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Decreased UV mutagenesis in cdc8, a DNA replication mutant of Saccharomyces cerevisiae

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DISCLAIMER

SUMMARY

A DNA replication mutant of yeast, cdc8, was found to decrease UV-induced reversion of lys2-1, arg4-17, tyr1 and ural. This effect was observed with all three alleles of cdc8 tested. Survival curves obtained following UV irradiation in cdc8 rad double mutants show that cdc8 is epistatic to rad6, as well as to rad1; cdc8 rad51 double mutants seem to be more sensitive than the single mutants. Since UV-induced reversion in cdc8 rad1 and cdc8 rad51 double mutants is like that of the cdc8 single mutants, we conclude that CDC8 plays a direct role in error-prone repair. To test whether CDC8 codes for a DNA polymerase, we have purified both DNA polymerase I and DNA polymerase II from cdc8 and CDC+ cells. The purified DNA polymerases from cdc8 were no more heat labile than those from CDC+, suggesting that CDC8 is not a structural gene for either enzyme.

INTRODUCTION

Ultraviolet light (UV)-induced mutations in Escherichia coli result from error-prone repair system(s) which may be constitutive and/or inducible (see Bridges, 1977; Lehmann and Bridges, 197 ; Radman, 1975; Witkin, 1976, for recent reviews). This error-prone repair activity is dependent on the lexA+ and recA+ functions (Bridges, Law and Munson, 1968; Bridges and Mottershead, 1971; Miura and Tomizawa, 1968; Witkin, 1969). In addition, DNA polymerase III, which is required for chain elongation, has also been implicated in UV-induced mutations (Bridges, Mottershead and Sedgwick, 1976; Bridges and Mottershead, 1978).

In the yeast Saccharomyces cerevisiae, UV mutagenesis is dependent on the RAD6 and REV3 genes. When these genes are mutant, no UV-induced mutations are greatly reduced at a wide variety of loci tested (Lawrence, et al, 1974; Lawrence and Christensen, 1976; Lemontt, 1971). The RAD6 gene is not only required for error-prone repair of UV damage, but also for error-prone repair of damage induced by a wide variety of diverse chemical agents (Prakash, 1974). Strains with temperature-sensitive DNA synthesis have been identified among the cell division cycle (cdc) mutants of yeast (Hartwell, 1971, 1973). Mutants in the CDC4 and CDC7 genes are blocked in initiation of DNA synthesis while mutants in the CDC8 and CDC21 genes show cessation of DNA synthesis upon incubation at the restrictive temperature and these two mutants are therefore classified as elongation mutants. Thus far, these four genes are the only ones identified in S. cerevisiae which specifi- cally block DNA synthesis without affecting synthesis of other macromolecules. CDC21 is now known to be the structural gene for thymidylate synthetase (Bisson and Thorner, 1977, Game, 1976). The cdc8 mutant is not defective in synthesis of deoxyribonucleotide precursors since no DNA synthesis is observed even in permeabilized cells held at the restrictive temperature and provided with doxyribonucleotide triphosphates (Hereford and Hartwell, 1971).

We wanted to determine whether gene functions required for DNA synthesis also play a role in mutagenic repair of UV damage in yeast. Our approach was to determine the effect of these cdc mutants which affect DNA synthesis on UV-induced mutations of nuclear genes. In this communication, we report the effect of cdc8 on UV-induced mutagenesis of nuclear genes in yeast.

METHODS

Strains

Strains A364A (a ade1 ade2 lys2-1 his7 tyr1 gal1 ura1 CDC+), strain 198 (cdc8-1 derivative of A364A) and other cdc mutant derivatives of A364A were obtained from Dr. Lee Hartwell whereas the cdc8-2 and cdc8-3 alleles used in this study were obtained from Dr. Dennis Livingston. The rad1 and rad6 mutants were obtained from Dr. Brian Cox and the rad52 mutant from Dr. Michael Resnick.

Markers desired were introduced into the various strains by standard techniques of yeast genetics.

Media

The following media were used: YPD, 1% Bacto-yeast extract, 2% Bacto-peptone, 2% dextrose, solidified with 2% Bacto-agar. Synthetic complete (SC) medium, used for viability determinations, consisted of 0.67% Difco yeast nitrogen base without amino acids, 2% dextrose, 2% Bacto-agar, adenine sulfate, L-arginine HCl, L-histidine HCl, L-methionine, L-tryptophan and uracil at 20 µg/ml, DL-homoserine at 100 µg/ml, L-isoleucine, L-leucine, L-lysine HCl and L-tyrosine at 30 µg/ml, L-valine at 150 µg/ml and L-phenylalanine at 50 µg/ml. Omission media used to score for revertants to prototrophy for arginine, lysine, tyrosine or uracil, consisted of the above constituents lacking one of the supplements at a time and were designated SC minus arginine (SC - arg), SC minus lysine (SC - lys), SC minus tryosine (SC - tyr) and SC minus uracil (SC - ura), respectively.

Irradiation

Survival curves: Cultures grown to stationary phase in liquid YPD at the permissive temperature of 25^{o} C were washed and plated on YPD plates.

Cells were irradiated with covers removed at a fluence rate of 0.1 and 1 $Jm^{-2}s^{-1}$. The radiation source and its dosimetry are given in Lawrence, et al., (1974). Plates were incubated in the dark at 25° C for 3 to 6 days before counting surviving colonies.

Mutation induction: cultures grown to stationary phase in liquid YPD at 25° C were washed and plated on SC for viability determinations and on various omission media for determining UV-induced reversion frequencies of a particular marker. Cells were irradiated on the surface of plates as described above. Plates were incubated at 25°C in the dark for 3 to 8 days before counting revertant colonies.

Growth of cells and purification of DNA polymerases

Cells of A364A (CDC+) and 198 (cdc8-1) were grown at 25° C to mid-exponential phase in 1 liter batches of YPD in 2.3 liter Fernbach flasks. Cultures were incubated with shaking at 36° C for 1 hour just before harvesting. A sample from each culture was withdrawn and spread on YPD plates for subsequent testing of the cdc phenotype. This was necessary to avoid cultures where a rare CDC+ revertant might arise and be selected for. Therefore, all cultures were routinely tested for reversion to the CDC+ phenotype. Cells were washed in buffer containing 20 mM Tris, pH 7.6 - 1 mM EDTA - 2 mM - 2-mercaptoethanol - 10% glycerol - 0.25 M mannitol - 0.05 mM PMSF and stored at -70° C.

DNA polymerases I and II were purified through the DEAE cellulose step as described by Chang (1977) with the following minor modifications. The cells were ruptured with glass beads in a Braun homogenizer as decribed by Wintersberger and Wintersberger (1970) instead of in an Eaton press. After

centrifugation at 24,000 x g for 30 min, the extract was diluted to give an A_{260} = 140 (50 g of cells typically yielded 120 ml of extract). Precipitation of nucleic acids was then carried out as described by Chang using 0.12% instead of 0.2% protamine sulfate. After fractionation by precipitation with ammonium sulfate and chromatography on phosphocellulose, the two DNA polymerases were separated by chromatography on DEAE cellulose. Fractions containing each DNA polymerase were pooled separately, concentrated by precipitation with ammonium sulfate, and stored at -20° C in 20 mM potassium phosphate buffer, pH 7.2 containing 0.1mM dithiothreitol and 50% glycerol. Although DNA polymerase II appears as a minor component when assayed using activated DNA, using the synthetic template poly dA: oligo dT, the two activities are present in nearly equal amounts. As described by Chang, we often observe two peaks of DNA polymerase I activity. Both of these activities are inhibited by antibody (generously provided by Dr. Chang) prepared against highly purified yeast DNA polymerase I.

RESULTS

Effect of cdc8 on UV-induced reversion

The effect of the cdc8 mutation on UV-induced reversion of various loci was examined by determining the frequency of revertants obtained at different loci following UV irradiation. The fluence response curves for reversion of lys2-1 to LYS+ in cdc8-1 and CDC+ haploid strains are given in Figure 1A and the survival curves for the same strains are given in Figure 1B. The frequency of revertants at all UV fluences is greatly reduced in the cdc8-1 haploid LP859-3B compared to the CDC+ strains, even at fluences where survival is still around 10% in the cdc8 strain. In order to ascertain that

the effect observed was in fact due to cdc8-1 and not to modifiers in the background of the strain, frequencies of UV-induced reversion were determined at two different fluences in several different CDC+ and cdc8-1 segregants obtained from the same cross that generated the strains used in Figure 1. Results of this segregation analysis are shown in Table 1. Reversion frequencies in cdc8-1 strains are much lower than in the CDC+ strains. Although the cdc8-1 strains are moderately UV-sensitive, reversion frequencies in CDC+ strains at a given survival level are higher than in cdc8-1 strains irradiated at a UV fluence resulting in the same survival. For example, CDC+ (LP859-1C) irradiated at 50 J/m^2 resulted in 33% survival and 143 LYS+ colonies per 10^7 survivors whereas a cdc8-1 segregant from the same tetrad (LP859-1B), when irradiated with 25 J/m^2 resulted in 33% survival but the frequency of reversion of lys2-1 is only 17 LYS+ colonies per 10^7 survivors. The data in Table 1 clearly show that the lowered reversion frequency of lys2-1 observed is due to the cdc8-1 allele itself and not to any background modifiers.

Other alleles of cdc8 were also tested for their effect on reversion of lys2-1. Figures 2 and 3 give the fluence response curves obtained for reversion and survival in cdc8-2 and cdc8-3 haploid strains, respectively. In haploids containing either the cdc8-2 or the cdc8-3 alleles, as was observed for cdc8-1 haploids, the frequency of reversion of lys2-1 is reduced substantially. At the highest UV fluence used in these experiments, survival in the cdc8 haploids is still about 10%. A one hour pulse at the restrictive temperature of $36^{o}C$ following UV irradiation with fluences of 25 J/m^2 or less does not appear to decrease the frequency of UV-induced reversion of lys2-1 or the

viability any further. Thus, all three alleles of cdc8 lower UV-induced reversion of lys2-1 in haploid yeast.

Since lys2-1 is an ochre-suppressible allele, we checked whether the cdc8 mutation affected reversion of other ochre-suppressible loci as well. UV-induced reversion frequencies at arg4-17 were determined in cdc8 and CDC+ strains. Mutation induction curves for reversion of arg4-17 to ARG+ and survival curves following UV irradiation in cdc8 and CDC+ strains are given in Figures 4A and 4B, respectively. At a fluence of 50 J/m^2, there are about 10 times as many revertants in the CDC+ haploids as there are in the cdc8 haploids. At very low UV fluences, there does not seem to be any difference in reversion frequency in CDC+ and cdc8 strains. Nevertheless, even though the data indicate that there is some induction of ARG+ revertants in the cdc8 strains, the level of induction is greatly reduced compared to the CDC+ strains. Although both lys2-1 and arg4-17 are ochre-suppressible, and spontaneous reversion of these alleles yields both suppressors as well as site revertants, UV-induced revertants are mainly site revertants (Lemontt, 1971).

