Cleavage of ATP by Mouse Uterine Chromatin after in vivo Administration of Oestradiol

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ATPase assay of the chromatin: Sonicated chromatin was incubated with 1 nm (50 nCi) 8-14C-ATP, to measure RNA synthesis in the template assay, we asked whether ATP itself might be attacked by chromatin-inherent enzymes under the conditions of the template assay. This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

Materials and Methods

Treatment of mice, chromat preparation and quantitation were performed as described in detail elsewhere.

ATPase assay of the chromatin: Sonicated chromatin was incubated with 1 nm (50 nCi) 8-14C-ATP, to measure RNA synthesis in the template assay, we asked whether ATP itself might be attacked by chromatin-inherent enzymes under the conditions of the template assay. 

Recently we found that the ability of mouse and rat uterine chromatin to act as template for in vitro RNA synthesis, using E. coli RNA polymerase, is inversely related to the ribonuclcease activity of the same chromatin. Intraperitoneal administration of oestradiol to mice decreases the apparent template activity compared with that of control chromatin. In rats, however, oestradiol increases the activity. Relatively high template activities are correlated with relatively low RNase activities, and vice versa. These observations suggested than an assay of the template activity of the chromatin may, therefore, not give a quantitative measurement of the sites free for transcription, hence of gene activity, as commonly assumed.

Using 14C-ATP to measure RNA synthesis in the template assay, we asked whether ATP itself might be attacked by chromatin-inherent enzymes under the conditions of the template assay. We now report that mouse uterine chromatin is indeed capable to degrade ATP to ADP, AMP, adenosine, inorganic phosphate and other still unknown phosphorylated byproducts has been demonstrated over a wide range of conditions of the template assay. The dependence of the reactions on the time course of incubation, on the chromatin quantity and on the temperature was studied. In vivo administration of oestradiol depresses the apparent ATPase activity of mouse uterine chromatin consistently by 10−35 per cent, depending on chromatin preparation, amount and incubation period. The data are compatible with the suggestion that mouse uterine chromatin may contain low quantities of ATPases which are firmly bound to the chromatin structure and which might interfere with the template assay under average conditions, depending on the chromatin preparation. The findings are discussed in view of the possible role of ATPases in uterine chromatin under oestradiol influence.

Results

All mouse uterine chromatin degrade 8-14C-ATP to some extent under the conditions of the template activity assay, i.e., using similar ATP and chromatin, as tested in vitro.

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tin quantities, buffer and incubation conditions. According to the time course of degradation (Fig. 1 a) first ADP, then AMP and adenosine are formed by chromatin from oestradiol-treated mice, whereas chromatin from untreated mice first produces ADP and AMP at about the same rate, and then adenosine (Fig. 1 b). Comparing the ATP metabolism reveals that in vivo hormone treatment reduces the apparent ATPase activity of the chromatin. Although the depression by about 20 per cent seems small and varies considerably between chromatin preparations, a difference has been consistently found with all chromatins tested (Table I).

Table I. The effect of intraperitoneal administration of oestradiol on the apparent ATPase activity of mouse uterine chromatin, as tested in vitro on several preparations.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Chromatin quantity (µg of chr.-DNA)</th>
<th>Incubation period (min)</th>
<th>Nanomol of ATP found (+)</th>
<th>Percent degradation after incubation inhibition with chromatin (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-14C-ATP</td>
<td>2</td>
<td>10</td>
<td>0.36</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>0.38</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>30</td>
<td>0.71</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>30</td>
<td>0.85</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>30</td>
<td>0.43</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>30</td>
<td>0.46</td>
<td>0.38</td>
</tr>
<tr>
<td>γ-32P-ATP</td>
<td>6</td>
<td>10</td>
<td>1.10</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>30</td>
<td>0.86</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>30</td>
<td>0.55</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>30</td>
<td>0.28</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Fig. 1. The time course of 8-14C-ATP degradation following incubation with 10 µg of DNA in the form of (a) chromatin from oestradiol-treated (+) and (b) untreated mice (—). Ordinate: Percentage of radioactivity input (50 nCi = 1 nM ATP). A = adenosine.

Fig. 2. The dependence of 8-14C-ATP degradation on the quantity of (a) chromatin from oestradiol-treated (+) and (b) untreated mice (—) following incubation for 30 min. Abscissa: Quantity of DNA in the form of chromatin; ordinate as in legend to Fig. 1.

Fig. 3. The time course of γ-32P-ATP degradation after incubation with 16 µg of DNA in the form of (a) chromatin from oestradiol-treated (+) and (b) untreated mice (—). I and II: Unidentified phosphates (see Fig. 4). Ordinate: Percentage of radioactivity input (4 µCi = 1.25 nM ATP).
ATP to give ADP and P\textsubscript{i} could be best demonstrated by the release of \textsuperscript{32}P\textsubscript{i} from \textsuperscript{32}P-ATP. According to Fig. 3 both chromatins rapidly degrade \textsuperscript{32}P-ATP to P\textsubscript{i} and other still unidentified products which are shown in Fig. 4. Again oestradiol causes a small but consistent reduction by about 15 per cent of the apparent ATPase activity of mouse uterine chromatin. It should be noted that the attack, apparently by nuclear enzymes, of \textsuperscript{32}P-ATP is more rapid than that of \textsuperscript{8}14C-ATP. Moreover, \textsuperscript{32}P-ATP is not degraded by incubation at 0 °C for 30 min, but \textsuperscript{8}14C-ATP is by about 13 per cent. This suggests the involvement of several chromosomal enzymes in the process of ATP metabolism. The dependence on the chromatin quantity of \textsuperscript{32}P-ATP degradation is shown in Fig. 5. Oestradiol reduces

