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VISCOELASTIC ANALYSIS OF HIGH MOLECULAR WEIGHT, ALKALI-DENATURED DNA FROM MOUSE 3T3 CELLS

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ABSTRACT Alkaline lysates of mouse 3T3 cells showed viscoelastic properties characteristic of very large molecules of single-stranded DNA. The viscoelastic retardation time and the sensitivity to low doses of nitrogen mustard and of X-irradiation suggest a molecular weight in excess of 10^{10} daltons. Contact-inhibited cells yielded larger single strands than actively growing cells.

We have recently obtained alkaline lysates of mouse 3T3 cells which exhibit viscoelastic properties characteristic of very high molecular weight material. Although there is as yet no assurance that the molecules being studied are isolated single strands (Cleaver, 1974; Simpson, et al., 1973; Jolley and Ormerod, 1974) or even are pure DNA, the retardation time and sensitivity to very low doses of nitrogen mustard and of X-irradiation all indicate an extensively strand-separated structure with a molecular weight in excess of 10^{10} daltons.

Mammalian cells lysed at pH > 12 release high molecular weight components which degrade over a period of several hours (Lett et al., 1970; Elkind and Kamper, 1970). With mouse cells we have found that if the pH of lysis and prolonged incubation is decreased to 11.7, stability is considerably enhanced (Fig. 1); i.e., retardation times are measurable even after 72 h, although the amount of viscoelastic recoil decreases with time. On the other hand, if the temperature is raised to 40° or the pH to 12.4, corresponding to an increase of fivefold in OH^- concentration, the viscoelasticity of these solutions decreases until none remains after overnight incubation.

Fig. 1 suggests a strikingly larger molecular weight of the single-stranded DNA obtained from contact-inhibited cells. The exact retardation times obtained for a given series of experiments depended on the precise growth characteristics of the cells being examined, which could vary somewhat from month to month. A significant difference was always observed between actively-growing and contact-inhibited cells, however, which could be due to a lower rate of replication and transcription in the contact-inhibited cells, or possibly an increased repair capacity or decreased nuclease level.

To determine if pH 11.7 is sufficiently alkaline to produce totally single-stranded

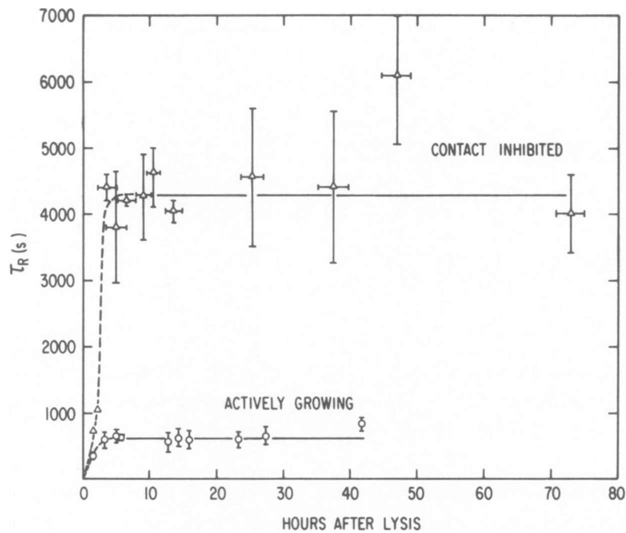

FIGURE 1 Viscoelastic retardation times for contact-inhibited and actively-growing mouse 3T3 cells lysed with alkaline detergent as a function of time after lysis. From 10^5 to 10^6 cells grown using standard conditions (37°, Dulbecco's modified Eagle's medium, CO_2 humidified atmosphere) were scraped from 50-mm plates with a rubber policeman (approximately 1/4 plate of well-dispersed, actively growing cells were scraped or 1/20 plate of confluent, contact-inhibited cells). Cells were centrifuged, resuspended in 3 ml cold 1 M NaCl, 10 mM Na_2HPO_4, transferred to the viscoelastometer chamber, and lysed by the addition of 0.3 ml of 4% sodium decyl sulfate, 0.16 M Na_4EDTA, 0.2 N NaOH. Following a temperature jump of 45° for 5 min, the lysate was returned to 25° and incubated for the indicated number of hours, before viscoelastic measurements were made. (Additional details of the viscoelastic technique are given in Uhlenhopp and Zimm [1975] and references therein.) The final salt concentration was 1.02 M Na^+, the pH 11.7, and the DNA concentration (based on the approximate number of cells lysed in the reaction volume of 3.3 ml) less than 1 μg/ml. Because of loss due to radial migration of large molecules during windup and relaxation (Shafer et al., 1974), each point represents a single relaxation for a single lysate; other incubation times required additional lysates. The vertical error bar indicates the maximum possible range of τ which could be obtained for a given relaxation curve; the horizontal error bar signifies the total time period during which the relaxation took place.

