NUCLEAR AND MITOCHONDRIAL DNA SYNTHESIS IN GAMMA RAY-RESISTANT AND -SENSITIVE SLIME MOLD AMEBAS

R. A. DEERING *and* D. S. JENSEN

From the Biophysics Department, The Pennsylvania State University, University Park, Pennsylvania 16802

ABSTRACT Uptake of [*methyl*-^{3}H]thymidine label from *Escherichia coli* 15T$^-$ into the DNA of *Dictyostelium discoideum* has been measured in control and [^{60}Co]-gamma-irradiated cells of the resistant strain NC-4 (D_{10}, colony-forming survival = 300 krad) and two sensitive daughter strains, γs-18 (D_{10} = 75 krad) and γs-13 (D_{10} = 4 krad). Nuclear (n) and mitochondrial (m) DNA were resolved by isopycnic CsCl gradients. The uptake of label into n-DNA during the immediate postirradiation period was selectively inhibited by irradiation, compared with uptake into m-DNA. For all three strains, the gamma ray dose to reduce the uptake into n-DNA to 37% of the control during the first hour after irradiation was 3 krad, whereas for uptake into m-DNA it was 75 krad. After the initial dose- and strain-dependent lag, uptake into n-DNA resumed. γs-18 showed longer lags in n-DNA synthesis and cell division than did NC-4. γs-13 resumed n-DNA synthesis and cell division after slightly shorter lags than for NC-4. The early postlag uptake into n-DNA in this strain was almost at the control rate and was accompanied by division until the cell number had nearly doubled. The rate of label uptake then declined, division stopped, and gradual cell lysis ensued. The postdelay response of γs-13 was almost independent of dose in the range of 10–100 krad. The response of γs-18 in these and earlier experiments is consistent with the viewpoint that it is sensitive because of a decreased rate of repair of DNA damage. However, the basis for the sensitivity of γs-13 seems to be more complex. This strain undergoes a premature but short-lived burst of n-DNA synthesis and division for what appears to be about one round of replication. Replication then ceases, even at very low doses, leading to greatly reduced probability of survival.

INTRODUCTION

The eukaryotic cellular slime mold, *D. discoideum*, is a useful model system for studying the effects of physical and chemical agents on cell function, replication, and differentiation, and for elucidating the role of repair processes in these responses. The vegetative ameboid cells rapidly ingest bacteria in suspension and can undergo exponential growth with a doubling time as short as 3 h. These cells have a log

phase DNA content of about 1.2×10^{-13} g, of which about 35% is mitochondrial and 65% nuclear (Sussman and Rayner, 1971; Firtel and Bonner, 1972), the latter distributed among seven chromosomes. Consequently, each chromosome has only about twice the complexity of the *E. coli* genome, making the cells of *D. discoideum* potentially simpler to study than those of many higher organisms.

The commonly used NC-4 strain displays unusual resistance to gamma rays, with a 10% survival dose (D_{10}) of 300 krad in air, suggesting effective repair capabilities (Deering, 1968). Several more sensitive mutants have been isolated from this parent strain (Deering et al., 1970), two of which, γs-18 (D_{10} = 75 krad) and γs-13 (D_{10} = 4 krad), will be further characterized here. Strain γs-13 is also much more sensitive to ultraviolet light and alkylating agents (Payez et al., 1972). Several lines of evidence have suggested that these sensitive strains have altered repair capabilities (Deering et al., 1970; Cleveland and Deering, 1972). A further understanding of these phenomena requires a knowledge of the molecular events occurring after treatment with damaging agents. This paper presents results on the rates of nuclear and mitochondrial DNA synthesis during a period of postirradiation incubation of NC-4, γs-18, and γs-13. It is shown that nuclear DNA synthesis is initially preferentially inhibited, compared to mitochondrial DNA synthesis; nuclear DNA synthesis later resumes after a lag dependent on the radiation dose and the strain.

MATERIALS AND METHODS

Growth

Vegetative log-phase amebas were grown in a suspension of 10^{10}/ml *E. coli* 15T⁻ in a nonnutrient buffer, phosphate-buffered saline (PBS), to a concentration of about 2×10^6/ml with vigorous aeration at 23°C (Deering et al., 1970). Cell concentrations were determined with a hemacytometer or Coulter counter (Coulter Electronics, Inc., Hialeah, Fla.). These cells were separated from the majority of the bacteria by differential centrifugation at 175 *g* and two washings with PBS.

Irradiation

The cells were irradiated at 2×10^6/ml in PBS with continuous aeration at 0°C in a [^{60}Co] Gammacell 200 source (Atomic Energy of Canada, Ltd. Ottawa, Ontario) at a dose rate of 8 krad/min. These suspensions were then mixed with an equal volume of labeled or unlabeled *E. coli* 15T⁻ at 2×10^{10}/ml and aerated in small bubbler tubes at 23°C.

Isotopic Labeling of Slime Mold DNA

Since our strains used bacteria as their sole source of nutrient, the *D. discoideum* DNA was labeled by allowing the cells to ingest, degrade, and reutilize the components of *E. coli* 15T⁻ labeled in its DNA with [*methyl*-^{3}H]thymidine. These bacteria were previously labeled in C-minimal medium (Roberts et al., 1955) containing 4 μg/ml total thymidine with [*methyl*-^{3}H]thymidine (Amersham Searle Corp., Arlington Heights, Ill.) at 0.6 Ci/mM. The stationary phase 15T⁻, having taken up about 85% of the label, were then washed, concentrated

to 5 × 10^{11}/ml in 15% glycerol in PBS, and stored at -53°C in small aliquots. In some experiments these bacteria were diluted with unlabeled 15T⁻ to reduce the specific activity of the *D. discoideum* DNA.

