THE ORGANIZATION AND REPAIR OF MAMMALIAN DNA

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1. Introduction

Although the use of alkaline sucrose gradients has become a popular tool for the measurement of DNA size, the use of neutral sucrose gradients has until recently been thought to be fraught with artifacts. Lange and Liberman [1] have developed a reliable automated neutral sucrose gradient system which yields precise size estimates of native mammalian DNA and measurements of double-strand break induction and repair.

In this review of my laboratory's work I propose to show (1) that large pieces of native mammalian DNA can be measured on our gradients, (2) the size of this DNA, (3) that this DNA consists of subunits joined together in tandem by linkages, (4) that double-strand breaks in the DNA subunits and cleavage of the linkages are both repaired with what appears to be a first order kinetics, dose-independent, rejoining rate. I shall also suggest two models, one for the organization of the DNA in the mammalian chromosome and the other for the explanation of cell survival curves in terms of DNA double-strand breaks.

2. Sedimentation of high molecular weight mammalian DNA

The DNA of exponentionally growing L5178Y mouse leukemia cells was uniformly labeled in the template strand (1–0.1 dpm/cell). This DNA sediments, with an apparent mol. wt. of 6.5×10^9, in a sharp, monodisperse ($M_w/M_n = 1.01$) peak, having traversed about 65% of the gradient length (GLT) after 1.5 hr at 30 000 rpm. The labeled cells have a growth rate within 20% of that of unlabeled controls.

A complete analysis of variance, *excluding errors due to any speed dependence phenomena,* was performed for $\hat{M}$, the geometric mean molecular weight (weight or number averaged), of any DNA measured by sedimentation in a neutral sucrose gradient. This technique yields 95% confidence (fiducial) limits (FL) of approximately ± 42% on our initial estimate of the molecular weight of the native DNA of L5178Y cells.

2.1. *Purity of the mammalian DNA*

Due to the large apparent size of the DNA we were measuring and the possibility it may not have been pure DNA, it was desirable to test the purity of our material. This was done in two ways, by density measurement and by protein and lipid labeling. Fractions containing the large DNA were collected from our neutral sucrose gradients and were rebanded on CsCl-density gradients. This DNA cosedimented with T_4 DNA showing the absence of any gross protein or lipid contamination under these conditions, i.e., less than a few tenths of a per cent contamination. The other method was to prelabel heavily the cells with ^{14}C-labeled amino acids and/or choline chloride in an attempt to label any proteins or lipids with which our DNA might be contaminated. No sign of contamination was detected, supporting our previous conclusion that our material is better than 99% pure.

2.2. *Speed dependence considerations*

Several laboratories have reported that for constant $\omega^2 t$, large DNAs sediment more slowly at higher

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rotor speeds than they do at lower angular velocities. Considerable confusion about this has developed in the literature as several reports of this phenomenon were published which subsequently proved to be due to (1) the use of improper assumptions in the calculation of number average molecular weights (cf. [2] for a detailed analysis of the source and the correction of this error), and (2) severe changes in gradient hydrodynamic properties during the longer spin [3].

2.2.1. Zimm-type speed dependence

Zimm [4] has developed a theoretical treatment of the forces on a radom coil configuration molecule, with free ends, undergoing centrifugation. His model predicts a rotor speed (ω) dependence of sedimentation distance. Chia and Schumaker [5] have recently shown that T_2 DNA centrifuged at 60 000 rpm behaves as predicted by Zimm's model. Examination of the sedimentation behavior of Dr Arthur Cole's data (personal communication) for the CHO cell DNA subunit also fit Zimm's model yielding an S° value of 119.8 (120 S); our large DNA revealed to us a very different type of speed dependence artifact.

