Role of karyoplasm in the emergence of capacity of egg cytoplasm to induce DNA synthesis in transplanted sperm nuclei

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SUMMARY

The behaviour of sperm nuclei was studied both in the cytoplasm of intact toad oocytes undergoing maturation and the cytoplasm of oocytes matured without germinal vesicles. The behaviour of the nuclei of pronase-treated sperm injected in the mature egg cytoplasm was shown to be exactly similar to that of the sperm nucleus after fertilization, i.e. they swelled, synthesized DNA, and divided. No changes in such sperm nuclei could be detected in the cytoplasm of the oocytes matured without germinal vesicles.

INTRODUCTION

In recent years Gurdon has extensively studied the mechanism of the cytoplasmic control of nuclear activity (Gurdon, 1967, 1968, 1969). One aspect of this problem is the cytoplasmic control of nuclear division and DNA synthesis. According to Gurdon 'DNA synthesis factor' appears during oocyte maturation, after passage of karyoplasm into the cytoplasm (Gurdon, 1967, 1968). The question of involvement of the karyoplasm in the origin of this factor remains open. We have attempted to answer it by studying the behaviour of the sperm nuclei in the cytoplasm of oocytes matured in the absence of karyoplasm, and in the cytoplasm of intact oocytes at successive stages of maturation.

MATERIALS AND METHODS

A piece of toad (Bufo viridis) ovary was excised and transferred into amphibian Ringer solution. Separate oocytes were detached from it with the aid of forceps. Germinal vesicles were removed from some oocytes by the method of Dettlaff, Nikitina & Stroeva (1964). The enucleated and the intact oocytes were cultivated in Ringer solution with progesterone (5 μg/ml) at 18–20 °C. Sperm of the common frog (Rana temporaria) treated with pronase (Koch Light Lab.) were used as a source of nuclei (Skoblina, 1974). Common frog sperm were used

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owing to the absence of toad males; frog oocytes are not suitable for such experiments as they do not tolerate microsurgery well enough. Some intact oocytes were transferred every 3 h to Ringer solution without progesterone and simultaneously injected with a sperm suspension treated with pronase, and with [H³]thymidine (0.04 μCi/oocyte, specific activity 22.4 Ci/m mole, Radiochemical Centre, Amersham). In 24–27 h the sperm suspension and thymidine were also injected into the oocytes which were maturing without germinal vesicles. To know exactly the stage of maturation at which the injections were made, 10–30 intact oocytes maturing under the same conditions were fixed in San-Felice fluid before each injection. Injected oocytes were fixed 2, 3, and 4 h after the injection of [H³]thymidine. Serial sections 6 μm thick were prepared. Some sections were treated with 1 mg/ml RNA-ase solution in phosphate buffer, pH 7.5 at 37 °C. Radioautographs were obtained as usual. The Feulgen reaction was carried out prior to the coating of sections with emulsion and after development they were counterstained with light green. From the oocytes fixed for stage determination, 243 were sectioned; the sperm suspension and [H³]thymidine were injected into 340 oocytes of three toad females, and in 300 oocytes the sperm nuclei were found.

RESULTS

Examination of sections showed that the number of sperm injected into an oocyte varied from 2 to 20. All the data refer to the behaviour of sperm nuclei in the animal cytoplasm. The transplanted nuclei in the same egg are sometimes at different stages of transformation; the tables include data on the most ‘advanced’ nuclei.

Behaviour of sperm nuclei in the cytoplasm of intact mature eggs and oocytes matured after the germinal vesicle removal

Most sperm nuclei were markedly swollen in the cytoplasm of intact mature eggs 2 h after injection (Fig. 1A). At the same time the first labelled mitoses, sometimes multipolar, occurred. Four hours after injection, swelling and dividing sperm nuclei were observed in practically all intact mature eggs (Table 1). All of them proved to be labelled (Figs. 2, 3). The label was retained when the sections were pretreated by RNA-ase prior to coating with emulsion. In none of the oocytes matured after germinal vesicle removal was there either swelling or labelling of the transplanted nuclei (Fig. 4, Table 1). Cytasters, occasionally numerous, were visible in the cytoplasm of these oocytes.

Behaviour of sperm nuclei injected into the cytoplasm of intact oocytes at successive stages of maturation

When the sperm suspension and [H³]thymidine were injected into the oocyte cytoplasm within the first 6 h of progesterone-induced maturation (from the stage of intact germinal vesicle to prometaphase of the first maturation division (I))
Figs. 1–4. Sperm nuclei in the toad egg cytoplasm (Figs. 1, 2, 3) and in the cytoplasm of oocytes matured without germinal vesicle (Fig. 4), 2 h after injection: 1, slide without emulsion, 2–4, radioautographs.

