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Membrane-DNA Attachment Sites in *Streptococcus faecalis* Cells Grown at Different Rates

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The M-band technique was used to assess the number of attachment points of DNA to the cell membrane of *Streptococcus faecalis* grown at three different rates. Cells were X irradiated in liquid nitrogen and then analyzed simultaneously for the introduction of double-strand breaks into the chromosome and the degree of removal of DNA from the cell membrane (M band). Consideration of the data from these experiments and of the topology of the bacterial chromosome resulted in a reevaluation of former quantitative models. Our results are consistent with a semiquantitative model in which the bacterial chromosome is organized around a core structure. We interpret our data to mean that the core is attached to the membrane and that the complexity of the core changes more drastically with growth rate than does the number of membrane-DNA attachment points. An alternative model in which RNA hybridizes with DNA containing single- and double-strand breaks is also discussed. In any event, the complexity of these interactions precludes a reliable estimate of the number of membrane-DNA attachment sites.

Based on electron microscopy and studies of cell fractions, it appears that the single circular chromosome found in bacteria is attached to its envelope (13, 21, 25) at multiple points (1, 10). The functional nature of these attachments is not fully known, but it has been proposed that they could provide a primitive mitotic apparatus by which a copy of the replicated chromosome could be segregated into each daughter cell at the completion of a cycle of cell growth (11). Specifically, evidence has been presented that various numbers of these attachment sites contain the origin (9, 19) and terminus (9) of the chromosome, also possibly the replication forks (13) and DNA-associated RNA polymerase molecules (10).

Dworsky and Schaechter (10) estimated the number of *Escherichia coli* membrane-DNA attachment sites to be between 13 and 19, from experiments which measured the number of X ray-induced double-strand breaks that are required to release half of the DNA from the membrane. Since some of these membrane-chromosome attachment sites may be part of the chromosomal replication machinery (i.e., origins, forks, or termini), and since the number of replication forks is a function of the bacterial growth rate (6), we expected that the number of membrane-chromosomal attachment sites would also be a function of the growth rate. Therefore, the methods of Dworsky and Schaechter were applied to cultures of *Streptococcus faecalis* growing at three rates, selected on the basis that the cultures should show progressive decreases in the number of origins, replication forks, and termini.

Examination of the data from these experiments led us to reexamine the models which Dworsky and Schaechter (10) used to interpret their data and to offer a new interpretation of their and our data. Our interpretation incorporates a previous idea that the procaryotic chromosome is organized around a core structure (30) and also questions the previous estimate for the number of membrane-DNA attachment sites. We also consider the possibility that the biphasic character of the data may be due to DNA-RNA hybridization. We provide simple methods for quantifying the complexity of the hypothesized core by using the M-band data, but we do not attempt a full-fledged mathematical model, since a large number of ad hoc assumptions would have to be made to develop such a model.

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MATERIALS AND METHODS

S. faecalis 9790 was grown in a chemically defined medium (26) at 37°C for at least six exponential mass doublings. Cells were labeled with [2-^{14}C]thymidine (0.5 μCi/ml) in the presence of unlabeled carrier at a concentration of 15 μg of thymidine per ml. For slowly growing cultures, the chemically defined medium was modified by omitting glutamine and limiting glutamate (29) to 20 μg/ml (80-min doubling time) and 10 μg/ml (120-min doubling time).

Cultures were rapidly chilled to 4°C in the midexponential phase at a concentration of approximately 7 $\times$ 10^8 cells per ml and harvested by centrifugation at 9,700 $\times$ *g* for 10 min. Cells were converted to protoplasts (bacteria with their cell walls removed) as previously described (23), except that 0.25 M sucrose was used as an osmotic stabilizer. After 30 min of exposure to lysozyme at 37°C, protoplast suspensions were rapidly frozen in 50-μl fractions and stored in liquid nitrogen until further use.

Protoplasts were exposed to radiation from a General Electric Maxitron 300 X-ray unit (General Electric Co., Milwaukee, Wis.) as previously described (20). Samples were maintained at liquid nitrogen temperature (−196°C) during and after irradiation. The dose rate was 15.04 kilorads (krads) min as measured with an r-meter (Victoreen Instrument Co., Cleveland, Ohio).

The amount of DNA attached to the membrane was measured by a modification of the M-band technique of Dworsky and Schaechter (10). Essentially, rapidly thawed protoplast suspensions were layered onto prechilled columns of buffered sucrose along with preformed magnesium-Sarkosyl crystals (20). M bands were formed by centrifugation at 30,000 $\times$ *g* for 20 min at 4°C and collected separately from the supernatant solution.

The same sample which had been thawed to form M bands was also simultaneously used to measure the molecular weight of double-strand DNA by the method of Burgi and Hershey (5), as described previously (20). Molecular weights at the modal point of radioactivity (M) were calculated by the method of Lydersen and Pettijohn (18): $M = (d/d_k)^{\alpha} M_k$, where d and d_k were the distance sedimented on neutral sucrose gradients of DNA from *S. faecalis* and λ phage (marker), respectively. α is 2.86, and the known molecular weight of phage λ DNA (M_k) is 3.1 $\times$ 10^7 (5). We include the molecular weight calculation for the sake of completeness, recognizing that this standard method is open to criticism due to the widely different weights of the λ and bacterial DNA. The analysis presented below does not hinge on the accuracy of the molecular weight calculation.

[^{14}C]thymidine was obtained from Amersham Corp. (Arlington Heights, Ill.). Sarkosyl NL-30 (sodium dodecyl sarcosinate) was a gift from CIBA-GEIGY Corp. (Greensboro, N.C.).

