Circular Dichroism and Ethidium Bromide Binding Capacity of Chromatin from Cells Temperature Sensitive for the Transformed Phenotype†

Toshinori Ide and Renato Baserga*

ABSTRACT: Clone H6-15/163 is a clone of cells, originally derived from SV-40 tranformed 3T3 cells, which express the transformed phenotype at low (32 °C) but not at high (39 °C) temperature. Chromatin was isolated from these cells grown at either temperature and studied by circular dichroism and for its ability to bind the intercalating dye, ethidium bromide. During the exponential phase of growth the chromatins of cells grown at either 32 or 39 °C are undistinguishable. Cessation of growth in confluent cultures results in marked changes in circular dichroism spectra and in ethidium bromide binding capacity of chromatin. The changes are much more pronounced at 39 °C (where the cells truly become quiescent) than at 32 °C (where cell proliferation continues although the number of cells per culture remains stationary). Temperature shifts and medium replacement also cause changes in chromatin structure, but the changes are again related to the extent of cell proliferation. It is concluded that the chromatin changes occurring in H6-15/163 cells and detectable by circular dichroism and ethidium bromide binding can be related to the proliferating activity of the cultured cells rather than to the expression of the transformed or untransformed phenotype.

Immunological and biochemical differences in chromatin and chromosomal proteins, between normal and neoplastic cells, have been reported by a number of authors (Wakabayashi and Hnilica, 1973; Yeoman et al., 1973; Zardi et al., 1973; Cholon and Studzinski, 1974; Weisenthal and Ruddon, 1973; Lin et al., 1974; Gronow and Thackrah, 1974; Biessmann and Rajewsky, 1975; Lea et al., 1975; Krause et al., 1975; Augenlicht et al., 1975). In these studies a comparison was made between animal tumors and tissues of origin, or between normal and transformed cells in culture, both approaches being open to possible criticisms (see Discussion). We have therefore extended these studies to a comparison of chromatin isolated from cultured cells that express the transformed phenotype at low (32 °C) but not at high (39 °C) temperature. The cell line used for these studies was clone H6-15, isolated from SV-40 transformed 3T3 cells and described by Renger and Basilico (1972). Chromatin isolated from these cells was studied by circular dichroism (CD) and for its ability to bind the intercalating dye ethidium bromide. Both of these methodologies have been extensively used in recent years to study the structure and function of isolated chromatin (Simpson and Sober, 1970; Shih and Fasman, 1970, 1971; Adler and Fasman, 1971; Johnson et al., 1972; Simpson, 1972; Hanlon et al., 1974; Wilhelm et al., 1970; Hjelm and Huang, 1974, 1975; Lurquin, 1974; Angerer and Moudrianakis, 1972; Lurquin and Seligy, 1972; Williams et al., 1972; Nicolini and Baserga, 1975). Our results show that at least by these methods, and with these cells, chromatin differences are related to the extent of cell proliferation in the cultures rather than to the expression of the transformed or untransformed phenotype.

Materials and Methods

Cell Culture. Cells used for these experiments were obtained through the courtesy of Dr. Claudio Basilico, New York University School of Medicine, New York. They were the clone H6-15 isolated and described by Renger and Basilico (1972). The clone is a temperature-sensitive mutant derived from SV-40 transformed 3T3 cells. The clone obtained from Dr. Basilico was recloned several times since the original culture had lost, in part, its temperature sensitivity for the transformed phenotype. The clone finally used in these experiments (clone H6-15/163) showed temperature sensitivity for the transformed phenotype as evidenced in Figure 1. The cells were grown in Dulbecco's modification of Eagles medium, supplemented with 10% calf serum. They were plated for most experiments in Blake bottles at a density of 3×10^3 cells/cm^2. Occasionally they were plated, at the same density in plastic 30-mm Petri dishes. The cells were grown either at 32 °C, at which temperature they express the transformed phenotype, or at 39 °C at which temperature they express the untransformed phenotype. Unless otherwise stated, the medium was changed twice a week after reaching confluence.

Cell Counts. The number of cells per culture was determined with a hemocytometer. The percentage of cells labeled by thymidine ^{3}H was determined as described in Table I, using [^{3}H]thymidine (New England Nuclear Corp.) with a specific activity of 6.7 Ci/mmol and NTB emulsion.

