DNA FRAGMENTATION IN A CLONAL LINE OF RAT PITUITARY TUMOR (GH_1 STRAIN)

R. L. CHURCH[1] and R. A. CONSIGLI[2]

Division of Biology, Subdivision of Molecular Biology and Genetics, Kansas State University, Manhattan, Kansas 66502

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It was observed that as much as 80% of the ^{3}H-thymidine labeled DNA of GH_1 clonal rat pituitary tumor cells disrupted and that small fragments of the DNA were released into the medium. The majority of the released fragments were not acid soluble. Newly replicated DNA was found to degrade at the same rate as long term labeled DNA. An active repair process must be functioning in these cells since they remain metabolically normal for indefinite periods of time.

The normal turnover of DNA in animal tissue is a subject of some dispute. Pelc (1-3) has presented evidence for both normal DNA synthesis and DNA turnover in a variety of tissues following the administration of ^{3}H-thymidine. DNA fragmentation, or solubilization has been found to occur as a result of viral infection (4,5), U.V. irradiation (6,7), bactericidal substances (8), FUdR treatment (9,10) or as a result of DNA replication (11-13).

Previous experiments in our laboratory investigating the incorporation of DNA precursors into the GH_1 cells led us to suspect that cellular DNA was undergoing fragmentation. To ascertain whether or not such a turnover of DNA was taking place, several experiments were conducted which will be presented in this report.

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[1]Present address: Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut 06032.

[2]Recipient of USPHS Career Development Award: CA 12056.

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MATERIALS AND METHODS

GH_1 clonal pituitary tumor cells (14) were maintained in Ham's F-10 medium (15) supplemented with 20% fetal calf serum and antibiotics. The cells were prelabeled for 4 days after seeding in Ham's medium supplemented with ^{3}H-thymidine (7.0 C/mM, 0.5 uc/ml) plus uridine (5 ug/ml), and deoxycytidine (5 ug/ml). After four days the medium containing ^{3}H-thymidine was removed, the cells washed 3 times with PBS (pH 7.2) and Ham's medium without ^{3}H-thymidine replaced on the cells. At various time periods thereafter, the medium was removed from the cells, centrifuged at 10,000 RPM in the Sorval HB4 rotor for 15 minutes to remove cellular debris, and the supernatant assayed for total and TCA-soluble radioactivity. The cells were washed with PBS, hydrolyzed with boiling 1 N NaOH, and an aliquot assayed for radioactivity. The TCA-soluble and insoluble radioactivity in the medium was compared to the cellular radioactivity to determine the percent of solubilized ^{3}H-thymidine labeled DNA.

In other experiments, GH_1 cells were pulse labeled for 30 minutes with ^{3}H-thymidine (7.0 C/mM, 50 uc/ml) and the label chased by addition of regular Ham's medium following removal of the ^{3}H-thymidine. At various times after removal of the label, the percent of ^{3}H-thymidine labeled DNA released into the medium was calculated as above. In addition, 30 minute pulse-labeled cells were also harvested and DNA fragmentation determined on sucrose gradients (5-20%, pH 7.2) following the technique of Hyodo, et al. (11). Fractions were collected on Whatman 3 MM filter discs (2.3 cm^2), washed in cold 10% TCA and acetone, air dried and placed in vials with 5 ml of nonaqueous scintillation fluid and radioactivity determined in a Packard liquid scintillation counter.

RESULTS AND DISCUSSION

It was observed that the ^{3}H-thymidine prelabeled GH_1 cells under-

went extensive breakdown or solubilization of their DNA. Five hours after replacing the ^{3}H-thymidine containing medium, 45% of the pre-labeled DNA had been degraded and was present in the medium. Forty-five hours after removing the ^{3}H-thymidine label, 80% of the DNA was degraded. As only 10% of the label in the medium was TCA-soluble, the ^{3}H-thymidine labeled DNA fragments released into the medium were at least 10 nucleotides in length.

It was of interest to determine if all labeled cellular DNA was fragmenting or if only newly replicated DNA was fragmenting. It was found that GH_1 cells which received a 30 minute pulse with ^{3}H-thymidine also fragmented their DNA (35%) within 2 hours after removal of the ^{3}H-thymidine pulse. The rate of fragmentation was identical to that observed in the long term (4 day) ^{3}H-thymidine labeling period. The low percentage of TCA-soluble ^{3}H-thymidine labeled DNA in the medium was also the same as in the long term label experiment. By using the sucrose gradient technique of Hyodo, et al. (11), it was possible to observe the size of the intracellular DNA molecules following the 30 minute pulse with ^{3}H-thymidine. It was found that at 2 hours after the pulse, the majority of the ^{3}H-thymidine label remaining in the cell was in the bulk DNA (fig. 1a). Twenty-four hours later, much of the ^{3}H-thymidine label was associated with the low molecular weight region of the sucrose gradient (fig. 1b). Forty-eight hours later (fig. 1c) nearly all of the newly replicated ^{3}H-thymidine labeled DNA had been degraded, and much of the label remained in the saline-sucrose layer of the gradient, indicating complete degradation into small fragments. Between 24 and 48 hours after pulse labeling, most of the labeled DNA found in the cells had degraded to small molecular weight pieces.