The effect of the cdc8 mutation on UV-induced reversion extends to non-suppressible markers as well. The induction curves for TYR+ revertants from tyr1 and the survival curves following UV irradiation in cdc8 and CDC+ strains are given in Figures 5A and 5B, respectively. The data show that reversion at this locus is also greatly diminished in the cdc8 haploid. This reduction in UV-induced mutations also segregates with the cdc8 gene. UV-induced reversion of tyr1 was substantially lower in five cdc8 segregants examined compared to two CDC+ strains obtained from the same cross (Table 2). Reversion of ura1 to URA+, another non-suppressible site, is also reduced in cdc8 haploid strains

(Table 3). All of these data indicate that the effect of the cdc8 mutation is to reduce UV-induced reversion at both the suppressible and non-suppressible loci tested.

UV-induced reversion frequencies of lys2-1 and tyr1 were determined in other cell division cycle mutants that have a temperature sensitive defect in DNA synthesis: cdc4-1, cdc7-4 and cdc21-1. The defect in cdc4-1 and cdc7-4 is not known, except that both of these mutants apparently can carry out chain elongation but not initiation of DNA synthesis at the restrictive temperature (Hartwell, 1971). Reversion frequencies for lys2-1 and tyr1 in cdc4 and cdc7 haploids were essentially like those in CDC+ strains; reversion in the cdc21 strain was only slightly reduced.

UV sensitivity and UV-induced reversion in cdc8 rad strains

In yeast, the radiation sensitive (rad) mutants isolated in various laboratories (Cox and Parry, 1968; Game and Mortimer, 1974; Resnick, 1969; Snow, 1967), comprise 32 distinct genetic loci and belong to three epistatic groups (Cox and Game, 1974) or pathways for repairing UV-induced damage in DNA. One group consists of 9 genes involved in excision of UV-induced pyrimidine dimers: RAD1, RAD2, RAD3, RAD4 (Prakash, 1975, 1977a; Resnick and Setlow, 1972; Unrau, Wheatcroft and Cox, 1971; Waters and Moustacchi, 1974), RAD10, RAD16 (Prakash, 1977b), RAD7, RAD14 and MMS19 (Prakash and Prakash, unpublished results). The second epistatic group consists of at least seven loci involved in error-prone repair of UV-induced damage: RAD6, RAD8, RAD9, RAD18, REV1, REV2 and REV3. All of these mutants reduce UV mutability at some, if not all, loci tested (Lawrence and Christensen, 1976; Lemontt, 1971). The third epistatic group consists of the genes RAD50 to RAD57.

Mutants at these loci were selected for sensitivity to X-rays and are only moderately UV-sensitive (Game and Mortimer, 1974). However, in combination with a rad3 mutant, defective in excision repair, enhanced UV sensitivity is observed (Cox and Game, 1974). A triple mutant haploid in which all three pathways are blocked is as UV sensitive as the double mutant uvrA recA in E. coli. Thus far, all yeast mutants which show lowered UV-induced mutation belong in the RAD6 error-prone repair epistatic group, and since the cdc8 mutant lowers UV-induced mutations at all the loci tested, we wanted to see whether the cdc8 mutant belonged to the previously identified epistatic group involved in error-prone repair or whether it represented a mutation in another mutagenic pathway. Double and single mutants of cdc8 RAD+, CDC+ rad6, CDC+ RAD+ and cdc8 rad6 were constructed by standard yeast genetic techniques and survival curves were obtained following UV irradiation by plating cells on YPD medium and incubating the plates at 25^{o} C. Figure 6 gives the results obtained. Since the rad6 mutant is quite sensitive to killing by UV, lower UV fluences than were used for mutation experiments were employed in these experiments. In this range of UV fluences, the cdc8 strains give survival curves with somewhat steeper slopes than the CDC+ strains. These survival curves represent the results obtained from strains generated from two different crosses. It can be seen that the sensitivity of the double mutant of cdc8 rad6 is only somewhat greater than that of the rad6 single mutant only at 10 J/m^2, however, the standard errors are large and the 95% confidence interval for the mean survival of rad6 mutants overlaps the mean survival of cdc8 rad6. We conclude that the cdc8 mutant probably belongs to the same epistatic group as rad6.

Since we noted that the sensitivity of cdc8 strains varied somewhat depending on the background, great care was taken to use many different strains to obtain the data given in the survival curves and to always use cdc8 haploids obtained from segregants used in a particular cross, i.e., in each case, the cdc8 survival curve given is obtained from cdc8 haploids generated from the particular cdc8 X rad cross.

If the function provided by the CDC8 gene were required in more than one pathway of repair, then the cdc8 mutant would be epistatic to more than one group. In order to determine whether this might be the case, cdc8 was crossed to rad1, a member of the group involved in excision of dimers, and to rad51, a member of the third epistatic group in yeast. Survival curves obtained for CDC+ RAD+, cdc8 RAD+, CDC+ rad1 and cdc8 rad1 strains are given in Figure 7. Double mutants of cdc8 rad1 appear to be somewhat more sensitive than rad1 single mutants. However, the 95% confidence intervals of the mean survival of rad1 mutants overlap the mean survival of the cdc8 rad1 mutants at all UV doses. It seems as if cdc8 also belongs to the rad1 epistatic group. Double mutants of cdc8 rad51 are more UV sensitive than the rad51 and cdc8 single mutants and the interaction appears additive (Figure 8). Experiments in which stationary cells of cdc8 haploids and cdc8 rad strains were incubated in YPD medium at 36^{o}C for 1 hour prior to UV irradiation had no effect on the survival curves obtained.

The UV-induced reversion frequency of lys2-1 in cdc8 rad1 and cdc8 rad51 double mutants is like the frequency found in cdc8 single mutants (Table 4).

DNA polymerases in cdc8 mutants

Since cdc8 mutants show cessation of DNA synthesis at the restrictive temperature (Hartwell, 1971), we have investigated the possibility that these mutants are defective in a DNA polymerase. Two DNA polymerases have

been purified from extracts of S. cerevisiae (Wintersberger and Wintersberger, 1970; Wintersberger, 1974; Helfman, 1973; Chang, 1977). Both enzymes are of high molecular weight (> 100,000) but they show no immunological cross-reactivity (Chang, 1977). In addition, the two enzymes can be distinguished on the basis of template specificity; DNA polymerase II is relatively inactive with a standard "activated" DNA, but functions well using poly dA:oligo dT as a substrate (Chang, 1977).

We have partially purified both DNA polymerase I and DNA polymerase II from cdc8-1 and from its parent strain. Cells were incubated for 1 hour at the restrictive temperature just before harvesting to maximize the possibility of detecting a defective enzyme. However, we found no effect of the cdc8 mutation on the activity of either DNA polymerase I or DNA polymerase II. The enzymes purified from cdc8-1 had essentially the same activitity, assayed at either 37°C or 45°C with either activated salmon sperm DNA or poly dA:oligo dT, as the enzymes from CDC+ strains (Table 5). Furthermore, the kinetics of heat inactivation of enzymes from the two strains was identical (Figure 9). Thus, the half life at 45°C for the enzyme from either cdc8-1 or CDC+ was 1 minute for DNA polymerase I and and 6 minutes for DNA polymerase II. We tentatively conclude that the cdc8 mutation does not affect the activity of either DNA polymerase I or DNA polymerase II.

DISCUSSION

The results presented here show that the cdc8 mutation results in decreased frequencies of UV-induced mutations of both suppressible and non-suppressible loci. These effects of lowered UV-induced mutations are apparent at the permissive temperature of 25° C, even though cdc8 is not defective in DNA synthesis at this temperature. Spontaneous mutation is

unaffected in cdc8 haploids (Newlon, Ludescher and Water, 1979). The reduction in UV induced mutations observed in cdc8 may be due either to a direct involvement of the CDC8 gene product in error-prone repair or to increased error-free repair. In yeast, UV damage can be repaired by an error free excision repair pathway controlled by RAD1 and several other loci (Lawrence and Christensen, 1976; Prakash, 1975), error-free recombinational repair controlled by RAD51 and other loci (McKee and Lawrence, 1979; Prakash, et al., 1979), or in an error-prone manner, controlled by RAD6, REV3 and several other loci (Lawrence and Christensen, 1976; Lemontt, 1971). Since the cdc8 rad1 and cdc8 rad51 double mutants show low UV-induced reversion frequencies similar to cdc8 single mutants, we conclude that the CDC8 gene function is involved in error-prone repair. Error-prone repair in yeast does not appear to be a major repair pathway contributing to survival from UV damage since cdc8 mutants are not particularly UV sensitive but show a large decrease in UV induced reversion at all of the loci tested. The rad6 mutants, which show no UV-induced reversion are very UV sensitive (Figure 6). The increased UV sensitivity of rad6 must derive from its largely error-free role in repair. In E. coli, error-prone repair may also not contribute greatly to UV survival since rnm mutants of lexA are not very UV sensitive but show no UV induced reversion (Volkert, George and Witkin, 1976).

According to current ideas on UV mutagenesis in E. coli, most of the photoproducts induced in DNA by UV irradiation are non-mutagenic (Bridges, 1977; Lehmann and Bridges, 197 ; Witkin, 1976). The lesions which are potentially mutagenic for the cell represent a minor fraction of the total photoproducts, and are thought to arise after DNA replication has occurred from templates containing unexcised dimers. DNA which is replicated following

UV irradiation contains gaps and most of the daughter strand gaps are repaired in a recombinational manner (Rupp, et al., 1971). Recombinational repair involving recA+, polA+ or polC+ is thought to repair the potentially non-mutagenic daughter strand gaps in an error-free way while the potentially mutagenic daughter strand gaps can be repaired either in an error-free manner, involving recA+, uvrA+, uvrB+, excision-dependent post-replication repair (Green, et al., 1977) or in an error-prone manner, involving recA+, lexA+ and polC+ (see Bridges, 1977; Lehmann and Bridges, 197 ; Sedgwick, 1976). In an excision-defective strain carrying a temperature-sensitive polymerase III mutation in E. coli, UV-induced revertants (suppressor mutations) to trp+ show no loss of photoreversibility when cells are held at the restrictive temperature of 43° C while photoreversibility for mutations is lost at the permissive temperature of 34° C (Bridges, Mottershead and Sedgwick, 1976), thus showing that polymerase III is involved in UV mutagenesis. Thus, DNA polymerases I and III, the products of the polA+ and polC+ (dnaE+) genes, respectively, play a role both in error-free and error-prone repair of UV damage.

