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The nucleosome repeat length increases during erythropoiesis in the chick

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ABSTRACT

During erythropoiesis in the chick, the nucleosome repeat length increases from 190 base pairs to 212 base pairs. This increase is correlated with a dramatic increase in the concentration of the red cell specific histone H5 (from 0.2 molecules per nucleosome to 1 molecule per nucleosome) and with no change in the concentration of H1 (1 molecule per nucleosome).

INTRODUCTION

The primitive line of red blood cells first appears in the blood islands of developing chick embryos 35 hours after fertilization. Over the succeeding 5 days, this population develops in the circulation as a homogeneous cohort of maturing erythroid cells. On day 3 of incubation, the cells are largely early polychromatophilic erythroblasts; on day 4, mid-polychromatophilic erythroblasts; and on day 6, immature reticulocytes. Over this period, the cells divide about 6 times, produce large quantities of Hb and globin mRNA, and begin to accumulate a red-cell specific histone, H5 (1-8). After day 6, no further DNA or RNA synthesis occurs and a new population of cells, the definitive series of red cells, enters the circulation, probably arising from the endothelial lining of the blood vessels.

Recently, it has become clear that the repeat distance of nucleosomes along the DNA is not constant. It is different from species to species (9-14) from tissue to tissue (13, 15), and in the case of Stylonychia, the repeat is even different between the macro and micro nuclei (16). The biological meaning of these very gross changes in nuclear organization is not known. Here I show that the repeat length increases during the development of the primitive chick erythrocyte lineage, and that this increase is correlated with an increase in the red cell specific histone, H5.

MATERIALS & METHODS

Cells, Nuclei, and Analysis of DNA Repeat Length

Developing red blood cells were isolated by vein puncture from the circulation of 3-16 day chick embryos as described previously (3). The cells were washed three times in SCC (.14 M NaCl, .01 M Tris-Cl, pH 7.1, .015 M NaCitrate) and then lysed with 0.5% Nonidet P40 in RSB (0.01 M NaCl, 0.005 M $MgCl_2$, 0.01 M Tris-Cl pH 7.4). Nuclei were pelleted by centrifugation and washed free of hemoglobin with RSB. For digestion with staphylococcal nuclease (Worthington), nuclei were usually suspended at a DNA concentration of 1mg/ml in RSB containing 10^{-4} M $CaCl_2$ and 10 µgm/ml staphylococcal nuclease. Digestion was for increasing periods of time at 37°C. The digestion was usually stopped by adding EDTA to 10mM, protease (Aldrich) to 200 µgm/ml, and SDS to 1%. After incubation at 37°C for 2 hours, the sample was mixed with an equal volume of sample buffer (25% glycerol, .005% bromphenol blue, 0.002M sodium acetate, 0.2 mM EDTA, 0.004 M Tris-Cl, pH 7.8), and loaded directly onto 3% acrylamide slab gels (acrylamide:bisacrylamide ratio of 300:8) made up in a running buffer containing 0.02M sodium acetate, 2 mM EDTA, 0.05M Tris-Cl, pH 7.8. Gels were preelectrophoresed for 15 minutes and samples run for about 4 hours at 140V. Gels were stained with 2 µgm/ml ethidium bromide in water. The bands were visualized by u.v. illumination and photographed through a red filter.

Our basic results were not changed if the DNA was isolated by phenol: chloroform extraction and ethanol precipitation, nor if the sample was pretreated with RNase or with proteinase-K. The presence or absence of spermidine (3mM) in our digestion system had no effect on the differences in repeat length observed, nor were these differences lost when the digestion occurred at 4°C instead of 37°C. One potential artifact that was encountered, however, was that the rate of migration increased slightly as the amount of DNA loaded onto the gel increased. As a consequence, most comparisons of repeat lengths were made over a number of DNA concentrations and over a range of concentrations where this effect was minimal (usually 5-15 µgm of DNA per channel).

For the determination of PCA (perchloric acid) soluble material after nuclease treatment, 1/10 volume of 70% perchloric acid was added to a given aliquot and the precipitate centrifuged at 2000 x g for 10 minutes. The amount of material in the supernatant was determined by absorbance at 260 nm. The amount of material remaining acid insoluble

was determined by heating the pellet to 95°C for 30 minutes and measuring the absorbance at 260 nm of the supernatant obtained after low speed centrifugation.

