

PROPERTIES OF THE GENOME IN NORMAL AND SV-40 TRANSFORMED WI-38 HUMAN DIPLOID FIBROBLASTS. III. TURNOVER OF NONHISTONE CHROMOSOMAL PROTEINS AND THEIR PHOSPHATE GROUPS.

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(Received in final form February 28, 1975)

Summary

The turnover of nonhistone chromosomal proteins and their phosphate groups was compared in normal and in SV-40 virus transformed WI-38 human diploid fibroblasts. Cells were pulse labelled with tryptophan-<sup>3</sup>H and <sup>32</sup>P for 30 minutes and the specific activities of tryptophan-<sup>3</sup>H and <sup>32</sup>P in the various molecular weight classes of nonhistone chromosomal proteins were determined during the first four hours following termination of labelling. While a rapid turnover of high molecular weight nonhistone polypeptides (142,000 to 200,000 Daltons) is evident after one hour in SV-40 transformed cells, the specific activities of these nonhistone chromosomal polypeptides are not significantly decreased in normal cells. In contrast, a rapid turnover of low molecular weight (30,000 to 51,000 Daltons) nonhistone chromosomal proteins occurs during the first hour in normal WI-38 cells with no corresponding decrease in the specific activities of these proteins in SV-40 transformed cells. There is no apparent net turnover of phosphate groups on nonhistone chromosomal proteins in either normal or SV-40 transformed cells four hours following pulse labelling. Rather, during the first four hours significant fluctuations are observed in the <sup>32</sup>P specific activities of defined molecular weight fractions. Taken together with previous reports of differences in the composition, synthesis and phosphorylation of nonhistone chromosomal proteins in normal and SV-40 transformed human diploid cells the present results further indicate the complex nature of the alterations in these proteins which accompany viral transformation.

Evidence is rapidly accumulating which indicates that chromosomal proteins play a key role in dictating structural and transcriptional properties of the genome in eukaryotic cells. Histones appear to be primarily involved in the repression of DNA-dependent RNA synthesis (1,9) and in the maintenance of

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chromatin structure (2,8,18). In contrast, recent findings from several laboratories suggest that nonhistone chromosomal proteins may interact with specific DNA sequences and thereby regulate the expression of defined gene loci (7,11, 12,21-23,26,30). Transformation of mammalian cells by DNA viruses results in phenotypic changes which are evident at the morphological as well as the biochemical levels (3,4,29). Since these viral induced cellular modifications reflect alterations in gene expression, one might anticipate modifications in the macromolecules which comprise the genome and interact with DNA to regulate its function. Several laboratories including our own have recently reported viral induced modifications in the protein components of the genome of human diploid cells (6,13-15,17,31). The histones and nonhistone chromosomal proteins of normal and SV-40 transformed WI-38 human diploid fibroblasts were compared and variations were observed in the compositions, rates of synthesis, acetylation, and phosphorylation of defined fractions. To achieve a more comprehensive understanding of viral-induced changes in chromosomal protein metabolism we have examined the rates of nonhistone chromosomal protein turnover as well as the turnover of phosphate groups added post-translationally to these polypeptides.

#### Materials and Methods

##### I. Cell Culture.

Human diploid WI-38 fibroblasts and SV-40 transformed WI-38 fibroblasts were grown in monolayer culture as previously described (13). All experiments were carried out utilizing exponentially-growing cells. The normal human diploid fibroblasts ranged from passage 28-32 since age-dependent modifications in the metabolism of chromosomal proteins have been observed in late passage cells (27).

##### II. Labelling with Radioisotopes.

###### a. Labelling with L-tryptophan-<sup>3</sup>H and <sup>32</sup>P.

Medium was removed from each flask and replaced with 15 ml of L-tryptophan-free and phosphate-free BME containing L-tryptophan-<sup>3</sup>H (5  $\mu$ Ci/ml, 1.65

