Homologies in Amino Acid Composition and Structure of Histone F2a1 Isolated from Human Leukaemic Cells

By LAXMAN S. DESAI AND GEORGE E. FOLEY

The Children's Cancer Research Foundation, and Harvard Medical School, Boston, Mass. 02115, U.S.A.

(Received 16 January 1970)

Histones F2a1 extracted from normal and neoplastic cells possess similar amino acid compositions. Tryptic and chymotryptic peptides of the F2a1 histones have identical chromato-electrophoretic R_F values. It is concluded that histones F2a1 from various sources have similar overall structures. The observed differences in the ratios of ϵ -*N*-monomethyl- and di- ϵ -*N*-methyl-lysine in the histones from normal and neoplastic cells may be of significance with respect to gene regulation.

Stedman & Stedman (1950) called attention to the general lack of knowledge of histone chemistry nearly two decades ago. Since then results have accumulated on gene regulation by histones, indicating that different species of histones exhibit different degrees of influence on DNA-dependent RNA synthesis (Bonner & Ts'o, 1964; Busch, 1965). There is some information on the possible physical relationship of histone-DNA complexes by X-ray diffraction (Zubay, 1964) and electron microscopy (Huxley & Zubay, 1961). However, it is still unclear as to whether all histones are aligned to neutralize the phosphate groups of DNA, or whether some histones are specifically linked to specific base sequences, as suggested by the close relationship described between G+C-rich components of DNA and histones (Crampton, Lipschitz & Chargaff, 1954; Skalka, Rasmussen & Luck, 1964). Elucidation of such problems is now possible since precise methods for the isolation and characterization of individual histone species have been developed (Johns, 1967; Mauritzen, Starbuck, Taylor, Saroja & Busch, 1967; Fambrough & Bonner, 1968). Extensive investigations on the primary structure of histones are being made, and some preliminary results have been reported (Desai, Ogawa, Mauritzen, Taylor & Starbuck, 1969; Sautiere, Starbuck, Roth & Busch, 1968; DeLange, Fambrough, Smith & Bonner, 1968).

The present paper describes comparative studies on the composition and structure of histones F2a1 extracted from human leukaemic lymphoblasts (CCRF-CEM cells), bovine lymphosarcoma, foetal calf thymus, calf thymus, and Novikoff hepatoma.

EXPERIMENTAL

Materials. Human leukaemic lymphoblasts (CCRF-CEM cells) were harvested from large-volume exponential-phase suspension cultures in Eagle's minimal essential

medium modified for suspension culture and supplemented with 10% (v/v) foetal calf serum as described previously (Foley *et al.* 1965; Foley, Lazarus, Farber, Uzman & Adams, 1967). Histones were extracted from these cells (40–50 g wet wt.) as described by Johns (1964). The histone fraction F2a1 (also referred to as Histone IV, GAR- and β 7 histones) was then isolated from CCRF-CEM cells and Novikoff hepatoma by a modified procedure (Johns, 1967), and purified by passage through Sephadex G-100 columns. The same fraction (F2a1) was isolated from foetal calf thymus, bovine lymphosarcoma, and calf thymus as described by Starbuck *et al.* (1969). The purity of all fractions was determined by gel electrophoresis and amino acid analysis.

Amino acid analysis. Histone samples (1 mg/0.1 ml) were hydrolysed as described previously (Desai *et al.* 1969). The hydrolysates were dissolved in 1 ml of sodium citrate buffer, pH 2.2 (Spackman, Stein & Moore, 1958), filtered through a Millipore filter (0.22 μ m), and analysed in a Beckman-Spinco 120 automatic amino acid analyser (Moore & Stein, 1963).

Cyanogen bromide cleavage. The F2a1 histone was treated with cyanogen bromide as described by Gross & Witkop (1962). The reacted product was analysed on a Sephadex G-100 column (145 cm $\times$ 2.5 cm) in 0.01 M-HCl. The extinction of the effluent was read at 230 nm.

Polyacrylamide disc electrophoresis. The purity and the species of the histones were examined by polyacrylamide gel electrophoresis as described by Fambrough & Bonner (1966).