Cell Incubations and Histone Analysis

4 or 5 day erythroblasts were suspended in supplemented (3) F12 medium (GIBCO). Incubations were for 7 hours with L-Leucine-^{3}H (38Ci/mmole, 100 μCi/ml) or L-Lysine-^{3}H (44 Ci/mmole; 100 μCi/ml), (New England Nuclear Corp.)

15% polyacrylamide sodium dodecyl sulfate gels were made using a modification of the procedure of Laemmli (23). The separating gel was made with an acrylamide:bisacrylamide ratio of 300:4 in 0.1% sodium dodecyl sulfate, 0.375 M Tris-Cl pH 8.8. The stacking gel was made 3% polyacrylamide with an acrylamide:bisacrylamide ratio of 300:8 in 0.1% sodium dodecyl sulfate, 0.125 M Tris-Cl pH 6.8. Gels were electrophoresed at 130 V for 6 hrs. using a buffer system of 0.38 M glycine, 0.05 M Tris, pH 8.8 and stained overnight with 0.1% Coomassie brilliant blue in 50% methanol. 10% acetic acid and diffusion destained with 5% methanol, 7% acetic acid. Nuclei were mixed directly with an equal volume of buffer containing 4% SDS, 20% glycerol, 10% mercaptoethanol, 0.001% bromphenol blue, 0.125 M Tris-Cl pH 6.8 (sample buffer) and boiled for one minute prior to loading onto the gel. Histones were eluted from the stained gel by incubating the homogenized gel slice in two successive washes of 0.5% SDS, 5 mM Na-phosphate, pH 6.8 at 37° for 24 hours.

RESULTS AND DISCUSSION

The nucleosome repeat length was compared for erythroblasts of 4-day old chick embryos and their progeny some two generations later at 6 days of development. Nuclei were isolated from both populations and digested with micrococcal nuclease, usually to 8-10% acid solubility. The DNA was then purified and separated on 3% polyacrylamide gels. Fig. 1 shows that 6-day old erythroblasts display a longer repeat than their ancestors. These differences remain for both longer and shorter periods of nuclease digestion (Fig. 1c, d); they are independent of the exact protocol of DNA isolation; they occur when digestion is carried out over a wide range of Mg^{+2} (1mM-10mM), Ca^{+2} (.1mM-10mM), or NaCl (1mM-100mM) concentrations or when digestion occurs at 4°C instead of 37°C. Table 1 summarizes the repeat lengths measured according to the method of Compton et al., (13). As a molecular weight standard a partial digest

