DNA Content of Mycoplasma-Modified FL Human Amnion Cells[1] (34527)

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Various effects of long-term exposure of the FL line of human amnion cells to mycoplasma have been reported previously (1-3). The changes have included alterations in cellular morphology, cultivation characteristics, and an increased resistance to mycoplasma. In addition, a distinct reduction in chromosome numbers, an increase in the frequency of aberrations, and the appearance of several new chromosome varieties were observed. Although various chromosome aberrations decreased in frequency, the chromosome numbers did not revert to higher numbers when mycoplasma was eliminated (4). There was a notable reduction in the tumor-producing capacity of the mycoplasma-modified cells of several strains compared to the parent FL line (5).

In view of this change of the malignant state of FL cells as a consequence of the mycoplasma infection, it was important to determine if the lower chromosome numbers could be correlated with a reduction in the amount of DNA per cell. The DNA contents of FL, primary amnion, and the mycoplasma-modified F 138cl cells are reported in this paper.

Materials and Methods. Preparation of cells. Primary human amnion cells were seeded in medium 512 (6) supplemented with 15% fetal bovine serum. Seven days later the medium was changed to McCoy's (7) with 10% agamma calf serum. Ten-day-old cultures were used for DNA determinations. The FL (8) and the mycoplasma-modified F 138cl (4) cells were grown in McCoy's medium with 10% agamma calf serum. They were detached in the logarithmic growth phase with 0.05% trypsin, washed twice with saline A (9), and frozen as a cell pellet at —20°. The primary amnion cells were removed with a 0.25% trypsin solution.

Extraction procedure. A modified Schmidt-Thannhauser procedure was used (10). The frozen samples were resuspended in 2.5 ml of a 5% solution of trichloracetic acid and incubated for 15 min at 4°. The supernate was discarded, and the samples were extracted twice with 100% ethanol for 10 min at room temperature. The remaining material was resuspended in 1 *N* NaOH for 1 hr at 37°. Standard solutions of calf thymus DNA (Sigma Chemical Co. Type 1) were prepared by two-fold serial dilutions in 1 *N* NaOH and were incubated for 1 hr at 37°.

DNA determination. The DNA contents of the alkaline digests were determined by a modified indole reaction (11, 12). A mixture of 1 ml of sample and 1 ml of 10% monochloroacetic acid was combined with 2 ml of freshly prepared indole–HCl reagent. The samples were placed in a boiling water bath for 10 min and cooled in running water. Four milliliters of iso-amyl acetate were added, and the samples were agitated for 15 sec. The iso-amyl acetate layer was removed after centrifugation for 3 min at 1500 rpm, and the procedure was repeated twice. The water layer was transferred to a cuvette, and the optical density at 490 mμ was determined in a Beckman DU spectrophotometer.

Results. The results of four individual experiments, including 18 cultures of each cell line and 4 cultures of primary amnion cells are summarized in Table I. The data show that the average DNA content of cells of the

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TABLE I. Comparison of DNA Content and Chromosome Numbers for FL,[a] F 138cl,[b] and Primary Amnion Cells.

Cell type	No. of samples	μg DNA/10^6 cells	Chromosome numbers
FL	18	26.7 ± 0.57	70–76 (range, 68–80)
F 138cl	18	27.1 ± 0.68	63–64 (range, 59–67)
Primary amnion	4	18.8 ± 0.82	46

[a] FL are spontaneously transformed amnion cells.

[b] The F 138cl line was derived from FL cells by long-term mycoplasma infection, subsequently eliminated.

mycoplasma-modified line was not different from cells of the FL line. Therefore, the reduced chromosome complement of the F 138cl cells does not reflect a lower DNA content. The reproducibility of the results of different experiments is reflected in the small deviation from the mean value. The amount of DNA in primary amnion cells was considerably lower than that of the two transformed cell lines. The difference in the number of chromosomes between spontaneously transformed amnion cells and normal cells is proportional to the difference in DNA content.

Conclusion. Previously reported values for DNA in continuously propagated cell lines, such as KB, HeLa, and FL, were significantly higher than those for normal cells (13). Our data also show an increase in DNA content for two transformed cell lines with malignant characteristics, both derived from normal amnion cells. However, the similarity in DNA content for FL and F 138cl cells indicates that mycoplasma modification, including the change in the malignant state and a reduction of approximately 15% of the chromosome number, cannot be explained by a loss of DNA, but may be interpreted by a rearrangement of the genetic material.

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