Isolation of Chromatin. Chromatin was isolated from detergent-clean nuclei. The nuclei in turn were isolated from the cell pellet harvested in ice-cold calcium magnesium-free Hank's salt solution by a modification of the procedure of Hymer and Kuff (1964). The Triton-clean nuclei were then lyzed in distilled water and chromatin was prepared by the method of Paul and Gilmour (1968). This includes a centrifugation through 1.7 M sucrose at 104 000 × 90 min in a SW50.1 rotor of a Spinco centrifuge. The combined use of a detergent to clean the nuclei and centrifugation through 1.7

† From the Department of Pathology and Fels Research Institute, Temple University School of Medicine, Philadelphia, Pennsylvania 19140. *Received August 28, 1975.* This work was supported by U.S. Public Health Service Research Grants CA-08373 and CA-12923 from the National Cancer Institute.

Table I: Labeling Index of H6-15/163 Cells.[a]

Day	32 °C (%)	39 °C (%)
1 (1–2 day)	98.1	98.2
	98.2	98.7
5 (5–6 day)	91.4	74.0
	91.7	68.2
8 (8–9 day)		6.0
		5.5
11 (11–12 day)	47.0	2.9
	47.8	2.4

[a] Cells were grown at either 32 or 39 °C for the times indicated in the first column. They were then exposed for 24 h to [^{3}H]thymidine (0.3 μCi/ml). The percentage of labeled cells was determined by autoradiography, using the methodology of Baserga and Malamud (1969).

M sucrose result in a chromatin pellet that is essentially free of contamination by cytoplasmic proteins (Dingman and Sporn, 1964; Wilhelm et al., 1972; Weisenthal and Ruddon, 1973; Augenlicht and Baserga, 1973; Bolund and Johns, 1973), ribonucleoproteins (Pedersen, 1974), and most nuclear membranes (Röwekamp and Sekeris, 1974; Harlow and Wells, 1975). The chromatin pellet was resuspended in 0.01 M Tris at pH 8 and used for further studies as unsheared chromatin.

Determination of Circular Dichroism. Circular dichroism was measured using a Jasco Model J-40 recording spectropolarimeter with CD capability only. Standardization of the instrument was performed according to the method of Simpson and Sober (1970), and with the modifications described by C. Nicolini et al. (manuscript submitted for publication). All measurements were carried out at 23 °C in a nitrogen atmosphere using a 1.0-cm fused quartz cell at a sample concentration of 1 O.D unit at 260 nm. Other details have been given in a paper by Nicolini and Baserga (1975). Corrections for light scattering were applied as described by Maizel et al. (1976). Results are expressed in terms of mean ellipticity (θ) where the dimensional units are in deg cm^2/dmol of nucleotide residue, assuming a mean molecular weight of a nucleotide as 330. Under the above operating conditions the overall error in repeat determinations of an identical sample was 4% in the 250–300-nm range.

Studies of Ethidium Bromide Binding to Chromatin by Spectropolarimetry. Ethidium bromide binding was measured using the methods of Dalgleish et al. (1971) and Williams et al. (1972), with the modifications introduced in this laboratory (Nicolini and Baserga, 1975). The measurement of ethidium bromide binding is based on the observation that DNA–ethidium bromide complexes demonstrate optical activity in the 290–600-nm region. Free ethidium bromide does not exhibit any optical activity over the 300–600-nm region. The ellipticity measured between 300 and 350 nm is related to the number of ethidium bromide molecules strongly bound to either DNA or chromatin (primary binding sites). Only intercalated dye molecules acquire optical activity (Aktipis et al., 1975; Parodi et al. 1975), with a maximum at 308 nm, while the weakly bound dye molecules, which interact with the phosphate groups of the DNA backbone, are not optically active. The results are expressed as mean ellipticity (θ) whose dimensional units are deg cm^2/dmol of nucleotide, as a function of the ratio added dye/nucleotide and at 308 nm (Nicolini and Baserga, 1975).

