CHANGES IN THE TEMPLATE ACTIVITY OF CHROMATIN FROM PIGEON ERYTHROCYTES AND ERYTHROBLASTS INDUCED BY PARTIAL SALT DEPROTEINIZATION

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1. Introduction

It is known that the maturation of avian erythroid cells is accompanied by the inactivation of their genome. The template activity of chromatin, isolated from mature avian erythrocytes is much lower than the template activity of chromatin prepared from avian liver and kidney [1] or calf thymus [2]. It has been suggested that serine-rich histone fraction (F2c or F V) is responsible for this genome repression [3, 4]. One of the arguments for this suggestion is the late appearance of this histone fraction in the course of erythroid cell maturation [3, 5]. This suggestion at the moment appears very probable but it is not yet proved. Seligi and Neelin [4] have studied the template activity of chick erythrocyte chromatin after stepwise acid extraction of lysine-rich and serine-rich histone fractions as described by Murray [6]. They have demonstrated that the removal of serine-rich histone fraction increases chromatin template activity from 1–2% to 40–50% as compared with the template activity of deproteinized DNA. This communication is concerned with the effect of stepwise salt extraction of chromatin proteins upon the template activity of chromatin from two types of pigeon erythroid cells – bone marrow erythroblasts and mature erythrocytes.

2. Methods

Suspensions of immature erythroid cells were obtained from pigeons injected with heavy doses of phenylhydrazine (4 injections daily, 15 mg/kg body weight each). This suspension contained 83% erythroid cells, which were represented by proerythroblasts (7%), basophilic erythroblasts (40%), polychromatophylic erythroblasts (20%), orthochromatic erythroblasts (8%) and erythrocytes (8%). This population of bone marrow cells will be referred to as "erythroblasts" although actually it contained 15–17% of myeloid cells and a certain amount of erythrocytes. It has been shown previously that this cell population synthesizes RNA 10 times more active than the mature erythrocytes of peripheral blood [7].

To prepare nuclei, cells were disrupted by hypotonic shock in 0.001 M Mg-acetate in 0.001 M Tris-HCl (pH 7.3) with three subsequent washings in 0.32 M sucrose containing 0.001 M Mg-acetate and 0.001 M Tris-HCl pH 7.3.

Chromatin was isolated from nuclei as described by Murray et al. [6] by 9 washings of nuclei with 0.14 M NaCl. The pellet of washed nuclei was rinsed several times with deionized water and left to swell in deionized water for 12–14 hr. Then water with washed out salt was removed and swollen chromatin was dispersed in the necessary volume of deionized water to give final DNA conc. of 200–250 μg/ml. Only freshly prepared chromatin preparations were used for the determination of template activity.

Stepwise removal of chromatin proteins was conducted by the extraction with increasing concentrations of NaCl [8]. For this purpose 5 M NaCl was added dropwise to the suspension of dispersed chromatin in 0.14 M NaCl with constant stirring. After further gentle stirring for 12–14 hr suspensions were centrifuged for 6 hr at 160,000 g. Each pellet was

gently suspended using a magnetic stirrer in the initial NaCl solution and again centrifuged for 6 hr at 160,000 *g*. To prevent pellet hardening in the course of centrifugation 1.7 M sucrose solution containing necessary concentration of NaCl was layered to the bottom of centrifuged tubes. The pellets of partially deproteinized chromatin were washed 3–4 times by deionized water, left overnight in water to swell and finally carefully dispersed in deionized water at the DNA conc. 100–200 μg/ml.

Histones were extracted from native and deproteinized chromatin by treatment with 0.2 N HCl (3 times) and fractionated by polyacrylamide gel electrophoresis as described by Johns [9]. DNA was prepared by phenol–detergent procedure [10]. Template activity was determined using *E. coli* RNA-polymerase prepared by modified Babinet isolation procedure [11, 12]. DNA was determined by Burton procedure [13], RNA by orcinol reaction [14] and protein by Lowry method [15].

3. Results

The composition of chromatin prepared from pigeon erythroid cell nuclei is presented in table 1. Fig. 1 presents the data on relative template activity of native chromatin prepared from erythroblasts and erythrocytes under the conditions of saturation with the enzyme. Template activity of chromatin from erythroblasts and erythrocytes corresponds to 12–14 and 2–3% of the template activity of deproteinized DNA, respectively.