Ci/mMole),  $^{32}\text{P}$  (100  $\mu\text{Ci/ml}$ ) and 2% fetal calf serum. After incubation for 30 minutes at  $37^\circ\text{C}$  the labelling medium was discarded and all monolayers were washed with 15 ml of warm ( $37^\circ\text{C}$ ) normal BME (containing tryptophan and phosphate). The cells which constituted the "pulse" samples were washed three times with 15 ml of cold ( $4^\circ\text{C}$ ) Earle's Balanced Salt Solution and nuclei were isolated immediately as described below. For all other flasks the cells were washed and reincubated in the same growth medium which covered the monolayers of cells prior to labelling. One, two, or four hours following resumption of incubation in growth medium "chase" samples were washed three times with cold ( $4^\circ\text{C}$ ) Earle's Balanced Salt Solution and nuclei were isolated. Each "pulse" or "chase" sample consisted of  $1.8 \times 10^7$  cells. Isotopes were obtained from the New England Nuclear Corporation, Boston, Massachusetts.

b. Labelling with Uridine  $^3\text{H}$  and Thymidine  $^{14}\text{C}$ .

Uridine 5- $^3\text{H}$  (58 Ci/mMole) and thymidine-methyl- $^{14}\text{C}$  (10 mCi/mMole) were added to growth medium covering monolayers of WI-38 cells to final concentrations of 3.3  $\mu\text{Ci/ml}$  and 0.1  $\mu\text{Ci/ml}$ , respectively. After incubation at  $37^\circ\text{C}$  for 30 minutes, isotope incorporation was terminated by pouring off the medium and washing the monolayers three times with 15 ml of cold ( $4^\circ\text{C}$ ) Earle's Balanced Salt Solution. Each sample consisted of  $1.8 \times 10^7$  cells.

III. Isolation of Nuclei and Chromatin.

Preparation of nuclei was carried out at  $4^\circ\text{C}$  as previously reported (24). Both cell lysis and washing of nuclei were carried out in the presence of 50  $\mu\text{g/ml}$  of 1-L-tosylamide-2-phenylethylchloromethyl ketone (TPCK) to inhibit proteolytic degradation (28).

Chromatin was isolated as previously described by Stein and Thrall(25).

IV. Polyacrylamide Gel Electrophoretic Fractionation of Chromosomal Proteins.

Chromatin was dissociated in 1.5 ml of 2% SDS, 5%  $\beta$ -mercaptoethanol, 0.0625 M Tris (pH 6.8), and then dialyzed for 12 hours against 0.1%  $\beta$ -mercaptoethanol, 0.0625 M Tris (pH 6.8) at  $22^\circ\text{C}$ . Sucrose and  $\beta$ -mercaptoethanol were added to final concentrations of 15% and 5%, respectively and 50  $\mu\text{l}$  aliquots

containing 50-75  $\mu$ g of proteins were electrophoresed on 10 x 0.6 cm, 8.75% polyacrylamide gels containing 0.2% Bisacrylamide, 1% SDS and 0.38 M Tris-HCl (pH 8.8). A 0.6 x 1 cm stacking gel was used containing 3% acrylamide, 0.08% Bisacrylamide, 1% SDS and 0.13 M Tris-HCl (pH 6.8). Electrophoresis was carried out for 8 hours at 1 ma/gel in a running buffer of 0.038 M Tris, 0.18 M glycine, 0.1% SDS (pH 8.4). Details of the procedure have been reported previously (16).

### Results

High Resolution Molecular Weight Fractionation of Nonhistone Chromosomal Proteins From Normal and SV-40 Transformed WI-38 Human Diploid Fibroblasts.

Polyacrylamide gel electrophoretic profiles of nonhistone chromosomal proteins from normal and SV-40 transformed WI-38 cells are compared in Figure 1. While the banding patterns are qualitatively similar for normal and SV-40 transformed WI-38 cells, differences in the amounts of protein present in specific molecular weight classes of nonhistone chromosomal proteins are evident.