The amebas utilized about 5% of the bacterial DNA label ingested, excreting the rest as acid-soluble material into the medium (Deering, unpublished). For pulse labeling, the amebas were grown in unlabeled 15T⁻ at 10^{10}/ml for the desired time after irradiation, washed free of the bacteria by three centrifugations, and then resuspended in the ^{3}H-labeled 15T⁻ for the required pulse; they were then washed twice to remove most of the labeled 15T⁻, resuspended in cold 15T⁻ for ½–2 h, washed twice more, and incubated again for another equal period in cold 15T⁻. This chasing procedure effectively removed virtually all labeled 15T⁻ DNA left in partially degraded intracellular bacteria, or as attached or cosedimenting clumps of bacteria. Merely washing the amebas three times before lysis was not sufficient to remove the contaminating *E. coli* DNA. For some bacterial preparations which exhibited considerable clumping, even the chasing did not completely remove all contaminating label. Other labeled bacterial preparations could be completely chased out by this procedure. Any small amount of contaminating bacterial DNA label was readily distinguishable from the slime mold DNA in the CsCl gradients. A small amount of *E. coli* DNA was often allowed to remain or was added as a density marker.

CsCl Gradients of Whole Cell Lysates

We combined the lysis and preliminary treatment procedure of Firtel and Bonner (1972) with the fixed-angle analytical isopycnic CsCl technique of Flamm et al. (1966). A somewhat similar method has been used by Sussman and Rayner (1971). An aliquot of the suspension containing 0.5–2 × 10^6 cells was washed in PBS to remove most of the bacteria, then washed twice in 7% ice-cold sucrose, and finally suspended in 1 ml of 0.1 M EDTA (pH 8). The number of cells was then determined by Coulter counter. To 0.77 ml of this suspension was added 0.23 ml 20% sodium lauroyl sarcosine (Sigma Chemical Co., St. Louis, Mo.), followed by the immediate addition of 0.27 g CsCl. After the CsCl dissolved, this solution was heated to 60°C for 10 min. If desired, the solution was frozen at this point for overnight storage. The procedure did not work if the cells were frozen before the lysis and initial CsCl addition, apparently because of enzymatic degradation of the DNA. Unlabeled carrier DNA (10 μg each of *E. coli* and *D. discoideum*) was then generally added, although later experiments indicated that this was not necessary for the success of the technique. CsCl and additional water were added to each sample to bring the density to 1.670 ± 0.003 (25°C) and to a final volume of 6.5 ml. Considerable care must be taken in the initial adjustment of the density. We used a micropycnometer (Szybalski and Szybalski, 1971), but index of refraction could be used if the proper correction were made for the presence of the relatively large amount of sodium lauroyl sarcosine and other components in the solution along with the CsCl. For our conditions, the initial index of refraction of the complex mixture was 1.3983 (25°C). A 6 ml portion was put into a cellulose nitrate tube, covered with mineral oil, and spun at 20°C for 64 h at 30,000 rpm in a 50 Ti fixed angle rotor.

50 10-drop fractions were collected from the bottom into 1 dram shell vials. Since the detergent collected near the top of the gradients, the drop size changed rapidly about three-fourths of the way up the gradient; each fraction was 0.13 ml up to fraction 32 (±2); they then changed rapidly to fractions of 0.055 ml from 38 to 50. Our DNA's were banded below fraction 30 so that the densities could be more readily determined and peak shape would not be distorted.

Scintillation Counting

The CsCl fractions were counted directly, by the toluene:triton emulsion method (Patterson and Greene, 1965), as follows. 0.6 ml of water was added to each sample, followed by 3.5 ml of scintillation fluid (per liter: 667 ml toluene, 333 ml Triton X-100, 5.5 g 2,5-diphenyloxazole [PPO], 0.5 g dimethyl 1,4-bis[2-(5-phenyloxazolyl)]benzene [POPOP]). The shell vials were capped, shaken to give a single clear phase, put into standard scintillation vials, and counted for 8 min each in a Nuclear Chicago 6801 scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.) at ambient temperature. These samples were severely quenched by the CsCl, but the counting efficiency as determined by the channels-ratio method did not change significantly along the gradients. The total label recovery was about 75% as determined by comparison with the counts of the initial CsCl solution before centrifugation. Trichloroacetic acid (TCA) precipitation of the fractions from the chased samples proved unnecessary.

Density Determinations

The densities of the two *D. discoideum* peaks were determined by using the *E. coli* DNA (ρ = 1.704, Vinograd et al., 1963) as the marker and then calculating their densities from measurements of the densities of collected fractions from representative gradients using the micropycnometer technique of Szybalski and Szybalski (1971). All plots except for Fig. 1 have been normalized to place the *E. coli* peak at fraction 10. Its actual location varied from about fraction 10 to 13, due to slight differences in the initial CsCl densities in different runs.