Usually chromatin template activity was determined under low ionic strength conditions (μ = 0.05).

Table 1
Chemical analysis of chromatins*.

	Chromatin from	
	Erythroblasts	Erythrocytes
DNA	1	1
RNA	0.21 ± 0.04	0.046 ± 0.002
Total protein	2.28 ± 0.11	1.36 ± 0.05

* Values expressed as mg based on 1.0 mg DNA. Each value is the average value from 6 experiments.

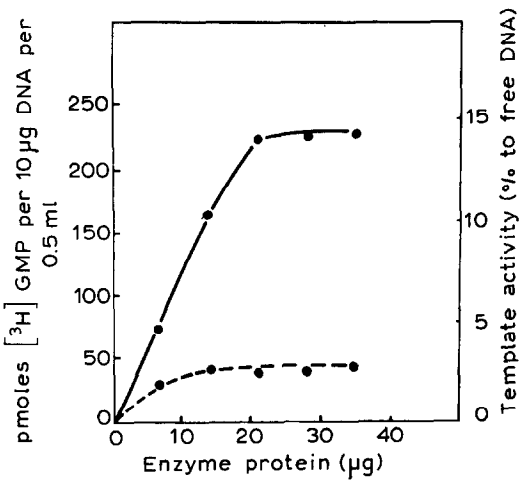

Fig. 1. Template activity of native chromatin prepared from erythroblasts (•–•–•) and erythrocytes (•--•--•). The incubation medium contained: 10 μg of deproteinized DNA, or chromatin (as DNA); 20 μM Tris-HCl (pH 7.9); 25 μM KCl; 1 μM Mg acetate; 0.15 μM $MnCl_2$; 2 μM β-mercaptoethanol; 0.1 μM each of CTP, ATP, UTP; 0.03 μM GTP; 0.4 nM [^{3}H] GTP (1.24 Ci/mM) and indicated amounts of *E. coli* RNA-polymerase (specific activity: 3000 units/mg protein). All in a final volume of 0.5 ml. The assays were incubated at 37° for 20 min after which 0.5 ml cold 10% trichloracetic acid was added, the acid-insoluble precipitates were collected by centrifugation, washed by cold 5% trichloracetic acid (2 times) and by cold absolute alcohol. The radioactivity was counted in gas-flow counter. (Maximal efficiency – approx. 40%).

It has been shown in several reports however, that addition of KCl to the final conc. 0.2–0.25 M in the assay mixture increases chromatin template activity [16].

Results presented in fig. 2 demonstrate that template activity of the chromatin from the erythroid cells depends on the ionic strength of the incubation mixture. Template activity of chromatin preparations from both sources increases with the increase of KCl concentration to 0.3 M in proportion to their initial activity although these chromatins are repressed to different extent. This result is in accordance with the data of De Bellis et al. [16] who have shown that the increase of ionic strength activates hetero- and euchromatin of rat liver to the same extent, that is their template activity in high ionic strength assay is proportional to their initial activity. In all subsequent

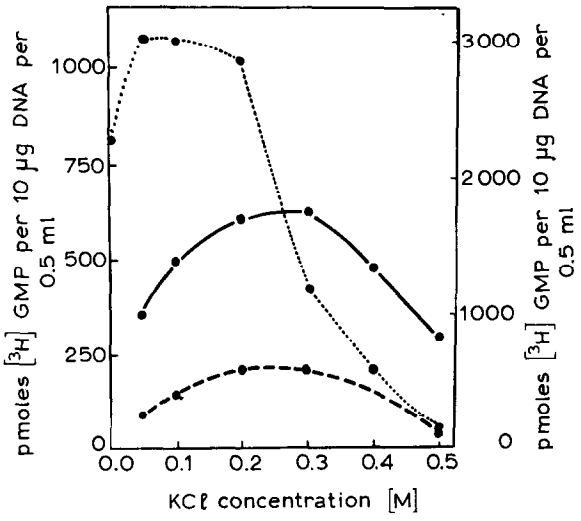

Fig. 2. Effects of KCl on the template activity of deproteinized DNA (•···•···•) and chromatins, prepared from erythroblasts (•—•—•) and erythrocytes (•--•--•). Assay conditions were as described in the legend to fig. 1 except that saturating amount (15 µg) of enzyme was added. Ordinate – incorporation of [^{3}H] GMP (left); chromatins as a template; (right): deproteinized DNA as a template.

