PIOTR CHOMCZYŃSKI, ANNA DRAGON and LECH ZWIERZCHOWSKI

HISTONE AND NON-HISTONE PROTEINS FROM RABBIT MAMMARY GLAND AT LATE PREGNANCY AND EARLY LACTATION*

Department of Biochemistry, Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jasłoźbiec, 05-551 Mroów, Poland

The content of non-histone protein in epithelial cell chromatin from rabbit mammary gland increased during late pregnancy and early lactation up to 30%, while the histone content remained unchanged. No qualitative changes, as judged by polyacrylamide-gel electrophoresis, were found during accumulation of non-histone proteins at the onset of lactation. The chromatin isolated at lactation, assayed either by polylysine or Toluidine Blue binding, contained slightly more available DNA-phosphate than the chromatin isolated at pregnancy.

Modifications of chromatin structure and changes in histone and non-histone protein components of chromatin during proliferation and differentiation of animal cells have been described by numerous workers (for reviews see LeStourgeon et al., 1974; Bradbury, 1975). In the present work, an attempt was made to demonstrate these changes in mammary epithelial cell chromatin during transition of mammary gland from non-secretory to secretory state. Accessibility of chromatin to Toluidine Blue and polylysine as well as the content and composition of histone and non-histone protein were determined.

MATERIALS AND METHODS

Reagents. The chemicals were purchased from the following sources: collagenase and deoxyribonuclease from Worthington (Freehold, N.J., U.S.A.); polylysine (poly-l-lysine, polymerization degree 77) from Miles Laboratories (Elkhart, Ind., U.S.A.); phenylmethylsulphonylfluoride and Toluidine Blue from Sigma (St. Louis, Mo., U.S.A.); calf thymus histone and bovine serum albumin from P.O.Ch. (Gląb, Poland).

* This work was supported by the Polish Academy of Sciences within the project 09.7.1

[23]
Great Popielno-White rabbits (6-8 months old) in their first pregnancy or first lactation were used.

Rabbits were killed by cervical dislocation. Mammary glands were excised, minced with scissors and treated with collagenase according to Topper et al. (1975). Mammary epithelial cells obtained by this treatment were homogenized and nuclei were isolated with the use of 1.8 M-sucrose, as described previously (Chomczyński & Topper, 1974).

Isolation of chromatin. Pure nuclei were extracted successively: once with 0.4% Triton X-100/0.25 M-sucrose/50 mM-Tris/HCl, pH 7.8/25 mM-KCl/5 mM-MgCl₂; three times with 75 mM-NaCl/24 mM-EDTA, pH 7; and twice with 5 mM-Tris/HCl, pH 8. All solutions contained 0.1 mM-phenylmethylsulphonylfluoride. After the extractions the nuclear pellet was suspended in water (pH 7) and gently shaken overnight. To remove aggregates, the obtained chromatin gel was centrifuged at 12,000 g for 20 min. The E₂₅₀/E₂₆₀ ratio of the final chromatin preparation was lower than 0.1 and the E₂₆₀/E₂₈₀ ratio was close to 1.7. The DNA content in chromatin was calculated from the value of E₂₆₀₀ assuming that E₁% is 220.

Binding of polylysine. A chromatin solution (20 - 30 µg of DNA/ml) was titrated with a polylysine solution, 0.75 mM in terms of lysine residues. At each titration point the reaction mixture was incubated at 4°C for 15 min, centrifuged at 12,000 g for 15 min and E₂₆₀ of the supernatant was measured. The amount of chromatin precipitated by polylysine was calculated from the difference in E₂₆₀ between the supernatant obtained and the initial chromatin solution (Simpson & Polacow, 1973).

Binding of Toluidine Blue. The procedure described by Izhaki (1971) was used. Chromatin (40 - 70 µg DNA/ml) was shaken with Toluidine Blue (final concentration 25 µM) at 4°C for 30 min. Insoluble dye-chromatin aggregates were removed by centrifugation, and E₆₅₀ of the supernatant was measured. The amounts of the dye bound to DNA phosphate in chromatin was calculated from the difference in E₆₅₀ between the control sample (no chromatin added) and the sample containing chromatin. Corrections were made for binding of Toluidine Blue to chromatin proteins which were determined after digestion of chromatin by deoxyribonuclease. The corrections were 8% and 16%, respectively, of the amount of the dye bound to the non-digested chromatin from pregnant and lactating rabbits.

