ALL-OR-NOTHING CHARACTER OF DNA DEGRADATION IN BACTERIA AFTER IONIZING RADIATION

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ABSTRACT The response of single cells of *Escherichia coli* B_{s-11} and 15JG151 to radiation-induced DNA degradation has been observed by autoradiography. For both cells it is concluded that the event which causes DNA degradation is of an all-or-nothing character. The unit which suffers degradation is not the whole cell, but each cell has between two and four such units. The results suggest that there is some resynthesis of degraded DNA. Evidence that this occurs is shown by examining the degradation of mass cultures of 15TAU/t3 and t7 below and above the permissive temperature for DNA synthesis. The results on the all-or-nothing character are in agreement with previous studies made by completely different techniques.

INTRODUCTION

Ionizing radiation, such as a fast electron generated by the interaction of gamma radiation with matter, produces large energy releases in very small regions. These average 55 eV, and since such an amount of energy far exceeds that of any chemical bond, the local action is clearly drastic. At the same time, such fast electrons traverse the overwhelming majority of atoms in their path without producing any effect at all. Accordingly it is to be expected that ionizing radiation should exert its action in an all-or-nothing way. If this is the case it is of great importance to establish it, for the essence of such action is that it can have no threshold; there cannot be less than one ionization, which already is capable of producing the radiobiological effect.

The demonstration of this all-or-nothing action has proved to be very difficult because living cells have means of response to damage of various kinds and the response may well confuse the findings in the system studied. The case of radiation-induced DNA degradation seems to be of the all-or-nothing character in one strain of *E. coli*, B_{s-11}, as suggested by Pollard and Tilberg (1972). The evidence was based on the observation of initial rates of degradation and on the distribution of the burst sizes of T7 phage, which uses only host DNA. The all-or-nothing nature of the process was clearly apparent. A legitimate criticism can be made, however, that the

phage multiplication process may involve more than the presence of host DNA and this is made more cogent by the finding (Marsden et al., 1972) that the all-or-nothing response of T4 infection to irradiated cells is not due to the degradation of the host DNA. Therefore it seemed sensible to examine the process of DNA degradation directly using autoradiography. Hildebrand and Polland (1969), on the basis of density gradient studies, suggested that degradation is all-or-nothing in 15JG151.

This direct examination has been attempted previously (for a review see Pollard, 1970). A major difficulty occurs in cells which contain a radiation-induced inhibitor of DNA degradation, for in such cells the DNA degradation is shut off at about 50%. This makes the statistical analysis very difficult. Cells such as B_{s-11} and JG151 do not contain the radiation-induced inhibitor and degrade nearly all their DNA. In this paper we give an account of observations on B_{s-11} and 15JG151 which provide evidence that the process of DNA degradation is elicited in an all-or-nothing fashion.

MATERIALS AND METHODS

Cells of B_{s-11} were from the stocks used by Pollard and Tilberg (1972) and originally obtained from Dr. J. R. White (University of North Carolina). Cells of 15JG151 were from Dr. L. Grady (Grady and Pollard, 1968). 15TAU/t3 was from Dr. Mary Osborn (Cambridge University) and 15TAU/t7 from Dr. Lehman (Stanford University).

For autoradiography of B_{s-11}, cells were grown in Roberts's C minimal medium supplemented with 2 g casamino acids/liter, 4 mg of deoxyadenosine, and 100 μCi of [^{3}H]thymine. Cells were grown for 3 h to a concentration of 3×10^8/ml. They were then washed by centrifugation and resuspension in medium containing cold thymine and chilled to ice temperature. Four samples of 5 ml each were taken, one for an unirradiated sample, and three for irradiation. Each sample was bubbled with oxygen for 1 min before irradiation, the control included. Doses of 4,000, 6,600, and 10,000 rad were given. The irradiation took place in a ^{60}Co Gammacell 200 (Atomic Energy of Canada, Ltd.). For the two highest doses, the samples were reoxygenated during irradiation. The total time for 10,000 rad was 1 min. After irradiation all samples were incubated for 100 min to allow the DNA to degrade. 1 ml samples from each of the four tubes were placed in 2 ml iced 10% trichloroacetic acid (TCA). 0.4 ml from each was also poured and spread on a plate covered with solidified agar. The TCA tubes were allowed to sit 15 min and then 1.5 ml of each of these samples was spread on agar plates. After the plates were dry the agar was cut into strips about 1 inch wide, lifted with a spatula, and a glass slide drawn across them. These were then coated with Kodak NTB-2 emulsion by dipping into liquid emulsion twice for about 3 s each. These slides were then allowed to decay in a light-tight pressure cooker containing drierite (W. A. Hammond Drierite Co., Xenia, Ohio).