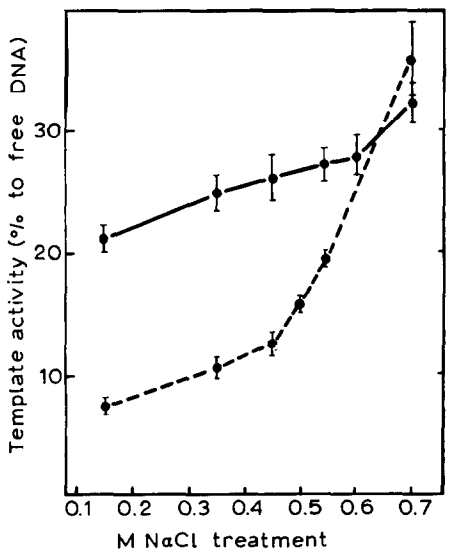

Fig. 3. Effects of NaCl treatment of erythroblasts (•—•—•) and erythrocytes (•--•--•) chromatins on the template activity. Assay conditions were as described in the legend to fig. 1 except that the reaction mixture contained 100 µM KCl and saturating amount (15 µg) of enzyme. Each point is the average value from 7 experiments (error bars represent standard deviation).

experiments determinations of template activity were conducted in the presence of 0.2 M KCl.

Polyacrylamide gel electrophoresis of acid soluble proteins prepared from native and partially deproteinized chromatin of pigeon erythroblasts and erythrocytes confirmed the results obtained earlier by other authors [5, 17].

Treatment with 0.55 M NaCl extracts F1 and probably a certain amount of F2 C histone from erythrocyte chromatin [3, 8, 17]. The latter histone (F2 C) is completely extracted by 0.7 M NaCl. After two extractions of erythroblast chromatin with 0.55 M NaCl and even 0.6 M NaCl a certain amount of protein (presumably F2 C) remains in chromatin preparations. Its presence is probably due to the contamination of the cell population by mature erythrocytes (see above). These trace amounts are also removed by extraction with 0.7 M NaCl.

Results presented in fig. 3 demonstrate the change of chromatin template activity after such treatments. In case of erythroblast chromatin template activity is increased gradually, as protein is removed by increasing NaCl concentrations. This is in agreement with the results of Smart and Bonner [18] who have shown that the increase of template activity of pea chromatin partially dehistonized by deoxycholate or NaCl treatment increases linearly in proportion to the amount of histone removed. In case of mature erythrocytes however no such linearity is observed in the course of stepwise deproteinization (fig. 3).

It is seen from the presented data that the curve showing the dependence of template activity on NaCl concentration used for protein removal has two distinct regions. A certain increase in the template activity is observed at low NaCl concentrations before 0.45 M. In this ionic strength region the increase of template activity of erythrocyte chromatin parallels to its increase in case of erythroblast chromatin. Probably this rise is due to the removal of trace amount of F1 histone from erythrocyte chromatin. In the range of NaCl concentrations 0.45–0.7 M the increase of template activity is more sharp and the difference between erythroblast and erythrocyte chromatin disappears. This is just that range of salt concentrations where the removal of F2 C histone from erythrocyte chromatin takes place.

These results enable one to consider the problem of involvement of different proteins extracted by NaCl in the inactivation of genome in the course of erythrocyte maturation. Under these conditions besides F1 and F2C histones a certain amount of non-histone proteins is removed from the chromatin. It has been shown that salt extracted non-histone proteins of chromatin do not restrict DNA transcription when added to RNA-polymerase reaction [19]. Moreover they can stimulate RNA synthesis [19] and neutralize the inhibitory action of histones [20]. In this connection it is improbable that template derepression observed in our experiments is connected with the removal of non-histone proteins.

The results obtained in this work provide an experimental verification for the suggestion that F2C histone fraction may be regarded as a factor responsible for additional genome inactivation at terminal stages of avian erythropoiesis. It is difficult to judge however, whether all loci active in erythroblasts are repressed by F2C histone or not. Taking into account the heterogeneity of this fraction [21] this seems possible "in principle".

It should be noted however that such interpretation of the results requires additional studies aimed to answer the question whether partial deproteinization activates just those loci which were inactivated in the course of erythrocyte maturation.

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