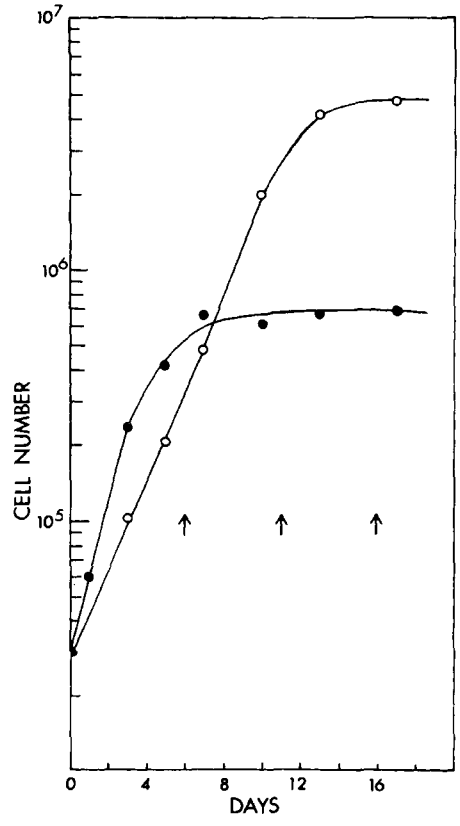

FIGURE 1: Growth of H6-15/163 cells at 32 °C (○) and 39 °C (●). Cells were plated in 30-mm Petri dishes at a density of 3 × 10^4 cells/dish. Arrows indicate medium change.

Analytical Procedures. DNA concentration of chromatin was determined by absorption at 260 nm in 0.2% sodium dodecyl sulfate using an extinction coefficient of 21 000/cm^2. Ethidium bromide was purchased from Sigma Chemical Corporation. Solutions were prepared in distilled water at 2 mg/ml and stored at 4 °C. The molar extinction coefficient of the drug was found to be 5600 at 480 nm.

Results

Growth of H6-15/163 Cells at 32 and 39 °C. Figure 1 shows the growth curves at 32 and 39 °C of the H6-15 clone used in these experiments (H6-15/163). At both temperatures the cells grow exponentially for 4 or 5 days, after which the cells stop growing at 39 °C while they continue to grow at 32 °C. The saturation density at the two temperatures was different by a factor of 6.85. In agreement with the results of Renger and Basilico (1972) we also observed that the cells stopped growing at 39 °C after confluence is reached (Table I). However, at 32 °C the cells continued to proliferate even after reaching confluence. Table I shows that 12 days after plating at 32 °C when the number of cells per culture is stationary, almost half of the cells are still synthesizing DNA, presumably replacing cells that die. On the contrary, at 39 °C, in confluent cultures, less than 3% of the cells are synthesizing DNA over a 24-h labeling period. This is an important point in comparing the chromatins from cells grown at different temperatures, since at 39 °C the cells are truly density inhibited, whereas at 32 °C the population is stationary (i.e., the number of cells does not increase further), but a certain amount of cell proliferation continues to go on, albeit at a reduced rate in comparison to the exponential phase.

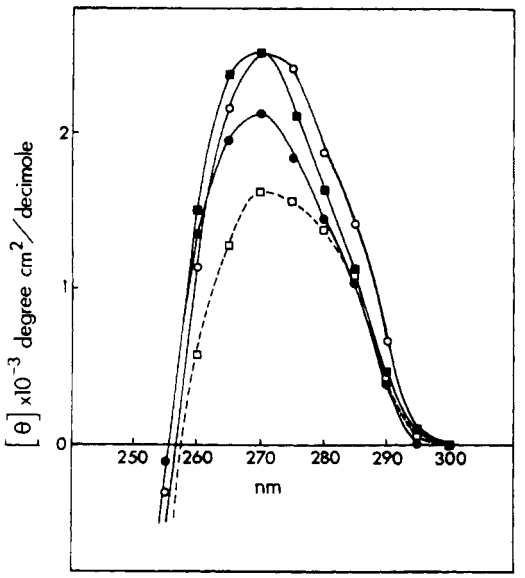

FIGURE 2: Circular dichroism spectra of chromatin from H6-15/163 cells grown at 32 °C. Cells were plated in Blake bottles at a density of 7×10^5 cells/Blake and harvested 1 day (●) 4 days (○), 9 days (■), and 14 days (□) after plating. Isolation of chromatin and determination of CD spectra are described in Materials and Methods.

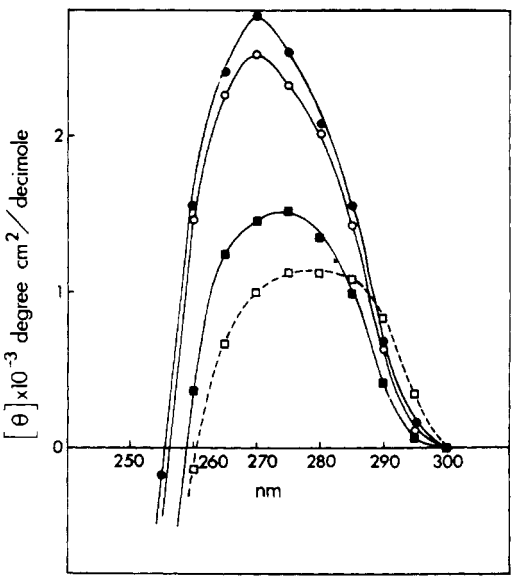

FIGURE 3: Circular dichroism spectra of chromatin from H6-15/163 cells. Condition were the same as in Figure 2, except that the cells were grown at 39 °C and were harvested 1 day (●), 2 days (○), 6 days (■), and 9 days (□) after plating.