N-Terminal amino acid analysis. Analyses were made by the modified methods described by Fraenkel-Conrat, Harris & Levy (1955), Fraenkel-Conrat & Tsung (1967) and Phillips (1958), and by using dansyl chloride. Dansylation of the peptides was accomplished as described by Gray & Hartley (1963). A sample (2 μ l) of the dansyl-peptide hydrolysate was applied to pre-coated silica gel thin-layer chromatographic plates [t.l.c.-plates, cellulose-pre-coated, abrasion resistant (without fluorescence indicator) 20 cm $\times$ 20 cm] for ascending chromatography and allowed to run for 2 h. The solvent systems used were benzene-pyridine-acetic acid (40:10:1, by vol.) and propan-2-ol-methyl acetate-ammonia (7:9:4, by vol.).

C-Terminal amino acid analysis with carboxypeptidase A.

Samples (1.0 μ mol) of protein or peptide were dissolved in 1.8 ml of 0.01 M-tris buffer, pH 7.5, and digested at 25°C or 37°C with 0.2 ml (0.015 μ mol) of carboxypeptidase A solution, prepared for use as described previously (Sautiere *et al.* 1968; Desai *et al.* 1969). Samples (0.2 ml) were removed at specific time-intervals during the first 24 h, further reaction being prevented by the addition of citrate buffer (0.5 ml), pH 2.2 (Spackman *et al.* 1958), and analysed in the amino acid analyser.

Edman degradation. Edman degradation was done as described by Dophidie, Moore & Stein (1967). A sample (0.1 μ mol) was removed after the reaction for amino acid analysis and the Edman degradation was repeated on the remainder of the material.

Tryptic hydrolysis. Whole F2a1 histone or peptides were dissolved to a concentration of approx. 1 μ mol/2 ml of deionized water, adjusted and maintained at pH 8.0 with 0.1 M-NaOH with a pH-stat. Trypsin (L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated; 0.1 mg/ μ mol) was added at the beginning of the reaction and the same amount of trypsin was again added to the reaction mixture after 1 h. The reaction was carried out for 2 h at 37°C and then stopped by the addition of acetic acid.

Chymotryptic hydrolysis. Chymotryptic (α -chymotrypsin, trypsin-free, Worthington Biochemical Corp., Freehold, N.J., U.S.A.) digestion of the whole F2a1 histone or peptides was done for 2 h in essentially the same manner as the tryptic hydrolysis, except that the reaction was carried out at 25°C. The digests were fractionated on a

column (50 cm $\times$ 0.9 cm) of Aminex A-4, by using a stepwise gradient (0.3 M, pH 4.0–0.5 M, pH 5.0) of pyridine-formate buffers. The column was equilibrated with 0.2 M-pyridine-formate buffer, pH 3.1 and run at 55°C with a flow rate of 60 ml/h. Separation was monitored with a Beckman-Spinco amino acid analyser and confirmed by ninhydrin reaction as follows: samples of each fraction were hydrolysed with a final concentration of 2.5 M-NaOH at 90°C for 2.5 h, adjusted to pH 5.5 with 45% (v/v) acetic acid and treated with ninhydrin (Spackman *et al.* 1958).

'Fingerprint mapping'. Whatman no. 3MM sheets were used for chromato-electrophoresis. Descending chromatography was run for 16 h in butan-1-ol-pyridine-acetic acid-water (15:12:3:10, by vol.) with Phenol Red as a marker. The chromatographic sheet was air-dried and subjected to high-voltage electrophoresis with arginine as a marker, with pyridine-acetate buffer, pH 3.6 (Katz, Dreyer & Anfinsen, 1959; Mauritzen *et al.* 1967). The chromato-electrophoretic sheets were dipped in ninhydrin-cadmium reagent (Liu, Stein, Moore & Elliott, 1965) to locate peptides (spots) and the arginine-containing peptides were identified with Sakaguchi reagent (Canfield & Anfinsen, 1963).

RESULTS

Amino acid composition of F2a1 histones. The amino acid compositions of various F2a1 histones are presented in Table 1. Although the overall composi-

Table 1. *Amino acid composition of the F2a1 histone from different tissues*

The values are expressed as mol of each amino acid/100 mol of amino acids recovered. The amounts of threonine, serine and tyrosine have been corrected for destruction during hydrolysis (Mauritzen *et al.* 1967). The values represent the average of five analyses.