Our results indicate that cdc8 mutants of yeast are not defective in either DNA polymerase I or DNA polymerase II. It is possible that in these mutants, a DNA polymerase is defective in vivo but not in vitro. In E. coli, some temperature-sensitive polC mutants produce a DNA polymerase III which is not thermolabile in vitro. However, these mutants do not immediately stop DNA synthesis at the restrictive temperature (Gefter, et al., 1971; Konrad, 1978). Since cdc8 is an immediate stop mutant (Hartwell, 1971), our finding that neither polymerase I nor polymerase II has increased thermolability strongly suggests that cdc8 is not a structural gene for either of these

enzymes. It is of course possible that cdc8 codes for third, as yet unidentified, DNA polymerase which is involved in both DNA replication and error-prone repair in yeast.

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FIGURE LEGENDS

Figure 1. (A) lys2-1 reversion fluence-response curves for CDC+ (A364A ● and LP859-3A ○) and cdc8-1 (LP859-3B □) strains following UV irradiation.

(B) Survival fluence-response curves for the same strains as in Figure 1A following UV irradiation.

Each curve represents the average of two to three replicate experiments for each strain.

Figure 2. (A) lys2-1 reversion fluence-response curve for CDC+ (LP1212-9A ●) and cdc8-2 strains (LP1212-3D □) following UV irradiation.

(B) Survival fluence-response curves for the same strains as in Figure 2A following UV irradiation.

Each curves represents the average of two to three replicate experiments for each strain.

Figure 3. (A) lys2-1 reversion fluence-response curves for CDC+ (LP1210-11A ●) and cdc8-3 (LP1210-1C □ and LP1210-2A ■) strains following UV irradiation.

(B) Survival fluence-response curves for the same strains as in Figure 3A following UV irradiation.

Each curve represents the average of two to three replicate experiments for each strain.

Figure 4. (A) arg4-17 reversion fluence-response curves for CDC+ (CL332-1B ○ and LP752-3D ●) and cdc8-1 (LP752-2B □ and LP752-4C ■) strains following UV irradiation.

(B) Survival fluence-response curves for the same strains as in Figure 4A following UV irradiation.

Each curve represents the average of two replicate experiments for each strain.

Figure 5. (A) tyr1 reversion fluence-response curves for CDC+ (LP752-3D ○) and cdc8-1 (LP752-3A ●) strains following UV irradiation.

(B) Survival fluence-response curves for the same strains as in Figure 5A following UV irradiation.

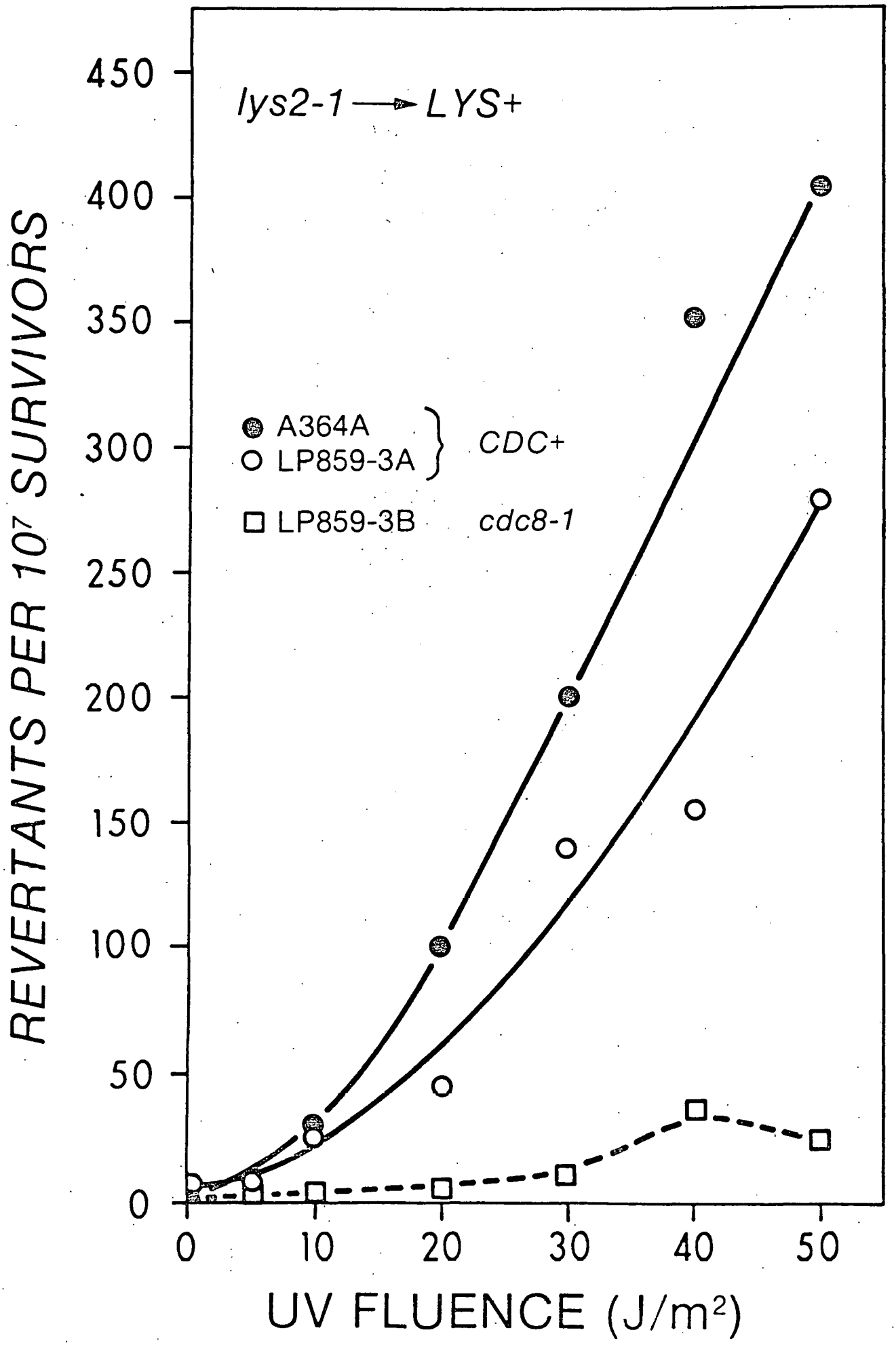
Figure 6. Survival curves following UV irradiation of CDC+ RAD+, ■ , (three strains from the LP-1305 cross); cdc8 RAD+, ○ , (one strain from the LP-1211 cross and 4 strains from the LP-1305 cross). CDC+ rad6, □ , (3 strain from the LP-1211 cross and 4 strains from the LP-1305 cross); and cdc8 rad6, ● , (3 strains from the LP-1211 cross and 2 strains from the LP-1305 cross). Each curve represents the average for the number of strains indicated.

Figure 7. Survival curves following UV irradiation of CDC+ RAD+, ■ , (one strain from the LP-1210 cross and one strains from the LP-1211 cross); cdc8 RAD+, ○ , (3 strains from the LP-859 cross); CDC+ rad1, □ , (3 strains from the LP-859 cross); and cdc8 rad1, ● , (2 strains from the LP-859 cross). Each curve represents the average for the number of strains indicated.

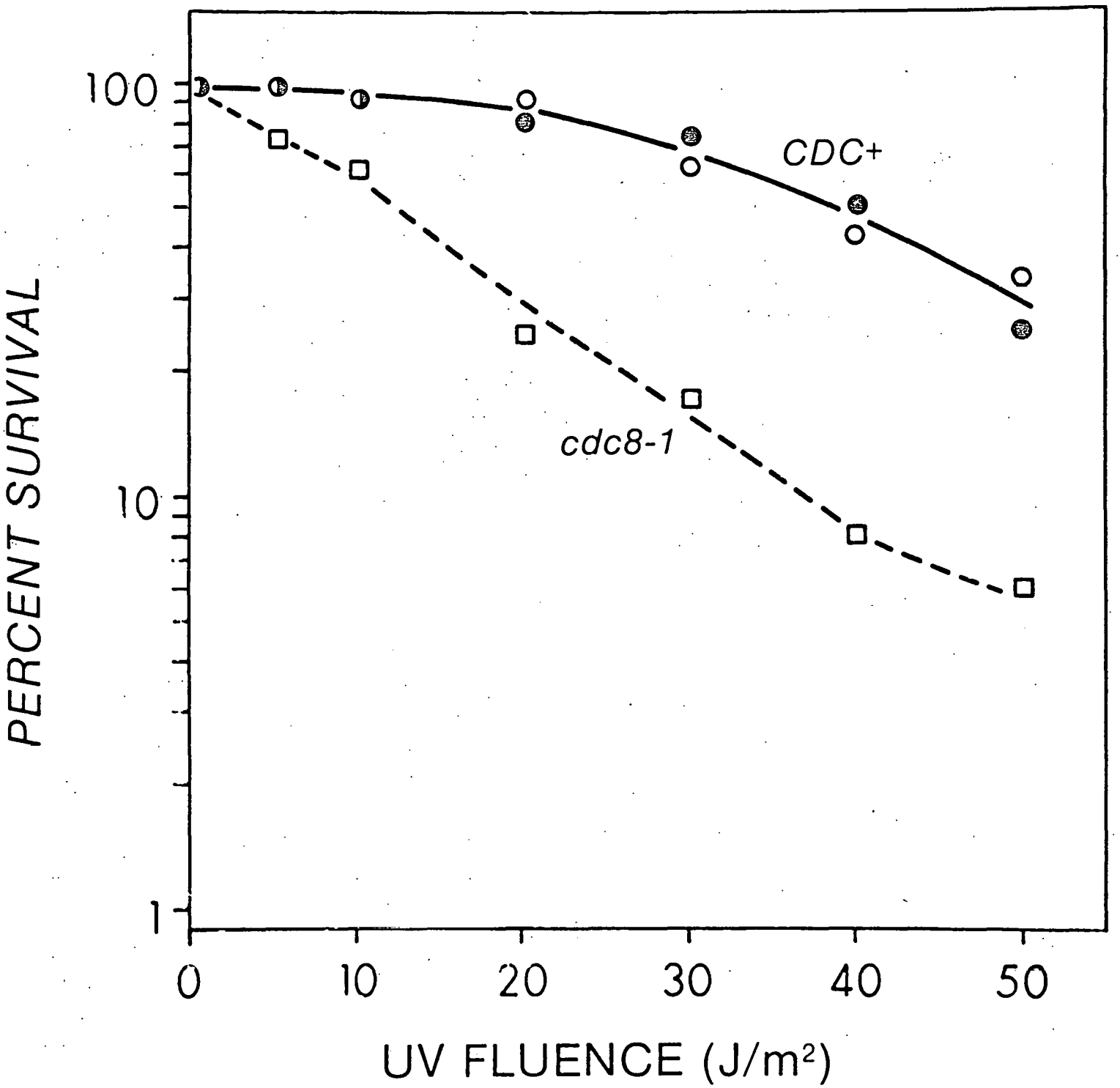
Figure 8. Survival curves following UV irradiation of CDC+ RAD+, ■ , (2 strains from the LP-1210 cross); cdc8 RAD+, ○ , (6 strains from the LP-1210 cross); CDC+ rad51, □ , (3 strains from the LP-1210 cross); and cdc8 rad51 strains, ● , (4 strains from the LP-1210 cross). Each curve represents the average for the number of strains indicated.

