Progesterone-induced in vitro maturation in oocytes of *Notophthalmus viridescens* (Amphibia Urodela) and some observations on cytological aspects of maturation

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**SUMMARY**

Maturation in vitro of oocytes of the newt, *Notophthalmus viridescens*, is inducible with progesterone after in vivo treatment of females with gonadotropin; few oocytes mature in vitro in the absence of such gonadotropin treatment. Chromosomes of most large oocytes of animals not receiving gonadotropin are still in the lambrush condition; chromosomes from gonadotropin-treated animals are shorter and the lateral loops are less profuse and somewhat retracted. The chromosome condition, then, can be correlated with susceptibility to progesterone induction of maturation in vitro. As maturation progresses, the germinal vesicle moves toward the surface and decreases in size, with an apparent loss of nuclear material from the centripetal end. Although lateral loops of most chromosomes disappear during the changes in the germinal vesicle, profuse loops develop during this period at the sphere loci, which were previously devoid of loops.

**INTRODUCTION**

The cytological changes associated with amphibian oocyte maturation have been the object of many studies, ranging back into the nineteenth century. Until the development of in vitro methods of inducing maturation, however, investigators were handicapped in obtaining oocytes in certain phases, and the whole process was somewhat unpredictable. Recently, the effectiveness of progesterone for inducing in vitro maturation has been demonstrated for oocytes of the anuran amphibians *Rana pipiens* (Wright, 1960, 1961; Masui, 1967; Schuetz, 1967; Smith, Ecker & Subtelny, 1968; Subtelny, Smith & Ecker, 1968; Smith & Ecker, 1969; Schuetz, 1971), *Xenopus laevis* (Horton, 1969; Brachet, Hanocq & Van Gansen, 1970; Serafin, 1970; Merriam, 1971; Thornton, 1971; Schorderet-Slatkine, 1972), *Bufo bufo* (Thornton & Evennett, 1969) and

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Discoglossus pictus (Bedate, Fraile, Lopez-Gordo & Calle, 1971). However, with the exception of preliminary reports by us (Barsacchi & Humphries, 1970; Barsacchi, 1971), reports of attempts to induce in vitro maturation with progesterone in urodele amphibians have been lacking. Indeed, studies of in vitro maturation in urodeles, using other methods, have been few, and little success has been reported (Nadamitsu, 1953; Lee & Humphries, 1961; Brachet et al. 1970). In view of the extensive work that has been done on the chromosomes, multiple nucleoli, and general meiotic phenomena in urodele oocytes (see, for references, Gall, 1954; Humphries, 1956; Callan, 1966; Miller & Beatty, 1969; Barsacchi, Bussotti & Mancino, 1970; Macgregor, 1972) and the potential usefulness of in vitro procedures in furthering such work, we undertook to develop an effective method for the oocytes of the urodele Notophthalmus viridescens. We present here our experience in the development of a method, together with the results of studies of certain aspects of the cytology of maturation.

MATERIALS AND METHODS

Adult female Notophthalmus viridescens were collected in North Carolina during the autumn or spring and used immediately or after storage in a refrigerator at 12 °C for periods ranging up to several months. Initially, ovaries were removed from females that had received no hormonal pretreatment. However, little success was obtained with in vitro maturation in these ovaries; therefore most females were pretreated. Pretreatment consisted of intraperitoneal injections of 50-75 units of chorionic gonadotropin (Antuitrin ‘S’, Parke-Davis) on alternate days until a maximum of 300 units had been administered. If egg deposition occurred prior to the administration of the maximum dose, pretreatment was stopped and the ovaries were removed for in vitro procedures. Ovaries removed from animals were dissected in Ringer’s solution. The largest oocytes were removed individually, with follicular layers intact, and placed in Ringer’s solution containing progesterone in concentrations of 0-2, 1, 2, 10 or 50 μg/ml. Usual concentrations were 2 or 10 μg/ml. Control oocytes were placed in Ringer’s solution. Periods of treatment ranged between 1 h and 2 h, but an exposure of 1 h was generally used. After exposure to progesterone, oocytes were washed briefly and returned to Ringer’s solution.

In experiments in which germinal vesicles were isolated from oocytes, the isolated nuclei were transferred into Ringer’s or into a medium made up of 0·1 m-KCl and 0·1 m-NaCl in a 5:1 mixture containing 0·001 m-CaCl₂. For experiments designed to test the effectiveness of progesterone on isolated nuclei or nuclear contents, progesterone was added to give a concentration of 10 μg/ml. Two sets of experiments were done. In the first set, females were pretreated with Antuitrin as usual. Nuclei and nuclear contents were then isolated from full-grown oocytes and incubated for 1 h or continuously in the above mentioned solutions containing progesterone. At the same time, full-
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grown oocytes were removed and incubated intact for 1 h in Ringer's containing progesterone as controls. In the second set of experiments, full-grown oocytes from pretreated females were incubated for 1 h in Ringer's with progesterone (10 μg/ml), then transferred to Ringer's. At this time and at 2 and 4 h after return of the oocytes to Ringer's, nuclei and nuclear contents were isolated and placed in one or the other of the salt solutions mentioned above. Full-grown intact oocytes of the same group served as controls. Study of chromosomes removed from living oocytes was done following the methods of Gall (1954) and Callan & Lloyd (1960), using an inverted phase-contrast microscope.