	Human leukaemic lymphoblasts (CCRF-CEM cells)	Novikoff hepatoma	Foetal thymus	Bovine lympho- sarcoma	Calf thymus
Lysine	9.9	10.5	10.1	9.9	9.8
ϵ -N-Methyl-lysine	0.65	0.63	0.77	0.30	0.15
Di- ϵ -N-methyl-lysine	0.35	0.37	0.23	0.60	0.60
Histidine	2.3	2.4	2.3	2.1	2.2
Arginine	13.5	12.6	13.3	13.5	13.0
Aspartic acid	5.3	5.7	5.5	5.5	5.4
Threonine	6.6	6.5	6.8	6.8	6.8
Serine	2.0	2.2	2.5	1.9	2.4
Glutamic acid	6.2	6.9	6.8	6.8	6.7
Proline	1.2	1.6	1.6	1.2	1.0
Glycine	16.5	16.1	16.1	17.0	16.8
Alanine	7.5	7.6	7.5	7.6	7.8
Cystine/2	0.0	0.0	0.0	0.0	0.0
Valine	7.8	7.9	7.6	7.4	7.7
Methionine	0.6	0.8	1.0	1.0	1.0
Isoleucine	5.3	5.0	5.5	5.3	5.3
Leucine	8.2	7.9	8.5	8.2	8.5
Tyrosine	3.0	2.8	2.1	3.6	3.1
Phenylalanine	1.6	2.2	1.8	2.0	2.0
Di- ϵ -N-methyl-lysine/ ϵ -N-methyl-lysine ratio	0.53	0.59	0.30	2.0	4.0
C-Terminal	Glycine	Glycine	Glycine	Glycine	Glycine
N-Terminal	Blocked	Blocked	Blocked	Blocked	Blocked

tions are similar, there are some striking differences in the dimethyl-/monomethyl-lysine ratio. The calculated molecular weight of these histones [based on one residue of methionine (cf. Table 1)] is about 11000.

End-group analysis. The amount of *N*-terminal amino acids, proline (0.001 μ mol), alanine (0.006 μ mol) and glycine (0.002 μ mol) (total amount of amino acids should be 1.25 μ mol) was very low. It was concluded that the major portions (90–93%) of *N*-terminal amino acids were blocked in all five of these histones. Further analyses indicated acetylserine to be the *N*-terminal amino acid in the order: acetylserine–glycine–arginine. The identical amino acids were found at the *N*-terminus of each histone.

Treatment of each histone with carboxypeptidase A for 40 min at 25°C released two residues of glycine. After 24 h digestion, threonine, leucine, tyrosine and phenylalanine were also released. Similar results were obtained in each instance, indicating that the *C*-terminal sequence was: glycine–glycine–CO₂H.

Cyanogen bromide cleavage. The F2a1 histone isolated from human leukaemic lymphoblasts (CCRF-CEM cells) was reacted with cyanogen bromide and the product was analysed by paper chromatography. The product contained two peptides (CNBr-1 and CNBr-2) that separated well upon elution with 0.01 M-hydrochloric acid from a column of Sephadex G-100 (2.5 cm $\times$ 145 cm). Paper and column chromatography revealed that the smaller peptide (CNBr-2) moved faster. The fluorodinitrobenzene and dansyl chloride procedures showed aspartic acid to be the *N*-terminal residue of this peptide. Carboxypeptidase A digestion showed glycine as the *C*-terminal residue of peptide CNBr-2. Thus, this peptide represents the *C*-terminal portion of the F2a1 molecule. Identical results were obtained with the other four histones.

Paper chromatography-electrophoresis of a tryptic hydrolysate of peptide CNBr-2 indicated the presence of five peptides, and ion-exchange chromatography of the tryptic digest on Aminex A-4 with stepwise elution with pyridine–formate buffer, also revealed five peaks. No differences were observed among the histones with respect to the electrophoretic mobilities of these tryptic peptides. Amino acid analyses of the peptide CNBr-2 indicated the presence of the following residues: 1 lysine, 2 arginine, 1 aspartic acid, 1 threonine, 1 glutamic acid, 4 glycine, 1 alanine, 2 valine, 2 leucine, 2 tyrosine, and 1 phenylalanine. Remarkably, peptide CNBr-2 from all five of these histones possessed an identical amino acid composition.