After suitable times for radioactive decay, varying from 2 to 8 days, the slides were developed in Kodak D-19 developer, fixed, and washed. The grains and the cells could be observed with a phase-contrast microscope.

The developed grains were easily visible. The cells of B_{s-11} are rather small and not so easy to see. Furthermore, especially on the unirradiated slides, there is some tendency for groups of cells to form. It was quite possible to find areas which contained mainly single cells and to count the grains above the cells. For both unirradiated and irradiated cells we found "zeros," cells with no grains above them. Cultures of B_{s-11} grown in minimal medium, even

with casamino acid supplement, show some cells which do not take up thymine in a 3 h period. The proportion is low, and far less than that in the irradiated case.

The procedure followed for 15JG151 was the same, except that this cell is an auxotroph for thymine. Accordingly 0.25 μCi/ml [^{3}H]thymine was used with no deoxyadenosine. The doses in this case were 6,600, 10,000, and 20,000 rad. A similar oxygenation procedure was followed.

The study of DNA degradation in mass culture for the temperature-sensitive cells was performed by labeling cells grown in C minimal medium with glucose accompanied by 50 μg arginine/ml, 30 μg uridine/ml, 50 μg proline/ml, 50 μg tryptophan/ml, 50 μg methionine/ml with 4 μCi [^{14}C]thymidine. These were grown after inoculation to 2×10^8 cells/ml, 20 μg/ml cold thymidine added and growth allowed to continue for 20 min more. Cells were then spun down and washed with C minimal plus thymidine. They were then resuspended in normal growth medium with 5 μg/ml cold thymidine. For irradiation they were bubbled with oxygen or nitrogen for more than a minute and irradiated in the ^{60}Co Gammacell 200.

After irradiation 1 ml samples were placed in 1 ml cold 10% TCA, allowed to stand 30 min, filtered through 0.45 μm Millipore filters (Millipore Corp., Bedford, Mass.), and washed with 3 ml cold 5% TCA plus 2 μg/ml thymine. The filters were glued to planchets and counted in a Nuclear-Chicago G-M counter (Nuclear-Chicago Corp., Des Plaines, Ill.).

RESULTS AND DISCUSSION

The distributions for one experiment with B_{s-11} are shown in Fig. 1. There is one striking result, which is the key to the whole of the findings. In the three irradiated cases there are a very large number of cells with no grains at all, while at the same time there are some cells with numbers of grains which overlap with the normal, unirradiated cells. The behavior expected if the degradation were to occur to the same extent, on the average, in all cells is a Poisson distribution about the average value. This would give a sharp peak at the points marked by the arrows and a relatively small number of zeros, as indicated in the parentheses. It is unequivocal that this average distribution does not occur and we therefore uphold the conclusion reached from the phage burst studies previously mentioned (Pollard and Tilberg, 1972).

The all-or-nothing phenomenon, equally definitely cannot apply to the whole of the DNA in a cell; instead there must be a small number of units, two or three, that are subject to the lesion and process which causes the DNA degradation. In Fig. 1 we show the analysis of the data on the basis that there are two such units per cell. We can proceed as follows. If the probability of a degradation-initiating lesion in one unit, for a given dose, is y, then the probability of total escape for that unit is e^{-y} and if there are two units, the probability that both will escape in the same cell is e^{-2y}. The probability for one hit and one miss is $2e^{-y}(1 - e^{-y})$ and of two hits is $(1 - e^{-y})^2$. This last can be made a basis for the calculation of y. For example, for the 6,600 rad case, after deducting the 69 zeros corresponding to the number found on the unirradiated case, there are 607 zeros in 921 cells. This gives $y = 1.72$. Then there should be 32 cells which have totally escaped and 280 cells which have received a hit in one of the degradation units. From the unirradiated case the average number

