# COMPARISON OF THE DNA REPEAT LENGTH IN $H_1$- AND $H_5$-CONTAINING CHROMATIN

## (Mature hen erythrocytes, immature chick erythroid cells and hen liver)

M. L. WILHELM, A. MAZEN and F. X. WILHELM
*Institut de Biologie Moléculaire et Cellulaire de CNRS, 15, Rue René Descartes, 67084 Strasbourg Cédex, France*

Received 17 May 1977

## 1. Introduction

The variability of the DNA repeat length in chromatin of eukaryotes is now well documented [1–11]. The size of the repeating unit ranges from 154 base pairs in *Aspergillus nidulans* [2] to 241 base pairs in the chromatin of sea urchin sperm [7]. As yet the origin of this phenomenon has not been explained but a few possible causes of the observed variability have been excluded [7–9]; indeed it has been shown that the rate of cell division, the stage in the cell cycle, the genomic activity, the phosphorylation of $H_1$ and the acetylation of histone $H_3$ and $H_4$ could not be correlated to the variation of the repeat length of chromatin. Noll [1] and Morris [2] have suggested that there may be a relationship between the structure of histone $H_1$ (as expressed by the content of basic amino acids) and the length of the DNA repeat in chromatin. As a test of this hypothesis Morris [3] has compared the structure of hen liver and hen erythrocyte chromatin in which a large fraction of the histone $H_1$ is replaced by the lysine rich histone $H_5$; the finding that the repeat length of hen erythrocyte chromatin was longer than that of hen liver chromatin has suggested that there may be a correlation between the increased length of the repeat of hen erythrocyte chromatin and the presence of $H_5$.

In the present work we have compared the DNA repeat length from mature erythroid cells of hen, immature erythroid cells of three-day old chicks [12] and hen liver chromatin. We show that the immature chick erythroid cell chromatin has the same repeat length as the hen liver chromatin, both being smaller than that of adult hen erythrocyte chromatin. Since the erythroid cells of chick contain the histone $H_5$ whereas the hen liver cells do not, our results suggest that there is no direct relationship between the presence of $H_5$ and the increased length of the repeating unit in the hen erythrocytes.

## 2. Materials and methods

Hen liver nuclei were prepared as described by Hewish and Burgoyne [13].

Nuclei of chick and hen erythroid cells were prepared either by the Hewish and Burgoyne procedure [13] or by the method described by Axel et al. [14].

The nuclei were digested with micrococcal nuclease (Worthington) in two ways according to the method of preparation used. When the Hewish and Burgoyne procedure was used the digestion buffer was 15 mM Tris–HCl (pH 7.4), 60 mM KCl, 15 mM NaCL, 15 mM 2-mercaptoethanol, 0.15 mM spermine, 0.5 mM spermidine, 0.3 M sucrose and 1 mM $CaCl_2$ whereas it contained 10 mM Tris–HCl (pH 8.0), 0.25 M sucrose, 1 mM $CaCl_2$ when the Axel et al. procedure was used.

Digestion to determine the DNA repeat was carried out at 37°C until 2–5% of the DNA was rendered acid soluble. Extensive digestion was obtained after 40–50% of the DNA was converted to acid soluble material.

Digestion was stopped by the addition of a five-fold excess of EDTA over the divalent ion concentration. Aliquots were precipitated with 5% perchloric acid to determine the amount of acid soluble material. The resistant fractions of DNA were extensively deproteinized with proteinase K (Merck) in the presence of 0.4% SDS, 0.8 M NaCl followed by an extraction with

 *North-Holland Publishing Company – Amsterdam*

phenol/chloroform/isoamylalcohol (24:24:1,v/v/v). The DNA was then precipitated with two vol. 95% ethanol. Polyacrylamide gels to determine the DNA repeat length contained 2.5% acrylamide and 0.5% agarose. The DNA fragments obtained after extensive micrococcal nuclease digestion were run in a Peacock and Dingman [15] gel system (6% acrylamide). All gels were run in an 12 cm long slab apparatus at room temperature. Acrylamide gels 2.5% were stained with 0.5–1 μg/ml ethidium bromide in running buffer for 3 h, illuminated with ultraviolet light and photographed through an orange filter. 6% acrylamide gels were stained with Stains-All (Eastman) 0.005% in 50% formamide, destained in water and photographed.