Tryptic hydrolysis. Tryptic hydrolysates of the histones were analysed by 'fingerprint mapping'. All 'maps' indicated the presence of about 30 peptides,

Fig. 1. Diagrammatic peptide 'map' of tryptic hydrolysis of F2a1 histone isolated from human leukaemic lymphoblasts (CCRF-CEM). Identical peptide 'maps' were obtained from F2a1 histones of various sources: bovine lymphosarcoma, Novikoff hepatoma, foetal calf thymus and calf thymus. Ar, Arginine marker; PR, Phenol Red marker; O, origin; E, electrophoresis; C, chromatography.

including brown- and yellow-staining peptides, in each histone and the R_F values and the electrophoretic mobilities of the tryptic peptides were essentially similar (Fig. 1). The number of peptides expected from the lysine and arginine content (Table 1) assuming a molecular weight of 11000 was 28–30.

Chymotryptic hydrolysis. The 'fingerprint maps' of the chymotryptic hydrolysate of these five histones were also similar. Six major peptides from each chymotrypsin-digested histone were obtained, and there was no difference in their electrophoretic mobilities, irrespective of the source of the histone, as indicated by the diagrammatic 'map' (Fig. 2a).

The remainder of the hydrolysate gave five peaks after passage through a column of Sephadex G-25, as indicated by the elution pattern in Fig. 3. The pattern of elution revealed an exact correspondence, and a marked uniformity among these peaks regardless of the source of the F2a1 histone. Each peak was recovered (as indicated by arrows, Fig. 3) and analysed (Table 2). In each instance, amino acid analyses showed that peak E (Fig. 3) contained only the *C*-terminal amino acids and their sequence is Gly-Phe-Gly-Gly-CO₂H. Peak C contained most of the methionine residues and therefore contains an overlapping peptide of CNBr-1 and CNBr-2 peptides from cyanogen bromide-cleaved histones.

Peak D, which appeared to possess a small portion of peak C, also contained some of the amino acids from the C-terminus of the F2a1 molecule. Analysis of peaks A and B indicated that peak A represented a mixture, largely with the components of peak B. Peak B contained ϵ -N-methyl-lysine, the only amino acid residue in the molecule which lies on the N-terminal of the F2a1 molecule, as well as many of the basic amino acids. Peak B represents a large peptide containing approximately 46 amino acids.

The peptide B₂ (Fig. 2b) contains one residue of histidine (only two residues are present in the whole molecule), and a trace of methionine; perhaps due to the fact that the peptides C₆ (Fig. 2c) and B₂ have the same electrophoretic mobilities. Peptide C₆ contains most of the methionine (1 residue), whereas the peptides D₂ and D₃ (Fig. 2d) correspond to peptides 4 and 5 in Fig. 2(a). The peptides D₂ and D₃ contain most of the aromatic amino acids. Peak E represented only one peptide, E₁ (Fig. 2e).

DISCUSSION

Amino acid analyses of F2a1 histones isolated from human leukaemic (CCRF-CEM cells), embryonic (foetal calf thymus), tumour (bovine lymphosarcoma, Novikoff hepatoma), and normal (calf thymus cells) indicate remarkable similarities with respect to their basic and acidic amino acid contents. The N-terminal amino acids are blocked, and glycine is the C-terminal amino acid. Similar observations have been reported with calf thymus and pea F2a1 histones (Phillips, 1963, 1968; DeLange *et al.* 1968, 1969; Sautiere *et al.* 1968), and with the arginine-rich histones isolated from different organs (Hnilica & Bess, 1965; Fambrough & Bonner, 1968). All of these histones contain evenly spaced peptides with basic amino acids which provide similar affinities for binding to DNA, suggesting that these F2a1 histones may exhibit a general function common to all cells; their role in gene regulation being determined by small structural differences, or differential degrees or rates of acetylation and methylation, as suggested by Vidali, Gershey & Allfrey (1968) and Gershey, Vidali & Allfrey (1968).

The remarkable similarities among the F2a1 histones from human leukaemic, embryonic and other neoplastic cells described above suggest that

Fig. 2. Diagrammatic peptide 'map' (a) of a chymotryptic hydrolysate of whole F2a1 histone. The same pattern was observed with chymotryptic hydrolysates of all histones from the different sources. The peptide 'maps' (b), (c), (d) and (e) are from corresponding peaks obtained from Sephadex G-25 (See Fig. 3). Ar, Arginine marker; PR, Phenol Red marker; E, electrophoresis; C, chromatography.