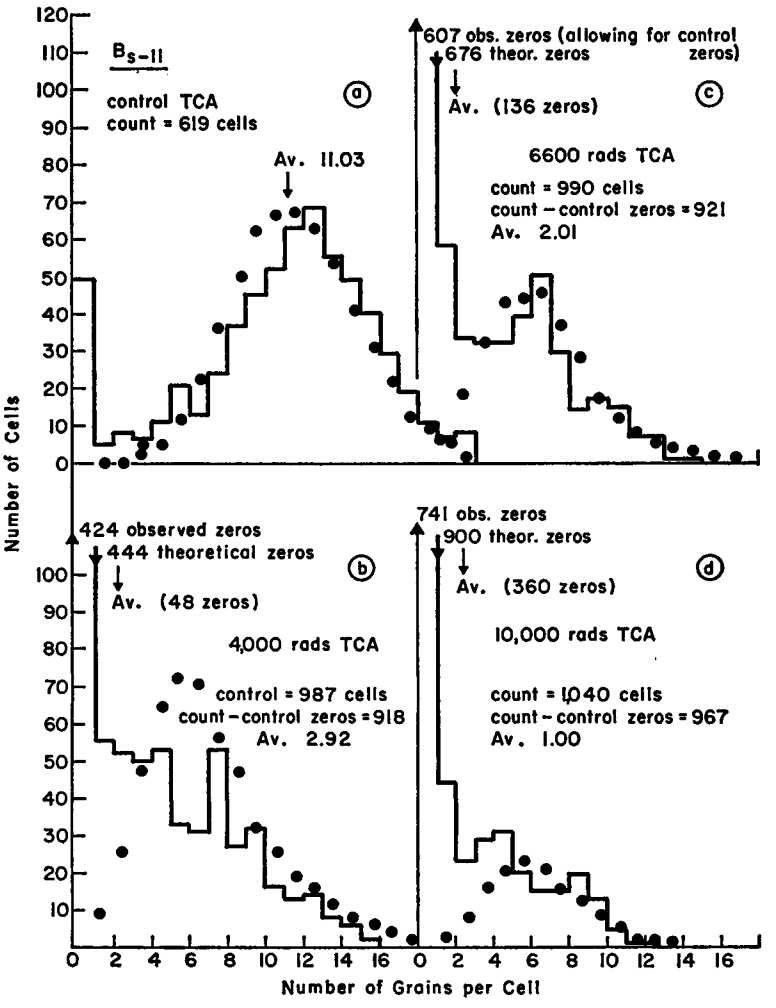

FIGURE 1 The distribution of B_{s-11} cells with different grain numbers for different treatments. All samples were oxygenated. The dots represent the theoretical distribution based on two degradation units per cell. The average value is shown by an arrow and the predicted number of zeros, using the average as a basis, is shown in parentheses. The observed number of zeros is indicated in each irradiated case, with the theoretical number of zeros given for comparison.

of grains is 11.03 per cell; for the one hit, one miss there should be half the number, or 5.5. It is now possible to calculate the theoretical distribution and it is shown in Fig. 1.

It is apparent that there is reasonable agreement for the high grain end, and of course, the number of zeros has been used to calculate y so the agreement there is automatic. The discrepancy is greatest for one- and two-grain cells. Our explanation for this is in part the resynthesis of degraded label by cells which still have some

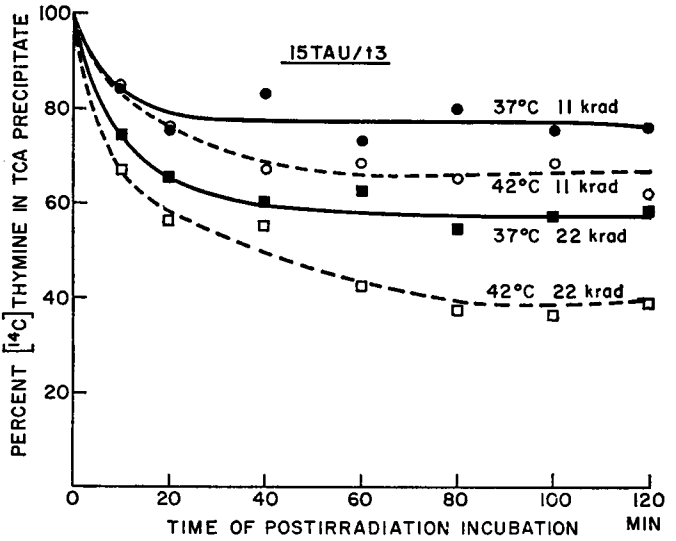

FIGURE 2 Evidence that resynthesis of degraded DNA can take place. The loss of TCA precipitable material after irradiation is shown for the temperature-sensitive strain TAU/t3. If the cells are kept above the permissive temperature the amount of degradation is greater.