RESULTS

To estimate the number of membrane-DNA attachment points in *S. faecalis* grown at different rates, we first used the method of Dworsky and Schaechter (10). This involved measuring the number of double-strand breaks introduced into the chromosome of *S. faecalis* by X radiation and the concomitant assessment of the fraction of DNA which was found attached to the membrane (M band) after the same X-ray dosage.

Figure 1 shows the dose response of DNA released from the M-band fraction after three separate cell suspensions which had grown at different rates were exposed to X radiation.

The doubling times of the three cultures of *S. faecalis* were 33, 80, and 120 min during midexponential growth, and these will be referred to as 33-min cells, 80-min cells, and 120-min cells, respectively. The initial percentage of DNA found in the M band from rapidly growing, unirradiated 33-min cells was about 86%, which is in agreement with data obtained from rapidly growing *E. coli* (10, 28). However, in the cells grown more slowly, only about 70% of the labeled DNA was initially found in the M band.

In a biphasic manner, DNA in all three cultures was released from the M-band fraction with increasing X radiation. At relatively low dosages of radiation there was a slow release until about 45 to 50% of the DNA had been removed from the M band. This was followed by a precipitous decline in the amount of DNA associated with the membrane. The inflection points of the curve of DNA loss from the M band were extrapolated visually and estimated at doses of 3,200 krads for 33-min cells, 2,750 krads for 80-min cells, and 2,300 krads for 120-min cells.

The decrease in double-strand molecular weight of DNA from cells grown at different rates and exposed to X radiation is shown in Fig. 2. These curves also appear to be biphasic in nature. The mean molecular weights of unirradiated DNA (0 krad) from 33-min, 80-min, and

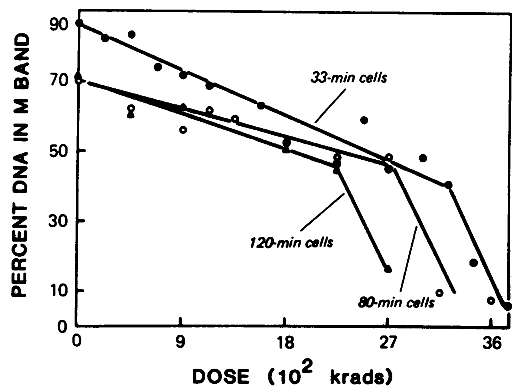

FIG. 1. Release of DNA from the M-band fraction after X irradiation of *S. faecalis* cells grown at three different rates. The DNA associated with the M band is expressed as a percentage of the total DNA.

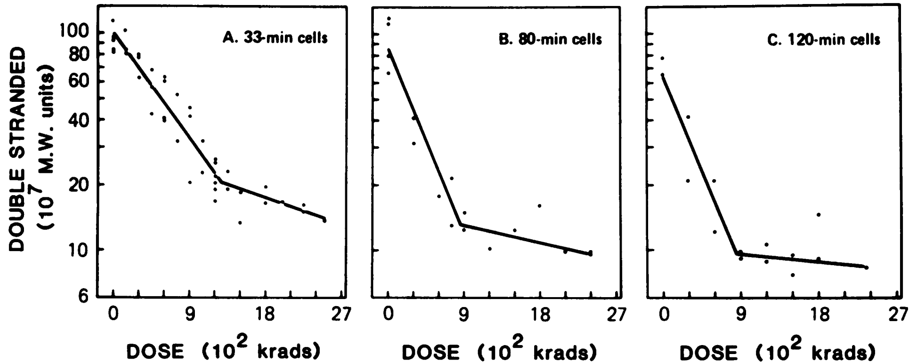

FIG. 2. Effect of X irradiation on the double-strand molecular weight of DNA. Molecular weights were calculated from sedimentation analysis by the method of Burgi and Hershey (5).

120-min cells were 9.14×10^8, 8.69×10^8, and 7.12×10^8, respectively. Over the same range of radiation, it appears that the double-strand molecular weights of DNA from 80-min and 120-min cells decreased more rapidly than those from 33-min cells.

At radiation doses above about 1,350 krads for 33-min cells and 900 krads for 80-min and 120-min cells, the introduction of double-strand breaks into the DNA backbone occurs at a slower rate than at lower radiation dosages. At these higher dosages, radiation-induced cross-linkings between different DNA strands and between DNA and protein become significant secondary events (3, 14) and would affect the measurement of molecular weight by sedimentation.

DISCUSSION

Method for interpreting the M-band data. The rationale used by Dworsky and Schaechter in calculating the number of membrane-DNA attachment points from the amount of DNA removed from M bands after various doses of radiation can be shown by the following example. Consider a suspension of bacteria which have a single chromosome that is 1 mm in length and that is attached to the cell membrane at exactly two points. For the circular chromosome discussed below, the attachment points are assumed to divide the chromosome into two equal lengths. The linear chromosomes discussed below may be imagined to be produced from circular ones, from a single random break. When the cells are disrupted and exposed to Mg-Sarkosyl crystals, hydrophobic membrane components bind to the crystals and cause the attached DNA to cosediment in sucrose gradients; thus an M band is formed (28). The exposure of such bacteria to X irradiation before disruption introduces random double-strand breaks into the backbone of the chromosome and results in a reduction in the amount of DNA that sediments with the M band. By knowing the average number of double-strand breaks per chromosomal length (μ) that are introduced by a given dose of radiation, it is possible to calculate the relative number of pieces of DNA of various lengths that are produced (viz., the relative number of DNA pieces having sizes of 0.1, 0.2, 0.3 . . . 1.0 mm). To do this accurately, one must know something about the topology of the chromosome before radiation (i.e., whether it was linear, circular, branched, etc.). In the Appendix, two equations (equations 2 and 3) are presented which allow the calculation of the relative number of randomly situated double-strand breaks which have been introduced into an initially linear or circular chromosome.