Division Lag Experiments

These methods were slightly modified from those used previously (Cleveland and Deering, 1972) in order to duplicate the isotope-uptake experiments using *E. coli* 15T$^-$. The cells were irradiated as for the uptake experiments, diluted with an equal volume of unlabeled *E. coli* 15T$^-$ at 2×10^{10}/ml, and aerated at 23°C. At intervals, total ameba count was determined with a Coulter counter. The presence of the irradiated medium during the postirradiation growth was not of importance as shown in experiments which removed the cells from the irradiated suspension medium and resuspended them in fresh unirradiated PBS for the growth with the bacteria.

Cell Viabilities

Colony-forming survival was determined for each sample used in the uptake experiments by plating with a lawn of *E. coli* B/r and counting the resulting clones (seen as clear plaques) after incubation (Deering et al., 1970). Survivals on *E. coli* B/r and 15T$^-$ have been shown to be the same (Khoury, this laboratory).

RESULTS

Labeling of Ameba DNA

Fig. 1 shows the CsCl gradients for the whole cell lysates of *D. discoideum* NC-4 cells grown on [^{3}H]thymidine-labeled *E. coli* 15T$^-$ for 3 h, washed by centrifugation to remove all apparent bacteria, and then lysed directly ("unchased") or chased in unlabeled 15T$^-$ for two periods of 1.5 h each ("chased"). The top panel is for un-

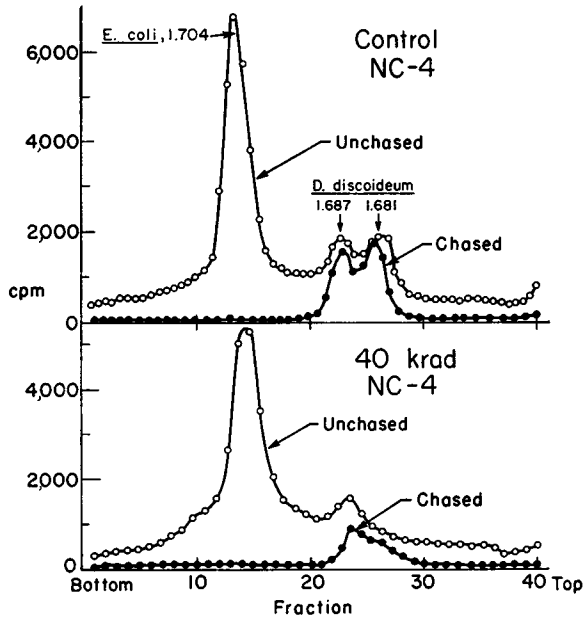

FIGURE 1 CsCl gradients of whole cell lysates of *D. discoideum* NC-4 grown on [*methyl*-^{3}H] thymidine-labeled *E. coli* 15T$^-$ for 3 h. Top, unirradiated; bottom, 40 krad. —○— washed but not chased; —●— chased for two periods of 1½ h each with unlabeled 15T$^-$ before lysis.

irradiated amebas and the bottom is for 40 krad of gamma rays given just before the start of the labeling. Even though repeated washings had removed all apparent bacteria (by microscopy) from the unchased samples, they still contained a large relative amount of labeled *E. coli* DNA (1.704 g/cm^3). The identity of the bacterial DNA peak was verified in initial experiments by cosedimenting ^{14}C-labeled *E. coli* DNA along with the slime mold preparations. The label attributable to the slime mold cells was in two peaks at densities of 1.687 g/cm^3 and 1.681 g/cm^3. These peaks were also present in the irradiated sample, but the pattern was altered, as we will discuss shortly. The labeled bacterial DNA was adequately chased out by our procedure in the control and irradiated samples up to doses of at least 100 krad.

The densities of the two slime mold peaks in our fixed-angle gradients agree closely but not exactly with those reported from analytical centrifugations by Firtel and Bonner (1972) for purified *D. discoideum* mitochondrial DNA (1.682 g/cm^3; 28% GC) and nuclear DNA (1.676 g/cm^3; 23% GC), obtained with an axenic derivative of NC-4, and with those reported by Sussman and Rayner (1971) provided allowance is made for their use of the *E. coli* marker density as 1.710 g/cm^3. A small satellite band of DNA (~5% of the total, of apparent nuclear origin) occurring 0.005 g/cm^3 to the heavy side of the mitochondrial DNA as reported by Firtel and Bonner (1972) was not detected in our gradients. Khoury and Deering (submitted for publication) have shown that the DNA of isolated nuclei bands at the position

of the upper peak in fixed-angle gradients identical with those used here. The slight differences between our densities and the previously published values probably result from the difficulties in accurately estimating absolute densities in fixed-angle preparative rotors; our band separation (0.006 g/cm^3) agrees with the published values. We conclude that the band at density 1.687 g/cm^3 is mitochondrial DNA (m-DNA) and that at 1.681 g/cm^3 is nuclear DNA (n-DNA).

For short pulses (up to 3 h) there was little evidence of incorporation of label into components other than m-DNA and n-DNA. With amebas collected after long duration labeling extending into the stationary phase, some label did band near the top of the gradients, possibly attributable to polysaccharide. Polysaccharide should band at 1.63–1.65 g/cm^3 (Firtel and Bonner, 1972) or at 1.666 g/cm^3 (Sussman and Rayner, 1971; for *E. coli* = 1.704 g/cm^3).