If the cells grown at 32 °C (transformed phenotype) are shifted to 39 °C (untransformed phenotype) there is a prompt increase in the shedding of cells from the surface and the number of cells per dish decreases modestly but appreciably (not shown). When cells grown at 39 °C are shifted to 32 °C after they have reached confluence, there is a marked increase in the population density but this only takes place 48 h or longer after the shift down. These observations are in agreement with the findings of Renger and Basilico (1973).

Circular Dichroism of Chromatin from H6-15/163 Cells Grown at either 32 or 39 °C. Figure 2 shows the circular dichroism spectra between 250 and 300 nm of chromatin isolated from H6-15/163 cells grown at 32 °C in different stages of growth. The positive ellipticity of chromatin decreases considerably when exponentially growing cells (days 1, 4, and 9) form confluent monolayers (day 14), in which the population density remains stationary. This is in agreement with the observations of Nicolini and Baserga (1975) and Nicolini et al. (1975) that chromatin from cells stimulated to proliferate has a higher positive ellipticity in circular dichroism spectra than chromatin isolated from resting or stationary cells. Figure 3 shows the circular dichroism spectra of chromatin from the same H6-15/163 cells grown at 39 °C. Again there is a marked decrease in the positive ellipticity of chromatin as the cell cultures reach confluence from a θ_{max} of 2800 on day 1 to θ_{max} of 1100 on day 9 (under these conditions the θ_{max} of protein-free DNA is approximately 8000). However, two differences can be noted between cells grown at 39 °C and cells grown at 32 °C, namely (1) the maximum positive ellipticity of chromatin from cells expressing the untransformed phenotype (39 °C), decreases at 6 days after plating, much earlier than the corresponding decrease that occurs in these cells grown at 32 °C and expressing the transformed phenotype, and (2) the maximum positive ellipticity of stationary cells at 32 °C does not reach the low values of confluent cells at 39 °C. However, as mentioned before, even at this late stage cells at 32 °C continue to synthesize DNA and divide while at 39 °C the cells are truly quiescent. The differences in circular dichroism spectra could then be easily attributed to the extent of cell proliferation rather than to an expression of the transformed phenotype.

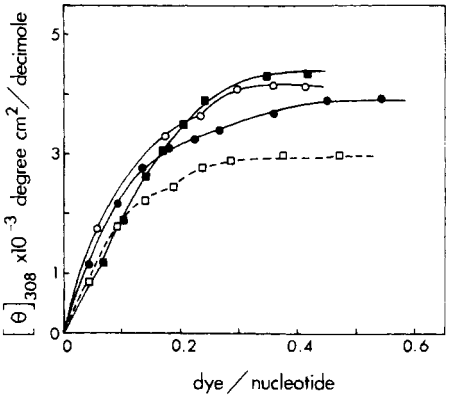

FIGURE 4: Ellipticity at 308 nm of ethidium bromide–chromatin complexes as a function of the ratio added dye/nucleotide. Chromatin was isolated from H6-15/163 cells grown at 32 °C and harvested 1 day (●), 4 days (○), 9 days (■), and 14 days (□) after plating. Other experimental details are given in Materials and Methods.

Ethidium Bromide Binding Studies. As mentioned in Materials and Methods the number of ethidium bromide molecules intercalating in chromatin was studied by spectropolarimetry using the methods of Williams et al. (1972) and Nicolini et al. (1975). Figure 4 shows the optical activity of ethidium bromide as measured at 308 nm at different dye/DNA ratios. The ordinate is a measure of the number of ethidium bromide molecules that are intercalated (Nicolini and Baserga, 1975). Figure 4 shows the binding of ethidium bromide to chromatin of H6-15/163 cells grown at 32 °C, and Figure 5 shows the same studies with H6-15/163 cells grown at 39 °C (under these conditions, the values for protein-free DNA are about four times the value for chromatin on day 1). Two things again should be noticed. (1) There is a decrease in the number of ethidium bromide molecules intercalating in chromatin when cells, either at 32 or