In Table 1, these equations have been used to calculate the relative number of DNA pieces of different lengths that would hypothetically result from an average of two double-strand breaks being introduced into an initially linear or circular chromosome. The larger number of small DNA pieces that results from a linear chromosome as compared with a circular chromosome is a consequence of the fact that one double-strand break is required to open the circular structure. To determine how many of the DNA pieces shown in Table 1 will sediment with the M band, the relative number of pieces of DNA in each size group must be multiplied by the probability that each piece will have at least one of the two membrane-DNA attachment points. In our example, if a circular chromosome is broken by two randomly situated double-strand breaks, the fragments with lengths greater than 0.5 mm will have a 100% probability of having at least one attachment point. For the fragments which are shorter than 0.5 mm, the probability of having attachment points follows the simple relation-

TABLE 1. Theoretical percentage of DNA in M band[a]

Length (l) of DNA pieces[b] (mm)	Midpoint	Linear chromosome			Circular chromosome		
		Relative no. of pieces[c]	Probability that a piece is attached[d]	Amt of DNA in M band[e]	Relative no. of pieces[f]	Probability that a piece is attached[d]	Amt of DNA in M band[e]
0.0–0.1	0.05	0.70	0.1	0.004	0.36	0.1	0.002
0.1–0.2	0.15	0.54	0.3	0.024	0.30	0.3	0.014
0.2–0.3	0.25	0.43	0.5	0.054	0.24	0.5	0.030
0.3–0.4	0.35	0.33	0.7	0.081	0.20	0.7	0.049
0.4–0.5	0.45	0.25	0.9	0.101	0.16	0.9	0.065
0.5–0.6	0.55	0.19	1.0	0.104	0.13	1.0	0.072
0.6–0.7	0.65	0.14	1.0	0.091	0.11	1.0	0.072
0.7–0.8	0.75	0.11	1.0	0.082	0.09	1.0	0.068
0.8–0.9	0.85	0.08	1.0	0.068	0.07	1.0	0.060
0.9–1.0	0.95	0.06	1.0	0.057	0.06	1.0	0.057
Original length		0.135	1.0	0.135	0.41	1.0	0.41
Fraction of DNA in M band				0.80[g]			0.90[h]

[a] Assumptions: (i) an average of 2 double-strand breaks (μ), (ii) two nonrandom attachments (α) per chromosome, and (iii) initial chromosome length (L) of 1.0 mm.

[b] Slight errors are introduced if the length classes are considered to have increments of 0.1 mm. The correct calculations consider infinitesimal length increments.

[c] Calculations based on equation 2 in the Appendix [$n(l)\Delta l$ with $\Delta l = 0.1$].

[d] Calculations based on the formula $\alpha \times l$.

[e] Number of pieces × length per piece × probability of attachment.

[f] Calculations based on equation 3 in the Appendix [$n(l)\Delta l$ with $\Delta l = 0.1$].

[g] The value obtained from equation 4 of the Appendix is 0.81.

[h] The value obtained from equation 6 of the Appendix is 0.89.

ship $\alpha \times l$, where α is the number of membrane-DNA attachment points on the unbroken chromosome ($\alpha = 2$ in this example), and l is the length of the subgroup of DNA pieces in question.

This equation has been used to calculate the probability of membrane attachments for each size class of DNA pieces presented in Table 1. It should be noted that this probability is independent of initial DNA topology and the number of double-strand breaks. If the attachment points in our example had been randomly arranged on the chromosome with an average of α per initial length, a different equation would have been used to calculate the probability of attachment ($1 - e^{-\alpha l}$); however, the process in all other respects would have been the same.

To calculate the amount of DNA in the M band, the relative number of DNA pieces in each size class is multiplied by the probability that each piece in this class contains at least one attachment point and by the length of the size class (Table 1); the total amount of DNA in the M band is the sum of the contributions from each length class.

To summarize, the relative number of pieces of DNA in each length class is a function of the unirradiated topology of the chromosome and of the average number of double-strand breaks per chromosomal length (μ), whereas the probability of these pieces having at least one attachment point is a function of the arrangement and number of such attachment points per chromosomal length (α). Therefore, in this model there is a clear numerical relationship between the fraction of DNA that sediments with the M band (F), μ, and α. In principle, one could construct tables, such as Table 1, showing the relationships of F, μ and α in cases where the DNA was assumed to be initially circular or linear and where attachment points were assumed to be randomly or nonrandomly arranged. With these calculations, either F, μ, or α could be predicted by having known values for two of these variables. Fortunately, these tables need not be constructed, since it is possible to derive equations relating F, μ, and α.

Four such equations are presented in the Appendix (see also references 5, 10, 18 and 28). They predict the relationships between F, μ, and α in situations where the initial chromosome is linear or circular and the attachment sites are regularly or randomly spaced. These equations go beyond those of Dworsky and Schaechter (10), who considered randomly and nonrandomly distributed attachment sites, but did not include the effect of the topology of the unirradiated chromosome. Our recalculation of the

relationships was motivated by our confusion as to whether their derivation applied to linear, circular, or arbitrarily shaped chromosomes.

Model cannot explain the data. The major problem in using any of these equations in the calculation of α is the experimental determination of μ. The usual method is to compare the molecular weight obtained from sedimentation data of the unirradiated chromosomes with that obtained from sedimentation data of the irradiated chromosomes. The position of the peak of DNA observed in the gradients is converted into an estimation of molecular weight by the equation of Burgi and Hershey (5) (or a modification of their equation). Unfortunately, the determination of molecular weights by this method is open to considerable error, especially when applied to DNA of high molecular weight.