![Fig. 4. Radioscan of a thin-layer chromatogram showing the labelled products of the reaction of \textsuperscript{32}P-ATP with chromatin from untreated mice. Incubation of 16 \mu g of chromatin-DNA for 30 min at 37 °C. I—III: Unidentified phosphates. Abscissa: Migration distance, start (S) to front (F): 17 cm.](image)

the capacity of mouse uterine chromatin to produce P\textsubscript{i} from \textsuperscript{32}P-ATP by approximately 35 per cent, using a concentration of 15.5 \mu g of chromatin-DNA per 60 \mu l of incubation mixture for a period of 30 min. Interestingly, hormone treatment increases the concentration of the unknown \textsuperscript{32}P-labelled by-products. Control experiments again demonstrated that ATP degradation and P\textsubscript{i} formation are due to the action of chromatin in the incubation mixture.

The temperature dependence of the apparent chromosomal ATPase activity is shown in Fig. 6. Again the hormone effect in this experiment agrees with the general observation that less P\textsubscript{i} is released from \textsuperscript{32}P ATP by chromatin from oestradiol-treated than by chromatin from untreated mice.

![Fig. 6. The temperature dependence of \textsuperscript{32}P-ATP degradation following the reaction with chromatin from oestradiol-treated (+) and untreated (−) mice. Incubation of 16 \mu g of chromatin DNA for 30 min at 37 °C. Ordinate as in Fig. 3.](image)

**Discussion**

Mouse uterine chromatin, prepared according to Marushige and Bonner \textsuperscript{6}, as modified by Teng and Hamilton \textsuperscript{7}, contains RNAse activity which may interfere with RNA synthesis in an in vitro assay of the template activity \textsuperscript{1, 2}. The studies reported here demonstrate that mouse uterine chromatin may, in addition, be capable to degrade ATP which is used in the template assay. Consequently, the question should be raised to which extent the apparent ATPase activity of mouse uterine chromatin might also interfere with the template test. It appears from Figs. 1 and 2 that under the average template assay conditions (about 1 \mu g of chromatin DNA, 37 °C, 10 min) less than 10 per cent of the input \textsuperscript{8}14C-ATP will be degraded by this particular chromatin preparation. However, the data of Table I suggest that other chromatin preparations may well reveal higher apparent ATPase activities. Since all chromatins were prepared by identical methods, it appears that the differences, from experiment to experiment, are mainly due to biological variations of the animal conditions. It should be noted that 'hormone' and 'control' chromatins were prepared simul-
taneously from the same strain of inbred mice, born on the same day and ovariectomized on the same day. But the experiments were repeated on other days with other populations of inbred mice of same age. Consequently, even standardized procedures cannot guarantee constant preparations. Moreover, other chromatin preparation procedures may well yield chromatins with different capacities to cleave ATP.

The experiments reported here do not characterize the molecular nature of the ATP hydrolysis by mouse uterine chromatin. The aim of the studies was only to find out whether mouse uterine chromatin is able, under the template assay conditions, to degrade ATP and to which extent and whether there exists an oestrogen influence on the ATP hydrolysis. The tested properties of the ATP cleavage are, however, compatible with the suggestion that it may be due to the action of nuclear ATPases. These must be firmly bound to the chromatin, since they endure the many washings of the chromatin purification procedure, which agrees with previous findings by SiEBERT\textsuperscript{8} on the close binding of nucleoside triphosphatases to mammalian nuclei.

The question may be raised whether the apparent ATPase activity of the isolated chromatin is also present in situ in the chromatin or may have been artificially attached to the chromatin by the preparation technique. Although the reported studies do not permit a decision, there is evidence for an intranuclear localization of ATPases\textsuperscript{8}. A similar question may be whether the oestradiol effect is related to real differences in the chromatin content of the ATPases. Again, a conclusive answer cannot be given. Yet, KARMAKAR and SPECTOR\textsuperscript{9} found a decrease of ATPase activity in microsomal and mitochondrial fractions in the uteri of oestradiol-treated rats, with the greatest drop in activity in the microsomal fraction (up to 45 per cent). The used chromatin preparation procedure effectively removes membrane particles, thus a main intracellular source of ATPases. But it cannot exclude the possibility that minute amounts of cytoplasmic phosphohydrolases are still preserved. – To detect the ATP hydrolysis by the small chromatin quantities, only the described radiochemical method was found to be sensitive enough, whereas enzymic methods\textsuperscript{10,11} were not.

At present we can only speculate as to the role of the apparent ATPase activity changes in the uterine chromatin due to hormone treatment. One of the earliest effects of oestradiol is an increase of water and sodium influx into the oestrous uterus and an increase of synthetic capacities of the chromatin, termed gene activity, accompanied by nuclear swelling. Since there is evidence to suggest that Na\textsuperscript{+}, K\textsuperscript{+}-stimulated ATPase is an essential mechanism by which cells maintain a low concentration of sodium\textsuperscript{12}, it appears that a decrease of ATPase activity should allow an increase of sodium influx into the chromatin. ATPase would thus be one of the few enzymes whose activity is depressed by oestradiol in contrast to the many stimulated by the hormone.

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