## 3. Results

### 3.1. *The DNA repeat length of chick and hen chromatin*

Figure 1 compares the DNA band pattern of immature chick erythroid cells, mature hen erythrocytes and hen liver nuclear chromatin after mild digestion with micrococcal nuclease. The rat liver nuclease digest shown in figure 1 was used to calibrate the size of the DNA fragments.

It appears that the fragments obtained from hen erythrocytes are longer than those of the other chromatin. This difference of length is also shown in fig.2 where a chick chromatin nuclease digest was flanked by two adult hen erythrocyte digests on the same gel slab. In fig.1 a digest of chick erythroid cells is flanked by two digests of hen liver nuclei to demonstrate that there is no difference of migration of the fragment of hen liver and chick erythroid cells chromatin.

Noll and Kornberg [16] have emphasized that a difference in the length of the fragments did not necessarily reflect a difference in the repeat length, since the degradation from the ends can be different in the various chromatins. This called for a method

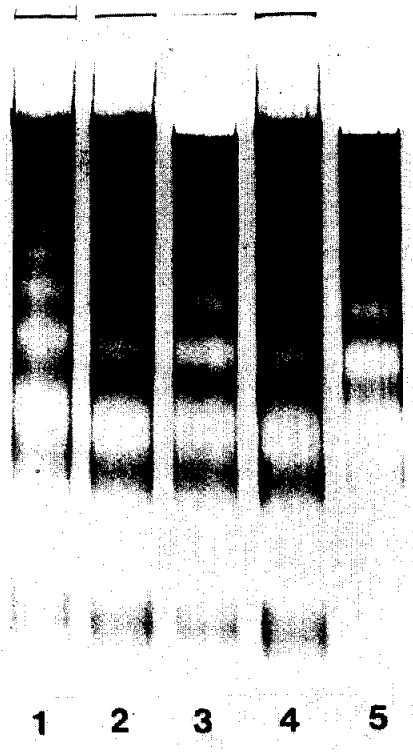


Fig.1. Polyacrylamide gel electrophoresis of micrococcal nuclease digests of chick and hen chromatin: (1) hen erythrocyte, (2) hen liver, (3) chick erythroid cells, (4) hen liver, (5) rat liver.

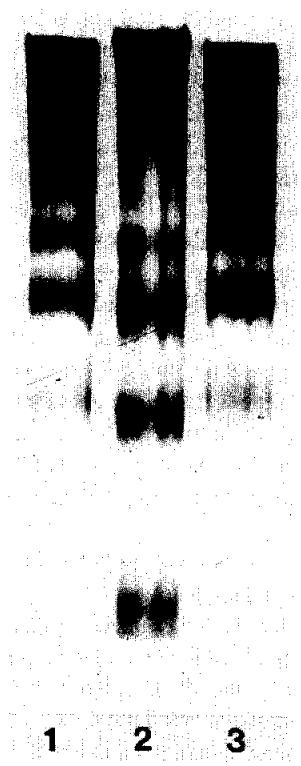


Fig.2. Comparison of the migration of the DNA fragments of hen erythrocytes and chick erythroid cells chromatin: (1) and (3) hen erythrocytes, (2) chick erythroid cells.