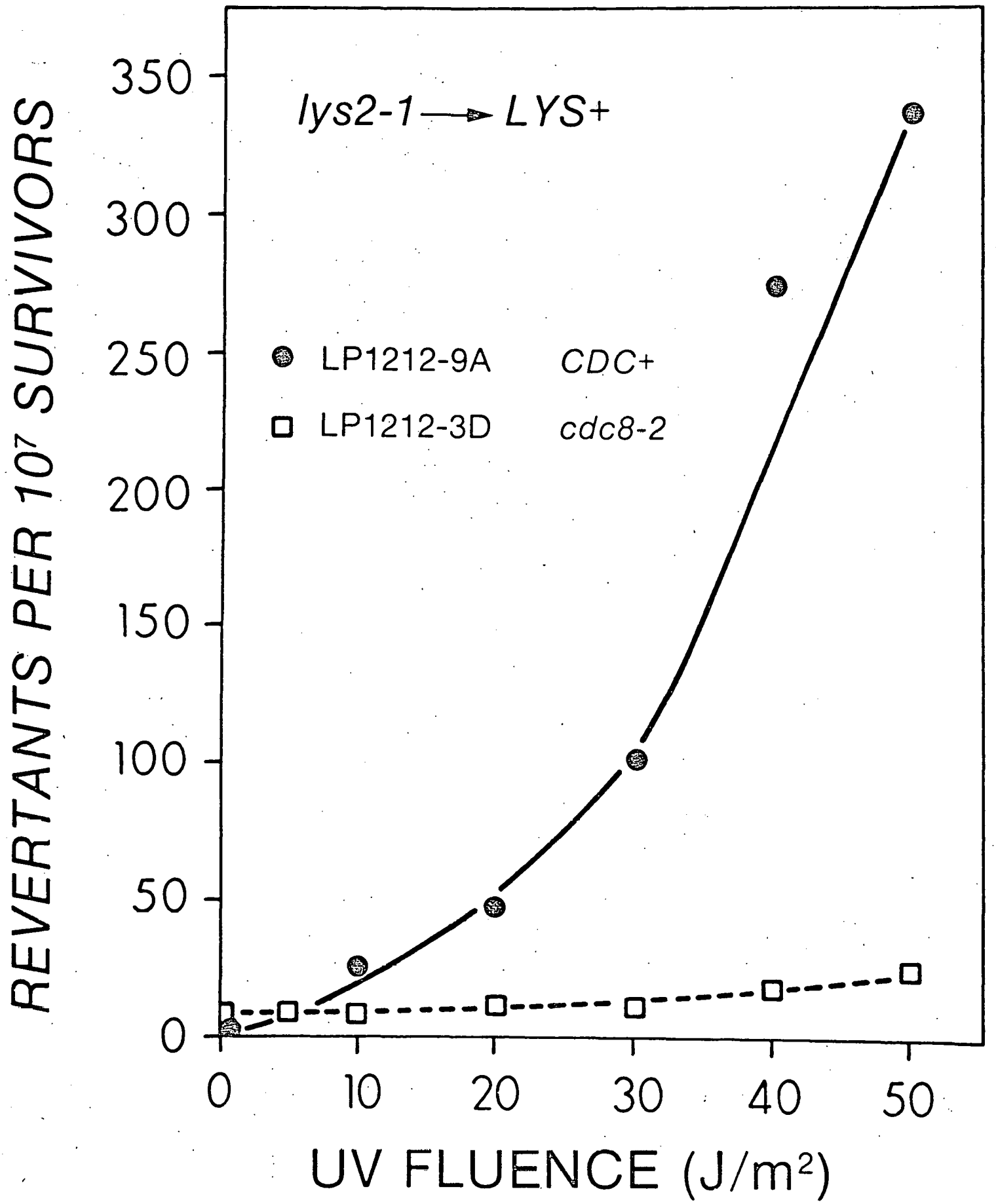
Figure 9. Heat inactivation of yeast DNA polymerases.

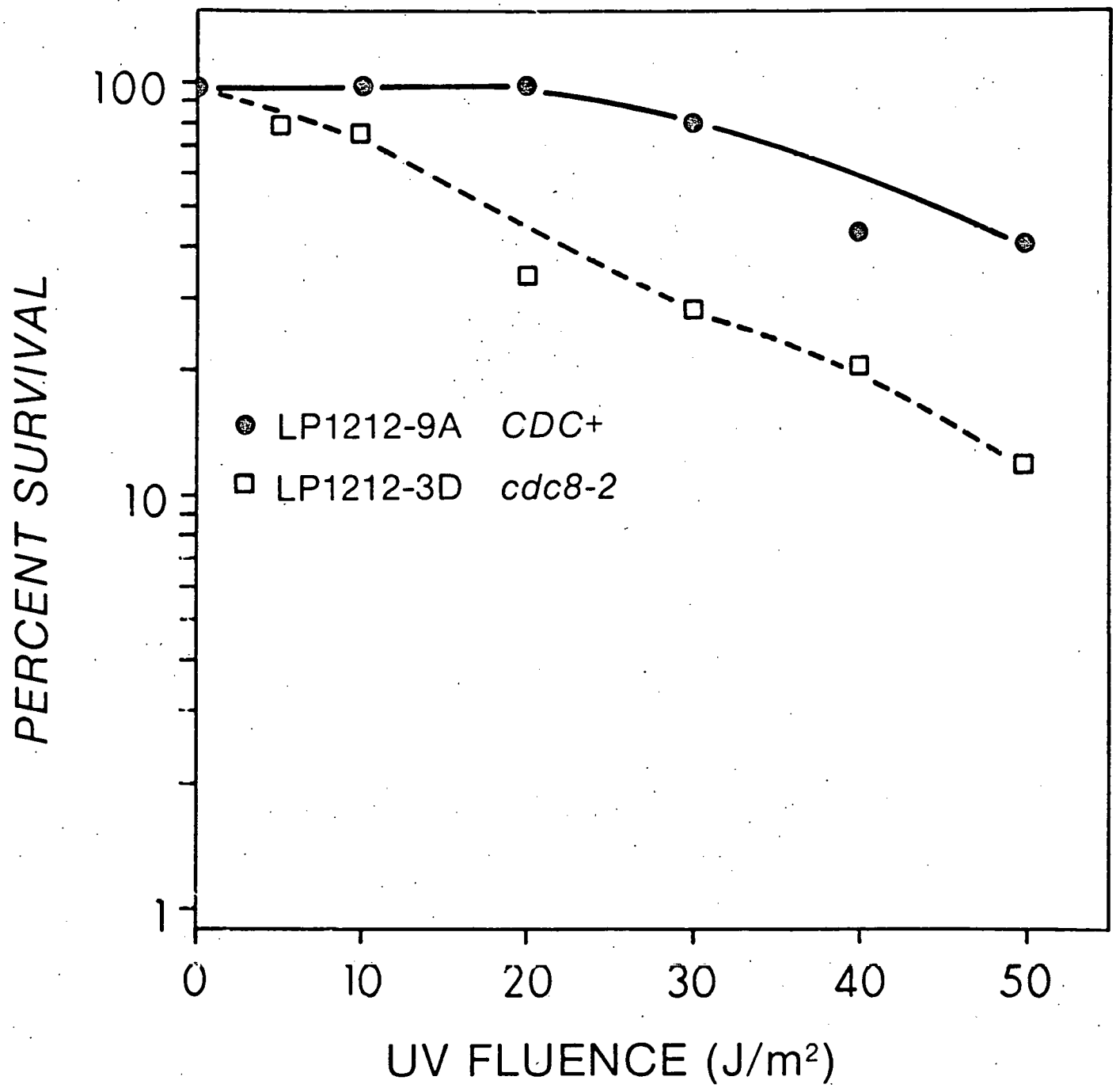
DNA polymerases were diluted to about 1 mg/ml in 20mM Tris, pH 7.5, 10mM 2-mercaptoethanol, 1 mg/ml bovine serum albumin and incubated at 45°C. At intervals, 5 µl samples were removed and immediately assayed for DNA polymerase activity at 37° using poly dA:oligo dT as described in Table 5. CDC+: ○ , □ ; cdc8: ● , ■ .