DNA, the melting temperature of calf thymus DNA was examined as a function of pH. Studier (1965) has shown that for viral DNA in 1 M Na^+, pH 11.5 is high enough to insure strand separation. Elson and Record (1974) have shown that for T2 DNA in 80% formamide strand separation is complete 15° above the T_m. Fig. 2 indicates that at pH 11.7 we are at least 20° above the T_m. Taken together, these facts suggest that the experimental conditions described in Fig. 1 should be adequate to accomplish strand separation.

Studies using low concentrations of the cross-linking agent nitrogen mustard ["HN_2," $(ClCH_2CH_2)_2NCH_3$] and low doses of X-irradiation tended to confirm the existence of single-stranded material. When actively-growing 3T3 cells were incubated with 0.4 μM HN_2 for 30 min at 37°, conditions which allow greater than 90% survival

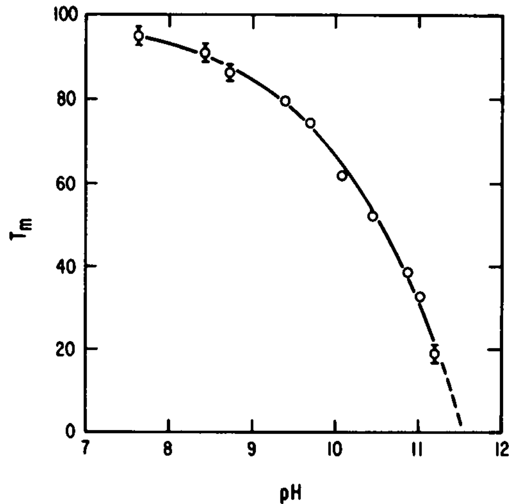

FIGURE 2 Melting temperature of calf thymus DNA as a function of pH in 1 M Na^+. Commercial calf thymus DNA (General Biochemicals Div., Mogul Corp., Chagrin Falls, Ohio) was dissolved in 1 M NaCl, 10 mM Na_2HPO_4, and various amounts of alkaline-EDTA-detergent solutions were added at 0° to obtain the indicated pH's. Melting curves were obtained using a recording spectrophotometer.

of Chinese hamster cells (Sakamoto and Elkind, 1969) and which should produce less than one cross-link per 10^{10} daltons of DNA (Elkind, personal communication), the retardation time of the lysates increased fivefold. This increase could be explained as the result of a covalent linkage binding two opposing DNA strands together, preventing their separation under denaturing conditions.

The effect of irradiation was more complex. Fig. 3 shows that low doses of X-rays increased the retardation time of lysates to twice that of the control, while higher doses sharply reduced τ until after 1 krad very little viscoelasticity remained. These doses were all in the biologically significant range; 100 rads should not be responsible for more than one or two breaks per 10^{10} daltons of DNA (Blok and Loman, 1973). Conceivably, the increase in τ at the two lowest doses could be due to an opening up of a circular or compacted structure or possibly to radiation-induced cross-linking (Blok and Loman, 1973). An alternative explanation for the effects of low X-ray doses and HN_2 treatment is an alteration of DNA metabolic processes resembling the onset of contact inhibition.

Although the experiments with HN_2 and X-irradiation did not prove that the DNA was exclusively single-stranded, they nevertheless emphasized the large size of the material being examined. Attempts to obtain a molecular weight estimate from retardation time relied on the viral DNA sedimentation data of Studier (1965), which were converted to $[\eta]$ using the equation of Scheraga and Mandelkern (1953). The resultant empirical relationship, $[\eta] = 5.63 \times 10^{-3} M^{0.77}$, when substituted into the equation of Zimm (1956), yielded the desired relation $M = 2.72 \times 10^8 \tau^{0.565}$. If it is assumed

