MODIFICATIONS IN THE CHROMOSOMAL PROTEINS OF SV-40 TRANSFORMED WI-38 HUMAN DIPLOID FIBROBLASTS

M. O. Krause* and G. S. Stein
Department of Biochemistry
University of Florida
Gainesville, Florida 32610

Received June 12,1974

SUMMARY

The composition and metabolism of chromosomal proteins associated with the genome of WI-38 human diploid fibroblasts and SV-40 transformed WI-38 fibroblasts were compared. While the five major histone fractions are present in normal and SV-40 transformed cells, variations in the relative contents and rates of synthesis of specific histone fractions are described. Differences in the protein contents and rates of synthesis of several molecular weight classes of nonhistone chromosomal proteins in normal and SV-40 transformed WI-38 cells are also reported.

Infection and transformation of eukaryotic cells with RNA and DNA viruses is reflected by modifications at the biochemical as well as morphological levels (1,2,3). Such changes include variations in growth control (4,5), cell surface architecture (6), cellular enzymes (7), and nuclear as well as plasma membrane associated antigens (8,9). Since these viral induced cellular changes reflect alterations in gene expression, one might anticipate modifications in the macromolecules which comprise the genome and interact with DNA to regulate its function.

The genome of eukaryotic cells is a nucleotprotein complex consisting primarily of DNA and two major classes of chromosomal proteins, histones and nonhistone chromosomal proteins. While the histones have been shown to be responsible for the repression of DNA dependent RNA synthesis (10,11), evidence is accumulating which indicates that nonhistone chromosomal proteins may recognize specific gene loci and play a key role in the regulation of gene readout (12-20). In the present studies, the chromosomal proteins associated

* Dr. Krause's permanent address is Department of Biology, University of New Brunswick, Fredericton, New Brunswick, Canada.

with the genome of normal and SV-40 transformed WI-38 human diploid fibroblasts were examined with respect to their composition and rates of synthesis.

MATERIALS AND METHODS

Human diploid WI-38 fibroblasts and SV-40 transformed WI-38 fibroblasts were grown in monolayer culture in Eagles Basal Medium (BME) supplemented with 10% fetal calf serum. All experiments were carried out utilizing exponentially growing normal and transformed cells. Criteria for exponential growth included determinations of cell growth rate and percentage of the cell population undergoing DNA replication. The normal human diploid fibroblasts utilized in these studies ranged from passage 28 to 32. This is an important consideration since age-dependent modifications in the metabolism of chromosomal proteins have been observed in late passage human diploid fibroblasts (21).

The synthesis of histones was assayed as follows: monolayers were labeled for 60 minutes at 37°C with arginine and lysine free BME containing 2% fetal calf serum, 2.5 μCi/ml l-arginine ^{3}H (20 Ci/mM) and 2.5 μCi/ml l-lysine ^{3}H (52 Ci/mM). Preparation of nuclei was carried out at 4°C and details of the procedure have been previously described (22). Cells were harvested by scraping with a rubber policeman, washed 3 times with Earle's balanced salt solution and lysed with 80 mM NaCl, 20 mM EDTA, 1% Triton X-100, pH 7.2. The lysing medium contained 50 μg/ml 1-1-tosylamide-2-phenyl-ethylchloromethyl ketone (TPCK) (23) to inhibit proteolytic degradation. Proteolysis of chromosomal proteins is frequently associated with actively proliferating transformed cells (24). Nuclei were pelleted by centrifugation at 1,000 x g for 4 min and washed 3 times with the lysing medium. This was followed by 2 washes with 0.15 M NaCl, 0.01 M Tris, pH 8.0. Nuclei isolated in this manner are free of cytoplasmic contamination when examined by phase contrast microscopy. Histones were extracted at 4°C for 60 min with 40 volumes of 0.25 normal HCl and the nuclei were re-extracted twice with 20 volumes of 0.25 normal HCl for 30 minutes. The pooled histone extracts were precipitated with 9 volumes of acetone for 12 hours at 4°C, washed with ethyl ether, evaporated to dryness, resuspended

in 0.9 normal acetic acid containing 15% sucrose and electrophoresed on 0.5 by 8.5 cm polyacrylamide gels by the method of Panyim and Chalkley (25).