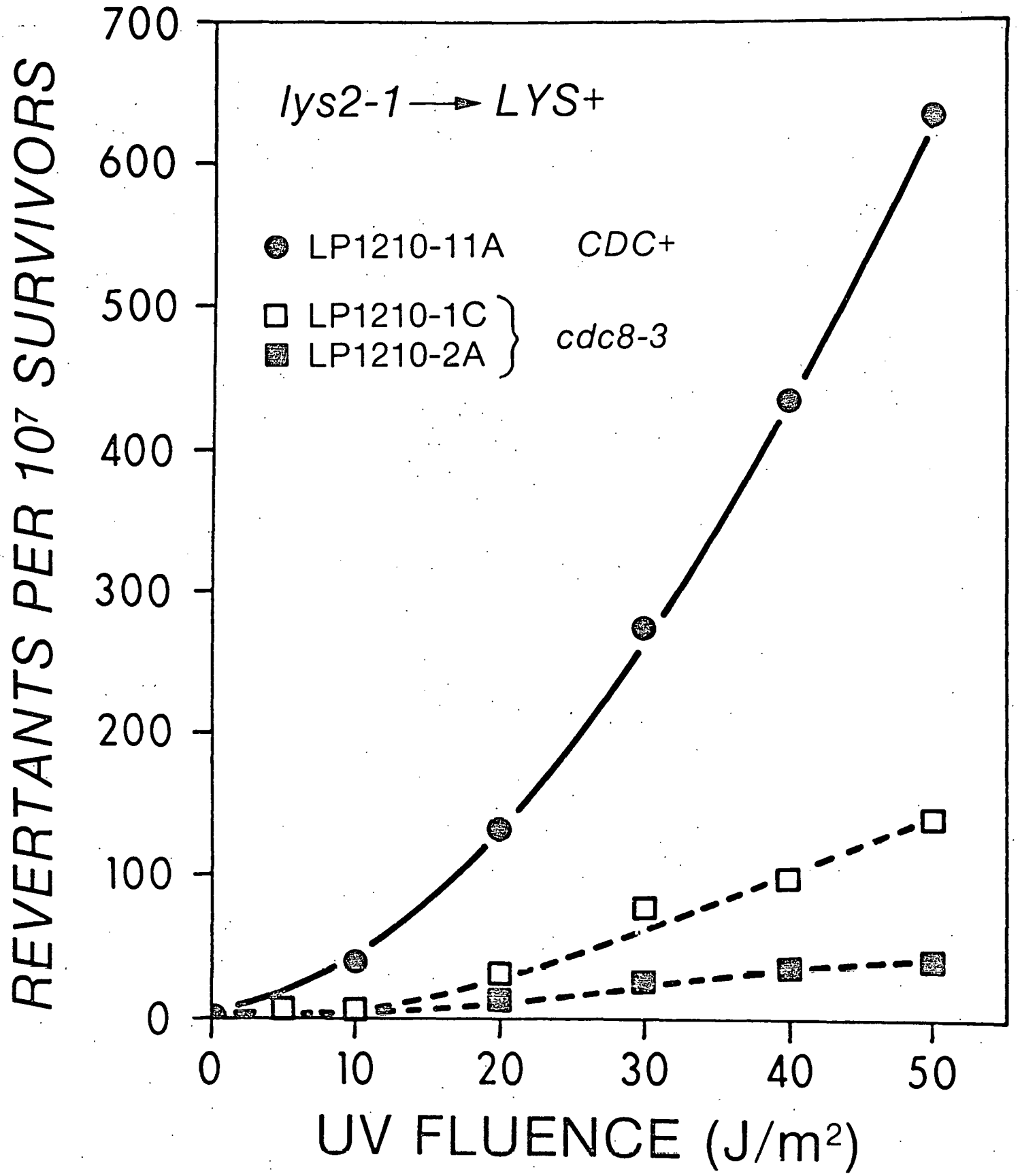

lys2-1 → LYS+
REVERTANTS PER 10⁷ SURVIVORS
UV FLUENCE (J/m²)
A364A
LP859-3A
CDC+
LP859-3B
cdc8-1
0
50
100
150
200
250
300
350
400
450
0
10
20
30
40
50

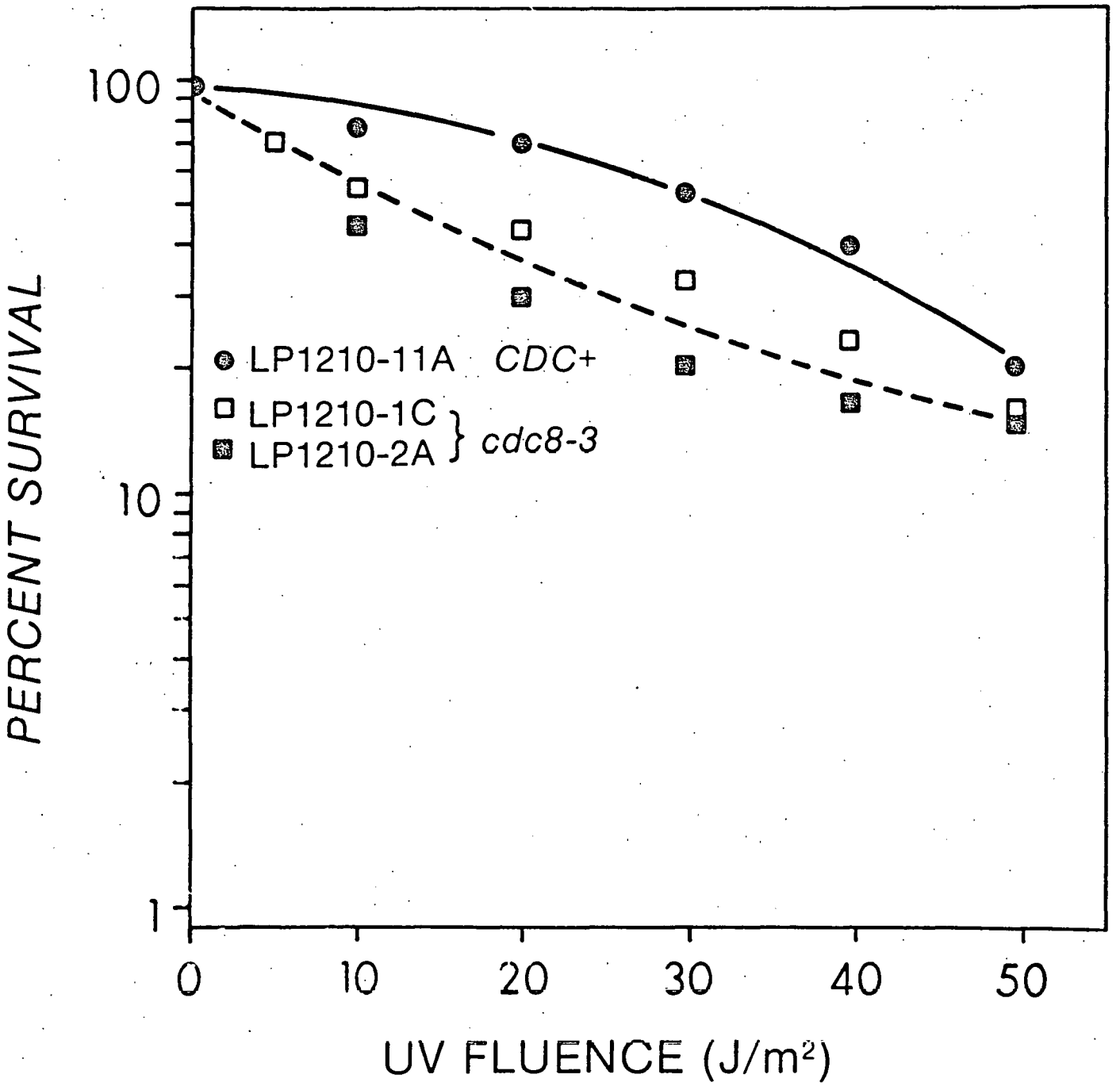
1B

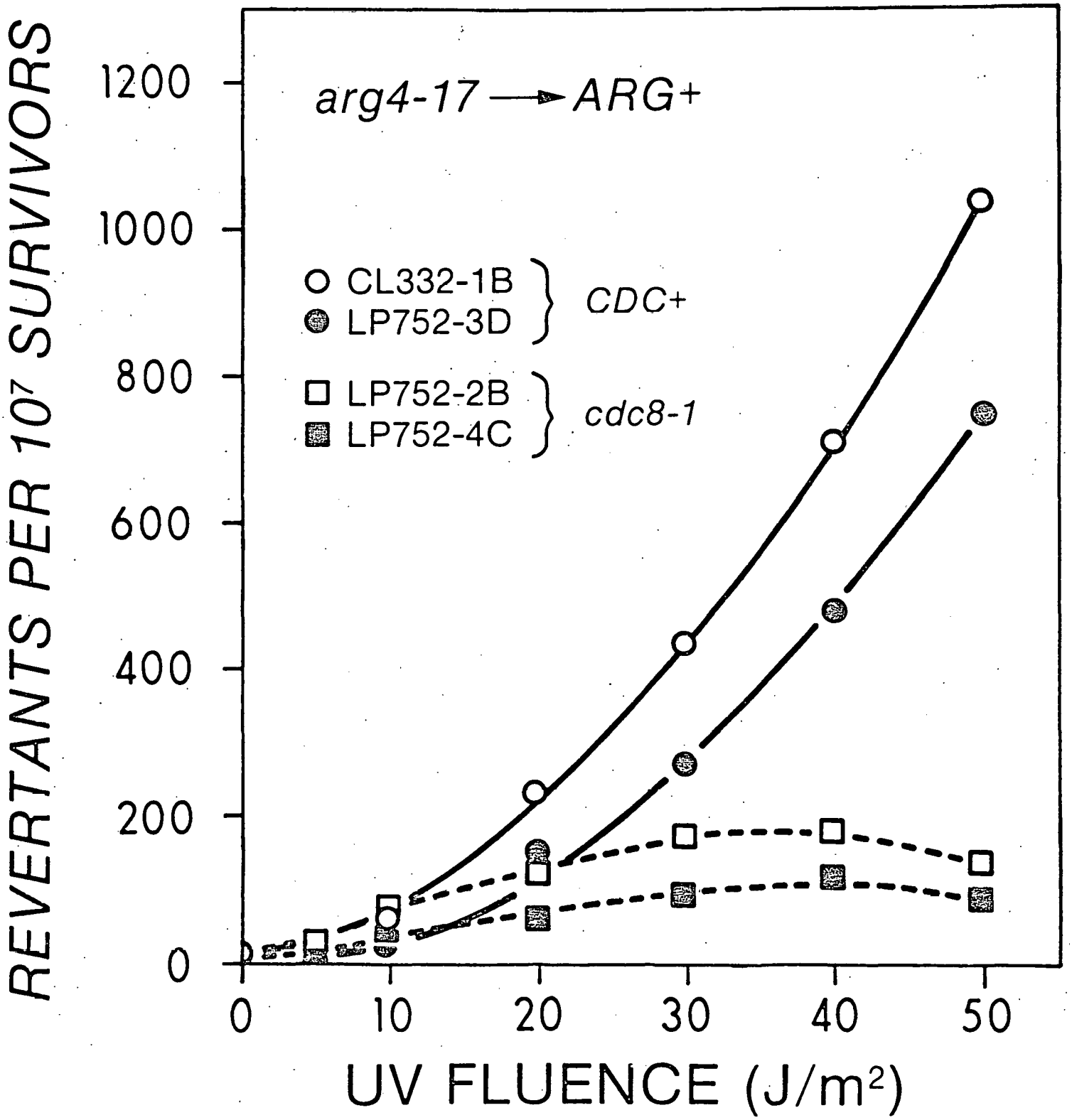

100
10
1
PERCENT SURVIVAL
CDC+
cdc8-1
0
10
20
30
40
50
UV FLUENCE (J/m²)