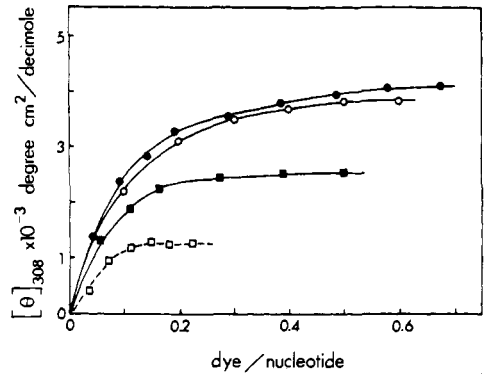

FIGURE 5: Same conditions as in Figure 4, except the cells were grown at 39 °C and harvested 1 day (●), 2 days (○), 6 days (■), and 9 days (□) after plating.

39 °C, shift from an exponential phase to a resting or stationary phase. This again is in agreement with the previous reports by Nicolini and Baserga (1975) and Nicolini et al. (1975), that the chromatin of cells stimulated to proliferate has more primary binding sites for ethidium bromide than chromatin isolated from G_0 cells. (2) The number of primary binding sites of stationary cells at 32 °C does not reach the low values of confluent cells at 39 °C.

Effect of Shift up and Shift down on Circular Dichroism Spectra and Ethidium Bromide Binding. In these experiments stationary cultures at either 32 or 39 °C were respectively shifted up or shifted down and the changes in chromatin were observed. Figure 6 shows the circular dichroism spectra of chromatin from cells grown for 6 days at 39 °C and shifted down to 32 °C. No changes are detectable in the circular dichroism spectra of chromatin isolated from cells 24 h after the shift down, but it should also be noted that after 24 h of a shift down there is no appreciable increase in the number of cells in the population. After 48 h, the chromatin of shifted cells shows a considerable increase in maximum positive ellipticity, as expected in cells that are stimulated to proliferate. Figure 7 shows the circular dichroism spectra of chromatins from cells grown for 9 days at 32 °C and shifted up to 39 °C. Under these conditions there is a considerable shedding of cells and cell proliferation decreases very rapidly (Renger and Basilico, 1973). Figure 7 shows that under these circumstances the maximum positive ellipticity of chromatin from shifted up cells is promptly decreased toward the direction of resting cells. The changes in ethidium bromide binding of chromatin of cells after temperature shift correspond completely to those in circular dichroism spectra of chromatin (data not shown).

Effect of Medium Change on CD Spectra and Ethidium Bromide Binding of Chromatin. Seventeen days after plating, the population density of H6-15/163 cells grown at 32 °C does not increase. However, a change of medium causes the cells to proliferate maintaining the cell density unchanged because of the shedding of cells from the surface. Figure 8 shows that after 1 day of medium change the maximum positive ellipticity of chromatin from 17 day old cells is as high as that from logarithmically growing cells. When H6-15/163 cells are grown at 32 °C for 14 days without medium change, the cells stop growing. Figure 8 indicates that under the circumstances the maximum positive ellipticity of chromatin decreases to the low value of cells confluent at 39 °C. The changes in number of ethidium bromide binding sites are comparable to those in the maximum

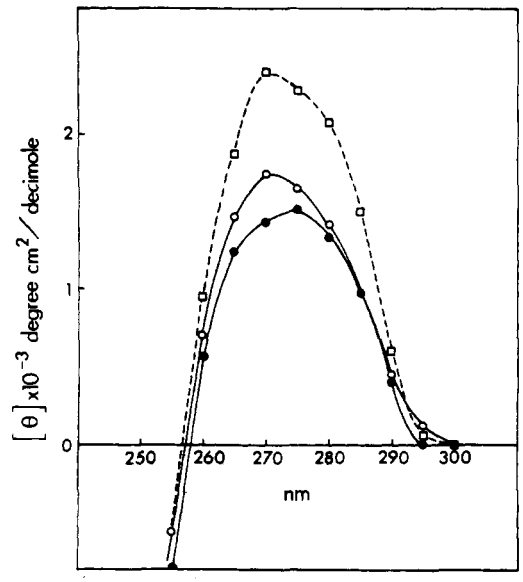

FIGURE 6: Effect of temperature shift down on circular dichroism spectra of chromatin isolated from H6-15/163 cells. (●) Cells grown at 39 °C for 6 days; (○) cells grown for 6 days at 39 °C and shifted down to 32 °C for 1 day; (□) same cells 2 days after shift down to 32 °C.