The synthesis of nonhistone chromosomal proteins in normal and SV-40 transformed WI-38 fibroblasts was assayed by the incorporation of l-tryptophane ^{3}H into chromosomal proteins. The monolayers were incubated in tryptophane free BME containing 2% fetal calf serum and 5 μCi/ml l-tryptophane ^{3}H (2.5 Ci/mM) for 60 minutes at 37°C. Cells were harvested and nuclei were prepared as described above. Chromatin was isolated as previously described by Stein and Thrall (26). The protein to DNA ratios of the chromatin preparations (normal and transformed cells) was 2.4 and the nonhistone chromosomal protein:histone ratios were 1.4. Chromatin was dissociated in 1% SDS, 1% β-mercaptoethanol, 0.01 M sodium phosphate pH 7.0, heated at 60°C for 60 minutes, dialyzed against 0.1% SDS, 0.1% β-mercaptoethanol, 0.1 M sodium phosphate pH 7.0 and chromosomal proteins were electrophoresed on 0.6 x 7.5 cm 7.5% polyacrylamide gels containing 0.1% SDS. A 0.6 x 2 cm stacking gel was used in SDS-electrophoretic fractionations and details of the procedure have been reported (27).

RESULTS AND DISCUSSION

Initially, the relative content and metabolism of the five principal histone fractions from exponentially growing normal and SV-40 transformed WI-38 human diploid fibroblast cells was examined. Cells were labeled with a mixture of l-arginine ^{3}H and l-lysine ^{3}H. Nuclei were isolated, histones were extracted and these basic chromosomal proteins were fractionated according to charge as well as molecular weight on acetic acid urea polyacrylamide gels as described in Materials and Methods. Figure 1 compares the polyacrylamide gel electrophoretic profiles of histones associated with the genome of normal WI-38 human diploid fibroblasts (1a) and such cells transformed by SV-40 virus (1b). The rates of histone synthesis in these cells are also shown. It is apparent that all five major histone fractions are present in normal and SV-40 transformed WI-38 cells, and that each fraction is actively synthesized. However, variations