Table 1
Sizes of the DNA fragments of chromatins from chick and hen cells

| Band | Rat liver[a] (base pairs) | Hen liver (base pairs) | Chick erythroid cells (base pairs) | Hen erythrocytes (base pairs) |
|---|---|---|---|---|
| 1 | 189 | 180 | 163 | 165 |
| 2 | 377 | 355 | 367 | 390 |
| 3 | 578 | 575 | 595 | 620 |
| 4 | 773 | 780 | 810 | 820 |
| 5 | 982 | 980 | 1010 | 1030 |
| 6 | 1175 | 1180 | 1175 | 1247 |
| Repeat length | | | | |
| | 196 ± 1 | 200 ± 5 | 202 ± 5 | 216 ± 5 |

[a]After Compton et al. [8]

of size determination eliminating the effect of degradation from the ends, which can be achieved by measuring the difference in size between the successive multiples as suggested by Noll and Kornberg [16].

The results of this analysis are given in table 1. It is clear that the DNA repeat length of mature hen erythrocyte is longer (216 ± 5 b. p.) than that of hen liver (200 ± 5 b. p.) and immature chick erythroid cells chromatin (202 ± 5 b. p.). The result for hen erythrocytes and hen liver is in agreement with that of Morris [3]. Four independent polyacrylamide gels have been measured to calculate the standard deviations given in table 1. The same value for the repeat length of immature chick erythroid cells was obtained with two independent samples of blood. We have also compared the repeat length obtained for nuclei prepared in two different ways (see material and methods) and we have not seen any difference according to the mode of preparation (data not shown).

### 3.2. *Extensive micrococcal nuclease digestion of erythroid cell chromatin*

Extensive micrococcal nuclease digestion of chromatin of mature hen erythrocytes and immature chick erythroid cells have been compared on 6% polyacrylamide slab gels (fig.3). We observe a common pattern of DNA fragments smaller than the nucleosome monomer with a major band centered around 135 b. p. Thus the nucleosome core appears to have essentially the same structure in both chromatins and is probably

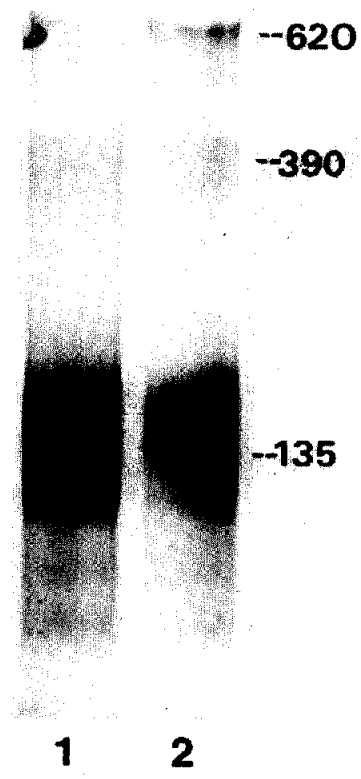


Fig.3. Extensive micrococcal nuclease digestion of (1) chick erythroid cells and (2) hen erythrocytes chromatin.

similar to the cores of all the eukaryotic cells studied up to now [8].

### 3.3. *Histone content of chick and hen nuclei*

The histones of chick and hen cells extracted from the nuclei with 0.25 N HCl were analysed by electrophoresis in SDS-polyacrylamide gels. As seen in fig.4a chick and hen erythroid cells both contain the specific histones $H_5$ whereas the hen liver cells do not. A faint band in the position of $H_5$ appears in the histones of hen liver nuclei and originates probably from a contamination of the hen liver cells by erythrocytes which is very difficult to avoid.

We have made a densitometer tracing of a photographic negative of the stained gel to estimate the amount of $H_5$ in the two erythroid tissues. It appears that the content of $H_5$ is slightly smaller in the nuclei of immature erythroid cells. This is in agreement with a previously observed variation of $H_5$ in erythroid cells [17]. However despite the small decrease in the content of $H_5$ in the chick erythroid cells, this histone remains the predominant lysine rich histone in these cells.

## 4. Discussion

In agreement with the results of Morris [3] and Compton et al. [8] we show that the number of base pairs per nucleosome can vary in two tissues from the same animal. Moreover we have found that it can vary for the same tissue at different stages of maturation.