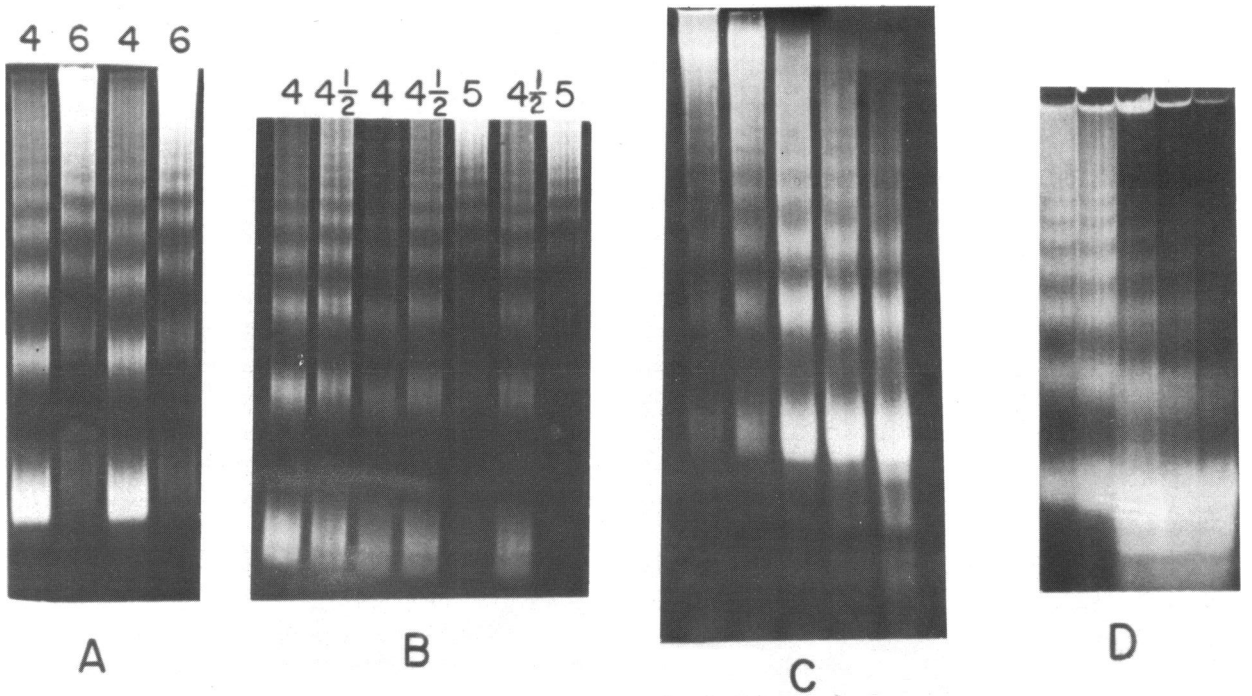

Figure 1
Increase in the nucleosome repeat length during primitive erythropoiesis in the chick embryo.

A) The top line shows the age of the red cells, either 4-day cells or 6-day cells. Nuclei were digested between 10 and 12%.
B) Nuclei were digested to 7-9%. The top line shows the age of the erythroblasts.
C) Time course of nuclear DNA digestion. 6-day cells were incubated with staph nuclease for increasing periods of time. Digestion from left to right: 9%; 16%; 21%; 32%; 38% acid solubility. Similar results have already been reported by Morris (1976b) for adult erythrocytes.
D) Time course of nuclear DNA digestion for 4 day cells.

TABLE 1

CHANGES IN NUCLEOSOME REPEAT LENGTH DURING CHICK EMBRYO ERYTHROPOIESIS

Red Cell Maturity	←Erythroblasts→				←Erythrocytes→				
Day of Development	3	4	5	6	8	10	12	16	Adult
Line of Red Cells	←Primitive→				←Definitive→			←Adult→	
Repeat: (Base pairs)	190±3	190±3	195±3	205±4	207±4		207±4	207±4	212
Moles H1 per Nucleosome		1	1	1				1	0.86
Moles H5 per Nucleosome		0.2	0.4	0.6				0.7	1

The repeat length is calculated as the average difference in base pairs between tetramers and trimers, pentamers and tetramers, etc. up to octamers after 8-10% digestion. The repeat obtained from adult red cell nuclei was used as an absolute standard. Measurements for 3, 4, 5, and 6 day cells are based upon 4 independent determinations yielding a standard deviation of ±3-4 base pairs: 8, 12 and 16 day measurements are based upon 2 determinations. Moles of H1 and H5 per nucleosome are taken from Table 3. Erythroblasts represent cells still actively engaged in RNA and DNA synthesis. Erythrocytes do not produce RNA or DNA.

from adult chicken erythrocytes was used. The repeat length in these cells is known to be 212 b.p. (15).

Table 1 (see also Fig. 1a, b) shows that for 3- and 4-day erythroblasts the repeat length is about 190 b.p.; for 5-day erythroblasts, the repeat increases slightly to about 195 b.p.; and for 6 day cells, the repeat is 205 b.p.; thus as this cohort of cells matures, the average repeat length increases gradually. (The gels could not resolve whether this

increase occurs in discrete steps of less than 6-10 b.p.). Despite the increase in repeat length that occurs as these cells mature, there is no marked increase in DNA band width (Table 2).

A number of investigators have suggested that differences in H1 are responsible for the differences in the repeat length between different tissues and different organisms. H1 is known to encompass as many as 10 primary sequence variants and the red cell specific histone, H5, which is similar but not identical to H1 (17) is sometimes considered still another variant (17, 18). The biological role of H5 during erythropoiesis is not well understood and the possibility that H5 may be responsible for the increase in nucleosome repeat length (15) during red cell maturation should be considered. Fig. 2a shows the nuclear proteins from 4-, 5- and 6-day red cells displayed directly on SDS acrylamide gels. It is clear that relative to the other histones H5 increases gradually over these 3 days of development (see also Table 3 below). During this period there is no apparent decrease in H1; thus, the total amount of H1 and H5 increases markedly. The molar amounts of H1 and H5 were determined using the radioactive labeling procedure previously employed to show that H2a, H2b, H3 and H4 are equimolar in these cells (19) (Fig. 2b). Table 3 shows the total ^{3}H-leucine or ^{3}H-lysine incorporation into H1, H5 and the sum of H2a, H2b, H3 and H4 for 4- and 5-day cells. When this incorporation is divided by the molar number of leucine or lysine residues the relative molar concentration of each histone class can be determined. In these cells, H1 is clearly