Fig. 3. Elution profile of chymotryptic hydrolysate of F2a1 histone isolated from human leukaemic cells (cf. text), from a column (2.5 cm × 145 cm) of Sephadex G-25, equilibrated and eluted with 0.2M-acetic acid (flow rate of 12.5 ml/h). There were no differences in the elution patterns of the chymotryptic hydrolysates of F2a1 histones isolated from the different sources.

Table 2. Amino acid composition of chymotryptic peaks of F2a1 histones of CCRF-CEM cells obtained from column chromatography on Sephadex G-25.

The values are expressed as mol of each amino acid/100 mol of amino acid recovered.

	Peaks				
	A	B	C	D	E
Lysine	4	9	3	1	0
ε-N-Methyl-lysine	0	1	0	0	0
Histidine	1	1	1	0	0
Arginine	4	6	3	1	0
Aspartic acid	2	4	1	1	0
Threonine	4	3	3	2	0
Serine	1	2	1	0	0
Glutamic acid	3	3	2	2	0
Proline	0	2	0	0	0
Glycine	6	8	3	2	3
Alanine	3	4	2	1	0
Cystine/2	0	0	0	0	0
Valine	4	4	3	1	0
Methionine	0	0	1	0	0
Isoleucine	1	4	1	0	0
Leucine	2	4	2	2	0
Tyrosine	0	1	1	1	0
Phenylalanine	0	0	0	0	1

such small structural differences might affect their role as possible gene regulators. Some structural analyses of various histone species have been reported (DeLange *et al.* 1969; Bustin, Rall, Stellwagen & Cole, 1969). For example, DeLange *et al.* (1969) have demonstrated that the overall structures of the F2a1 histone (or histone IV) of pea and calf thymus are essentially identical, except that the pea histone lacks ε-N-methyl-lysine and

contains one more residue of arginine and isoleucine than does calf thymus histone IV. It is of interest that the only remarkable difference observed among the histones considered above was in the ε-N-monomethyl-lysine/ε-N-dimethyl-lysine ratio, differences which may be of significance with respect to their biological activity.

Other experiments in which the histones considered above were added to exponential-phase cells in culture consistently showed that different species of histones exhibit different degrees of inhibitory activity, a biological function that may be related to the control of gene activity, which reflect differences in affinities between these histones and different recipient cells. In the case of human lymphocytic cells for example, a particular concentration of histone or histone fraction is more inhibitory for its isologous cells as opposed to homologous recipient cells, and indeed, may exhibit a growth-stimulatory effect on certain homologous recipient cells (Desai & Foley, 1970). The biological mechanisms underlying these observations are not yet known, but it is possible that the regulation of gene function by histones may involve something more than simple binding to DNA.

These studies were supported in part by research grants C-6516 from the National Cancer Institute and FR-05526 from the Division of Research Facilities and Resources, National Institutes of Health; the Albert and Mary Lasker Foundation, N.Y.; and the Alvan T. and Viola D. Fuller Cancer Research Unit Grant, American Cancer Society (Mass. Division) Inc. G.E.F holds Research Career Award 1-K6-CA-22,150 from the National Cancer Institute, National Institutes of Health. We thank Dr H. Maeda for the amino acid analyses and Dr J. A. Meienhofer (both of this Foundation) for constructive advice and criticism.