Morris [3] has argued that the difference in the repeat length of the hen liver and hen erythrocyte chromatin could be related to the replacement of $H_1$ by $H_5$ in the hen erythrocytes. Our results show that it is probably not so simple, since the immature erythroid cells of chicks which do contain histone $H_5$ have the same repeat as the hen liver chromatin.

Extensive micrococcal nuclease digestion has shown that the same nucleosome core was present in all the chromatins; thus the difference in length of the DNA repeat seems to originate from a difference of structure of the DNA that forms the link between two adjacent nucleosomes. There is now evidence that the lysine-rich histone $H_1$ (or $H_5$) is associated with that linker [16] and it has been suggested that the length of the linker could be directly correlated to the number of basic amino-acid residues in the lysine rich histone [1,2].

It is very difficult to accept this assumption for the hen tissue, especially in view of the recent reevaluation of the primary structure of $H_5$ ([18] and

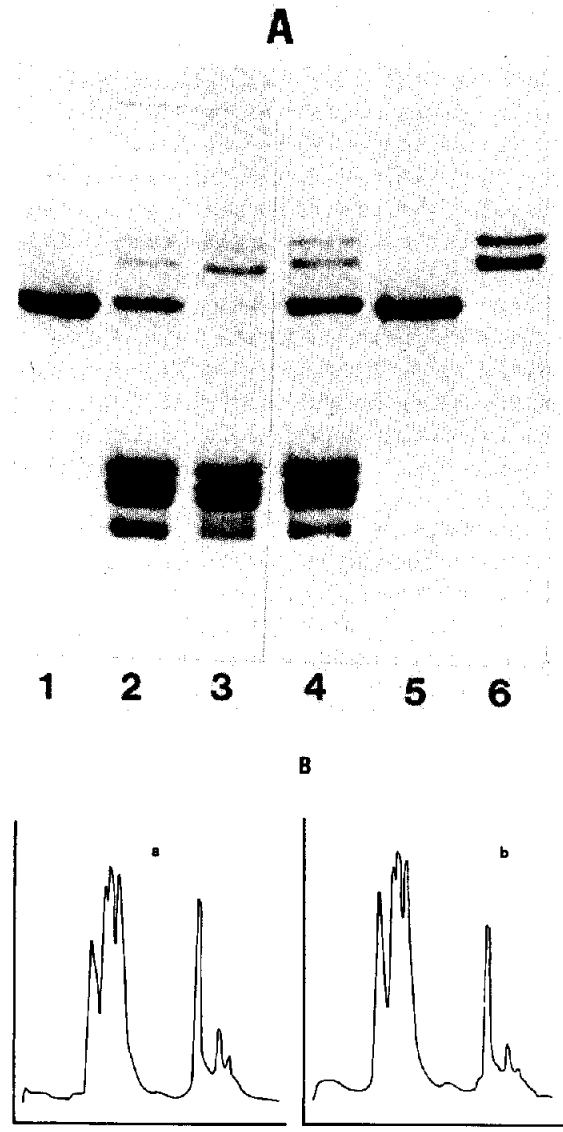


Fig.4. (A) Comparison of the histones of hen erythrocytes, chick erythroid cells and hen liver: (1) $H_5$ of hen erythrocytes, (2) hen erythrocytes histones, (3) hen liver histones, (4) chick erythroid cells histones, (5) $H_5$ of hen erythrocytes, (6) $H_1$ of hen erythrocytes. (B) Densitometer tracing of the histones of hen erythrocytes (a) and chick erythroid cells (b).

P. Sautière, personal communication); indeed it has been shown that $H_5$, which has a length of 185 residues, contains 62–62 lysines + arginines as compared to 60 lysines + arginines for histones $H_1$ [19]. On the other hand, the work of Crane-Robinson et al. [20] and Bradbury et al. [21] has shown that it is probably only the C-terminal half of $H_1$ (residue 100–216) or $H_5$ (residue 94–185) which interacts with the DNA; the comparison of the number of lysines + arginines in these two segments establishes again that there is not a big difference between the two histones: 45 lys + arg in $H_5$ and 44–46 lys + arg in $H_1$.