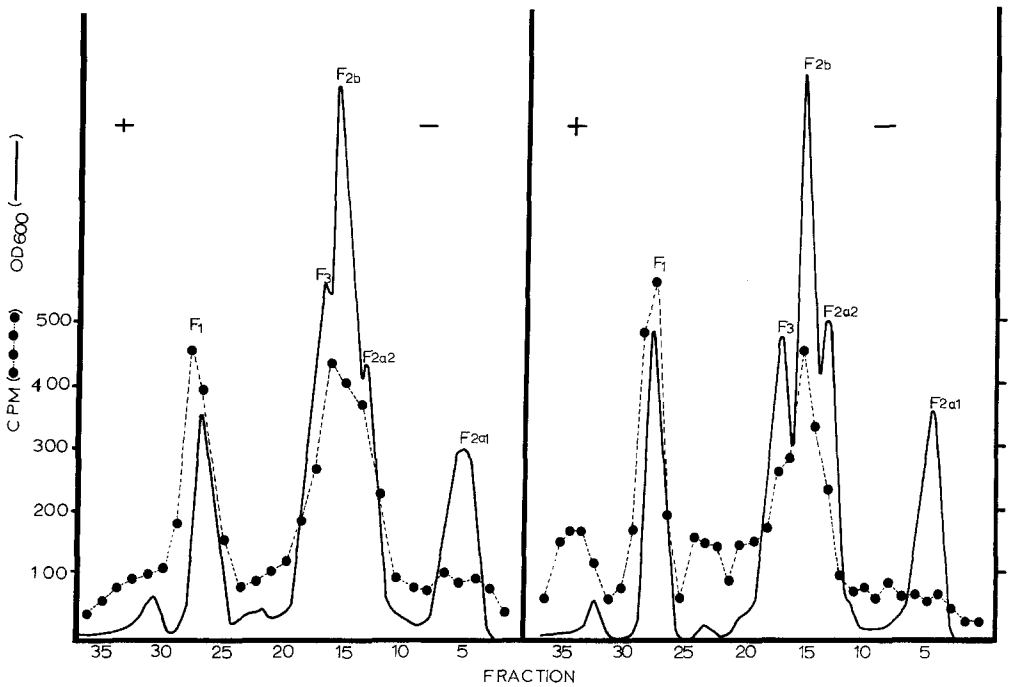

Figure 1

Electrophoretic fractionation of histones from normal WI-38 fibroblasts (1a) and SV-40 transformed WI-38 fibroblasts (1b) on acetic acid urea polyacrylamide gels. The electrophoretic banding pattern (————) and distribution of radioactivity throughout the gels (●---●---●) are shown.

in the relative amounts and rates of synthesis of specific histone fractions are evident. These results are summarized in Table I. Specifically, there is an increase in the relative amount of F2A2 and a decrease in the relative amount of F3 in SV-40 transformed WI-38 cells. Corresponding changes in the relative rates of synthesis can only be observed in F1; a relative increase in ^{3}H amino acid incorporation into F1 parallels the relative increase in the protein content of this histone fraction in SV-40 transformed cells. The other histone fractions show comparable levels of ^{3}H amino acid incorporation in the two cell types with the exception of F2A1 which exhibits relatively lower ^{3}H amino acid incorporation in the transformed cells. However, a comparison of ^{3}H amino acid incorporation as a function of relative protein content in each fraction (B/A x 100) demonstrates that there is an increase in the relative specific activity of F3 and a decrease in F2A2 and F2A1 in SV-40 transformed

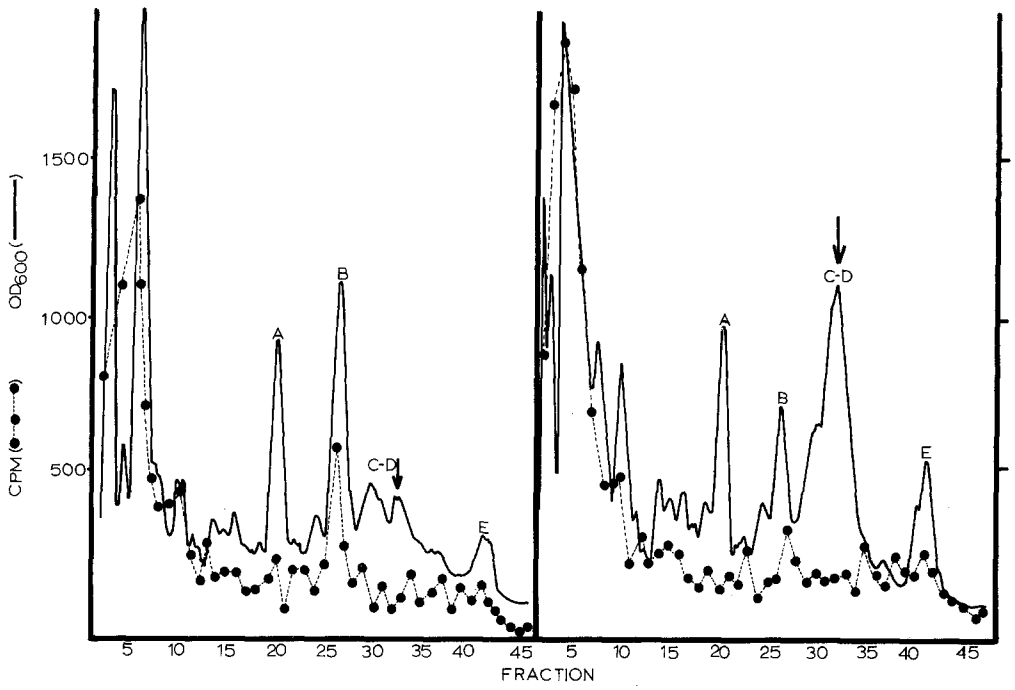

Figure 2

Electrophoretic fractionation of chromosomal proteins from normal WI-38 fibroblasts (2a) and SV-40 transformed WI-38 fibroblasts (2b) on SDS polyacrylamide gels. The electrophoretic banding pattern (——————) and distribution of radioactivity throughout the gels (●---●---●) are shown.