TABLE 2

DNA BAND WIDTHS FOR TRIMER AND TETRAMER DNA FRAGMENTS

	Trimers (W 3/4)	Tetramers (W 3/4)
4 day	99 b.p.	110 b.p.
6 day	94 b.p.	108 b.p.
14 day	90 b.p.	104 b.p.

Band widths were obtained from gels similar to those shown in Fig. 1 by tracing the negative with a Joyce Loebl densitometer. W 3/4 is the peak width at 3/4 of the maximum peak height as defined by Lohr et al., (1977). Each value is an average of 4 different experiments from independent digestions.

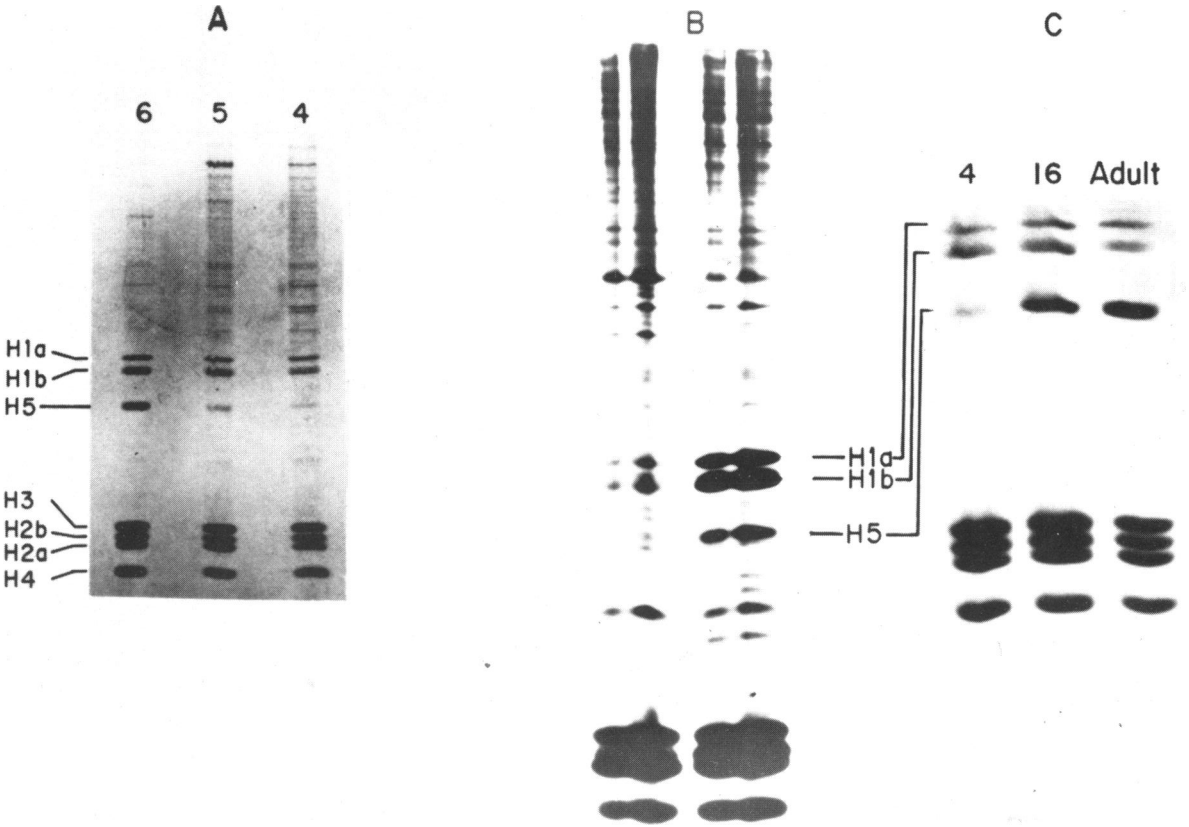

Figure 2

Nuclear proteins during primitive erythropoiesis of the chick. Experimental procedures are described in the legend to Table 2.