Where chasing was inadequate and an *E. coli* DNA peak remained, some additional label also appeared at the top. This is believed to be due to a few intact *E. coli* cells which were not completely lysed by the Sarkosyl treatment.

All experiments have indicated that the labeled DNA was stable during the chase period. Formic acid hydrolysis and chromatography of the labeled *D. discoideum* DNA showed that the ^{3}H still remained in thymine (Guialis, this laboratory).

For most experiments, we used a pulse-labeling period of 1 h, starting at various times after irradiation, followed by two chase periods of ½–1½ h each. The uptake counts for these experiments were normalized to cpm/10^6 cells at time of lysis. Fig.

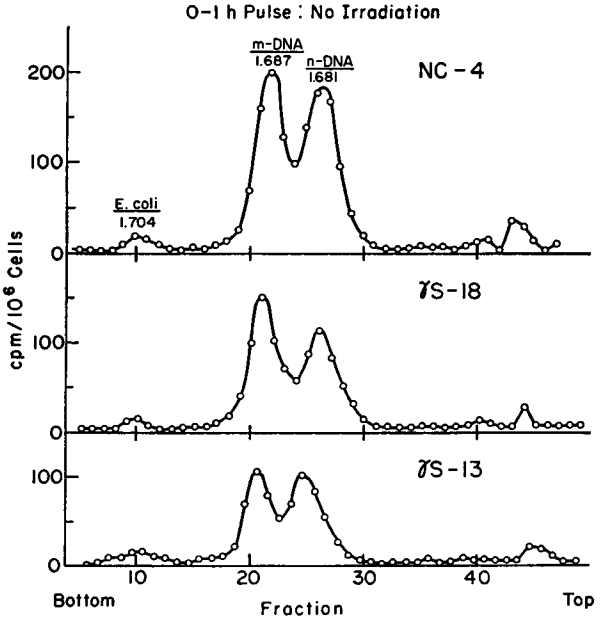

FIGURE 2 CsCl gradients of whole cell lysates of NC-4, γs-18, and γs-13 showing positions of *E. coli* 15T$^-$ marker and nuclear (n-) DNA and mitochondrial (m-) DNA peaks.

TABLE I

TIME-COURSE OF UPTAKE INTO NC-4 DNA

Uptake period	Total uptake	% Label in n-DNA
h	*cpm**	
0.17	408	43
1.25	4,175	57
3.0	9,683	61
6.0	16,991	60
24‡	56,624	70

* Initial cell concentration, 2.3×10^6/ml: 0.77 ml/sample.
‡ Already into stationary phase.

TABLE II

COLONY-FORMING SURVIVALS

Gamma ray dose	Fractional survival		
	NC-4	γs-18	γs-13
krad			
10	1	0.9	10^{-2}
40	0.95	0.6	10^{-4}
100	0.85	10^{-2}	10^{-5}

2 compares the gradients for 1-h pulse labeling of unirradiated NC-4, γs-18, and γs-13. A small bacterial DNA peak remained as a useful marker. The *D. discoideum* DNA peaks appeared at the same densities in all three strains. The total uptake was somewhat less for γs-13 because of its slightly slower growth rate (5 h doubling compared with 3 h for NC-4 and γs-18).

An analysis of the peak widths has indicated that the n-DNA peak is wider than the m-DNA peak, probably as a result of greater heterogeneity of base composition of the n-DNA (see Sussman and Rayner, 1971). By fitting characteristic curve shapes to our various gradients, it has been possible to estimate the amount of label in the m-DNA and n-DNA components.

Table I shows the total label taken into NC-4 DNA for various uptake periods, and the proportion of the label in the n-DNA. For the most extended labeling period (24 h), 70% of the label was in n-DNA, in close agreement with the estimates of Sussman and Rayner (1971) and Firtel and Bonner (1972) for the relative amount of n-DNA in their axenic strains. However, for the shorter pulses, a greater proportion of the label was found in the m-DNA, indicating a slightly more rapid labeling of this component. For 1 h pulse labeling of control cells, the proportion of label found in the n-DNA was 50 ±4% for NC-4, γs-18, and γs-13.

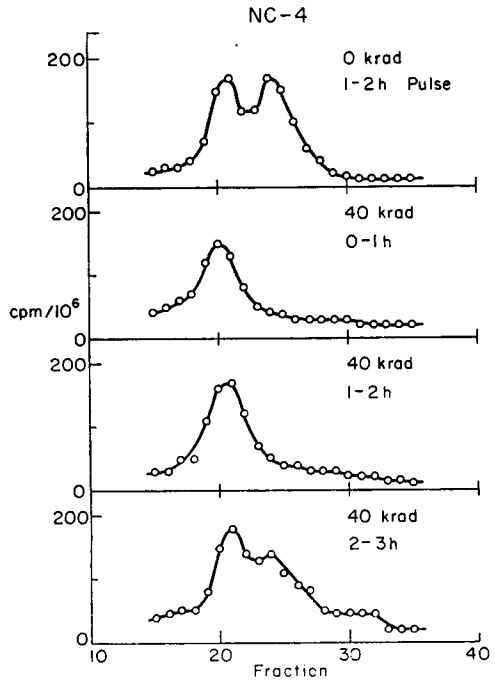

FIGURE 3 Gradients for 1 h labeling pulses given in the indicated interval, for NC-4 control and 40 krad. Only *D. discoideum* DNA region plotted.