Determination of histone and non-histone protein content. Chromatin (50 µg DNA/ml) was extracted twice with 10 vol. of 0.2 M-H₂SO₄ at 0°C for 1 h. The content of histone in the extract and of the non-histone protein in the residue were determined by the method of Lowry et al. (1951). Total calf thymus histones and bovine serum albumin were used as standards for histones and non-histone proteins, respectively.

Isolation of phenol-soluble non-histone proteins for gel electrophoresis. The procedure was based on the method of Teng et al. (1971) with slight modifications described by LeStourgeon et al. (1973). Chromatin was extracted twice with 0.25 M-HCl, twice with a chloroform/methanol solution (first 2:1, v/v and then 1:1, v/v) containing 0.25 M-HCl, and washed with ethyl ether. The chromatin residue was suspended in 0.1 M-Tris/HCl buffer, pH 8.2, containing 10 mM-EDTA and 0.14 M-2-mercaptoethanol. An equal volume of phenol previously saturated with the
above buffer solution was added to the chromatin suspension and the resulting emulsion was allowed to stand overnight at 4°C. After centrifugation the clear phenol phase was dialysed against 10 mM-phosphate buffer, pH 7.2, containing 0.14 M-2-mercaptoethanol, 0.1% sodium dodecyl sulphate and 0.25 M-sucrose.

Electrophoresis of proteins. Electrophoresis of histones was performed in 7% acrylamide gel with 0.9 M-acetic acid and 2.5 M-urea at pH 2.5, according to the method of Panyim & Chalkley (1969). Samples of histones (100 μg of protein) obtained by acid extraction were subjected to electrophoresis in 7.5×0.9 cm gels at 2.5 mA per gel.

Electrophoresis of non-histone proteins was performed in 8.75% acrylamide gel with 0.1% sodium dodecyl sulphate, according to Laemmli (1970). Samples (100 μg of protein) were applied to 9×0.9 cm gels, and electrophoresis was run at 0.5 mA per gel.

The gels were stained with 1% Amido Black solution in acetic acid/methanol/water (1:1:8, by vol.), destained with acetic acid/methanol/water (1:3:6, by vol.), then scanned at 600 nm in a Carl Zeiss (Jena) densitometer.

RESULTS

Polylysine and Toluidine Blue binding. Polylysine and Toluidine Blue binding were used to evaluate structural changes in the DNA-protein complexes (Itzhaki, 1971; Clark & Fenselinfeld, 1971) in epithelial cell chromatin of rabbit mammary gland on day 24 of pregnancy (P chromatin) and day 7 of lactation (L chromatin). The differences in the binding ability of the two chromatin preparations were slight and statistically insignificant (Table 1) both with polylysine and Toluidine Blue. In the experiments on polylysine and Toluidine Blue binding to deproteinized DNA, no differences were observed in the binding ability between the DNA isolated at pregnancy and at lactation. The ratio of lysine residues or Toluidine Blue bound to DNA phosphate was 1:1 within experimental error (±5%).

Table 1

Binding of polylysine and Toluidine Blue to the mammary gland epithelial chromatin

The results are mean values from nine chromatin preparations ± S.E.M. Chromatin from pregnant (P) and lactating (L) rabbits was used.

<table>
<thead>
<tr>
<th>Chromatin</th>
<th>Polylysine bound</th>
<th>Toluidine Blue bound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmole/μmole of DNA phosphate</td>
<td></td>
</tr>
<tr>
<td>P chromatin</td>
<td>0.43±0.05</td>
<td>0.47±0.03</td>
</tr>
<tr>
<td>L chromatin</td>
<td>0.49±0.04</td>
<td>0.52±0.04</td>
</tr>
</tbody>
</table>
Histone and non-histone protein contents in the mammary gland epithelial chromatin

The results are mean values from eight chromatin preparations ± S.E.M. Chromatin from pregnant (P) and lactating (L) rabbits was used.