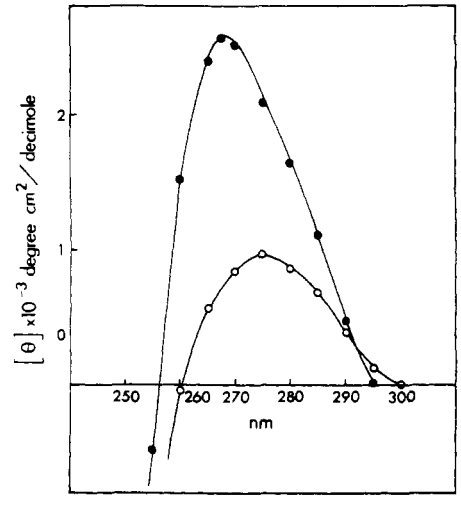

FIGURE 7: Effect of temperature shift up on circular dichroism spectra of chromatin from H6-15/163 cells. (●) Cells grown for 9 days at 32 °C; (○) same cells 1 day after shift up to 39 °C.

positive ellipticity of chromatin (not shown). In confluent culture at 39 °C, however, neither the number of cells, the maximum positive ellipticity, nor the number of binding sites for ethidium bromide are changed by the renewal of medium (not shown).

These results indicate again that the CD spectrum and the number of ethidium bromide binding sites of chromatin essentially reflect the proliferating state of cells.

Discussion

The two methodologies described in this manuscript, circular dichroism spectra and ethidium bromide binding capacity, have been used extensively in the past few years in studies of chromatin structure and function (see references in the introduction). It is now well established that in G_0 cells stimulated to proliferate there is an increase in the maximum positive ellipticity of chromatin in circular dichroism spectra (Nicolini and Baserga, 1975; Nicolini et al.

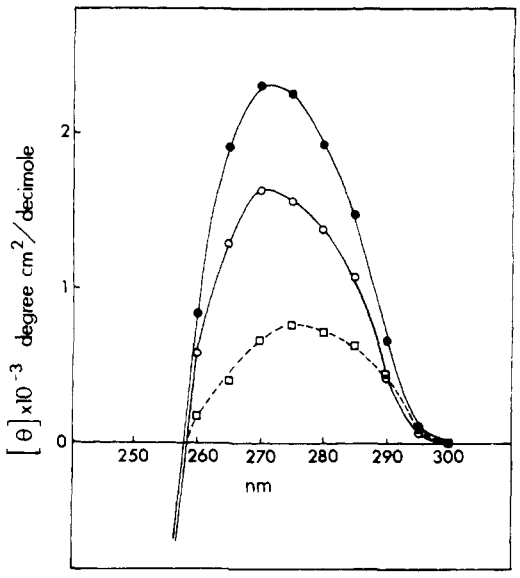

FIGURE 8: Effect of medium change on circular dichroism spectra of chromatin from H6-15/163 cells. Cells were grown at 32 °C for 14 days without medium change (□) or with a change of medium at day 11 (○). (●) Cells grown with medium change for 16 days, and 1 day after medium change. See Materials and Methods for experimental details.

1975), and in the capacity of chromatin to intercalate ethidium bromide molecules (Nicolini and Baserga, 1975; Nicolini et al. 1975). It has also been shown that an increased binding of acridine orange and other intercalating dyes can also occur in nuclei of cells stimulated to proliferate (Ringertz and Bolund, 1969; Darzynkiewcz et al. 1969; Zetterberg and Auer, 1970; Smets, 1973; Alvarez, 1974). In this respect our own results clearly confirm that in chromatin of proliferating cells the maximum positive ellipticity of circular dichroism spectra and the ability to intercalate ethidium bromide molecules are increased. Dramatic changes in both parameters occur when the cells expressing the untransformed phenotype (39 °C) become resting or when cells expressing the transformed phenotype (32 °C) become stationary.

Interestingly, several reports in the literature seem to indicate that the extent of ethidium bromide (or acridine orange) intercalation is proportional to transcriptional activity (Seligy and Lurquin, 1973; Sankaran and Pogell, 1973; Richardson, 1973; Nicolini and Baserga, 1975). However, we would like for the moment to avoid an interpretation of either the CD spectra or the ethidium bromide binding data, and simply reiterate that they change drastically with growth rates. The changes are not just a reflection of the number of cells in DNA synthesis (compare the data of Table I and Figure 3), but more likely of the number of cells in the proliferative cycle (G_1, S, G_2, and M). Similarly, we would like to avoid at this point any comment on the chromatin component responsible for the CD changes, whether RNA, as stated by Hjelm and Huang (1975), or protein (Nicolini et al. 1975).