This observation of DNA fragmentation or solubilization is an interesting phenomenon since the GH_1 cells continue to grow and multiply even while turning over DNA. Possibly an active DNA repair process occurred since

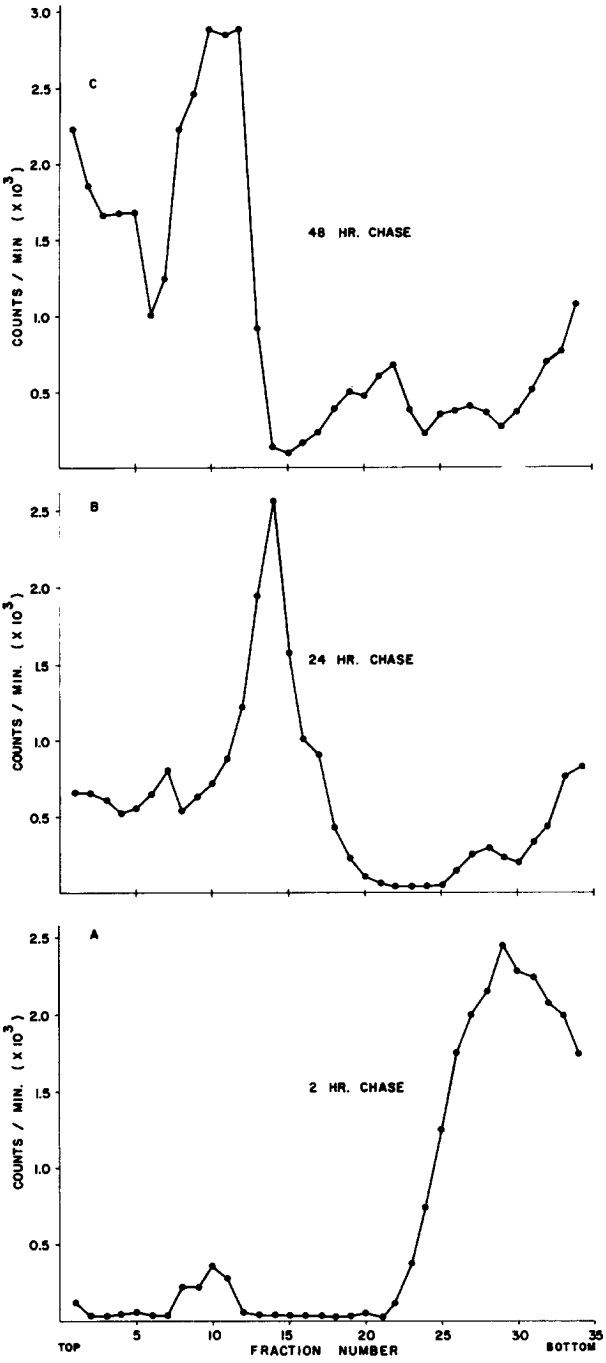

Fig. 1. Sedimentation profiles of cell DNA in neutral sucrose gradients (5-20%). GH_1 cells were pulsed with ^3H-thymidine (50 uc/ml) for 30 minutes, then maintained in Ham's medium lacking ^3H-thymidine and the label chased for various time periods. Centrifugation was at 35,000 r.p.m. at 20°C for 4 hours in the Spinco SW 41 rotor.

a. Harvested 2 hours after chase.
b. Harvested 24 hours after chase.
c. Harvested 48 hours after chase.

the GH_1 cells remained healthy and continued to grow. RNA and protein synthesis continued apparently unaffected by this DNA turnover phenomenon. Others (16) have shown that the DNA repair process is very sensitive to actinomycin D treatment. It is of interest that the GH_1 cells are extremely sensitive to actinomycin D, with a concentration of 0.05 ug/ml of actinomycin D being sufficient to kill the GH_1 cells. This concentration is 200 to 250 times lower than the concentration required to elicit a similar response in primary mouse embryo cultures or HeLa cells.

Bell's (17) description of "I-DNA" found in the cytoplasm of eukaryotic cells is similar to the intracellular degradation products found in GH_1 cells in this report. "I-DNA" may constitute DNA fragments from chromosomal turnover. Müller, et al. (18) concluded that Bell's observations were caused by artifacts of cell homogenization. However, the technique used in this report allows direct application of cells to the sucrose gradient (11) and isolation of intact DNA with minimum shear. Therefore, it is concluded that GH_1 cell DNA fragments appear as a result of normal DNA turnover. The observation that the GH_1 cells have a rather long generation time (40 to 50 hours) may be associated with this aspect of DNA solubilization, either as a cause or effect of solubilization.

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