2.2.2. Speed dependence due to a lysis layer and subsequent change in gradient shape

We found that centrifugation of our large DNA piece at lower angular velocities (ω = 50–10 000 rpm) yields an increased sedimentation distance for $\omega^2 t$ = constant. The apparent mol. wt. of this DNA is 1.7×10^{10}, the DNA content of 1/8 of a chromatid. At these lower speeds, no further tendency to increased sedimentation distance was found as ω was reduced from 10 to 5000 rpm. In collaboration with Dr Resnick we were able to demonstrate that this speed dependence was due to an initial non-linearity in the gradient caused by the addition of the lysis layer. This non-linearity disappeared with time and approximately 12 hr after addition of the lysis layer had completely disappeared. Thus, large DNA sedimented at high ω saw a steeper initial slope and, therefore, sedimented more slowly than it should have if the slope were linear throughout. A smaller DNA or the same large DNA sedimented at lower ω would see a less steep initial slope, or at low enough ω ($<$ 10 000 rpm) would see only the linear gradient and, therefore, would not be subject to this artifact [6]. Special gradients which are free of this artifact have been made (C. S. Lange et al., in preparation). For reasons not yet completely understood, this large DNA which we observe does not obey the Zimm model for speed dependence, although its subunits do.

3. The ionizing radiation dose response for DNA double-strand breakage

Asynchronous log phase L5178Y cells were irradiated with ^{60}Co γ-rays to accumulate doses of 2.4 krad to 40.4 krad. Between these doses the number of DNA double-strand breaks induced (or molecular weight reduction as seen in a graph of $1/M_n$ vs dose) was found to increase linearly with dose. When corrected for Zimm type speed dependence, the inverse variance weighted least squares regression line yielded a zero dose intercept of 8.0×10^8, not the unirradiated DNA size (1.7×10^{10}).

3.1. *Initial evidence for DNA subunits*

The molecular weight distribution at 2.4 krad (the lowest non-zero dose used) deviated only slightly from monodispersity. Yet, at least 20 breaks would be required to go from mol. wt. 1.7×10^{10} to 8.0×10^8. However, only 5 breaks, randomly distributed, are needed to reduce a monodisperse distribution into a Gaussian (random) distribution [7]. Therefore, the breakdown from 1.7×10^{10} to 8.0×10^8 could not have been by random breakage in a continuous DNA molecule, but must have been due to *specific* preferential *breakage* at *evenly spaced* sites along the DNA, i.e., between naturally occurring subunits. These subunits which we observe are the same size (120 S = 8×10^8) as those deduced by Zimm's model applied to Cole's data for CHO cells (see 2.2.1 above). They are also the double stranded equivalent of Lett's 165 S single-stranded subunit [8,9], which is apparently the largest *single-stranded* piece of DNA obtainable from mammalian cells. All larger pieces, in alkaline gradients, are probably incompletely denatured, or in neutral gradients, are multiples of this natural subunit. Our data show that about 21 of these subunits are linked together in tandem to make up a one-eighth of a chromatid piece.

4. The repair of DNA double-strand breaks, linkage breaks and the kinetics of repair

Exponentially growing L5178Y cells, with their

DNA uniformly labeled in the template strand, were irradiated with ^{60}Co γ-ray doses of 2.4 or 40.4 krad, and then incubated at 37°C for various periods of time before cell lysis on the gradient and DNA size measurement. The number of double-strand breaks (DSB) decreases exponentially with post-irradiation incubation time after either dose. The rate of rejoining is the same at both doses (parallel slopes) so that a dose-independent apparent rejoining rate can be determined to be T_{37} = 81 min. Correction of the raw data (obtained at 30 000 rpm) for speed dependence yields an estimate of T_{37} = 56 min. This result is in excellent agreement with the 52 min value reported [10] for the rate of DSB rejoining in *M. radiodurans*. Thus, both mammalian cells and *M. radiodurans* may have similar if not the same repair system(s). Other data [10] suggest that this repair is enzymatic. If this is the case, then the great resistance of *M. radiodurans* could be the result of having a great excess of repair molecules, relative to induced breaks, as compared with other cells. Mammalian cells could well have a similar number of repair molecules, but their possession of a 1000-fold larger genome and therefore that times as many more breaks per rad would yield proportionately fewer repair molecules per break and hence their greater sensitivity. It is also noteworthy that the repair we observed for the first 5 hr after irradiation with 40.4 krad was mainly DSB rejoining in the subunit DNA, whereas after exposure to 2.4 krad the rejoining observed was mainly linkage restitution (or replacement). Thus, both types of repair seem to take palce at the same rate.