Survival

For reference in relation to our other results, Table II gives the survival of colony-forming ability for our three strains at the gamma ray doses used here.

Gamma Ray Effect on Pulse Uptake

Fig. 3 shows typical gradients for 1 h pulse uptake periods for an NC-4 control and 40 krad sample, starting at different times after irradiation. The chase for each was two 1½-h periods. Only the *D. discoideum* DNA regions of the gradients are plotted. The *E. coli* DNA peaks (when present) were normalized to fraction 10 in this and subsequent plots.

For the pulse periods 0–1 h and 1–2 h after 40 krad, little if any uptake of label into the n-DNA occurred, whereas uptake into the m-DNA persisted. Uptake into the n-DNA resumed between 2 and 3 h after the irradiation.

Since uptake of label into slime mold DNA required intracellular breakdown of the bacterial DNA and subsequent reincorporation of its components, it was important to determine which of these steps was limited by the radiation. The observation that the ^3H label of the bacterial DNA was still incorporated into the mitochondrial DNA at a high rate during a 1 h pulse after doses up to 40 krad (Fig. 3) indicated

that degradation of the bacterial DNA still occurred to an extent sufficient to supply at least the m-DNA precursor pool. The intracellular bacterial DNA also chased out adequately in irradiated samples (Fig. 1), indicating continued degradation of the bacterial DNA. In addition, we have shown for all three strains that at 100 krads the bacterial DNA label was still introduced into a TCA-soluble intracellular pool, being delayed behind the control by only about ½ h. Considering that label appears in *D. discoideum* DNA in times as short as 10 min (Table I) and that there is normally an excess of DNA precursors available to the amebas (leading to excretion of 95% of the label), it is unlikely that a significant decrease in the pool of DNA precursors results from radiation doses in the range used here. We will therefore assume that the uptake of label into the *D. discoideum* n-DNA and m-DNA is a measure of the rates of synthesis of those components and that the availability of precursor is not the rate-limiting step.

To further explore in a quantitative way the differential effect of gamma rays on n-DNA synthesis during an immediate 1 h postirradiation labeling pulse, numerous CsCl gradients were done on each of the three strains at several doses ranging from 1 to 150 krad; the amount of uptake into n-DNA and m-DNA was estimated from the gradient profiles for each strain and dose. The results are plotted in Fig. 4. No strain differences are apparent. The dose to reduce uptake into n-DNA to 37% of its control value was 3 krad; for m-DNA, it was 75 krad. Thus, the blockage of n-DNA synthesis during the first hour postirradiation is about 25 times more sensitive to gamma rays than is m-DNA synthesis.

Fig. 5 shows some 1 h pulse uptake results for γs-18 and γs-13, starting at various times after irradiation. For γs-18 given 40 krad, the lag in n-DNA synthesis was about 3 h, slightly greater than that of the parent NC-4 strain. For γs-13, the lag at

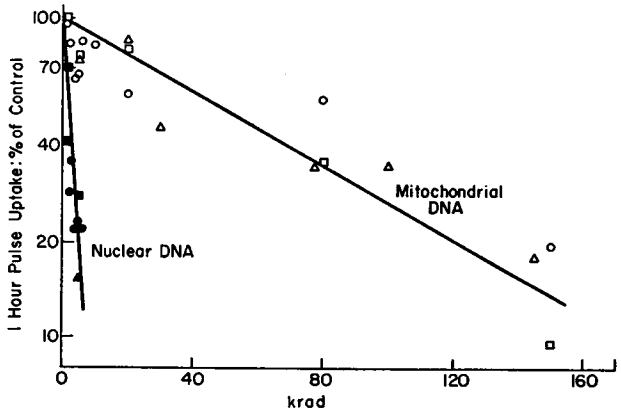

FIGURE 4 Relative uptake of label into n-DNA and m-DNA during the first hour after irradiation for a series of doses. Each point is an average from at least two CsCl gradients. Solid points, n-DNA; open points, m-DNA. Strain NC-4 (●, ○); γs-18 (■, □); γs-13 (▲, △).