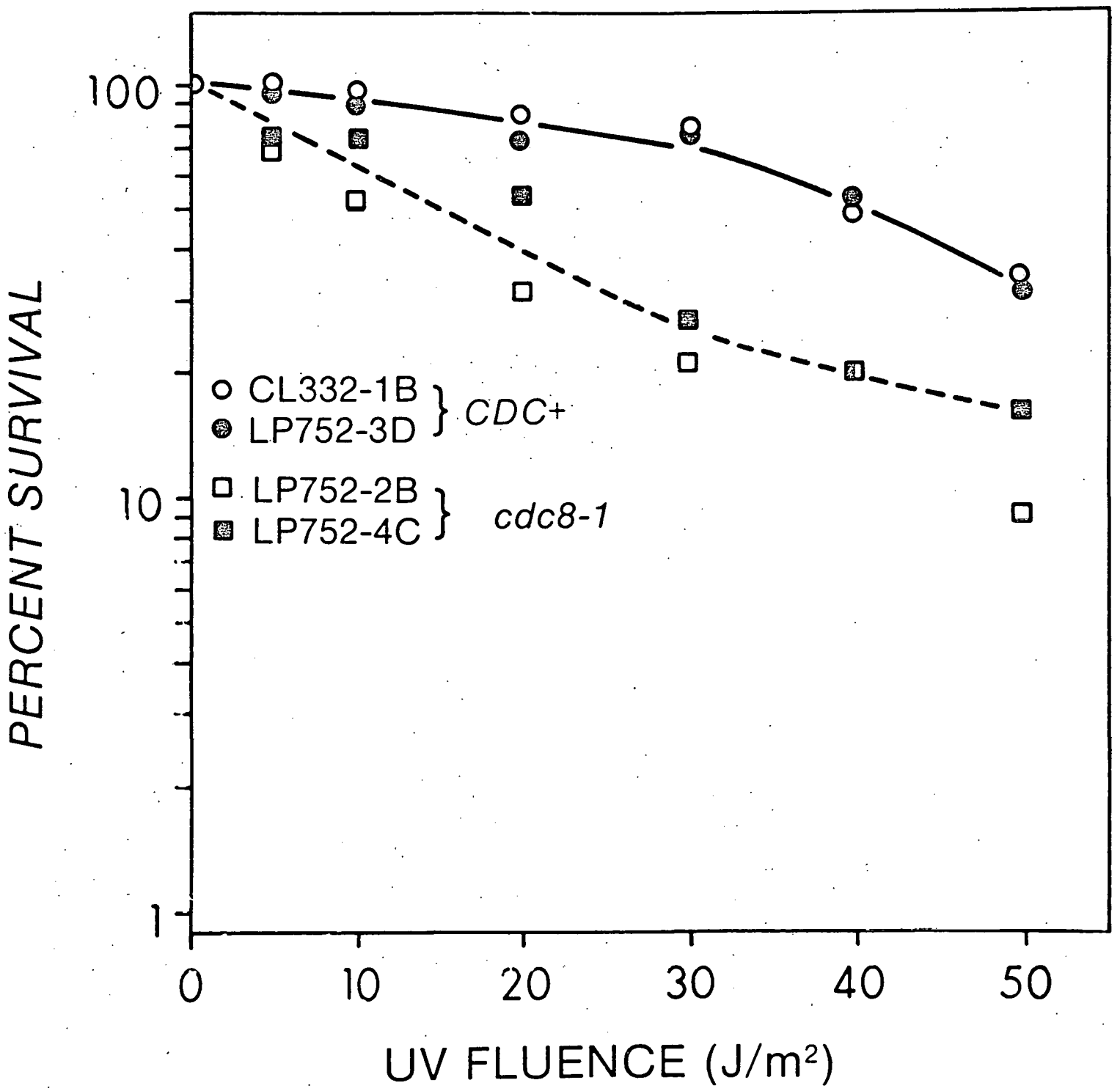

lys2-1 → LYS+
LP1212-9A CDC+
LP1212-3D cdc8-2
REVERTANTS PER 10⁷ SURVIVORS
UV FLUENCE (J/m²)
0
50
100
150
200
250
300
350
0
10
20
30
40
50

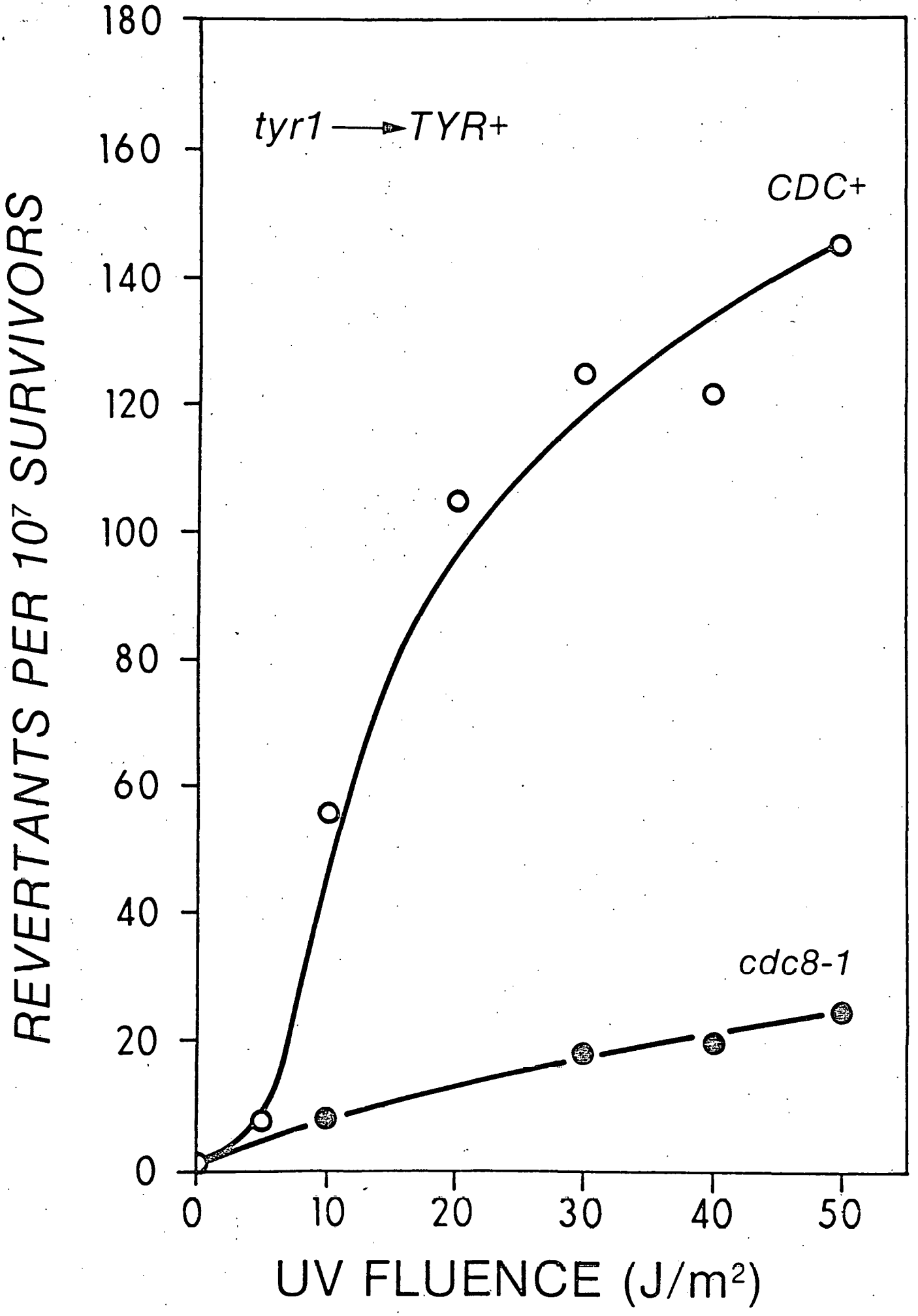

100
10
1
PERCENT SURVIVAL
LP1212-9A CDC+
LP1212-3D cdc8-2
0
10
20
30
40
50
UV FLUENCE (J/m²)

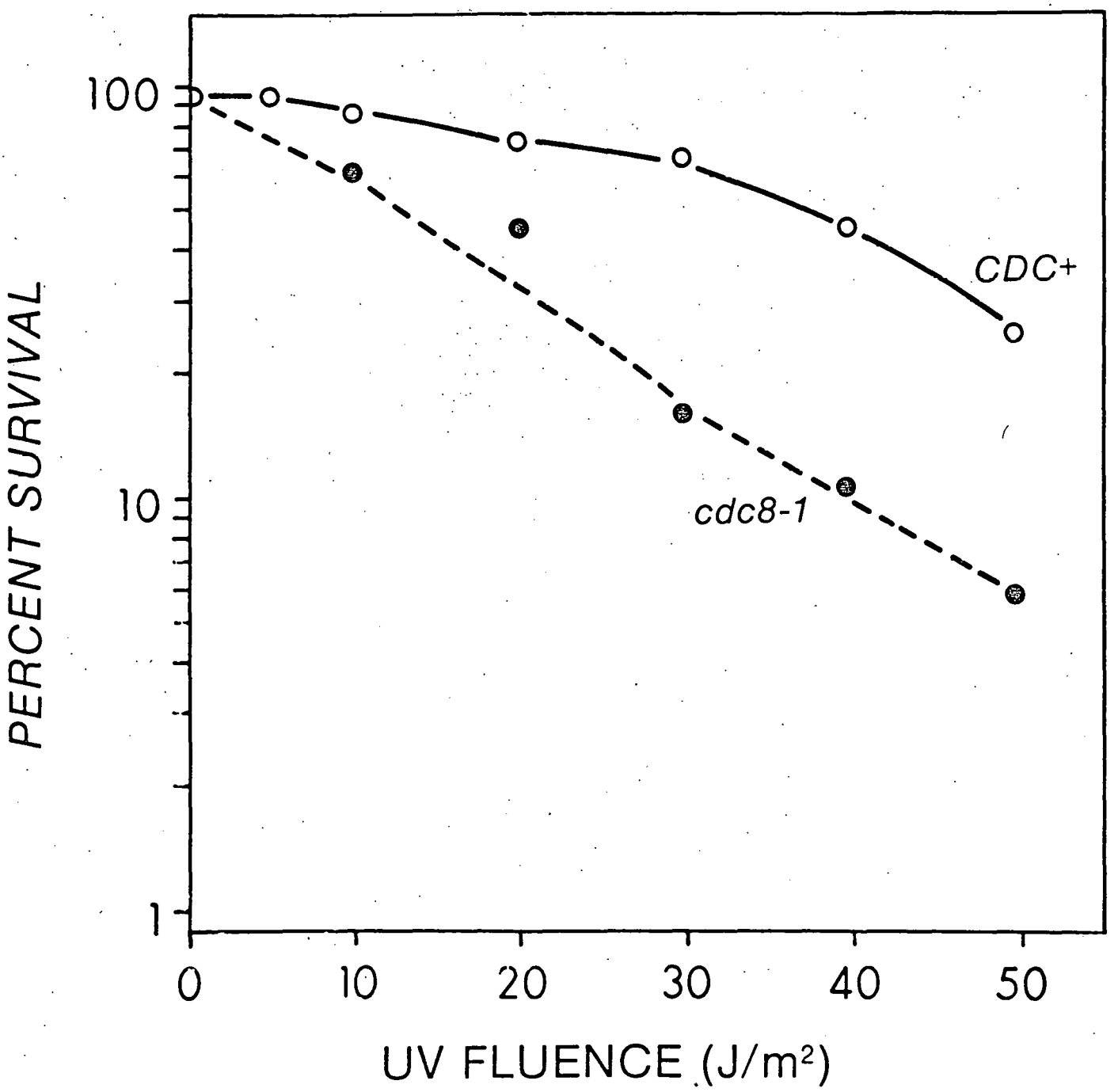

lys2-1 → LYS+
LP1210-11A CDC+
LP1210-1C
LP1210-2A
cdc8-3
REVERTANTS PER 10⁷ SURVIVORS
UV FLUENCE (J/m²)
0
100
200
300
400
500
600
700
0
10
20
30
40
50