ability to synthesize residual DNA. This could be either repair replication or an undamaged and functional growing point. Experiments with the temperature-sensitive DNA synthesizing cell TAU/t3 indicated that about 10% of the degraded DNA is resynthesized (Pollard, 1970, p. 79). The data indicating the presence of resynthesis are shown in Fig. 2. Cells of 15TAU/t3 were labeled with [^{14}C]thymine, irradiated with various doses of ^{60}Co gamma radiation, and the culture divided. One fraction was incubated at 37°C, the other at 42°C, above the permissive temperature. The amount of degradation is clearly more if DNA synthesis cannot proceed. A summary of the data in these experiments is given in Table I. The comparison between oxygenation and nitrogenation of the culture during irradiation is shown. This particular temperature-sensitive mutant also carries an inducible inhibitor of DNA degradation so the percent degraded does not exceed 75%. Experiments with TAU/t7 using rifampicin to prevent the transcription of the inhibitor gave a similar ratio of amount degraded with and without DNA synthesis present.

These experiments are important in the interpretation of the data on JG151. They also offer evidence that DNA synthesized after irradiation is not degraded to the same extent as preexisting DNA.

Once the value of y has been calculated for one dose it should be possible to determine the distributions for the other doses without reference to the data and then to check against the findings. Thus the value of y for the 4,000 rad case should be 1.05 and for 10,000 rad 2.6. Using these figures the first check is the numbers of zeros: for 4,000 rad there should have been 444; 493 were observed; for 10,000 rad the figure predicted is 900; 823 were found. The theoretical distributions found for these two

TABLE I

POSTIRRADIATION DNA DEGRADATION IN 15TAU/t3 ABOVE AND BELOW THE PERMISSIVE TEMPERATURE FOR DNA SYNTHESIS

Irradiation conditions	Incubation temperature	Percent degraded after 2 h
	°C	
5,500 rad, O_2	37	4
	42	12
11,000 rad, O_2	37	19
	42	39
22,000 rad, O_2	37	42
	42	60
33,000 rad, O_2	37	63
	42	72
5,500 rad, N_2	37	0
	42	8
11,000 rad, N_2	37	1
	42	10
22,000 rad, N_2	37	7
	42	17
33,000 rad, N_2	37	12
	42	29
66,000 rad, N_2	37	49
	42	59

The decreased degradation while DNA synthesis proceeds suggests that resynthesis of degraded DNA occurs.

cases are shown. It can be seen that if credence is given to the concept of resynthesis giving some unaccounted for (in the analysis) grains in the single- and double-grain cases, the agreement is very reasonable. In these two cases the number of zeros is a test of the theory, not a figure fixed by the data.

The results for 15JG151 permit the same conclusion to be reached. The data are not so easy to interpret because this cell ceases degradation at the level of about 8% in a mass culture experiment (Grady and Pollard, 1968). Therefore the class of fully degraded cells will probably include single, double, and triple grain counts as well as zeros. This introduces some uncertainty in the best method of analysis. In spite of this difficulty, it is not hard to see that the all-or-nothing character is again apparent. Fig. 3 shows the results of one experiment. The unirradiated appear at the upper left. The average number of grains counted was 13.8 and the Poisson distribution for that number is shown. The agreement is reasonable. The upper right is the 10 krad case and in this instance the value of the parameter y has been derived by taking the zeros and one grain counts to give the double hit case. After we deducted 10 zeros for probable dead cells, there were 293 cells counted. The value found for y is 0.55, leading to 52 cells expected to have totally escaped and 170 hit in one