REFERENCES

- Bonner, J. & Ts'o, P. (Eds.) (1964). *The Nucleohistones*, p. 307. San Francisco: Holden-Day Inc.
- Busch, H. (1965). *Histone and Other Nuclear Proteins*, p. 123. New York: Academic Press Inc.
- Bustin, M., Rall, S. C., Stellwagen, R. H. & Cole, R. E. (1969). *Science, N.Y.*, **163**, 391.
- Canfield, R. E. & Anfinsen, C. B. (1963). In *The Proteins*, vol. 1, p. 311. Ed. by Neurath, H. New York: Academic Press Inc.
- Crampton, C. F., Lipschitz, R. & Chargaff, E. (1954). *J. biol. Chem.* **206**, 499.
- DeLange, R. J., Fambrough, D. M., Smith, E. L. & Bonner, J. (1968). *J. biol. Chem.* **243**, 5906.
- DeLange, R. J., Fambrough, D. M., Smith, E. L. & Bonner, J. (1969). *J. biol. Chem.* **244**, 319.
- Desai, L. S. & Foley, G. E. (1970). *Abstr. 10th int. Cancer Cong., Houston*, p. 796.
- Desai, L. S., Ogawa, Y., Mauritzen, C. M., Taylor, C. W. & Starbuck, W. C. (1969). *Biochim. biophys. Acta*, **81**, 146.
- Dophidie, T. A. A., Moore, S. & Stein, W. H. (1967). *J. biol. Chem.* **242**, 1833.
- Fambrough, D. M. & Bonner, J. (1966). *Biochemistry, Easton*, **5**, 2563.
- Fambrough, D. M. & Bonner, J. (1968). *J. biol. Chem.* **243**, 4434.
- Foley, G. E., Lazarus, H., Farber, S., Uzman, B. G. & Adams, R. A. (1967). In *21st Annual Symp. Fundamental Cancer Research: The Proliferation and Spread of Neoplastic Cells, Houston*, pp. 65-97. Baltimore: The Williams and Wilkins Co.
- Foley, G. E., Lazarus, H., Farber, S., Uzman, B. G., Boone, B. A. & McCarthy, R. E. (1965). *Cancer, N.Y.*, **18**, 522.
- Fraenkel-Conrat, H., Harris, T. L. & Levy, A. L. (1955). *Meth. biochem. Analysis*, **2**, 359.
- Fraenkel-Conrat, H. & Tsung, C. M. (1967). In *Methods in Enzymology*, vol. 11, p. 151. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Gershey, L., Vidali, G. & Allfrey, V. G. (1968). *J. biol. Chem.* **243**, 5018.
- Gray, W. R. & Hartley, B. S. (1963). *Biochem. J.* **89**, 379.
- Gross, E. & Witkop, B. (1962). *J. biol. Chem.* **237**, 1836.
- Hnilica, L. S. & Bess, L. G. (1965). *Analyt. Biochem.* **12**, 421.
- Huxley, H. E. & Zubay, G. (1961). *J. biophys. biochem. Cytol.* **11**, 273.
- Johns, E. W. (1964). *Biochem. J.* **92**, 55.
- Johns, E. W. (1967). *Biochem. J.* **105**, 611.
- Katz, A. M., Dreyer, W. J. & Anfinsen, C. B. (1959). *J. biol. Chem.* **234**, 2897.
- Liu, T. Y., Stein, W. H., Moore, S. & Elliott, S. D. (1965). *J. biol. Chem.* **240**, 1143.
- Mauritzen, C. W., Starbuck, W. C., Taylor, C. W., Saroja, I. S. & Busch, H. (1967). *J. biol. Chem.* **242**, 2240.
- Moore, S. & Stein, W. H. (1963). In *Methods in Enzymology*, vol. 6, p. 819. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Phillips, D. M. P. (1958). *Biochem. J.* **68**, 35.
- Phillips, D. M. P. (1963). *Biochem. J.* **87**, 258.
- Phillips, D. M. P. (1968). *Biochem. J.* **107**, 135.
- Sautiere, P., Starbuck, W. C., Roth, C. & Busch, H. (1968). *J. biol. Chem.* **243**, 5899.
- Skalka, K., Rasmussen, P. S. & Luck, J. M. (1964). *Fedn Proc. Fedn Am. Socs exp. Biol.* **23**, 526.
- Spackman, D. H., Stein, W. H. & Moore, S. (1958). *Analyt. Chem.* **30**, 1190.
- Starbuck, W. C., Ogawa, Y., Quagliarotti, J. C., Taylor, C. W., Jordan, T. & Busch, H. (1969). *Fedn Proc. Fedn Am. Socs exp. Biol.* **28**, 2254.
- Stedman, E. & Stedman, E. (1950). *Nature, Lond.*, **166**, 780.
- Vidali, G., Gershey, L. & Allfrey, V. G. (1968). *J. biol. Chem.* **243**, 6361.
- Zubay, G. (1964). In *The Nucleohistones*, p. 95. Ed. by Bonner, J. & Ts'o, P. San Francisco: Holden-Day Inc.