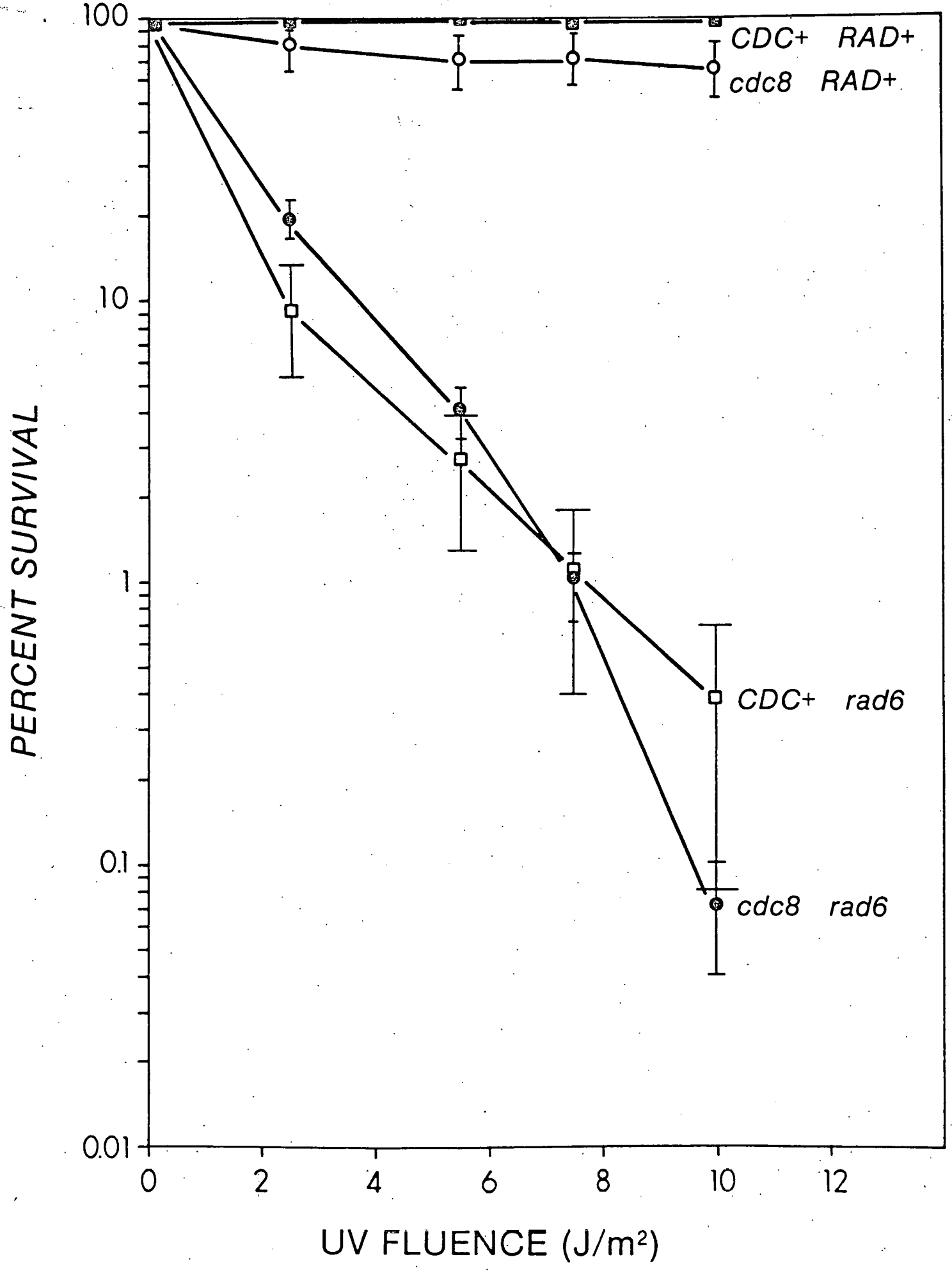

100
10
1
PERCENT SURVIVAL
LP1210-11A CDC+
LP1210-1C
LP1210-2A
cdc8-3
0
10
20
30
40
50
UV FLUENCE (J/m²)

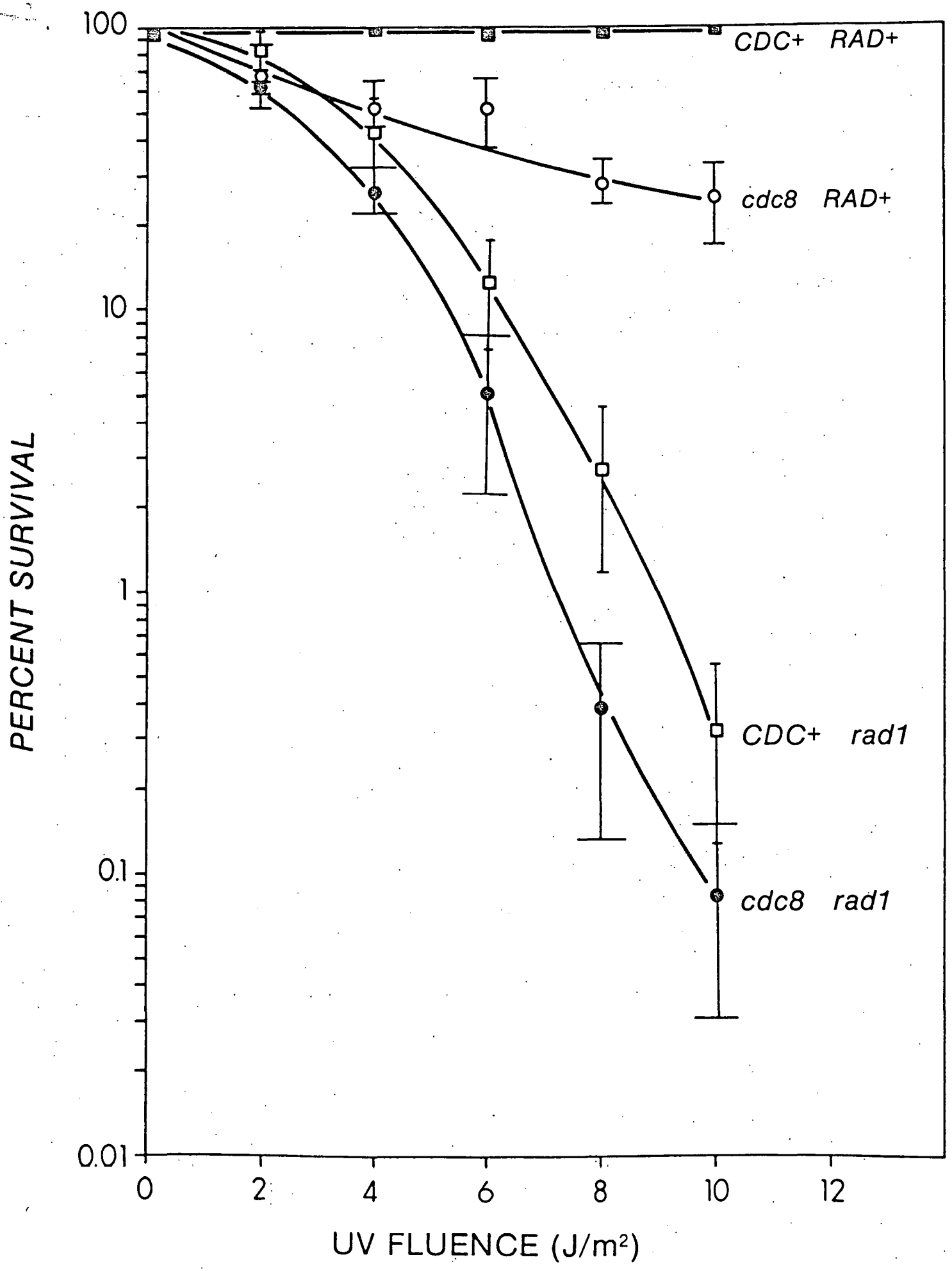

REVERTANTS PER 10^7 SURVIVORS
1200
1000
800
600
400
200
0
arg4-17 → ARG+
CL332-1B
LP752-3D
CDC+
LP752-2B
LP752-4C
cdc8-1
0
10
20
30
40
50
UV FLUENCE (J/m²)