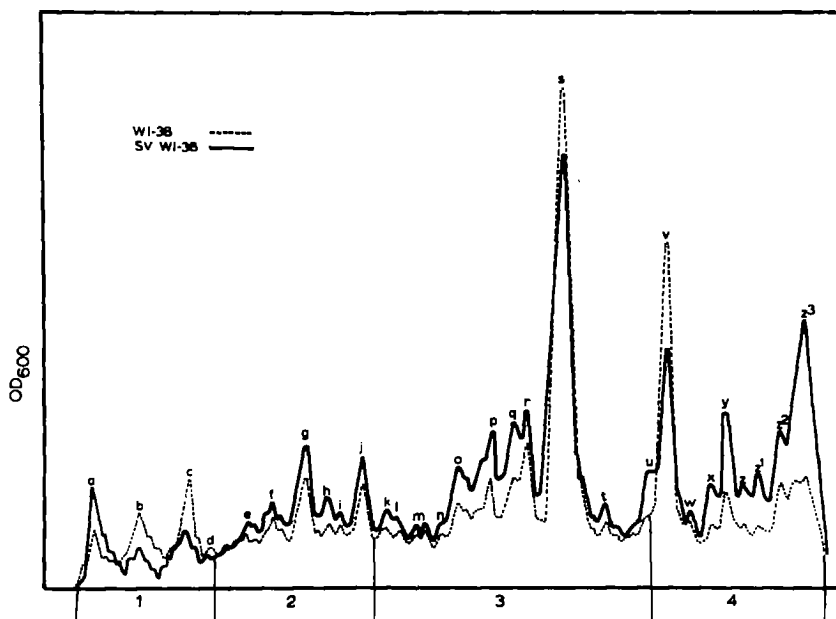


Fig. 1

Comparison of the absorbance scans (O.D. 600) of normal (dashed line) and SV-40 transformed (solid line) WI-38 cells.

The data in Table 1 indicate that the relative amount of nonhistone chromosomal protein present in the 30,000 to 51,000 molecular weight region of the gels is 1.5-fold greater for SV-40 transformed WI-38 cells than for normal WI-38 cells.

TABLE 1

	<u>MOLECULAR WEIGHT RANGE</u>			
	200,000- 142,000	142,000- 100,000	100,000- 51,000	51,000- 30,000
	<u>REGION OF GEL</u>			
	1	2	3	4
	Percent Protein			
WI-38 cells	11	17	49	22
SV-40, WI-38 cells	7	17	44	31

*Relative Amount of Protein in Various Molecular Weight Classes of Non-histone Chromosomal Proteins from Normal and SV-40 Transformed WI-38 Cells. The proteins were fractionated in SDS gels, which were then stained with 0.25% Coomassie Brilliant Blue and scanned at 600 nm. The areas under the four arbitrarily selected regions of the gel were integrated and compared in order to estimate the relative amount of protein in each. Within the narrow range of protein concentrations used in these electrophoretic fractionations the amount of stain bound (absorption) varied in a linear fashion with the protein concentration applied to the gels as determined by the method of Lowry (18). The molecular weight limits of each region were estimated using various protein molecular weight markers as references.*

In contrast, the relative amount of nonhistone chromosomal protein present in the 142,000 to 200,000 molecular weight region of the gels is 1.6-fold greater for normal than for SV-40 transformed cells. From the absorbance scans of the gels (Figure 1) it is apparent that only peaks B, C, S and V are more pronounced in normal WI-38 cells and that all remaining peaks are more pronounced in SV-40 transformed cells. It should be noted that each peak consists of several protein bands. These variations in specific molecular weight classes of nonhistone chromosomal proteins associated with the genome of normal and SV-40 transformed WI-38 human diploid fibroblasts are consistent with previous differences obtained by a lower resolution electrophoretic fractionation of nonhistone chromosomal polypeptides (13,14). However, with the increased