If one examines the kinetics of break induction vs cell survival of reproductive integrity, it rapidly becomes obvious that if unrepaired double-strand breaks are lethal, and we have every reason to believe that they are [11], then almost all double-strand breaks are repaired. The rad dose required to produce an average of one double-strand break per DNA subunit (8×10^8) is about 2.5 krad. However, to produce an average of one single double-strand break in the subunit DNA of an eighth of a chromatid unit (1.7×10^{10}) requires only about 120 rad. This also appears to be the dose needed to break a linkage. Thus 120 rad is sufficient to cause a break (either in a linkage or in the DNA) in a single eighth of a chromatid unit. Since there are some 320 such units per G_1 phase cell or 480 per log phase cell, the probability of finding a cell in which no such unit had been broken is far too small to correlate with lethality (D_o = 80 rad); to require that all such units be broken to produce lethality requires much too high a dose. However, if the cell has such a single unit which cannot be repaired but must remain intact for the cell to survive, then this damage could account for about 1/2 to 2/3 of the lethal events. The requirement that only one out of 320 such units be unrepairable is not extreme if that unit contains the genes which code for inducible repair enzymes. In *M. radiodurans*, inhibition of protein synthesis prevents repair of double-strand breaks, i.e., the repair system must be induced. Thus, if this hypothesized unrepairable repair unit were undamaged, all breaks would be repaired, but if it were broken, then no breaks would be repaired and cell lethality would ensue. An assumed misrepair frequency of about 3×10^{-3}, which is rather high for a mutation frequency, would also correlate with lethality (.e. 2/3 of the lethal events). For full details and the results summarized in sections 3 and 4 see Lange et al. [12]. Corry and Cole [13] have demonstrated qualitatively that DNA double-strand breaks in CHO cells are repaired. See also our report [14].

5. Further evidence for linkages

In order to examine more closely the nature of the repairable intersubunit linkages, special 'enzyme' gradients were constructed. They differ from the usual gradients in that after cell lysis on the top of the gradient, the DNA is centrifuged into a denser (5% sucrose) zone lying between the lysis layer and the linear gradient. By prior loading of this zone with the appropriate enzyme(s), one can test for the existence of a particular structure in the linkage(s). Our data show that RNase A has no effect (therefore, single-stranded RNA is not an essential constituent of the linkage) but DNase-free (tested on T_4 DNA) pronase and trypsin completely reduce the 1.7×10^{10} DNA to the 8×10^8 subunits. Therefore, the linkages must contain peptide bonds, i.e., probably proteins.

Mercaptoethanol has the same effect as the proteases; therefore, disulfide bonds must also play an essential role in the structure of the linkage. Further work on this problem is now in progress.

6. A model for the organization of DNA in the mammalian chromosome

The data we obtained in the above sections 3–5 strongly indicate that mammalian chromosomal DNA comes in about $1-2 \times 10^{10}$ eighth of a chromatid packages. In the L5178Y cell there would then be about 320 such large molecules per G_1 phase cell or about 480 (450–520) per average log phase exponentially growing cell. Both the size estimate ($1-2 \times 10^{10}$) and the number of such molecules per log phase cell ($480 \pm 8\%$ S.E.M.) have been confirmed [15] using a totally different technique, the viscoelastic retardation time method [16].

Each of the 1.7×10^{10} molecules have been shown by us to consist of about 21 DNA subunits of about 8×10^{8} each, joined together in tandem by disulfide-containing protein linkages which are labile to proteases (pronase and trypsin), mercaptoethanol, alkali [9], and low doses of radiation; radiation and alkali damage are additive [9]. This model and a discussion of the Sobell [17,18], the Dounce–Hilgartner [19,20], and the Stubblefield [21] models will be presented in full elsewhere (C. S. Lange and D. F. Liberman, in preparation).

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