A) Coomasie blue stained gel from 4-, 5- and 6-day nuclei.
B) Nuclear proteins from 4-day cells labeled with ^{3}H-leucine (left) or ^{3}H-lysine (right) and visualized by fluorography (21).
C) Coomasie blue stained gel from 4-day, 16-day and adult nuclei.

TABLE 3

MOLAR STOICHIOMETRY OF H1 AND H5 DURING ERYTHROPOIESIS

	H^3-lysine CPM			H^3-leucine CPM			Coomasie Blue (OD600)		
	H1a H1b	H5	H2a H3 H2b H4	H1a H1b	H5	H2a H3 H2b H4	H1a H1b	H5	H2a H3 H2b H4
4 Day Cells	10,999	2,002	21,517	6,734	1,431	60,108	0.33	0.06	1.08
Molar Ratio	1.0	0.2	2.0	1.0	0.2	2.0	1.64	.32	2.0
5 Day Cells	15,008	6,185	28,372	8,898	3,209	74,157	0.40	0.14	1.15
Molar Ratio	1.0	0.4	2.0	1.0	0.37	2.0	1.72	.66	2.0
6 Day Cells	-	-	-	-	-	-	0.37	0.20	1.23
Molar Ratio	-	-	-	-	-	-	1.50	.90	2.0
Molar Ratio Corrected							1.0	0.60	2.0
16 Day Cells	-	-	-	-	-	-	0.33	0.20	1.10
Molar Ratio	-	-	-	-	-	-	1.50	1.0	2.0
Molar Ratio Corrected							1.0	0.7	2.0
Adult Cells	-	-	-	-	-	-	.08	.082	0.30
Molar Ratio							1.30	1.46	2.0
Molar Ratio Corrected							0.86	1.0	2.0

The incorporation of ^{3}H-lysine and H^3-leucine into the various histone classes was determined by homogenizing the gel slice and eluting the protein. For Coomasie blue stained proteins a similar procedure was used, however, the concentration of bound dye was determined optically. For both methods, the molar ratio (relative to the nucleosome) of (H1a and H1b) and of H5 was determined relative to the sum of H2a, H2b, H3 and H4. The calculation was based upon the known molecular weights and the known amino acid composition of each histone (22). Since 6-day cells do not synthesize histone, the radioactivity measurement could not be used. In order to accurately make this determination for 6-day cells, the dye binding characteristics of the histones must be established. For 4- and 5-day cells, it is apparent that H1 and H5 bind about 50% more dye per unit mass than the inner histones assuming that the labeling procedure is more reliable. For 6-day cells, the molar ratio of the histones is corrected for the increased dye binding capacity of H1 and H5.

present in half molar concentrations relative to the inner histones, which are equimolar to each other. Thus, there is an average of 1 H1 and 0.2 H5 molecules per nucleosome for 4-day cells and 1 H1 and 0.4 H5 molecules per nucleosome for 5-day cells.

In order to estimate the concentration of the various histone classes for 6-day cells (in contrast to 3, 4, and 5-day cells, 6-day cells do not synthesize DNA or histones) the Coomasie blue binding characteristics for the different histone classes were determined by normalizing the stoichiometric determination obtained using the stain to that obtained for the labeling procedure in 4- and 5-day cells. This is necessary since H1 and H5 bind more dye per molecule than the other histones (Table 3). For 6-day cells an estimate of 1 H1 and 0.6 H5 molecules per nucleosome is obtained. Thus, Table 3 shows that the concentration of H1 is unchanged between 4 days and 6 days, however, there is a large gradual increase in the amount of H5 over this period. This confirms previous results of Moss et al., (16) and Appels & Wells, (20) for these primitive red cells.

Table 1 and Table 3 also show that mature erythrocytes from a second line of red cells, the definitive line, which gives rise to the circulating cells of 16 day embryos has a repeat of 207 b.p. with H1 present in about 1 copy per nucleosome and H5 present in 0.7 copies per nucleosome. A third line of cells present in the circulation from adult birds has a repeat of 212 b.p. and 0.86 molecules of H1 and 1 molecule of H5 per nucleosome. Thus, the repeat and the H5 content appear to be different not only at various stages in the development of a single red cell lineage, but also in the mature erythrocyte derived from different red cell lineages.

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