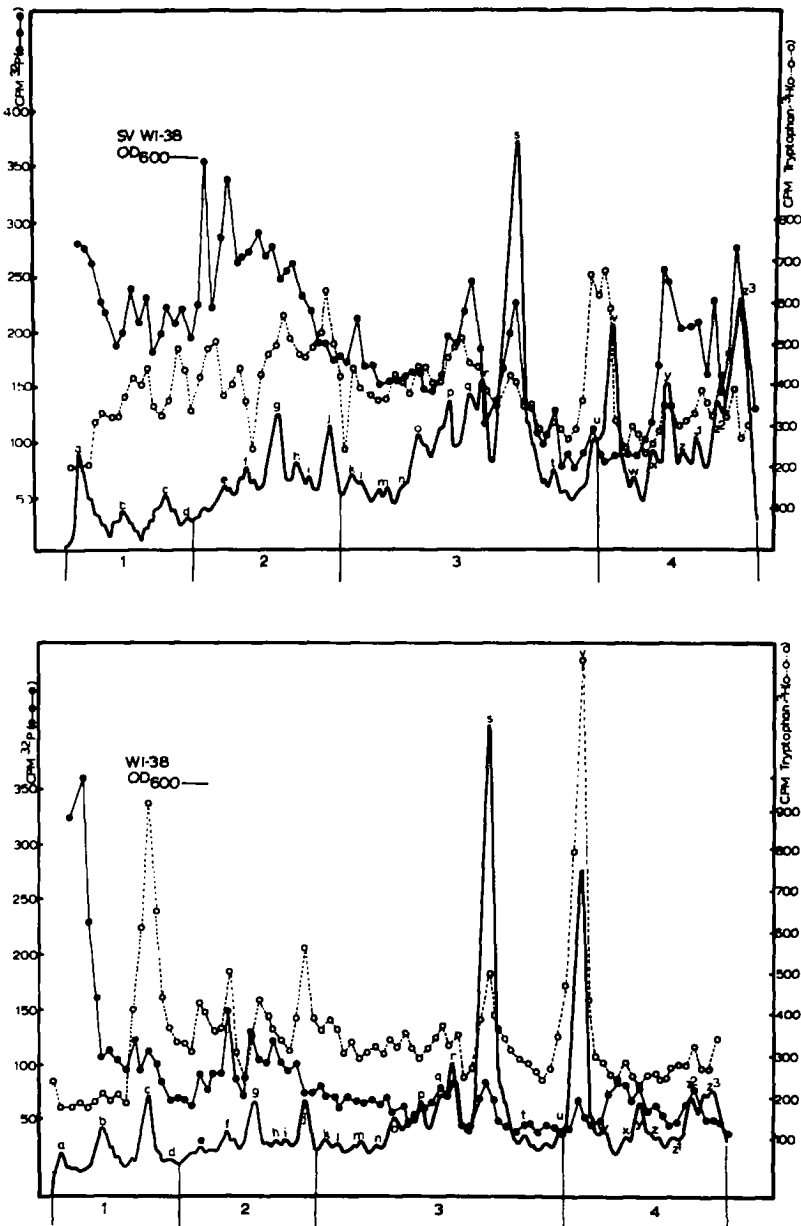


Fig. 2 (a) above, 2 (b) below

Radioactivity profiles for tryptophan- $^3\text{H}$  labelled (open circles) and  $^{32}\text{P}$  labelled (closed circles) nonhistone chromosomal proteins of normal (a) and SV-40 transformed (b) WI-38 cells, fractionated as described in "Materials and Methods". The respective absorbance scans are illustrated as solid lines.

degree of band separation afforded by the present method such variations in nonhistone chromosomal polypeptides are more definitively pronounced.

Turnover of Nonhistone Chromosomal Proteins and Their Phosphate Groups in Normal and SV-40 Transformed WI-38 Human Diploid Fibroblasts.

The turnover of nonhistone chromosomal proteins and their phosphate groups in normal and in SV-40 transformed WI-38 cells was examined by pulse-labelling cells with L-tryptophan- $^3\text{H}$  and  $^{32}\text{P}$  for 30 minutes and then determining the specific activities of tryptophan- $^3\text{H}$  and  $^{32}\text{P}$  in nonhistone chromosomal proteins immediately, as well as 1, 2 and 4 hours following termination of labelling. In Figure 2 the incorporation of tryptophan- $^3\text{H}$  and  $^{32}\text{P}$  into the various molecular weight classes of nonhistone chromosomal polypeptides immediately following pulse labelling is shown. A significant increase in the phosphorylation of most molecular weight classes of nonhistone chromosomal proteins is evident in the SV-40 transformed cells. That  $^{32}\text{P}$  incorporation reflects solely nonhistone chromosomal protein phosphorylation and does not represent nucleic acid synthesis was supported by the absence of significant levels of radioactivity in these gels when normal and SV-40 transformed WI-38 cells were labelled with thymidine- $^{14}\text{C}$  and uridine- $^3\text{H}$ . Some nucleic acids were found to enter the stacking gel (not shown) but only background levels of radioactivity could be detected throughout the separating gel. The incorporation of tryptophan- $^3\text{H}$  into these nonhistone chromosomal proteins indicates their rates of synthesis. While the specific activities of tryptophan- $^3\text{H}$  in peaks C and V are greater in normal compared to SV-40 transformed cells, the specific activities of tryptophan- $^3\text{H}$  in most nonhistone polypeptides which migrate in the 30,000 to 51,000 (region 4) and in the 51,000 to 100,000 (region 3) molecular weight regions of the polyacrylamide gels shown in Figure 2 are greater in SV-40 transformed cells. With the exception of peak A,  $^{32}\text{P}$  incorporation through all 4 regions of the gel is considerably higher in SV-40 transformed cells. Figures 3 and 4 show the specific activities of tryptophan- $^3\text{H}$  (Figure 3) and  $^{32}\text{P}$  (Figure 4) in the nonhistone chromosomal proteins of