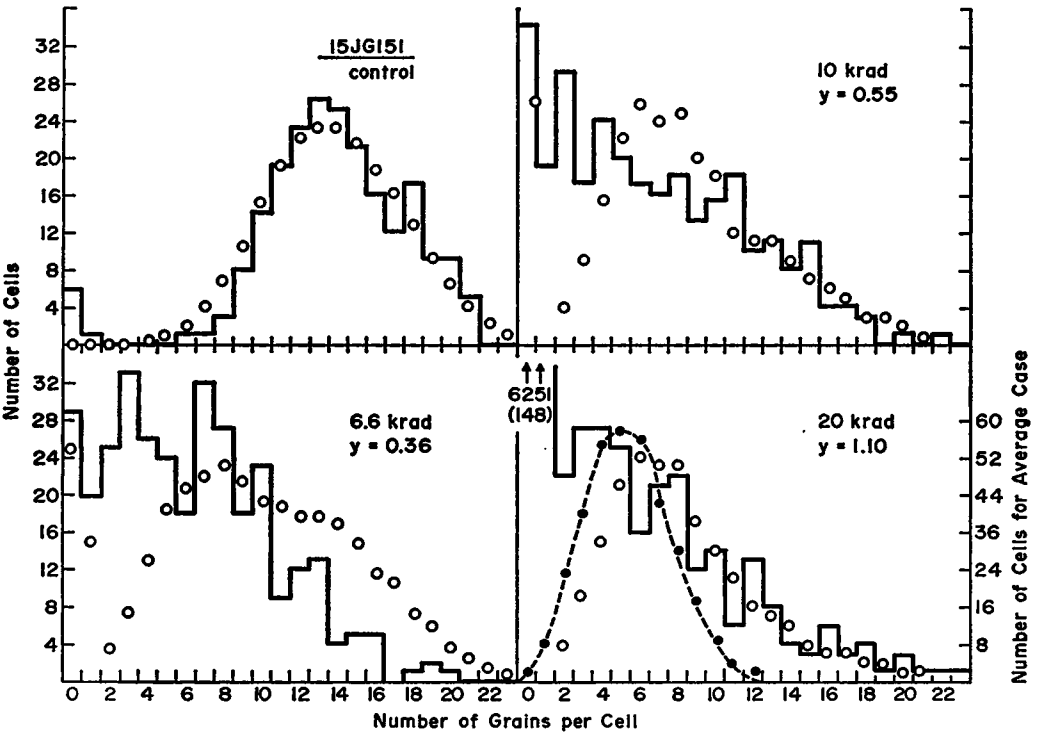

FIGURE 3 The distribution of JG151 cells with different grain numbers for different treatments. The doses given are as indicated. The theoretical distributions as indicated in Fig. 1 are shown. In the lower right the distribution for average degradation spread through all cells is shown as a dotted line. The scale has been changed as indicated on the right.

genome but escaping in the other. By using 13.8 grains for the total escape and 6.9 for the one hit case, the predicted line is shown as round dots. The agreement is fair, except that 2, 3, and 4 are present in excess. With this value of y for the 10,000 rad case the whole curves for the 6,600 rad and 20,000 rad cases can be calculated. Agreement is now also required for the 0 and 1 totals and the predicted values.

The 6,600 rad case shows about the expected number of zeros and ones, but falls short for the high numbers. (For comparison the distribution expected for the average in all cells is shown. This is a very bad fit, because it predicts no increase at zero and 1 but rather a very large number in the region of the average. There are also too many cells with high grain counts.) The basic result shows more clearly for the 20,000 rad case. Here the fit at the upper end is quite good. The numbers of zeros and ones (113 found) fall rather below the 148 predicted. Nevertheless the basic shape is in agreement, with the same exception for the 2, 3, and 4 cell cases. The distribution for the average, which is shown as a dotted line, with the scale changed as indicated, is not easily reconciled with the data. It predicts almost no zeros and ones and none in the high grain count region. Thus, in a more qualified

way, we feel that the results from 15JG151 fit the same pattern of all-or-nothing DNA degradation. This has already been suggested by Hildebrand and Pollard (1969), using the technique of observing cells in density gradient sedimentation. Two classes of cells were found after irradiation. The light (and degraded) cells could be separated from normal, undegraded cells. The findings on JG151 thus confirm the earlier conclusions.

These two kinds of cells are representative of a class which has been "cured" of an episomal factor that transcribes an inhibitor of radiation-induced DNA degradation. Where such an episome exists, findings on DNA degradation are extremely hard to interpret. The induction of the defective prophage (if that be the episomal factor) is presumably all-or-nothing and caused by radiation. Thus two processes are taking place after radiation. Because of this complication, the character of radiation-induced DNA degradation has been hard to elucidate.

The analysis of degradation in terms of two degradable units per cell is almost certainly oversimplified. The work of Cooper and Helmstetter (1968) and Kubitschek and Freedman (1971) has established that cells of *E. coli* carry increasing numbers of genomes per cell if their division rate increases. By using the formula given (Kubitschek and Freedman [1971]) with our approximate division time of 35 min the expected number of genomes per cell is 2.9. If there is a relation between the number of genomes and the degradation units then perhaps three units per cell would be better for a theoretical analysis. We have attempted this, and the fit in the three- and four-grain cases is better. We do not believe the data are sufficiently accurate to enable a good enough estimate to be made of this number for real use in describing cells. We believe it lies between two and four. It is remarkable that a process started in a cell by a relatively small and confined insult should on the one had result in the massive degradation of a large part of the DNA and on the other hand none. Obviously more knowledge of the structure of the cell is needed.

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