cells. Another consistent difference in the histones of the two cell types is reflected by the relative mobilities of F3, F2B, and F2A2 in the gels resulting in an increased resolution of the histone fractions from transformed cells. Since mobilities in this system are a function of both charge and molecular configuration, the observed differences are likely to be ascribed to post-synthetic regulatory modifications (24).

Figure 2 compares the SDS polyacrylamide gel electrophoretic profiles of 1-tryptophane ^{3}H labeled chromosomal proteins from normal (2a) and SV-40 transformed WI-38 human diploid fibroblasts (Fig. 2b). Since histones do not contain tryptophane residues, the distribution of radioactivity in the gels solely reflects nonhistone chromosomal protein synthesis. A marked increase in the synthesis of high molecular weight nonhistone chromosomal proteins in

TABLE I

Normal and SV-40 transformed WI-38 human diploid fibroblasts were labeled with a mixture of l-arginine ^{3}H and l-lysine ^{3}H. Histones were extracted from isolated nuclei and electrophoresed on polyacrylamide gels by the method of Panyim and Chalkley (25). Procedures are described in Materials and Methods. The electrophoretic banding patterns and distributions of radioactivity in each histone fraction are indicated in Figure 1. The relative amounts of protein in the histone fractions (column A) were determined by integrating the area in each band. The rates of synthesis (column B) were determined by ^{3}H amino acid incorporation into each histone fraction. The range of values did not exceed 5%.

Relative Amounts and Synthetic Activities of Histone Fractions from Normal and SV-40 Transformed WI-38 Human Diploid Fibroblasts.

	WI-38 Cells			SV-40 Transformed WI-38 Cells		
Histone Fractions	A Percent of Total Histones	B Percent of Total cpm	B/A x 100	A Percent of Total Histones	B Percent of Total cpm	B/A x 100
F_1	15	33.9	226	18	38.7	215
F_3	27	28.4	105	20	26.4	132
F_{2b}	33	21.9	66	30	21.8	72
F_{2a2}	8	8.4	105	14	8.2	59
F_{2a1}	17	7.4	43	18	4.9	27

SV-40 transformed cells is evident. This is suggested by an elevated level of tryptophane ^{3}H incorporation into nonhistone chromosomal proteins which migrate between fractions 1-10 (greater than 100,000 MW). SV-40 transformed cells also exhibit a decreased synthesis of nonhistone chromosomal proteins which migrate between fractions 25-30. Optical density scans of the gels at 600 nm shown in Figure 2 indicate the relative protein contents of each molecular weight class of nonhistone chromosomal protein. Consistent with variations in the synthesis of high molecular weight nonhistone chromosomal proteins in normal and SV-40 transformed fibroblasts, there are differences in the banding patterns of nonhistone chromosomal proteins which migrate between the fractions 1 and 18. Differences in peak A and B are also apparent. While

B is more pronounced than A in normal fibroblasts, peak A is more pronounced than B in SV-40 transformed cells. In addition, modifications in nonhistone chromosomal proteins which migrate between fractions 27 and 45 are also evident. Peaks C-D and E are significantly increased in the SV-40 transformed cells. It should be indicated that several lines of evidence suggest that peak D (indicated by arrow) represents F1 histone.

