X-ray-irradiated donor DNA (Table 5). Samples of these lysates were sedimented in neutral sucrose gradients to measure double-stranded breaks and, after denaturation, in alkali sucrose gradients to measure single-stranded breaks in the donor DNA.

The sedimentation values of the unintegrated donor DNA peaks present on the gradients from all the lysates were calculated (Table 6). In general, X-ray doses such as 20 and 50 krads which enable us to see changes of several orders of magnitude in a biological assay, are not great enough to affect greatly our physical measurements. Only in one case, that of *com*⁻48, is there a significant drop in sedimentation value with varying X-ray dose. This is consistent with our previous suggestion, with regard to *com*⁻48, that donor DNA in this strain may be inactivated further by a nuclease which preferentially works at single-stranded lesions; this would account for the finding that the change in sedimentation values in neutral sucrose is greater than that in alkali sucrose.

The denaturation of DNA from donor-recipient complexes of *dab*⁻ mutants as recipients demonstrated previously that whatever donor DNA has associated with the recipient has done so in covalent linkage (19). Treatment of these lysates with alkali and sedimentation in sucrose density gradient showed that donor atoms remained associated with recipient DNA. However, there was a reduction in the amount of X-ray-irradiated DNA associated in *dab*⁻strains: a 30% reduction with a dose of 50 krads in the case of Rd and *com*⁻48 and a 20% reduction with the same dose in *com*⁻60. This reduction indicated that, in wild-type cells, transformation (82% reduction at 50 krads; Table 5) was two to three times more sensitive than integration (30% reduction with the same dose). In *com*⁻48 and 60, transformation was even more sensitive (100 and 99.5% reduction, respectively, at 50 krads) than association (30 and 20% reduction, respectively). This indicated that the transforming sensitivities of *com*⁻48 and 60 could not be interpreted in terms of a reduction in association. *Com*⁻78 did not associate sufficient amounts of donor DNA in this experiment, even in the case of unirradiated DNA, for any conclusions to be made about the effects of X-ray-irradiation on integration.

TABLE 6. *Sedimentation values of the unintegrated X-ray-irradiated donor DNA present in the lysates*

Strain	Dose (krads)	In neutral sucrose		In alkali sucrose	
		S^a	$\Delta S_N{}^b$	S^a	$\Delta S_A{}^b$
Control[c] (untransformed lysate + DNA)	0	35		21	
	20	32		20	
	50	32	3	20	1
Rd	0	32		22	
	20	30		21	
	50	29	3	21	1
com⁻48	0	35		21	
	20	34		20	
	50	27	8	18	3
com⁻60	0	33		21	
	20	29		20	
	50	30	3	19	2
com⁻78	0	29		21	
	20	29		19	
	50	29	0	19	2

[a] T4 phage DNA, with a known value of 63*S*, (kindly provided by D. Frankel) was used to calculate the above values by separate sedimentation, in both neutral and alkali sucrose gradients.

[b] ΔS_N and ΔS_A were calculated by subtracting *S* values at 50 krads from *S* values at 0 krads.

[c] The untransformed lysate was a mixture of lysates prepared from competent cultures without added donor DNA. Irradiated DNA samples were added after lysis and just before sedimentation.

DISCUSSION

The major properties of class 3 mutants are summarized in Table 7, which includes both the physical and biological fates of transforming DNA determined in the accompanying paper (19) and the responses of class 3 mutants to radiation as determined in the present investigation. Because these data were discussed in detail above, only the main findings are summarized below.

The average transformation frequencies of class 3 *com*⁻ mutants are shown in Table 7,

column 2. These vary from 1 to 0.001% of the Rd control. Column 3 shows that the low transformation frequencies in these mutants do not result from failure of DNA uptake, since this is seldom less and more often greater than the amount of DNA taken up by the Rd parent. The biological fate of donor DNA at 60 min (column 4) is reduced in all mutants compared to Rd, suggesting that, in some cases, it is not being replicated and that, furthermore, in some cases, it is being inactivated. Column 5 shows the virtual absence of biologically active recombinant DNA in all of these mutants, and column 6 shows the variable amount of donor DNA which can be associated. On the basis of the results presented in columns 5 and 6, we designate these mutants as either *dad*$^-$ (absence or reduced association) or *dab*$^-$ [physically normal (apparently) association but with an absence of biologically active recombinant DNA; column 7].

The radiation sensitivities of these mutants are summarized in Table 7, columns 8–12. Three mutants are highly UV-sensitive in terms of colony-forming ability: *com*$^-$40, 60, and 78 (column 8). None of the mutants is more sensitive than wild type to UV-irradiated transforming DNA; in fact, six of the mutants show considerably greater resistance (column 9). Two of the mutants (*com*$^-$40 and 60) are slightly more sensitive to X-ray in colony formation (column 10), whereas most of the mutants show some degree of sensitivity to X-ray-irradiated transforming DNA.