<table>
<thead>
<tr>
<th>Chromatin</th>
<th>Histone</th>
<th>Non-histone proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/mg of DNA</td>
<td></td>
</tr>
<tr>
<td>P chromatin</td>
<td>1.20±0.10</td>
<td>0.87±0.09</td>
</tr>
<tr>
<td>L chromatin</td>
<td>1.16±0.09</td>
<td>1.13±0.08</td>
</tr>
</tbody>
</table>

Histone and non-histone proteins. The content of histones in chromatins studied was practically unchanged on transition from pregnancy to lactation, whereas the content of non-histone proteins in L chromatin was by about 30% higher than in P chromatin (Table 2). The resolution patterns obtained by polyacrylamide-gel electrophoresis were identical for the non-histone proteins from P and L chromatin (Fig. 1) which indicates that the increase in the non-histone protein content was not accompanied by binding of new protein species to deoxyribonucleoprotein. The resolution pattern of the rabbit liver non-histone proteins included in Fig. 1, differed from that obtained for the mammary gland non-histone proteins.

Electrophoresis of histones showed no differences between the samples isolated from P and L chromatin. The lack of changes in the histone content and composition in the mammary gland at pregnancy and lactation is in agreement with an earlier raport by Stellawagen & Cole (1968).

Fig. 1. Densitogram of electrophoretic separation of non-histone proteins isolated from the mammary gland epithelial chromatin at pregnancy (P) and lactation (L), and from liver (Lv). On each gel, 100 µg of protein was applied and electrophoresis was carried out as described in the Materials and Methods section.
DISCUSSION

Barker & Ludwick (1974) reported that in vitro the template activity of the chromatin isolated from rat mammary gland increased considerably during late pregnancy and early lactation. This is consistent with the RNA synthesis in vivo in mouse mammary gland (Banerjee et al., 1971; Barankiewicz et al., 1975) and with the RNA synthesis in vitro in the nuclei isolated from rabbit mammary gland (Chomczyński et al., 1974). The period of late pregnancy and early lactation seems to be the most important phase of the mammary gland development, especially as concerns activation of RNA synthesis. The mechanism of changes in chromatin template activity during activation of transcription in eukaryotic cells has not so far been fully elucidated. The general scheme involving condensed and extended segments in chromatin (the latter are thought to be preferentially transcribed) is widely accepted. Assayed by polylysine binding, the condensed segments have less available DNA phosphate than the extended segments (Simpson & Polacow, 1973). We have found that the amount of polylysine-available DNA phosphate in mammary epithelial chromatin does not change significantly during activation of the RNA synthesis at early lactation, and the most evident modification of mammary epithelial chromatin is the increase in the non-histone protein content observed at lactation. The non-histone protein fraction is supposed to contain specific activators of gene transcription (Stein et al., 1974). In agreement with this hypothesis, the observed increase in non-histone proteins in the chromatin from the 7-day lactating mammary gland epithelial cells may be related to the activation of RNA synthesis. In most cases, quantitative changes in non-histone proteins are accompanied by qualitative changes, as judged by polyacrylamide-gel electrophoresis (LeStourgeon et al., 1974). However, we have found no changes in the non-histone protein composition during accumulation of these proteins in the mammary epithelial chromatin at the onset of lactation. On the other hand, Kadohama & Turkington (1973) have reported that epithelial chromatin isolated from lactating mammary gland contains a different set of non-histone proteins as compared with that from virgin mammary gland. In view of these findings it may be suggested that changes in the non-histone protein composition occur at early- or mid-pregnancy.

REFERENCES

22, 11-18.

HISTONY I BIAŁKA NIEHISTONOWE GRUCZÓŁU MLECZNEGO KRÓLKA
W OKRESIE PÓŻNEJ CIĄŻY I WCZESNEJ LAKTACJI

Streszczenie

Zawartość białek niehistonowych w chromatynie komórek nablonkowych gruczołu mlecznego królika jest wyższa o 30% w 7 dniu laktacji w porównaniu z 24 dniem ciąży, podczas gdy zawartość histonów w chromatynie nie ulega zmianie. Stosując elektroforezę w żelu poliacrylamidowym stwierdzono, że wykazanemu wzrostowi zawartości białek niehistonowych nie towarzyszą zmiany jakościowe tej frakcji. Przeprowadzając wiązanie polilizyny i błektu toluidynowego do chromatyny wykazano, że ilość wolnych grup fosforanowych DNA wzrasta nieznacznie w okresie laktacji.

Received 12 May, 1977.