As an alternate approach to determining whether the Dworsky and Schaechter model for membrane-DNA attachment points fits our observations, we used a procedure that avoids the estimation of μ from sucrose gradients.

It was assumed that double-strand breaks are introduced into the chromosome at most as a second-order process that follows to the equation: $\mu = a + bd + cd^2$ with $a, b, c \geq 0$, where μ is the linear density of double-strand breaks, a is related to the initial number of double-strand breaks, b is related to the number of initial single-strand breaks, c is related to the rate of formation of single-strand breaks (which in turn controls the rate of double-strand breaking), and d is the dose of radiation as shown in Fig. 1 (8).

This equation includes as a special case the possibility that there is a linear relation between X-ray dose and double-strand breaks (4) and approximates the theoretical possibility that the dose response may go as some noninteger power less than 2 (16). The values of a, b, and c corresponding to the experimental density of double-strand breaks may be determined by substituting the above expression for μ into the equations relating μ and the percentage of DNA in the M band. By systematically varying these three parameters, we should find some combination of numerical values that causes the predicted values of the M band data to most closely match the experimental values shown in Fig. 1. We were surprised to find that for each of the four models there was no combination of values for a, b, and c that fit the data over the entire range of X-ray doses. The results of one such attempt are shown graphically in Fig. 3. Here the M band data obtained from the culture with a 33-min doubling time is shown in all four panels. In Fig. 3A and B, by using the equation for nonrandom attachment points and an initially circular chromosome, it is possible to arrive at values for a, b, c, and α that allow a good fit of the initial portion of the data where the decrease in the fraction of DNA found in the M band per unit dose is slight or, alternatively, that fit the rapid decrease phase observed at high radiation doses. However, no single set of variable values allows a good fit of data obtained at both high and low doses. Figures 3C and D show a similar fit assuming a random distribution of attachment points. The fit was not improved by using the equations that apply to an initially linear chromosome. The conclusion is that these types of models are inconsistent with the biphasic structure of the M band data (Fig. 1).

Core models. A new proposal is that the data in Fig. 1 are in agreement with a model in which the chromosome is organized into a series of loops which are connected at a series of sites to a radiation-sensitive core. This model is virtually identical to the one proposed by Worcel and Burgi (30) and Kleppe et al. (12) some years ago for the organization of the DNA of *E. coli* and is consistent with the looped organization seen in electron micrographs of the chromosomal DNA of *E. coli* (7). The principal difference between this model and our proposal is that, although in both views the DNA must be organized around a core structure, some of the loops of our model, as well as the core itself, may be associated in

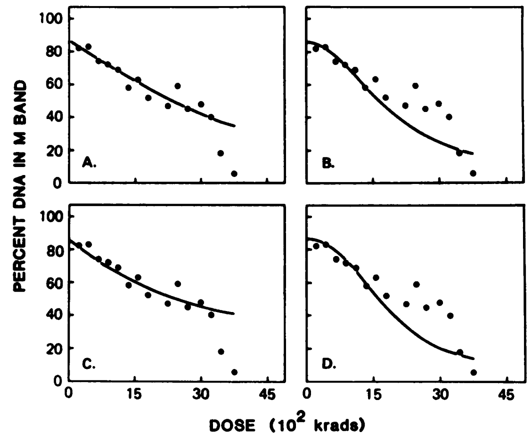

FIG. 3. Graphic display of an attempt to fit equations to actual data points obtained as DNA is released from the M-band fraction after X irradiation of 33-min cells. The data points in all four panels are identical to those from the 33-min cells in Fig. 1. The fraction of DNA in the M band was calculated with equations 6 (A and B) and 7 (C and D) in the Appendix, assuming that the number of double-strand breaks (μ) goes at most as the square of the dose (d): $\mu = a + bd + cd^2$. The parameters a, b, c, and α were systematically varied. For no combination of parameter values was it possible to simultaneously fit the upper and lower portions of the data. Similar results were obtained with equations 4 and 5 and data points for 80-min and 120-min cells.

some way with the membrane. Our interpretation accounts for the biphasic character of the M band data and provides a crude measure of the complexity of the hypothesized core under different growth conditions, but cannot provide any details about its structure. We have not attempted a full-fledged mathematical model of the biphasic character of the data, since a large number of ad hoc assumptions would have to be made to develop such a model.

The models considered by Dworsky and Schaechter (10) may be converted to core models if we imagine that most of the membrane-DNA attachment sites are gathered into a single point, the core. So long as the core remains intact and attached to the membrane, the Dworsky and Schaechter approach is correct, and the slight reduction in DNA observed along the upper portion of Fig. 1 might be used to estimate the number of attachment sites (the number of loops plus the number of peripheral attachment sites). If the estimate is to be based on the radiation dose required to reduce the membrane-attached DNA to half its original value (10), the upper portion of the curve will generally have to be extrapolated into the region of the curve in Fig. 1 that shows an abrupt drop. It is likely that others (1, 10) did not observe this rapid detachment of DNA from the M band because of the relatively lower doses of X irradiation that they used.

The abrupt drop in the amount of DNA associated with the membrane occurs when the core disintegrates or simply detaches from the membrane, due to radiation damage. The kinetic details of the drop provide semiquantitative information about the structure of the core. From the data, we can read the radiation dose at the midpoint of the drop (T) as well as the approximate range of radiation dose over which the drop occurs (Δ). Our data show that the position of the drop increases as the doubling time decreases (Fig. 1); but the slope of the drop is independent of the doubling time.