Not included in Table 7 are our results on the physical fate of X-ray-irradiated transforming DNA which demonstrate that irradiation facilitates a further breakdown of DNA in the *com*$^-$48 mutant.

Whether any or all of these unusual properties of the mutants are related to their transformation deficiency is not certain. We are presently looking at other properties of these mutants (e.g., ability to excise dimers, recovery from radiation sensitivity in liquid holding, absence or modification of DNA ligase and DNA polymerase). Since DNA polymerase is involved in the repair of UV (16) and X-ray

TABLE 7. *Major properties of class 3 com*$^-$ *mutants*[a]

Strains	% Transformation[b]	Donor recovery[c]	Recovery index[d]	Recombinants[e]	Association of DNA[f]	Classification[g]	UV^s_{col}[h]	UV^r_{DNA}[i]	X^s_{col}[j]	X^s_{DNA}[k]	Classification[l]
Rd	1	100	1.5	2,512	50		1.0	1.0	1.0	1.0	
com$^-$ 4	10^{-4}	300	0.54	12	0	*dad*$^-$	0.69	12.3	1.0	1.3	UV^r_{DNA}
com$^-$ 13	10^{-3}	74	0.71	14	0	*dad*$^-$	1.2	1.1	1.0	1.5	
com$^-$ 38	10^{-3}	197	0.39	2	12	*dad*$^-$	0.69	7.8	1.0	1.0	UV^r_{DNA}
com$^-$ 40	10^{-5}	110	0.13	0	47	*dab*$^-$	11	1.8	2.2	4.8	$UV^s_{col}\ X^s_{col}\ X^s_{DNA}$
com$^-$ 48	10^{-5}	325	0.34	2	38	*dab*$^-$	0.72	5.2	1.0	5.3	$UV^r_{DNA}\ X^s_{DNA}$
com$^-$ 60	10^{-4}	82	0.31	6	44	*dab*$^-$	11	0.52	1.7	4.5	$UV^s_{col}\ X^s_{col}\ X^s_{DNA}$
com$^-$ 63	10^{-2}	275	0.77	15	0	*dad*$^-$	0.78	1.2	1.0	2.0	
com$^-$ 67	10^{-3}	110	1.0	76	3	*dad*$^-$	0.90	1.3	1.0	1.7	
com$^-$ 78	10^{-4}	108	0.72	3	14	*dad*$^-$	18	3.5	1.0	2.8	$UV^s_{col}\ UV^r_{DNA}\ X^s_{DNA}$
com$^-$ 93	10^{-5}	265	0.66	3	7	*dad*$^-$	0.75	5.1	1.0	1.8	UV^r_{DNA}
com$^-$101	10^{-4}	200	0.74	5	4	*dad*$^-$	0.75	5.7	1.0	1.9	UV^r_{DNA}

[a] Data in the first six classification columns are taken from the accompanying paper (19).

[b] Percentage of *com*$^-$ cells transformed to *nov*^{r} with Rd *nov*^{r} DNA compared to Rd cells.

[c] Donor (*nov*^{r}) activity at 10 min in the lysates (% of Rd).

[d] Donor (*nov*^{r}) activity in lysates (60 min:20 min ratios).

[e] Number of recombinants (*str*^{r} *nov*^{r}) in 60-min lysates.

[f] Percentage of donor DNA associated with recipient.

[g] Derived from data in preceding five columns. Donor association defective, *dad*$^-$; donor association biologically defective, *dab*$^-$.

[h] Ultraviolet (UV) sensitivity of colony formation (from Table 1). 37% Survival dose ratio (Rd:*com*$^-$).

[i] UV resistance of transforming DNA (from Table 2). % Survival ratio (*com*$^-$:Rd) at 5,000 ergs/min^2.

[j] X-ray sensitivity of colony formation (from Table 3). 37% Survival dose ratio (Rd:*com*$^-$).

[k] X-ray sensitivity of transforming DNA (from Table 4). 37% Survival dose ratio (Rd:*com*$^-$).

[l] Derived from data in preceding four columns. Abbreviations: UV^s_{col}, UV sensitivity of colony formation compared to Rd; X^s_{col}, X-ray sensitivity of colony formation compared to Rd; UV^r_{DNA}, increased resistance of UV-irradiated DNA in recipient cell compared to Rd; X^s_{DNA}, increased sensitivity of X-ray-irradiated DNA in recipient cell compared to Rd.

(14, 25) lesions, the sensitivity of *com*$^-$40 and 60 to UV and X-ray irradiation and their inability to transform may be explained, in these mutants, in terms of altered or reduced polymerase activity.

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