normal and SV-40 transformed WI-38 cells at various times following pulse labelling. In Figure 3 the most significant differences in the rates of nonhistone chromosomal protein turnover in normal and in SV-40 transformed WI-38 cells are apparent in regions 1 and 4. In region 1 a two-fold decrease occurs in the specific activity of nonhistone chromosomal proteins within one hour following pulse labelling in SV-40 transformed cells while no turnover is evi-

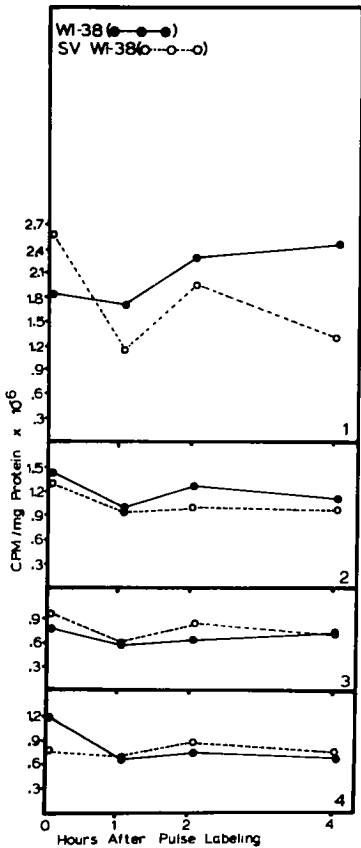


Fig. 3 (left)

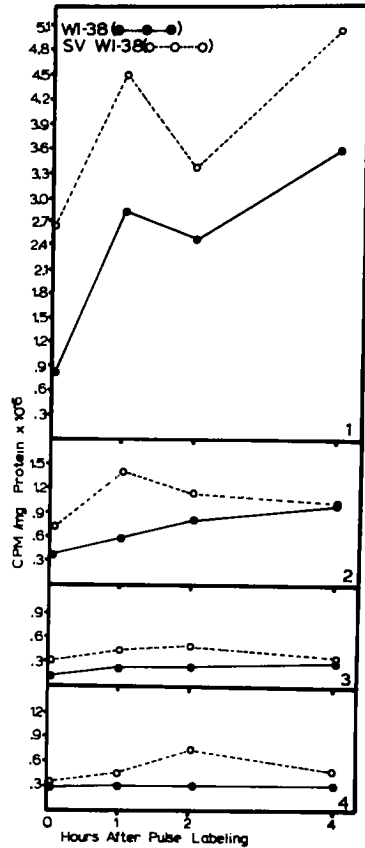


Fig. 4 (right)

The turnover of tryptophan-<sup>3</sup>H labelled nonhistone chromosomal proteins from normal (closed circles) and SV-40 transformed (open circles) WI-38 cells, in the four discrete molecular weight regions defined in Table 1.

The turnover of <sup>32</sup>P labelled nonhistone nuclear proteins from normal (closed circles) and SV-40 transformed (open circles) WI-38 cells, in the four molecular weight regions defined in Table 1.



dent in this region in normal WI-38 cells. In region 4 a two-fold decrease occurs in the specific activity of nonhistone chromosomal proteins within one hour following pulse labelling in normal WI-38 cells while no turnover is evident in this region in SV-40 transformed cells.