The ratio Δ/T may be regarded as the coefficient of variation of a multistage stochastic process corresponding to the disintegration of the core. The number of stages in the stochastic process can be taken to be a measure of the complexity of the core and can be estimated from the coefficient of variation. In the Appendix, we consider three models for the multistage stochastic process.

In the first model, the core is destroyed when N independent sites are hit by radiation. If this is true, then $N \cong e^{\{[1.28/(\Delta/T)] - 0.57\}}$. In the second model, the core undergoes N conformational changes during the course of its destruction, with each successive conformational change resulting from the same average radiation dose. If this is true, then $N = 1/(\Delta/T)^2$. In the third model, the core again undergoes N conformational changes as it is destroyed, but each successive stage of destruction requires a smaller radiation dose than the previous one. If this is true, then $N \cong 4/3 \times [1/(\Delta/T)^2]$. For the 33-min, 80-min, and 120-min cells, we used Fig. 1 to estimate Δ/T; these values are 0.13, 0.15 and 0.18, respectively. Then for the first model, the values of N are 10,700, 2,900, and 700 for the 33-min, 80-min, and 120-min cells, respectively; for the second model the values of N are 59, 44, and 30, respectively; and for the third model, the values of N are 79, 59, and 40, respectively. Since we see no reason that the number of radiation-sensitive sites in the core should increase exponentially as the population doubling time decreases, the first model seems implausible. In other words, the destruction of the core involves something more complicated than simply the accumulation of radiation hits at independent sites. It is more likely that the core-membrane complex is destroyed in stages, analogous to a building that is being shot at with bullets (models 2 and 3).

Apart from this general observation, the only other conclusion that the core model allows us to draw is the following: since the dose-response curves of DNA removed from the membrane (M band; Fig. 1) for 33-min, 80-min, and 120-min cells differ greatly at higher radiation doses (>2,200 krads), but not at lower doses, a change in growth rate has a more drastic effect on the complexity of the core than on the number of membrane-DNA attachment sites.

Caveats. None of the models for interpreting M band data takes into account the fact that an exponentially growing population consists of cells having a variety of ages, each cohort differing possibly with regard to the number and types of membrane-DNA attachment sites. A more complete model would incorporate the existence of an age-dependent chromosome structure that is more complicated than the linear or circular alternatives (e.g., the existence of theta-shaped chromosomes). Our experience with the simpler models suggested that although the chromosome topology affects the structure of the predicted curves, further assumptions about the dynamics of chromosome topology would not provide an explanation for the biphasic character of the data.

One must recognize not only the complications that were ignored by the models, but also the possibility that some of the models' assumptions may be wrong. It was assumed that a double-strand break occurring in a linear piece of DNA results in two physically separate new pieces. The possibility exists, however, that RNA may hybridize to frayed DNA at single-

and double-strand breaks, thereby bandaging DNA pieces which would otherwise come apart. This possibility is minimized by the fact that our cells were irradiated at liquid nitrogen temperatures (−196°C) and were rapidly thawed and lysed for M-band and molecular weight analyses; but it might nevertheless occur since the DNA and RNA are in such close proximity in the unlysed protoplasts. If such DNA-RNA hybridization does in fact occur, the rate of fragmentation of DNA should go as the third power of radiation dose (two DNA single strands plus one RNA single strand), which might account for the abrupt drop in the dose-response curve. Such abrupt drops have been observed in in vitro experiments involving DNA-RNA hybrids which were X irradiated (22).

The last point to be considered is why the abrupt decrease in M band-associated DNA at high radiation doses has not been described previously. Two factors seem to be important. First, the range of radiation doses used in this study is very large; second, the methodology used in this study differs from that employed previously by others. Here protoplasts were irradiated at liquid nitrogen temperatures and under anaerobic conditions. Both factors are known to reduce the yield of double-strand breaks per krad dose (2) and also may result in other differences in the manner in which intact protoplasts are affected by massive radiation doses.

APPENDIX

Early literature on the mathematics of randomly degraded polymers is reviewed by Tanford (27). He gives an expression for the distribution of lengths of a randomly broken polymer, assuming that breaks occur only at discrete sites (monomers). However, if the polymer is assumed to be a continuum, breaks may occur anywhere along its length. Rupp and Howard-Flanders (24) made this assumption and calculated the expected distribution of DNA fragment sizes resulting from random breaks. Litwin et al. (17) criticized the Rupp and Howard-Flanders results on the grounds that those results are correct only if the number of breaks is large; they estimate the percentage error using formulas derived by Litwin (15).

The Dworsky and Schaechter (10) calculations for the number of membrane-DNA attachment sites assume that the expected distribution of DNA fragments follows the Rupp and Howard-Flanders model. Therefore, the Dworsky and Schaechter calculations have general applicability only if the number of double-strand breaks is large.

In this appendix we show that the Rupp and Howard-Flanders and Dworsky and Schaechter results are correct only for circular polymers with uniformly distributed attachment sites. First, we give a general equation for the expected distribution of lengths of a randomly broken polymer. Then, we solve this equation for linear and for circular polymers. These results are combined with two membrane-DNA attachment models to give four equations for interpreting M band data. Finally, we derive expressions for the degradation kinetics of chromosomes with radiation-sensitive cores.

Expected number of pieces of DNA in each length class and topology class. Suppose that an initial population of DNA consists of a very large number of identical pieces, each having total length L. This population is irradiated until the average number of double-strand breaks is μL per initial piece. Let $n(l;\mu,L)\Delta l$ denote the expected number of linear fragments having length in the range l to $l + \Delta l$, with the understanding that the experiment is parameterized by particular values of μ and L.