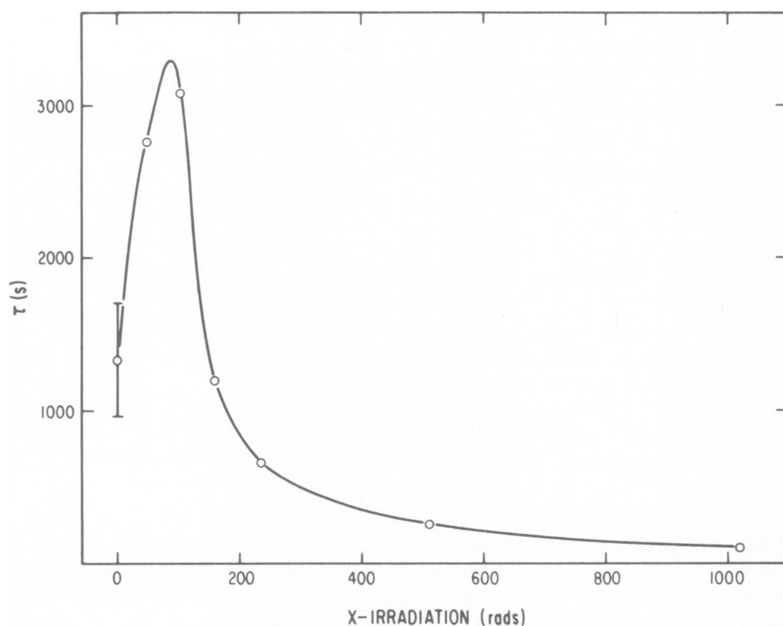

FIGURE 3 X-irradiation of mouse 3T3 cells. Medium was withdrawn from plates of contact-inhibited 3T3 cells and replaced with 3 ml of cold 0.075 M NaCl, 0.075 M Na_3citrate, pH 7.2. Covered plates were irradiated at 0° with 280 kVp X-rays, then harvested and lysed as in Fig. 1. Lysates were incubated 3 h at 25° before measurement. The retardation time for control, unirradiated cells is the average of five separate determinations and is lower than the retardation time observed for the different aliquot of contact-inhibited cells described in Fig. 1 (see text).

that the molecules being examined in contact-inhibited 3T3 lysates are linear, single-stranded, random coils, the molecular weight calculated using this equation is $3–4 \times 10^{10}$ daltons.

The amount of DNA in the larger mouse chromosomes is about 2×10^{11} daltons, suggesting either that single-stranded DNA exists intracellularly in units smaller than entire chromosome lengths, or that some fragmentation took place before our measurements could be made. We feel, however, that too much uncertainty still surrounds these preliminary results to permit drawing any firm conclusions. In any case, viscoelastometry seems to be useful for characterization of DNA of this size. Even if the structure being examined is not exclusively single stranded, the viscoelastic properties respond to modification of DNA resulting from treatment of mammalian cells with low doses of drugs and radiation.

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REFERENCES

BLOK, J., and J. LOMAN. 1973. *Curr. Top. Radiat. Res. Q.* **9**:165.
CLEAVER, J. E. 1974. *Biochem. Biophys. Res. Commun.* **59**:92.
ELKIND, M. M., and C. KAMPER. 1970. *Biophys. J.* **10**:237.
ELSON, E. L., and M. T. RECORD. 1974. *Biopolymers.* **13**:797.
JOLLEY, G. M., and M. G. ORMEROD. 1974. *Biochim. Biophys. Acta.* **353**:200.
LETT, J. T., S. KLUCIS, and C. SUN. 1970. *Biophys. J.* **10**:277.
SAKAMOTO, K., and M. M. ELKIND. 1969. *Biophys. J.* **9**:1115.
SCHERAGA, H. A., and L. MANDELKERN. 1953. *J. Am. Chem. Soc.* **75**:179.
SHAFER, R. H., N. LAIKEN, and B. H. ZIMM. 1974. *Biophys. Chem.* **2**:180.
SIMPSON, J. R., W. A. NAGLE, M. C. BICK, and J. A. BILLIE. 1973. *Proc. Natl. Acad. Sci. U. S. A.* **70**:3660.
STUDIER, F. W. 1965. *J. Mol. Biol.* **11**:373.
UHLENHOPP, E. L., and B. H. ZIMM. 1975. *Biophys. J.* **15**:223.
ZIMM, B. H. 1956. *J. Chem. Phys.* **24**:269.