There is no evidence for turnover of phosphate groups on nonhistone chromosomal proteins in either normal or transformed cells four hours following pulse labelling with  $^{32}\text{P}$ . Rather, Figure 4 indicates that during this period either significant amounts of phosphate are added to nonhistone chromosomal proteins which migrate in the 142,000 to 200,000 and 100,000 to 142,000 molecular weight regions of polyacrylamide gels or additional phosphorylated nonhistone chromosomal proteins become associated with the genome. In addition, fluctuations in the  $^{32}\text{P}$  specific activities of nonhistone chromosomal proteins, particularly those in the 142,000 to 200,000 molecular weight range, are evident in normal and in SV-40 transformed cells during the first four hours following pulse labelling. In all molecular weight regions of the gels,  $^{32}\text{P}$  specific activities are lower in normal than in SV-40 transformed cells after 1 and 2 hours of chase; however, in regions 2, 3 and 4 the values reach similar levels in both cell types after 4 hours of chase.

#### Discussion

The present studies demonstrate that there are differences in the rates of turnover of defined molecular weight classes of nonhistone chromosomal polypeptides in normal as compared to SV-40 transformed WI-38 human diploid fibroblasts. Such variations in the rates of nonhistone chromosomal protein turnover are apparent within one hour following pulse labelling with tryptophan- $^3\text{H}$ . There is no net turnover of label in any of the major molecular weight classes of nonhistone chromosomal proteins four hours following pulse labelling of normal and SV-40 transformed WI-38 cells with  $^{32}\text{P}$ . While  $^{32}\text{P}$  incorporation reflects phosphorylation of preexisting nonhistone chromosomal proteins associated with the genome, it may at least in part also represent phosphorylation of newly synthesized nonhistone chromosomal polypeptides and phosphorylated pro-

teins from other cellular compartments which are added to the genome. Interpretation of these findings may be complicated by the re-utilization of tryptophan-<sup>3</sup>H and <sup>32</sup>P.

Fluctuations in the <sup>32</sup>P and tryptophan-<sup>3</sup>H specific activities of nonhistone chromosomal proteins, particularly those in the 142,000 to 200,000 molecular weight ranges, are evident in normal and in SV-40 transformed cells during the first four hours following pulse labelling. There are several possible interpretations of these findings. Fluctuations in tryptophan-<sup>3</sup>H and <sup>32</sup>P radioactivity in the nonhistone chromosomal proteins may reflect re-utilization of label. The sources of labelled amino acid can be breakdown of nuclear or cytoplasmic protein. <sup>32</sup>P can be derived from degraded nucleic acids or phosphorylated proteins, as well as from phosphate groups selectively removed from phosphorylated polypeptides. Increases and decreases in the tryptophan-<sup>3</sup>H and <sup>32</sup>P specific activities of nonhistone chromosomal proteins may also be attributable to exchange of nonhistone chromosomal polypeptides and phosphorylated nonhistone chromosomal polypeptides between the genome and the nucleoplasm or cytoplasm. Such nucleoplasmic and cytoplasmic pools of nonhistone chromosomal proteins have been previously reported (5,10,24), and evidence has been presented which suggests that exchange of nonhistone chromosomal proteins between such pools and the genome does in fact occur. These findings clearly necessitate defining the genome as a macromolecular complex consisting of "stable" and "dynamic" components. DNA and histones comprise the stable elements while the nonhistone chromosomal proteins are, at least in part, in a state of flux (24). The present results are consistent with this concept.

Earlier evidence has indicated differences in the composition, synthesis, and phosphorylation of nonhistone chromosomal proteins in normal and SV-40 transformed WI-38 human diploid fibroblasts (6,13,14,17,20,31). Taken together with the present results which show significant differences in the turnover of nonhistone chromosomal proteins and their phosphate groups it is apparent that complex alterations in nonhistone chromosomal protein metabolism occur when

human diploid cells are transformed by SV-40 virus. Whether these changes in the proteins associated with the genome are directly responsible for the morphological and biochemical modifications which accompany transformation by DNA tumor viruses remains to be established.

#### Acknowledgement

The authors are indebted to Rochelle Filker for testing the linearity of protein concentrations and Coomassie blue staining and to Jeudi Davis for cultivation of the cells. These studies were supported by the following research grants: GB38349 from the National Science Foundation; GM20535 from the National Institutes of Health; F73UF from the American Cancer Society and A4433 from the National Research Council of Canada.

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