For observations on preserved oocytes, material was fixed in Bouin's picric-formol or by freeze-substitution and embedded in Tissuemat. Serial sections of 10 μm thickness were cut and were stained with Harris's acid hematoxylin and fast green.

RESULTS

In vitro induction of maturation

Progesterone treatment of oocytes taken from animals not previously treated with gonadotropin seldom resulted in maturation. In experiments using oocytes from six animals, only 9 out of 75 oocytes matured after a 1 h exposure to progesterone and only 1 oocyte matured out of 84 continuously incubated in progesterone. All of these 10 oocytes had been taken from animals recently collected from nature during the breeding season (Table 1). In contrast, pretreatment of animals with gonadotropin, followed by treatment of the isolated oocytes with progesterone and subsequent incubation in Ringer's solution, resulted in maturation in 89% (169 of 189) of the oocytes so treated (Table 2). As expected, following pretreatment with gonadotropin, some control oocytes (not exposed to progesterone in vitro) also matured. Incubation of oocytes in progesterone for 1 h, followed by return to ordinary Ringer's, was more effective than continuous incubation in progesterone (Table 3). In this experiment, all oocytes treated for 1 h matured, while only about half of the continuously treated oocytes matured. When using pretreated animals, maturation was induced by all tested concentrations of progesterone, and exposure times as short as 1 min were effective. Maturation was ordinarily not accompanied by ovulation, but in some animals ovulation did occur (cf. Table 3). The first maturation spindle usually appeared at about 12 h after initiation of progesterone treatment, although the times varied for different oocytes, even in the same ovary. Maturation to metaphase II was complete at about 20 h.

When nuclei or nuclear contents were isolated and exposed to progesterone, or when nuclei were removed from oocytes treated with progesterone and incubated in salt solutions, no maturational changes were observed. Intact oocytes taken from the same animals and treated with progesterone matured as usual.
Table 1. In vitro induction of oocyte maturation by progesterone without previous gonadotropin treatment of the females\textsuperscript{‡}

<table>
<thead>
<tr>
<th>Female designation</th>
<th>Oocytes incubated in progesterone for 1 h</th>
<th>Oocytes incubated continuously in progesterone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number incubated</td>
<td>Number matured</td>
</tr>
<tr>
<td>Q\textsuperscript{*}</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>R\textsuperscript{*}</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>S\textsuperscript{†}</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>T\textsuperscript{*}</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>U\textsuperscript{†}</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>V\textsuperscript{*}</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Totals</td>
<td>75</td>
<td>9</td>
</tr>
</tbody>
</table>

* Females recently collected in nature.
† Females previously kept in the laboratory at 12 °C.
‡ Full-grown oocytes were incubated in Ringer's containing progesterone at a concentration of 10 μg/ml for 1 h, then kept in Ringer's.

Table 2. In vitro induction of maturation by progesterone in oocytes taken from animals pretreated with gonadotropin\textsuperscript{*}

<table>
<thead>
<tr>
<th>Female</th>
<th>Progesterone-treated oocytes</th>
<th>Control oocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number treated</td>
<td>Number matured</td>
</tr>
<tr>
<td>E</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>G</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>O</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>P</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
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<td>6</td>
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<td>9</td>
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<td>5'</td>
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<tr>
<td>6'</td>
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<td>9</td>
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<td>9</td>
</tr>
<tr>
<td>10</td>
<td>29</td>
<td>26</td>
</tr>
<tr>
<td>12</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Totals</td>
<td>189</td>
<td>169</td>
</tr>
</tbody>
</table>

* Females, either recently collected in nature or kept in the laboratory at 12 °C, were treated with a series of injections totalling 200 or 300 i.u. of Antuitrin over a period of 7 days. Full-grown oocytes were incubated in Ringer's containing progesterone at concentrations of 0·2 μg/ml (♀♀ 10 and 12), 2 μg/ml (♀♀ 4, 5, 6 and 9) and 10 μg/ml (♀♀ G, O, P, 5', 6'). Concentrations between 1 and 50 μg/ml were tested for ♀ E. Time of incubation was 1 h, followed by Ringer's. Control oocytes were incubated in Ringer's.
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Table 3. In vitro induction of oocyte maturation by limited or continuous exposure to progesterone*

<table>
<thead>
<tr>
<th>Female</th>
<th>Oocytes incubated in progesterone for 1 h</th>
<th>Oocytes incubated continuously in progesterone</th>
<th>Control oocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number incubated</td>
<td>Number matured</td>
<td>Number incubated</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>5†</td>
<td>4</td>
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<td>2</td>
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<td>10</td>
<td>9</td>
</tr>
<tr>
<td>N</td>
<td>—</td>
<td>—</td>
<td>15</td>
</tr>
<tr>
<td>Totals</td>
<td>33</td>
<td>33</td>
<td>44</td>
</tr>
</tbody>
</table>

* Females had been kept in the laboratory at 12 °C. Treated with a total 200 or 300 i.u. of Antuitrin in a series of injections over 7 days. Full-grown oocytes were incubated in Ringer's containing progesterone at a concentration of 10 μg/ml. Control oocytes were incubated in Ringer's.