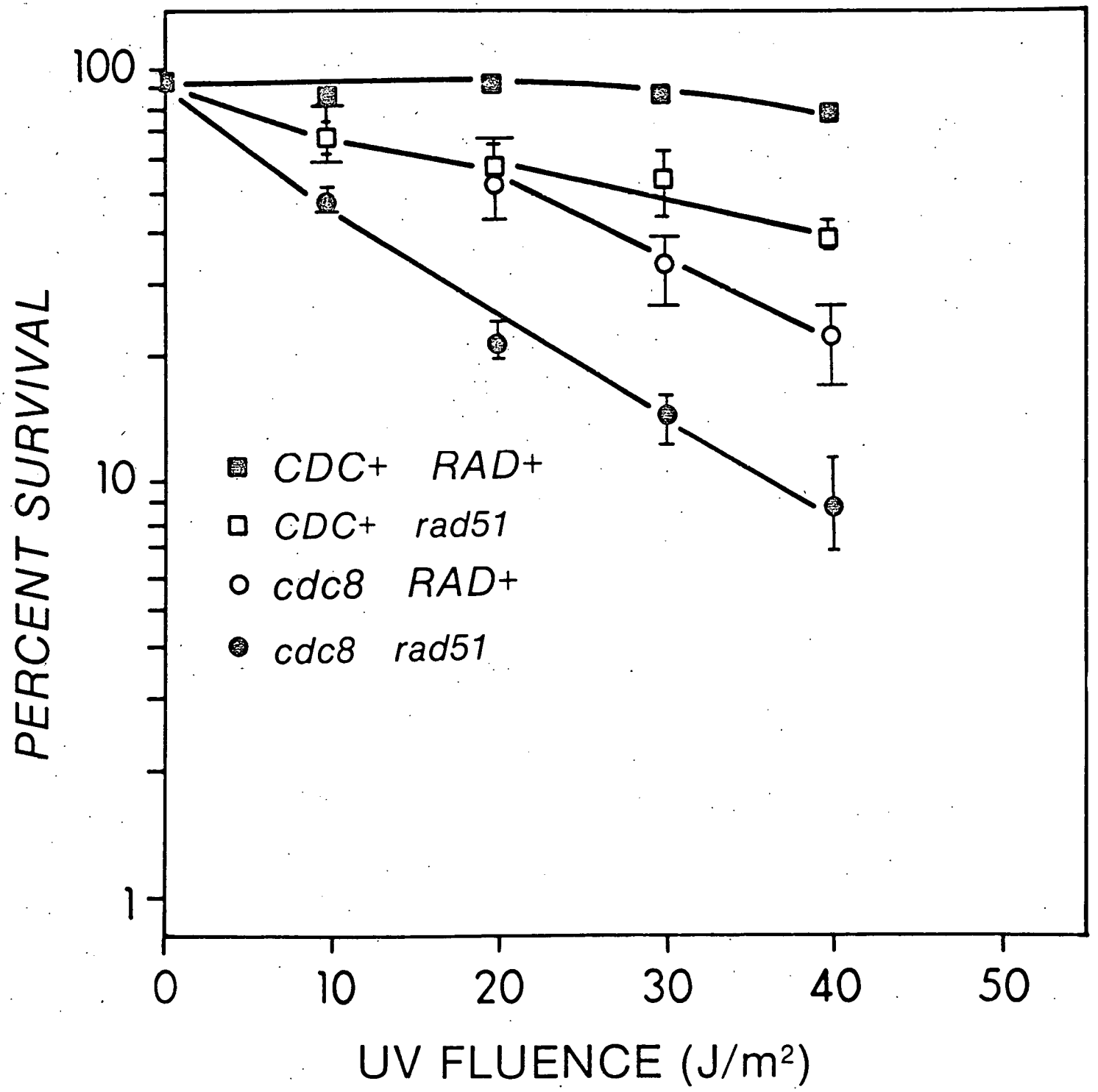

100
10
1
PERCENT SURVIVAL
CL332-1B
LP752-3D
CDC+
LP752-2B
LP752-4C
cdc8-1
0
10
20
30
40
50
UV FLUENCE (J/m²)


tyr1 ⟶ TYR+
CDC+
cdc8-1
REVERTANTS PER 10⁷ SURVIVORS
UV FLUENCE (J/m²)
0
20
40
60
80
100
120
140
160
180
0
10
20
30
40
50


100
10
1
PERCENT SURVIVAL
CDC+
cdc8-1
0
10
20
30
40
50
UV FLUENCE (J/m²)


100
10
1
0.1
0.01
PERCENT SURVIVAL
0
2
4
6
8
10
12
UV FLUENCE (J/m²)
CDC+ RAD+
cdc8 RAD+
CDC+ rad6
cdc8 rad6


100
10
1
0.1
0.01
PERCENT SURVIVAL
0
2
4
6
8
10
12
UV FLUENCE (J/m²)
CDC+ RAD+
cdc8 RAD+
CDC+ rad1
cdc8 rad1


100
10
1
PERCENT SURVIVAL
CDC+ RAD+
CDC+ rad51
cdc8 RAD+
cdc8 rad51
0
10
20
30
40
50
UV FLUENCE (J/m²)

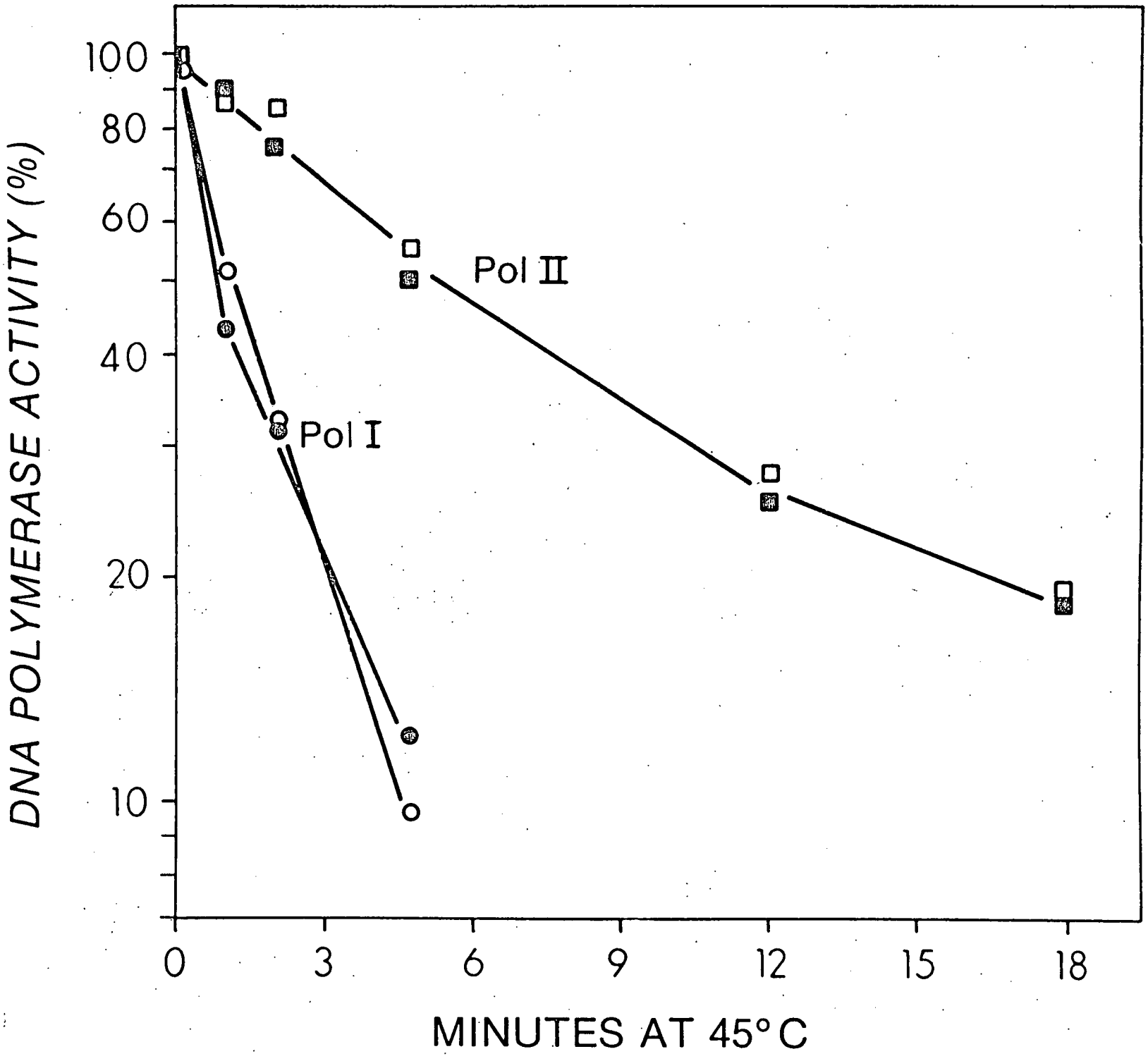

DNA POLYMERASE ACTIVITY (%)
100
80
60
40
20
10
Pol II
Pol I
0
3
6
9
12
15
18
MINUTES AT 45° C

TABLE 1

Revertants of *lys2-1* → *LYS+* 10^7 survivors in *CDC+* and *cdc8-1* haploids.

	UV fluence (J/m^2)		
	0	25	50
CDC+ strains			
LP859-1C	24(100)	71(67)	143(33)
-1D	4(100)	64(65)	224(34)
-2A	7(100)	68(85)	209(37)
-2B	5(100)	74(100)	176(61)
3A	4(100)	158(73)	348(42)
3C	2(100)	189(53)	242(53)
5B	4(100)	63(82)	189(50)
5D	43(100)	150(100)	516(53)
6B	2(100)	35(67)	72(48)
6D	3(100)	50(100)	139(62)
cdc8-1 strains			
LP859-1A*	3(100)	18(18)	13(6)
-1B*	4(100)	17(33)	55(8)
-2C	2(100)	0(2)	0(0.3)
-2D	34(100)	51(8)	
-3B	5(100)	15(13)	0(5)
-5A	3(100)	4(23)	15(6)
-5C	0(100)	14(2)	
-6A	7(100)	10(32)	18(14)
-6C	8(100)	54(36)	19(13)

Percent survival is given in parenthesis.
*Average of two experiments.

TABLE 2

Revertants of *tyr1* → *TYR+* per 10^7 survivors in *CDC+* and *cdc8-1* haploids.

	UV fluence (J/m^2)	
CDC+ strains	0	25
LP752-3D	8(100)	75(82)
-10B	3(100)	48(95)
cdc8-1 strains		
LP752-2D	4(100)	5(4)
-3A	3(100)	13(25)
-4D	3(100)	0(4)
-7D	3(100)	4(12)
-8A	7(100)	5(10)

Percent survival is given in parenthesis.

TABLE 3

Revertants of *ura1* → URA+ per 10^7 survivors in *CDC+* and *cdc8-1* haploids.

	UV fluence (J/m^2)		
	0	25	50
CDC+ strains			
LP859-1C	1(100)	9(87)	48(27)
-1D	0(100)	25(65)	38(34)
-2A	0(100)	18(85)	24(37)
-3A	1(100)	13(82)	20(50)
-3C	0(100)	14(53)	31(53)
-5B	0(100)	21(82)	48(50)
cdc8-1 strains			
LP859-1A	1(100)	0(11)	0(3)
-1B	2(100)	0(17)	0(6)
-2C	0(100)	0(2)	0(0.3)
-2D	1(100)	0(8)	
-3B	0(100)	0(13)	0(5)
-5A	0(100)	0(23)	0(16)
-6A	0(100)	0(32)	0(14)
-6B	1(100)	1(36)	0(13)

Percent survival is given in parenthesis

TABLE 4

UV-induced reversion of *lys2-1* (revertants/10^7 survivors) in *rad* and *cdc8 rad* double mutants

Strain	UV fluence (J/m^2)					
	0	2	4	6	8	
rad1	9	25	54	96	270	
cdc8-2 rad1	2	5	10	12	17	
	0	10	20	30	40	50
rad51	4	51	172	363	401	678
cdc8-3 rad51	0	6	9	24	12	11

TABLE 5. Effect of temperature on the activity of yeast DNA polymerases.

DNA polymerase I and II were assayed using activated salmon sperm DNA or poly dA:oligo dT as described by Chang (1977). Incubation was for 15 minutes at 37°C or 45°C. One unit is defined as 1 nmole of total nucleotides polymerized per hour.

Enzyme	Assay Conditions					
	Salmon sperm DNA			poly dA:oligo dT		
	37 C	45 C	45 C/37 C	37 C	45 C	45 C/37 C
	Units m/g			Units/mg		
pol I CDC+	374	415	1.11	117	79	0.68
pol I cdc8	459	534	1.16	110	88	0.80
pol II CDC+	170	292	1.72	401	411	1.02
pol II cdc8	232	420	1.81	489	547	1.12