The mass action kinetic equation describing $n(l;\mu,L)\Delta l$ is

$$\frac{\partial n}{\partial \mu}\Delta l = -nl\Delta l + \Delta l \int_l^\infty dl' Q(l',\mu) \qquad (1)$$

where the first term on the right-hand side represents loss through breakage of fragments of size l to $l + \Delta l$ at a rate proportional to the total amount of DNA in that class; and where the second term represents the increase through breakage of longer pieces at distances between l and $l + \Delta l$ from a free end. $Q(l',\mu)dl'$ is the number of free ends defining fragments of length l' to $l' + dl'$, at the instant that the breakage average is μ per unit DNA length. For example, if a DNA molecule were in the shape of a Y with all three of its arms having lengths greater than l', then the molecule would contribute three free ends to $Q(l',\mu)dl'$, but if a DNA molecule were in the shape of a circle or a theta, it would contribute 0 free ends to Q.

If all initial DNA molecules are linear with length L, the number of such molecules as a function of μ is simply $e^{-\mu L}$, where the initial number of molecules is defined to equal one unit. Then

$$\int_l^\infty Q(l',\mu)dl' = 2\left[\int_l^L n(l';\mu,L)dl' + e^{-\mu L}\right]$$

and equation 1 becomes

$$\frac{\partial n}{\partial \mu}\Delta l = -nl\Delta l + 2\Delta l\left[\int_l^L n(l';\mu,L)dl' + e^{-\mu L}\right]$$

which has as its solution

$$n(l;\mu,L)\Delta l = \mu e^{-\mu l}[2 + \mu(L - l)]\Delta l \qquad (2)$$

If all initial molecules are circular with length L, then the numbers of circular (C) and linear (N) fragments of length L as a function of μ are given by the equations

$$\frac{dC}{d\mu} = -LC \qquad \frac{dN}{d\mu} = L(C - N)$$

or

$$C = e^{-L\mu} \qquad N = \mu L e^{-L\mu}$$

where C is initially 1. Then

$$\int_l^\infty dl' Q(l',\mu) = 2\left[\int_l^L n(l';\mu,L)dl' + \mu L e^{-L\mu}\right]$$

and equation 1 becomes

$$\frac{\partial n}{\partial \mu}\Delta l = -nl\Delta l + 2\Delta l\left[\int_l^L n(l';\mu,L)\mathrm{d}l' + \mu L e^{-L\mu}\right]$$

The solution to this equation is

$$n(l;\mu,L)\Delta l = \mu^2 L e^{-l\mu}\Delta l \quad (3)$$

Recovery of sedimented DNA. (i) Membrane attachment models. Consider a circular chromosome of length L with αL regularly arranged membrane attachment sites. Then, the distance between adjacent sites is $1/\alpha$. When the DNA is fragmented, all pieces of length greater than $1/\alpha$ will contain at least one attachment site. Let l denote the length of a fragment. If $l \le 1/\alpha$, the probability that it contains one attachment site is αl, and the probability that it contains no attachment site is $1 - \alpha l$.

If the initial fragment is linear rather than circular, the same formula applies if we imagine that the linear DNA piece was formed through a single, random break in the circular DNA described above.

If the membrane attachment sites have a random rather than a uniform arrangement, the number of attachment sites on the circular or linear fragment of length L has a Poisson distribution with average αL. Then, for fragments of length l, the probability that there is one or more attachment sites is $1 - e^{-\alpha l}$, and the probability that the fragment possesses no attachment sites is $e^{-\alpha l}$.

(ii) Recovery of DNA in various length ranges. To obtain the amount of DNA in any length class, we need only multiply the total number of fragments by the length of the fragments in that class. So, the results of the previous section may be combined with the results of the previous paragraphs to yield the following formulas for the fraction of membrane-bound DNA.

For initially linear DNA with regularly arranged membrane attachment sites, the amount of the total DNA which is attached to membrane and which has the indicated length is calculated as follows:

$$\mu l \times \alpha l[2 + \mu(L - l)]e^{-\mu l}\mathrm{d}l \qquad l \le \frac{1}{\alpha}$$

$$\mu l[2 + \mu(L - l)]e^{-\mu l}\mathrm{d}l \qquad \frac{1}{\alpha} \le l < L$$

$$Le^{-\mu L} \qquad l = L$$

The fraction of the total DNA which is membrane bound for $L \equiv 1$ is calculated as follows:

$$\alpha\mu \int_0^{1/\alpha} \mathrm{d}l\, l^2 e^{-\mu l}[2 + \mu(1 - l)] + \mu\int_{1/\alpha}^1 le^{-\mu l}[2 + \mu(1 - l)]\mathrm{d}l + e^{-\mu}$$

$$\cdot = 2\left(\frac{\alpha}{\mu} - \frac{\alpha}{\mu^2}\right) + e^{-\mu/\alpha}\left\{\left(\frac{\mu^2}{\alpha^2} + \frac{2\mu}{\alpha} + 2\right)\cdot\left(\frac{\alpha}{\mu^2} - \frac{\alpha}{\mu}\right) + \frac{\mu}{\alpha} + 1\right\} \quad (4)$$