Table 1. *Behaviour of sperm nuclei in the cytoplasm of eggs and oocytes matured in vitro without germinal vesicle*

<table>
<thead>
<tr>
<th>Recipients</th>
<th>State of oocyte nucleus at the moment of injection</th>
<th>Total number of oocytes containing sperm nuclei</th>
<th>Oocytes in which sperm nuclei:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Remained intact</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Eggs Enucleated oocytes</td>
<td>Metaphase II (15)*</td>
<td>66</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>52</td>
<td>52</td>
</tr>
</tbody>
</table>

* In this table and the next the number of oocytes examined for stage determination is indicated in parentheses.
Table 2. Behaviour of sperm nuclei in the cytoplasm of toad oocytes at successive stages of maturation

<table>
<thead>
<tr>
<th>Duration of cultivation in Ringer with progesterone before injection (h)</th>
<th>State of oocyte nucleus at the moment of injection</th>
<th>State of oocyte nucleus at the moment of fixation</th>
<th>Total number of oocytes containing sperm nuclei</th>
<th>Oocytes in which sperm nuclei:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>0</td>
<td>Intact GV (25)*</td>
<td>Intact GV (21)</td>
<td>21</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>GV at the surface (20)</td>
<td>GV at the surface (3), prometaphase I† (15)</td>
<td>28</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>Prometaphase I (10)</td>
<td>Prometaphase I (7), polar body I (3), prometaphase II‡ (1)</td>
<td>21</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>Prometaphase I (3), prometaphase II (1)</td>
<td>Polar body I (4), prometaphase II (29)</td>
<td>20</td>
<td>85</td>
</tr>
<tr>
<td>12</td>
<td>Polar body II (2), prometaphase II (6)</td>
<td>Prometaphase II (10)</td>
<td>19</td>
<td>43</td>
</tr>
<tr>
<td>15</td>
<td>Metaphase II (9)</td>
<td>Metaphase II (27)</td>
<td>41</td>
<td>11</td>
</tr>
<tr>
<td>18</td>
<td>Metaphase II (8)</td>
<td>Metaphase II (10)</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>21</td>
<td>Metaphase II (7)</td>
<td>Metaphase II (7)</td>
<td>25</td>
<td>36</td>
</tr>
</tbody>
</table>

* GV, germinal vesicle. † I, first maturation division. ‡ II, second maturation division.

no marked changes occurred in the nuclei, and they did not incorporate [H³]thymidine (Table 2). With injection of sperm suspension and [H³]thymidine 9 h after transfer of oocytes to the progesterone solution, the first swelling and labelled nuclei appeared only 4 h after injection. Only prometaphase and metaphase II (second division) were found in the oocytes at this time. The behaviour of the sperm nuclei injected into oocytes 12 h after the beginning of hormonal treatment and later, was similar to that in the intact mature eggs (Table 2). With a single exception all swelling and dividing nuclei were labelled (Table 2). With injection of the sperm suspension 12 h, 15 h, 18 h or 21 h after the beginning of
DISCUSSION

The transplantation of pronase-treated sperm from the common frog into intact mature eggs of the toad showed that sperm nuclei swelled, synthesized DNA, and began dividing. DNA synthesis was first detected at the metaphase II stage. DNA synthesis in brain nuclei transplanted into maturing *Xenopus laevis* oocytes was observed for the first time by Gurdon (1967). The stage of oocyte maturation at which DNA synthesis began was not mentioned, but it appeared to be the second maturation division (nuclei were transplanted at metaphase I stage, their transformation was observed 90 min later). Ziegler & Masui (1973) observed the swelling of brain nuclei (which, according to Gurdon, is a prerequisite of the beginning of DNA synthesis) only in mature *Xenopus* eggs after their activation. Hence, DNA synthesis in sperm and somatic nuclei transplanted into maturing oocytes appears to have begun at a similar stage of maturation.

The absence of changes in the sperm nuclei in the oocytes matured without the germinal vesicle, showed that the karyoplasm is indispensable for the cytoplasm to acquire the capacity to induce both the transformation of sperm nuclei into pronuclei and DNA synthesis in them. But since there exists a considerable time gap between the germinal vesicle breakdown and the beginning of nuclear transformation, it is highly probable that certain processes occurring in the cytoplasm after its mixing with the karyoplasm during maturation are essential for the appearance of 'DNA synthesis factor'.

One of the candidates for the role of 'DNA synthesis factor' was DNA polymerase (Gurdon & Speight, 1969; Gurdon & Woodland, 1969; Grippo & LoScavo, 1972). Grippo and his co-authors have determined the activity of DNA polymerase in extracts from intact primary and mature oocytes and oocytes matured without the germinal vesicle, and have shown that a new DNA polymerase appears in the oocytes during maturation and that it is absent both in primary oocytes and those matured without germinal vesicle (Grippo *et al.* 1974). But Ford and Woodland have recently calculated that the rise in DNA polymerase activity per cell occurring during oocyte maturation is insufficient to account for the difference in DNA synthesis between these two cell types. They have suggested therefore that factors other than DNA polymerases are involved in the control of DNA synthesis during oocyte maturation (Ford & Woodland, 1975).
REFERENCES


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