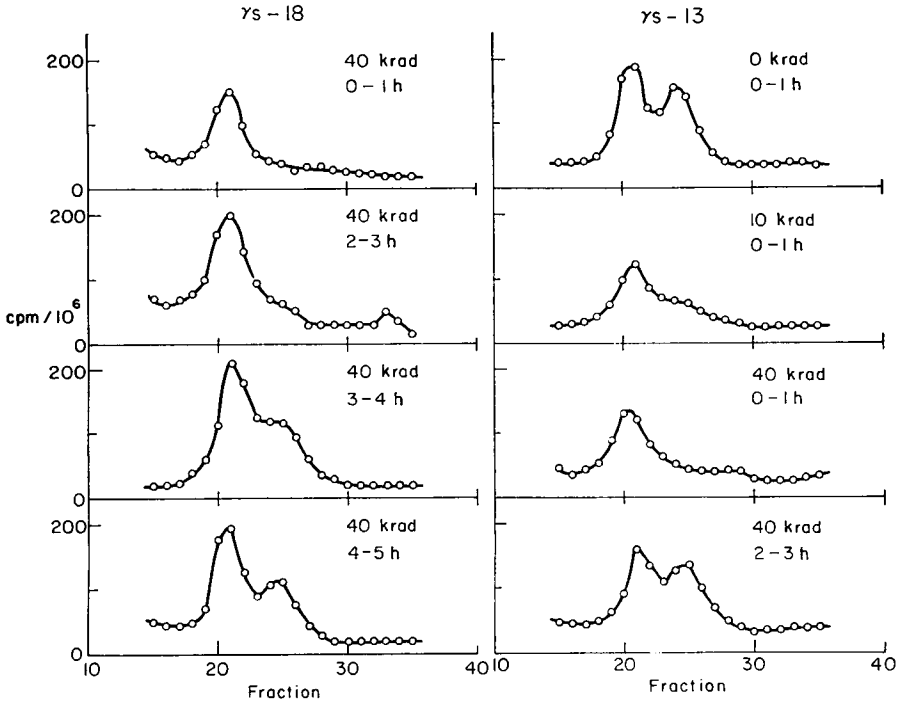

FIGURE 5 1 h pulse uptakes for interval indicated, after 0, 10, or 40 krad to γs-18 and γs-13.

10 krad was less than 1 h and for 40 krad less than 2 h. Other results not shown here indicate that the γs-13 n-DNA lag for 40 krad was about 1½ h, slightly less than for NC-4.

Uptake of label into n-DNA and m-DNA after 100 krad is shown in Fig. 6 for 1 h pulses starting either 2 h or 8 h after irradiation for all three strains. Only γs-13 resumed n-DNA synthesis within the 3 h period after the irradiation. The 8–9 h pulse indicates that NC-4 had returned to a normal uptake rate by that time. γs-13 was still synthesizing n-DNA also, but at a reduced rate and with a broader peak, possibly indicative of lower molecular weight. Nuclear DNA synthesis did not noticeably resume in γs-18 within this 9 h postirradiation period.

Figs. 3, 5, and 6 have presented uptake normalized to 10^6 cells at the time of lysis, that is, the number of cells present after the end of the chase period. This normalization was necessary due to the somewhat variable loss of cells during the numerous centrifugations involved in the chasing. The gradients in these figures are therefore *not* precisely normalized to an equal number of cells present during the uptake period, since the different irradiated samples increased in cell number by different amounts during the chase period, depending on the dose, strain, and time at the start of the pulse. However, using information from growth curves after irradiation, it is possible to correct these gradients to approximately the same

numbers of cells during uptake. If this is done, all of these gradients are nearly on the same relative basis (within ±25%) with the exceptions of γs-18 at 40 krad, 0–1 h; γs-18 at 100 krad, 2–3 h; and γs-13 at 100 krad, 8–9 h pulse. The uptake in these should be reduced by a factor of about two to make them roughly comparable with the others on an equal cell basis.

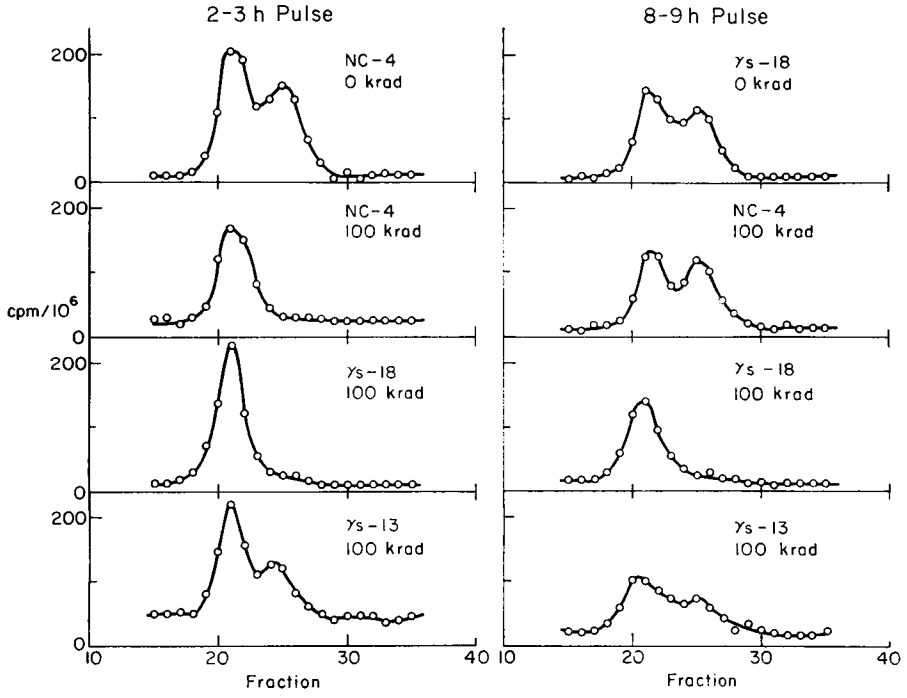

FIGURE 6 Uptake during the 2–3 h or 8–9 h interval after irradiation for the three strains after 100 krad.