Lin et al. (1974) reported differences in circular dichroism spectra of chromatin and in non-histone chromosomal proteins of WI-38 human diploid fibroblasts and their SV-40 transformed counterparts 2RA cells. As mentioned in the introduction, differences in chromatin and non-histone chromosomal proteins between normal and neoplastic tissues have also been reported in several other systems by a number of authors using immunological or biochemical methods. Most of these studies were vitiated by the fact that the comparison was done between a tumor and its tissue of origin, which has a different cellular composition than the tumor. Using SV-40 transformed cell lines does not greatly improve the situation since the cell lines that have been transformed are, by the time they are studied, completely different cell lines from the original cell culture. In the present experiments we have used a cell line which is capable of expressing the transformed or the untransformed phenotype depending on the temperature at which the cells are grown. Although this experimental model is not totally free of criticisms itself it seems to offer a reasonably good method to investigate differences in chromatin and/or its components that possibly relate to the expression of the transformed phenotype. As pointed out by Renger and Basilico (1972) in their original paper, H6-15 cells at 32 °C are transformed on the basis of 3 different criteria, one of which, anchorage independence, is a characteristic that correlates consistently with in vivo tumorigenicity (Shin, personal communication). These criteria have been preserved in our clone, and it should be noted, the phenotypes are expressed also when the cells are growing exponentially (Renger and Basilico, 1973). On this basis our conclusions can be summarized as follows. (1) Circular dichroism spectra are markedly influenced by the proliferating state of the cells. The maximum positive ellipticity of chromatin decreases markedly when cells, either at 32 or 39 °C shift from an exponential phase to a resting or a stationary phase. (2) Similar drastic changes occur in ethidium bromide binding capacity of chromatin when cells, again either at 39 or 32 °C, shift from an exponential phase of growth to a resting or stationary phase. (3) There are chromatin differences between cells at 32 °C and cells at 39 °C in their resting or stationary phase, but these differences can be largely explained by the fact that cells at 32 °C are still dividing and synthesizing DNA, even when they have reached a stationary state, while at 39 °C the cells are truly quiescent. (4) During the exponential phase of growth the circular dichroism spectra of chromatins are undistinguishable whether chromatin is isolated from cells at 32 or at 39 °C, although the cells are already expressing their transformed or untransformed phenotype (Renger and Basilico, 1972, 1973). (5) The ability of chromatin to intercalate ethidium bromide also is the same in cells at 32 or 39 °C, during the exponential phase of growth. (6) Temperature shifts and medium changes that cause variations in growth rates produce corresponding variations in chromatin structure.

A reasonable interpretation of these results is that circular dichroism spectra and ethidium bromide binding capacity of chromatin essentially reflect the proliferating state of the cells, and are not related to transformation.

In conclusion our studies show that variations in growth rates cause dramatic changes in the structure of chromatin, as determined by circular dichroism or ethidium bromide binding capacity. In addition, they indicate the necessity of comparing chromatins of transformed and untransformed cells that are in the same state of proliferating activity before any difference can be attributed to the transformed state.

References

Adler, A. J., and Fasman, G. D. (1971), *J. Phys. Chem. 75*, 1516–1526.