The evidence presented demonstrates significant variations in chromosomal proteins associated with the genome of normal and SV-40 transformed WI-38 human diploid fibroblasts. Differences in the composition and metabolism of histones as well as nonhistone chromosomal proteins are reported. These results are consistent with findings of Zardi, et al. (28) which indicate immunological variations in chromatin from normal and transformed human diploid fibroblasts. Taken together with evidence which suggests that chromosomal proteins play a key role in regulation of DNA dependent RNA synthesis, one can speculate that the observed modifications in histones and nonhistone chromosomal proteins of SV-40 transformed WI-38 human diploid fibroblasts may be functionally related to alterations in gene expression associated with viral transformation. Such reasoning is further supported by recent findings that changes in the synthesis of nonhistone chromosomal proteins occur following infection of several types of eukaryotic cells with oncogenic RNA and DNA viruses (29-31).

REFERENCES

1. H. M. Temin, Ann. Rev. Microb. 25 (1971) 609.
2. T. L. Benjamin, Current Topics in Microb. and Immun. 59 (1972) 107.
3. C. Moscovici and P. K. Vogt, Virology 35 (1968) 487.
4. G. Todaro, H. Green and B. Goldberg, Proc. Nat'l. Acad. Sci. 51 (1964) 66.
5. C. D. Scher and G. Todaro, Exptl. Cell Res. 68 (1971) 479.
6. K. D. Noonan and M. M. Burger, Current Topics in Membrane Science (Academic Press, New York), in press.
7. D. Bull, A. T. Taylor, D. M. Austin and O. H. Jones, Virology 57 (1974) 279.
8. H. M. Temin and H. Rubin, Virology 6 (1958) 669.
9. J. S. Butel, S. S. Teuethia, N. Biswal and M. Benyesh-Melnick, In Vitro 6 (1971) 335.
10. R. C. Huang and J. Bonner, Proc. Natl. Acad. Sci. 54 (1965) 960.
11. V. G. Allfrey, V. Littau and A. E. Mirsky, Proc. Nat'l. Acad. Sci. 49 (1963 414.
12. R. S. Gilmour and J. Paul, FEBS Letters 9 (1970) 242.
13. C. Teng, C. Teng and V. G. Allfrey, J. Biol. Chem. 246 (1971) 3597.
14. T. C. Spelsberg and L. Hnilica, Biochem. J. 120 (1970) 435.

15. L. J. Kleinsmith, J. Heidema and A. Carroll, Nature 226 (1970) 1025.
16. G. S. Stein and J. Farber, Proc. Nat'l. Acad. Sci. 69 (1972) 2918.
17. G. S. Stein, S. C. Chaudhuri and R. Baserga, J. Biol. Chem. 247 (1972) 3918.
18. G. S. Stein and R. Baserga, Adv. Cancer Res. 15 (1972) 287.
19. G. S. Stein, T. C. Spelsberg and Kleinsmith, L. J., Science 183 (1974) 817.
20. T. C. Spelsberg, J. A. Wilhelm and L. S. Hnilica, Sub-Cell. Biochem. 1 (1972) 107.
21. G. Stein and D. Burtner, in press, Exptl. Cell Res.
22. G. S. Stein and T. W. Borun, J. Cell Biol. 52 (1972) 292.
23. R. Taber, R. Wertheimer and J. Golrick, J. Mol. Biol. 80 (1973) 367.
24. M. O. Krause and G. S. Stein, in press, J. Cell Biol.
25. S. Panyim and R. Chalkley, Biochem. 8 (1969) 3972.
26. G. S. Stein and C. L. Thrall, FEBS Letters 34 (1973) 35.
27. J. Bhorjee and T. Pederson, Biochem. 12 (1973) 2766.
28. L. Zordi, J. Lin and R. Baserga, Nature New Biol. 245 (1973) 211.
29. G. S. Stein, G. Moscovici, C. Moscovici and M. Mon, FEBS Letters 38 (1974) 295.
30. G. Rovera, R. Baserga and Defendi, V., Nature New Biol. 237 (1972) 240.
31. L. Ledinko, Virology 54 (1973) 294.

These studies were supported by the following research grants: GB38349 from the National Science Foundation; F73UF from the American Cancer Society and A4433 from the National Research Council of Canada.