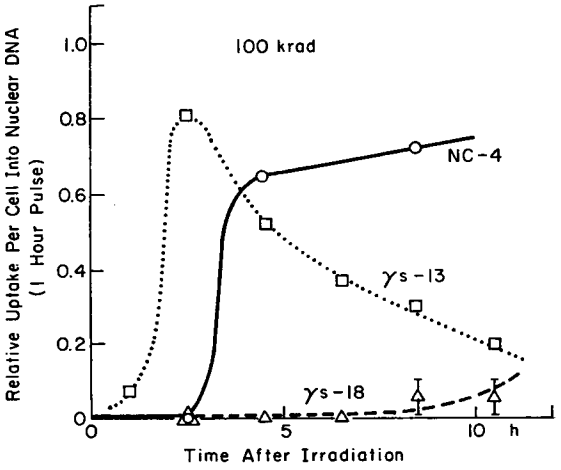

FIGURE 7 Relative magnitude of uptake per cell into n-DNA for a 1 h pulse with midpoint at the indicated time after irradiation, for NC-4, γs-18, and γs-13 given 100 krad.

In order to give a more quantitative comparison of pulse uptakes normalized to the same numbers of cells during uptake, careful corrections have been applied to a series of gradients done after 100 krad. The results are shown in Fig. 7 for uptake into n-DNA during a 1 h pulse with its midpoint at the plotted time. Nuclear DNA synthesis resumed in NC-4 by about 4–5 h. Only slight resumption occurred in γs-18 by 10 h. As noted earlier, γs-13 showed the shortest lag in n-DNA synthesis. However, this new synthesis was only temporary, with the rate of uptake per cell subsequently sharply declining to become very low by 10 h and virtually zero by 16 h (not shown here).

Postirradiation Cell Division

It has been reported (Cleveland and Deering, 1972) that the radiation-induced division lags for all three strains growing on *E. coli* B/r were very nearly the same, although the postlag growth kinetics were radically different. A further analysis of those and additional results on B/r and of subsequent experiments with growth on $15T^-$ indicate that there are small but reproducible differences among the lags of these strains, with the effect being rather pronounced at 100 krad. Fig. 8 presents

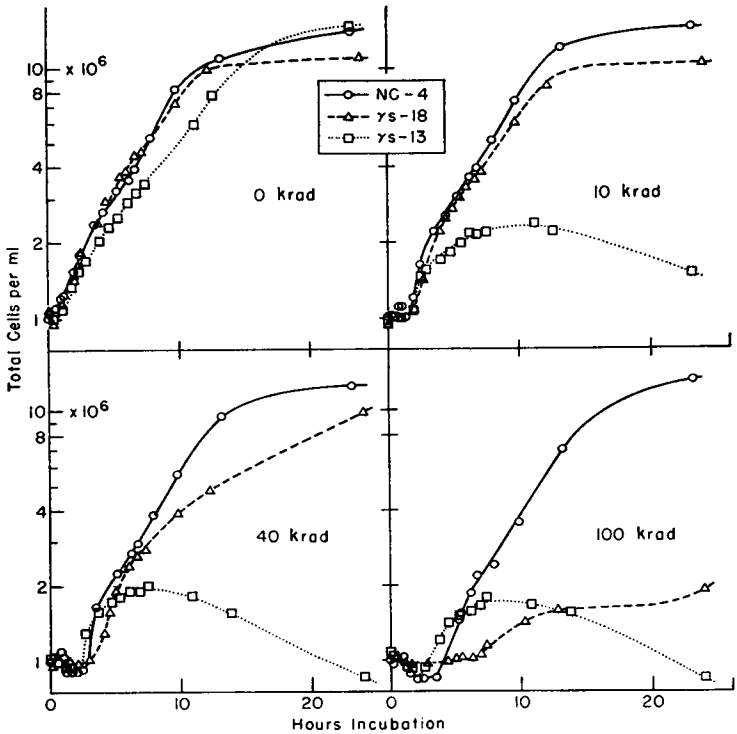

FIGURE 8 Postirradiation growth curves, showing total cells per milliliter as a function of time for all three strains after 0, 10, 40, or 100 krad.

postirradiation curves for *D. discoideum* growth on *E. coli* 15T$^-$ after 0, 10, 40, and 100 krad of gamma rays.

The control curves for all three strains were approximately exponential up to 10^7/ml. γs-13 grew slightly more slowly than the other two strains. Growth ceased at a little over 10^7/ml due to depletion of the bacteria. Little if any initial lag was evident for these controls.

NC-4 had a distinct dose-dependent lag, followed by an abrupt return to continued exponential growth.

γs-18 given 10 krad showed a similar response. However, at 40 and 100 krad, it exhibited a longer lag than NC-4. Its postlag growth was also slower than for the control. Both of these effects were very pronounced at 100 krad, where an approximately 7 h lag was followed by very little subsequent division. The beginning of an ncrease at about 24 h was due to the progeny of the few surviving cells.

γs-13 showed a very different response, even at doses as low as 10 krad. After a lag that was somewhat less than for NC-4, the cells initially resumed division at a rate faster than the control, but after increasing by about a factor of two (almost independent of dose in the range 10–100 krad), division ceased at about 9 h after irradiation. A gradual lysis of cells then commenced.

A comparison of n-DNA synthesis lags and division lags revealed that in all cases (with the possible exception of γs-18 at 100 krad) cell division began about ½–1 h after the resumption of n-DNA synthesis. For γs-13, postlag cell division ceased in conjunction with the decreased rate of uptake into n-DNA.