Aktipis, S., Martz, W. W., and Kindelis, A. (1975), *Bio-*

chemistry 14, 326-331.
Alvarez, M. R. (1974), *Exp. Cell Res. 83,* 225-230.
Angerer, L. M., and Moudrianakis, E. N. (1972), *J. Mol. Biol. 63,* 505-521.
Augenlicht, L. H., and Baserga, R. (1973), *Arch. Biochem. Biophys. 158,* 89-96.
Augenlicht, L. H., Biessman, H., and Rajewsky, M. F. (1975), *J. Cell. Physiol. 86,* 431-438.
Baserga, R., and Malamud, D. (1969), Autoradiography. Techniques and Applications, New York, N.Y., Hoeber, p 17.
Biessmann, H., and Rajewsky, M. F. (1975), *J. Neurochem. 24,* 387-393.
Bolund, L., and Johns, E. W. (1973), *Eur. J. Biochem. 40,* 591-598.
Cholon, J. J., and Studzinski, G. P. (1974), *Cancer Res. 34,* 588-593.
Dalgleish, D. G., Peacocke, A. R., Fey, G., and Harvey, C. (1971), *Biopolymers 10,* 1853-1863.
Darzynkiewcz, Z., Bolund, L., and Ringertz, N. R. (1969), *Exp. Cell Res. 56,* 418-424.
Dingman, C. W., and Sporn, M. B. (1964), *J. Biol. Chem. 239,* 3483-3492.
Gronow, M., and Thackrah, T. M. (1974), *Chem. Biol. Interact. 9,* 225-236.
Harlow, R., and Wells, J. R. E. (1975), *Biochemistry 14,* 2665-2674.
Hanlon, S., Johnson, R. S., and Chan, A. (1974), *Biochemistry 13,* 3972-3981.
Hjelm, R. P., and Huang, R. C. C. (1974), *Biochemistry 13,* 5275-5283.
Hjelm, R. P., and Huang, R. C. C. (1975), *Biochemistry 14,* 1682-1688.
Hymer, W. C., and Kuff, E. L. (1964), *J. Histochem. Cytochem. 12,* 359-363.
Johnson, R. S., Chan, A., and Hanlon, S. (1972), *Biochemistry 11,* 4347-4358.
Krause, M. O., Kleinsmith, J., and Stein, G. S. (1975), *Exp. Cell Res. 92,* 164-174.
Lea, M. A., Koch, M. R., and Morris, H. P. (1975), *Cancer Res. 35,* 1693-1697.
Lin, J.-C., Nicolini, C., and Baserga, R. (1974), *Biochemistry 13,* 4127-4133.
Lurquin, P. F. (1974), *Chem. Biol. Interact. 8,* 303-313.
Lurquin, P. F., and Seligy, V. L. (1972), *Biochem. Biophys. Res. Commun. 46,* 1399-1404.
Maizel, A., Nicolini, C., and Baserga, R. (1976), *Exp. Cell Res.* (in press).
Nicolini, C., and Baserga, R. (1975), *Chem. Biol. Interact. 11,* 101-116.
Nicolini, C., Ng, S., and Baserga, R. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 2361-2365.
Parodi, S., Kendall, F., and Nicolini, C. (1975), *Nucleic Acids Res. 2,* 477-486.
Paul, J., and Gilmour, R. S. (1968), *J. Mol. Biol. 34,* 305-316.
Pedersen, T. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 617-621.
Renger, H. C., and Basilico, C. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 109-114.
Renger, H. C., and Basilico, C. (1973), *J. Virol. 11,* 702-708.
Richardson, J. P. (1973), *J. Mol. Biol. 78,* 703-714.
Ringertz, N. R., and Bolund, L. (1969), *Exp. Cell Res 55,* 205-214.
Röwekamp, W., and Sekeris, C. E. (1974), *Arch. Biochem. Biophys. 160,* 156-161.
Sankaran, L., and Pogell, B. M. (1973), *Nature (London), New Biol. 245,* 257-260.
Seligy, V. L., and Lurquin, P. F. (1973), *Nature (London), New Biol. 243,* 20-21.
Shih, T. Y., and Fasman, G. D. (1970), *J. Mol. Biol. 52,* 125-129.
Shih, T. Y., and Fasman, G. D. (1971), *Biochemistry 10,* 1675-1683.
Simpson, R. T. (1972), *Biochemistry 11,* 2003-2008.
Simpson, R. T., and Sober, H. A. (1970), *Biochemistry 9,* 3103-3109.
Smets, L. A. (1973), *Exp. Cell Res. 79,* 239-243.
Wakabayashi, K., and Hnilica, L. S. (1973), *Nature (London), New Biol. 242,* 153-155.
Weisenthal, L. M., and Ruddon, R. W. (1973), *Cancer Res. 33,* 2923-2935.
Wilhelm, F. X., Champagne, M. H., and Duane, M. P. (1970), *Eur. J. Biochem. 15,* 321-330.
Wilhelm, J. A., Groves, C. M., and Hnilica, L. S. (1972), *Experientia 28,* 514-515.
Williams, R. E., Lurquin, P. F., and Seligy, V. L. (1972), *Eur. J. Biochem. 29,* 426-432.
Yeoman, L. C., Taylor, C. W., Jordan, J. J., and Busch, H. (1973), *Biochem. Biophys. Res. Commun. 53,* 1067-1076.
Zardi, L., Lin, J.-C., and Baserga, R. (1973), *Nature (London), New Biol. 245,* 211-213.
Zetterberg, A., and Auer, G. (1970), *Exp. Cell Res. 62,* 262-270.