For initially linear DNA with randomly arranged attachment sites, the amount of the total DNA which is attached to membrane and which has the indicated length is calculated as follows:

$$(1 - e^{-\alpha l})l\mu e^{-\mu l}[2 + \mu(L - l)]\mathrm{d}l \qquad l < L$$

$$L(1 - e^{-\alpha L})e^{-\mu L} \qquad l = L$$

The fraction of total DNA which is membrane bound for $L \equiv 1$ is calculated as follows:

$$(1 - e^{-\alpha})e^{-\mu} + \mu\int_0^1 le^{-\mu l}(1 - e^{-\alpha l})[2 + \mu(1 - l)]\mathrm{d}l$$

$$= (1 - e^{-\alpha})e^{-\mu} + 1 - e^{-\mu} - \frac{(2 + \mu)\mu}{(\alpha + \mu)^2}\cdot\{1 - e^{-(\alpha+\mu)}(\alpha + \mu + 1)\} + \frac{\mu^2}{(\alpha + \mu)^3}\cdot\{2 - e^{-(\alpha+\mu)}([\alpha + \mu]^2 + 2[\alpha + \mu] + 2)\} \quad (5)$$

For initially circular DNA with regularly arranged membrane attachment sites, the amount of the total DNA which is attached to membrane and which has the indicated length is calculated as follows:

$$\alpha l\mu l\mu L e^{-l\mu}\mathrm{d}l \qquad \frac{1}{\alpha} \le l$$

$$\mu l\mu L e^{-\mu l}\mathrm{d}l \qquad \frac{1}{\alpha} \le l < L$$

$$L(1 + \mu L)e^{-\mu L} \qquad l = L$$

The fraction of the total DNA which is membrane bound for $L \equiv 1$ is calculated as follows:

$$\alpha\mu^2 \int_0^{1/\alpha} l^2 e^{-\mu l}\mathrm{d}l + \mu^2\int_{1/\alpha}^1 le^{-\mu l}\mathrm{d}l + (1 + \mu)e^{-\mu}$$

$$= 2\frac{\alpha}{\mu}(1 - e^{-\mu/\alpha}) - e^{-\mu/\alpha} \quad (6)$$

For initially circular DNA with randomly arranged attachment sites, the amount of the total DNA which is attached to membrane and which has the indicated length is calculated as follows:

$$\mu^2(1 - e^{-\alpha l})lLe^{-l\mu}\mathrm{d}l \qquad l < L$$

$$L(1 - e^{-\alpha L})(1 + \mu L)e^{-L\mu} \qquad l = L$$

The fraction of the total DNA which is membrane bound for $L \equiv 1$ is calculated as follows:

$$\mu^2\int_0^1 le^{-l\mu}(1 - e^{-\alpha l})\mathrm{d}l + (1 - e^{-\alpha})(1 + \mu)e^{-\mu}$$

$$= 1 - e^{-\mu}(1 + \mu) - \left(\frac{\mu}{\mu + \alpha}\right)^2 \cdot [1 - e^{-(\alpha+\mu)}(1 + \alpha + \mu)] + (1 - e^{-\alpha})(1 + \mu)e^{-\mu} \quad (7)$$

Degradation of a chromosome having a radiation-sensitive core. The abrupt release of DNA from the membrane may be explained by the following model. Some of the DNA is directly attached to the membrane, but most of the DNA is attached to a central core which is in turn membrane bound. If the core suffers enough damage by X rays, it disintegrates and releases most of the DNA from the membrane. The following submodels may be used to estimate the number of radiation hits or stages of destruction needed to destroy the core. In one submodel, there are N sites in the core which must be hit before the core is destroyed. In a second submodel, the chromosome undergoes N sequential stages of destruction, with the average radiation dose required for each stage being the same. In a third submodel, there are again N sequential stages, but each stage takes a smaller radiation dose to occur, on the average, than the previous one.

The parameter N in each of these models is to be estimated from the coefficient of variation of random process which is completed after the accumulation of a (random) total radiation dose D: $D = d_1 + d_2 + d_3 + \ldots + d_N$. We assume that each of the individual doses (d_i; $i = 1, 2, \ldots, N$) has an exponential distribution with an average proportional to i^β, where the exponent β has values -1, 0, and $+1$ for the submodels 1, 2, and 3, respectively. Then, the average value of D is simply proportional to

$$\sum_{i=1}^{N} i^\beta$$

and the standard deviation for D is proportional to

$$\sqrt{\sum_{i=1}^{N} i^{2\beta}}$$

and the coefficient of variation equals

$$C = \frac{\sqrt{\sum_{i=1}^{N} i^{2\beta}}}{\sum_{i=1}^{N} i^\beta}$$

These summations are standard. For model 1

$$C \cong \frac{\pi/\sqrt{6}}{0.57 + \ln(N)} \quad (\beta = -1)$$

For model 2

$$C = \frac{1}{\sqrt{N}} \quad (\beta = 0)$$

For model 3

$$C = \frac{\sqrt{N(N + 1)(2N + 1)/6}}{N(N + 1)/2} \quad (\beta = +1)$$

These equations may be inverted to give N as a function of C. For model 1

$$N \cong \exp\left(\frac{\pi/\sqrt{6}}{C} - 0.57\right)$$

For model 2

$$N = \frac{1}{C^2}$$

and for model 3

$$N = \frac{\left(\frac{4}{3C^2} - 1\right) + \sqrt{\left(1 - \frac{4}{3C^2}\right)^2 + \frac{8}{3C^2}}}{2}$$