DISCUSSION

The large relative amount of m-DNA (~35%) makes *D. discoideum* a good organism for comparing DNA synthesis in the nucleus and mitochondrion. The differential effect of gamma rays on uptake of label into m-DNA and n-DNA suggests at least some degree of independence for these two processes, a conclusion in agreement with previous published work on other organisms (Guttes et al., 1967; Nass, 1969; Grossman et al., 1969). This response of *D. discoideum* to gamma rays appears analogous to that reported for the preferential inhibition of n-DNA synthesis by cycloheximide in an axenic strain of this organism (Sussman and Rayner, 1971) and in yeast by Grossman et al. (1969). In contrast, ethidium bromide preferentially inhibits m-DNA synthesis in this organism (Firtel and Bonner, 1972; Sussman and Rayner, 1971). The greater gamma ray sensitivity of the nuclear DNA synthesis could mean in terms of radiation target theory that the "sensitive target" for n-DNA synthesis is about 25 times that for m-DNA synthesis, or that some feature of the control of these syntheses is differentially altered by the radiation. Possibly the result implies a much larger replicating unit for the n-DNA, a result not totally unexpected from an examination of the sizes of the n-DNA and m-DNA in *D. discoideum* (Khoury and Deering, submitted) and in other organisms.

The chemical treatments that have preferentially diminished n-DNA synthesis (Sussman and Rayner, 1971; Grossman et al., 1969) have also led to considerable reduction of m-DNA synthesis. With 10 krad of gamma rays *D. discoideum* m-DNA synthesis is only slightly depressed, even though little if any n-DNA is synthesized during the first hour after irradiation. This provides a very useful method for selectively labeling the m-DNA of this organism for use in other experiments, particularly in strain NC-4 where such a dose allows subsequent resumption of n-DNA synthesis, followed by apparently normal growth and virtually 100% cell survival.

At present, our knowledge of the details of the relative modes of DNA synthesis in nuclei and mitochondria in *D. discoideum* does not allow a more complete interpretation of the molecular events underlying the preferential inhibition of n-DNA synthesis by ionizing radiation. It has been reported (Chang and Looney, 1966; Baugnet-Mahieu et al., 1970) that m-DNA synthesis in rat liver cells is less sensitive to radiation than is n-DNA synthesis. The differential effect in those cases appears to be considerably less than that reported here for *D. discoideum.* The results are harder to interpret due to the use of whole-body irradiation and the relatively smaller proportions of mitochondrial DNA.

The results on the relative radiation-induced lags in n-DNA synthesis in NC-4, γs-18, and γs-13 are somewhat surprising, particularly for the very sensitive γs-13 strain. We have previously postulated that γs-18 and γs-13 may be sensitive because of deficiencies in repair (Deering et al., 1970), using repair in the most general sense until further results were available at the molecular level. On the simplest premise, repair-defective cells might be expected to have a longer induced lag in DNA synthesis if repair occurred only very slowly or not at all.

The intermediately sensitive strain, γs-18, meets these expectations; it shows lags in both DNA synthesis and division which are longer than for the resistant NC-4. One possible conclusion is that some form of repair is occurring more slowly in γs-18 and that it is capable of reversing only a limited amount of damage. This viewpoint is supported by our previous observations of a slower split-dose recovery for γs-18 (Deering et al., 1970), and a greatly extended period of caffeine sensitivity (up to 10 h) after irradiation as compared to NC-4 (about 3 h) (Deering, unpublished observations). Thus, our present results are consistent with the hypothesis that γs-18 is capable of repair, but at a reduced rate. The nature of the critical ionizing radiation lesion(s) which must be repaired in these cells is still unknown.

The complex response pattern of the very sensitive γs-13 strain is not as readily interpretable in terms of simple repair concepts. As reported earlier (Deering et al., 1970), this strain has only a 50% control plating efficiency, it shows no split-dose recovery and no oxygen effect, and its survival is increased by postirradiation incubation in caffeine, in contrast to the sensitization of NC-4 and γs-18 by this chemical. As shown here, it has a *shorter* lag in n-DNA synthesis and in division than does its resistant parent. The amount of uptake into n-DNA in this early postirradiation burst of synthesis is an appreciable fraction of that of a control culture

for a similar pulse period, making it unlikely that this is entirely some form of repair synthesis. The cells approximately double, and then division and uptake into DNA cease. The postirradiation division amounts to almost exactly one cell doubling, which appears to be nearly independent of dose from 10–100 krad. This response suggests that this strain undergoes one postirradiation round of replication but that some step in reinitiation is then permanently blocked, or the template is inadequate, even at doses as low as 10 krad. The molecular basis for this is not yet evident. One can speculate that the control of DNA synthesis is altered in this mutant such that semiconservative synthesis (possibly not normal) (*a*) resumes before repair can be completed, or (*b*) starts in addition to or instead of valid repair, and that the consequences are the synthesis of incorrect daughter strands. Alternatively, some initiation promoter or inhibitor might be under altered control.

γs-13 may be sensitive not because of a repair deficiency in the usual sense, but because it does not wait for repair to be completed before initiating extensive replication, i.e., a possible case of "premature fixation" of damage. (This suggests the existence of a class of radiation-sensitive mutants which might be called "imp" mutants ["impatient"].) Verification of such possibilities awaits the results of further experimentation.

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