Finally it is difficult to predict the way $H_1$ or $H_5$ binds to the DNA, since all the basic amino acids are certainly not bound to the phosphate groups of DNA [22].

If, however, the lysine rich histones play a role in the size of the repeat, it is possible that they do not always interact in the same way the DNA depending upon the state of chemical modification of their amino acid side chains; this, for example, could be the case for the histone $H_5$ in chick and hen erythroid cells [23].

In conclusion, our results clearly show that there is no direct relationship between the amino-acid sequence of histone $H_1$ (or $H_5$) and the DNA repeat length of chromatin. We should now look at several other factors (non-histone proteins, chemical modifications of the histones etc.) to try to understand the variability of the DNA length contained in the nucleosome.

**Acknowledgements**

We thank Professor P. Chambon for providing a rat liver digest. This work was supported by grant No. 75.7.0187 from the DGRST.

**References**

[1] Noll, M. (1976) Cell 8, 349–355.
[2] Morris, N. R. (1976) Cell 8, 357–363.
[3] Morris, N. R. (1976) Cell. 9, 627–632.
[4] McGhee, J. D. and Engel, J. D. (1975) Nature 254, 449–450.
[5] Sollner-Webb, B. and Felsenfeld, G. (1975) Biochemistry 14, 2915–2920.
[6] Thomas, J. O. and Furber, V. (1976) FEBS Lett. 66, 274–280.
[7] Spadafora, C., Bellard, M., Compton, J. L. and Chambon, P. (1979) FEBS Lett. 69, 281–285.
[8] Compton, J. L., Bellard, M. and Chambon, P. (1976) Proc. Natl. Acad. Sci. USA 73, 4382–4386.
[9] Lohr, D., Corden, J., Tatchell, K., Kovacic, R. T. and Van Holde, K. E. (1977) Proc. Natl. Acad. Sci. USA 74, 79–83.
[10] Rill, R. L., Nelson, D. A., Oosterhof, D. K. and Hozier, J. C. (1977) Nucleic Acids Res. 4, 771–789.
[11] Johnson, E. M. , Littau, V. C., Allfrey, V. G., Bradbury, E. M. and Matthews, H. R. (1976) Nucleic Acids Res. 3, 3313–3329.
[12] Godet, J. (1970) Expt. Cell.Res. 60, 157–165.
[13] Hewish, D. R. and Burgoyne, L. A. (1973) Biochem. Biophys. Res. Commun. 52, 504–510.
[14] Axel, R., Cedar, H. and Felsenfeld, G. (1973) Proc. Natl. Acad. Sci. USA 70, 2029–2032.
[15] Peacock, A. and Dingman, C. (1967) Biochemistry 6, 1818–1827.
[16] Noll, M. and Kornberg, R. D. (1977) J. Mol. Biol. 109, 393–404.
[17] Mazen, A. and Champagne, M. (1972) Biochemie 54, 1273–1279.
[18] Crane-Robinson, C., Briand, G., Sautière, P. and Champagne, M. (1977) Eur. J. Biochem. in press
[19] Bustin, M. and Cole, D. R. (1968) J. Biol. Chem. 243, 4500–4505.
[20] Crane-Robinson, C., Danby, S. E., Bradbury, E. M., Garel, A., Kovacs, A. M., Champagne, M. and Daune, M. (1976) Eur. J. Biochem. 67, 379–388.
[21] Bradbury, E. M., Cary, P. D., Chapman, G. E., Crane-Robinson, C., Danby, S. E., Rattle, H. W. E., Boublik, M., Palau, J. and Aviles, F. J. (1975) Eur. J. Biochem. 52, 605–613.
[22] Touvet-Poliakow, M. C., Daune, M. P. and Champagne, M. H. (1970) Eur. J. Biochem. 16, 414–423.
[23] Tobin, R. S. and Seligy, V. L. (1975) J. Biol. Chem. 250, 358–364.