For sufficiently small C, the expression for model 3 is nearly

$$N \cong \frac{4}{3C^2}$$

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LITERATURE CITED

1. **Abe, M., C. Brown, W. G. Hendrickson, D. H. Boyd, P. Clifford, R. H. Cote, and M. Schaechter.** 1977. Release of *Escherichia coli* DNA from membrane complexes by single-strand endonucleases. Proc. Natl. Acad. Sci. U.S.A. **74:**2756–2760.
2. **Achey, P. M., D. Billen, and H. P. Beltranena.** 1971. Single strand breaks in gamma-irradiated $\phi\chi$174 DNA induced by exposure to alkali. Int. J. Radiat. Biol. **20:**501–504.
3. **Bohne, L., T. Coquerelle, and U. Hagen.** 1970. Radiation sensitivity of bacteriophage DNA. II. Breaks and cross-links after irradiation in vivo. Int. J. Radiat. Biol. **17:**205–215.
4. **Bonura, T., C. D. Town, K. C. Smith, and H. S. Kaplan.** 1975. The influence of oxygen on the yield of DNA double-strand breaks in x-irradiated *Escherichia coli* K-12. Radiat. Res. **63:**567–577.
5. **Burgi, E., and A. D. Hershey.** 1963. Sedimentation rate as a measure of molecular weight of DNA. Biophys. J. **3:**309–321.
6. **Churchward, G., E. Evangeline, and H. Bremer.** 1981. Growth rate-dependent control of chromosome replication initiation in *Escherichia coli*. J. Bacteriol. **145:**1232–1238.
7. **Delius, H., and A. Worcel.** 1974. Electron microscopic visualization of folded chromosome of *Escherichia coli*. J. Mol. Biol. **82:**107–109.

8. **Dertinger, H., and H. Jung.** 1970. Molecular radiation biology. Springer-Verlag, New York.
9. **Doyle, R. J., U. N. Streips, S. Imada, V. S. C. Fan, and W. C. Brown.** 1980. Genetic transformation with cell wall-associated deoxyribonucleic acid in *Bacillus subtilis*. J. Bacteriol. **144:**957–966.
10. **Dworsky, P., and M. Schaechter.** 1973. Effect of rifampin on the structure and membrane attachment of the nucleoid of *Escherichia coli*. J. Bacteriol. **116:**1364–1374.
11. **Jacob, P., S. Brenner, and F. Cuzin.** 1964. On the regulation of DNA replication in bacteria. Cold Spring Harbor Symp. Quant. Biol. **28:**329–348.
12. **Kleppe, K., S. Övrebö, and I. Lossius.** 1979. The bacterial nucleoid. J. Gen. Microbiol. **112:**1–13.
13. **Leibowitz, P. J., and M. Schaechter.** 1975. The attachment of the bacterial chromosome to the cell membrane. Int. Rev. Cytol. **41:**1–28.
14. **Lett, J. T., K. A. Stacey, and P. Alexander.** 1961. Cross-linking of dry deoxyribonucleic acids by electrons. Radiat. Res. **14:**349–362.
15. **Litwin, S.** 1969. Distribution of radioactive recovery in randomly cut and sedimented DNA. J. Appl. Prob. **6:**275–284.
16. **Litwin, S.** 1974. Distribution of shortest distance between random cuts in opposite strands of DNA. J. Appl. Prob. **11:**363–368.
17. **Litwin, S., E. Shahn, and A. W. Kozinski.** 1969. Interpretation of sucrose gradient sedimentation pattern of deoxyribonucleic acid fragments resulting from random breaks. J. Virol. **4:**24–30.
18. **Lydersen, B. K., and D. E. Pettijohn.** 1977. Interactions stabilizing DNA tertiary structure in *Escherichia coli* chromosome investigated with ionizing-radiations. Chromosoma **62:**199–215.
19. **Nagai, K., W. Hendrickson, R. Balakrishnan, H. Yamaki, D. Boyd, and M. Schaechter.** 1980. Isolation of a replication origin complex from *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **77:**262–266.
20. **Parks, L. C., D. T. Dicker, A. D. Conger, L. Daneo-Moore, and M. L. Higgins.** 1981. Effect of chromosomal breaks induced by x-irradiation on the number of mesosomes and cytoplasmic organization of *Streptococcus faecalis*. J. Mol. Biol. **146:**413–431.
21. **Pettijohn, D. E.** 1976. Prokaryotic DNA in nucleoid structure. CRC Crit. Rev. Biochem. **4:**175–201.
22. **Robev, S., and Z. Marinova.** 1967. Radiobiology x-radiation sensitivity of DNA—ability to form specific molecular hybrids with isologous mRNA. Nature (London) **213:**935.
23. **Roth, G. S., G. D. Shockman, and L. Daneo-Moore.** 1971. Balanced macromolecular biosynthesis in "protoplasts" of *Streptococcus faecalis*. J. Bacteriol. **105:**710–717.
24. **Rupp, W. D., and P. Howard-Flanders.** 1968. Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli* following ultraviolet irradiation. J. Mol. Biol. **31:**291–304.
25. **Ryter, A.** 1968. Association of the nucleus and the membrane of bacteria: a morphological study. Bacteriol. Rev. **32:**39–54.
26. **Shockman, G. D.** 1962. Amino acids, p. 567–673. *In* F. Kavanagh (ed.), Analytical microbiology. Academic Press, Inc., New York.
27. **Tarlord, C.** 1961. Kinetics of macromolecular reactions, p. 611–624. *In* Physical chemistry of macromolecules. John Wiley & Sons, Inc., New York.
28. **Tremblay, G. Y., M. J. Daniels, and M. Schaechter.** 1969. Isolation of a cell membrane-DNA-nascent RNA complex from bacteria. J. Mol. Biol. **40:**65–76.
29. **Toennies, G., L. Iszard, N. B. Rogers, and G. D. Shockman.** 1961. Cell multiplication studied with an electronic particle counter. J. Bacteriol. **82:**857–866.
30. **Worcel, A., and E. Burgi.** 1972. On the structure of the folded chromosome of *Escherichia coli*. J. Mol. Biol. **71:**127–147.