† Maturation was accompanied by ovulation.

The oocytes during maturation

Observations on fixed and sectioned oocytes, on intact living oocytes, and on isolated nuclei and nuclear contents of living oocytes allow a general description of some of the changes that occur during maturation. The most obvious early change was the movement of the germinal vesicle toward the surface of the animal pole, leaving behind a conical transparent area, free of yolk (Fig. 1A). During the period of migration and later, after the more superficial position was reached, the germinal vesicle underwent a gradual decrease in size. Nuclei at the superficial location varied in diameter from 340 to 170 μm. A membrane was still present about these nuclei, and was separable with fine forceps in a dissection of the oocytes (Fig. 1B). During the changes mentioned, the membrane appeared to enclose completely the nuclear contents, but it is not possible to say that such membranes are without breaks. The decrease in volume, along with the appearance of the clear, conical, 'tail', suggest that material moves from the germinal vesicle. The isolated nuclear content of these small germinal vesicles, observed under phase contrast, revealed condensed chromosomes surrounded by a compact group of spheroid bodies of uncertain nature. Only a few typical nucleoli and spheres were evident in these germinal vesicles (Fig. 1C). At this stage the nuclear contents were very stiff and did not disperse in the medium ordinarily used for dispersion and study of lampbrush chromosomes. In some oocytes, spindle formation appeared to commence near or upon the nuclear membrane (Fig. 1D). At breakdown of the germinal vesicle, fragments of nuclear membrane could sometimes be seen surrounding the clear area previously occupied by the vesicle. Observations on the condensation of chromosomes and their alignment on the first
meiotic metaphase spindle revealed that there is some lack of synchrony in both processes (Figs. 2A, B). The emission of the first polar body and the formation of the second meiotic spindle occurred as previously described (Humphries, 1956) (Figs. 2C, D).

The changes occurring in the lampbrush chromosomes, as observed by the methods of Gall (1954) and Callan & Lloyd (1960) require special consideration. The largest oocytes taken from animals not pretreated with gonadotropin contained chromosomes in the typical lampbrush condition, while those from pretreated animals had chromosomes in a more shortened condition, with fewer and less well developed lateral loops (Figs. 3A, 4A). As maturation proceeded, the loops continued to retract, and both chromosomes and nucleoli became concentrated in the center of the germinal vesicle. The nucleoli were spherical, and appeared to contain large ‘vacuoles’ (Figs. 3B–D). Gall (1954) described the location of spheres at subterminal positions on lampbrush chromosomes 10 and 5. Through use of his maps and descriptions it was possible to identify the chromosomes with precision (Figs. 3A, 4A). We observed, in some oocytes undergoing maturation, loops expanding from the chromosome sites where the spheres were attached (Fig. 4B). In other oocytes, apparently in a more advanced stage of maturation, the chromosomes lacked spheres and extensive loops expanded from the sphere loci (Fig. 4C). Detached spheres were occasionally seen near the chromosomes, sometimes associated with the loops at the sphere loci (cf. Fig. 4D). In many of the maturing oocytes one or two large spheres, with a central ‘vacuole’ containing a globule, appeared free in the nuclear sap; they sometimes showed rounded protuberances. Chromosome 7 carries the nucleolus-organizing region (Gall, 1954). During our studies we noted that nucleoli were also inserted in an intercalary position on the short arm of one of the longest bivalents, probably number 11. This may be, then, another nucleolus-organizer chromosome.

**FIGURE 1**

Photomicrographs of oocytes of *Notophthalmus viridescens* during induced *in vitro* maturation. The oocytes were incubated for 1 h in Ringer's solution containing progesterone (10 μg/ml), then transferred to Ringer's. (A, B, D) Sections of 10 μm. Fast green stain. (C) Phase-contrast photomicrograph. (A) 3 h from the beginning of incubation. The germinal vesicle is at the animal pole. Lampbrush chromosomes and nucleoli are in the center of the nucleus. × 155. (B) 5 h from the beginning of incubation. The germinal vesicle has decreased in size; a nuclear membrane is still present. × 300. (C) 7 h from the beginning of incubation. Nuclear content isolated from a germinal vesicle of about 170 μm in diameter. The chromosomes are condensed and surrounded by many spheroidal bodies. × 400. (D) 9 h from the beginning of incubation. The spindle initiates to form near the nuclear membrane. × 475.
DISCUSSION

Our results show that in Notophthalmus oocytes the in vitro induction of maturation is more readily achieved when the females are treated previously with gonadotropin. A similar conclusion can be drawn from experiments carried out in other species of newts (Batistoni & Barsacchi, 1974). In contrast, the experience of investigators using anurans (loc. cit.) shows that gonadotropin pretreatment is not necessary for induction of in vitro maturation of a high proportion of large oocytes in these species. The contrast may be interpreted to mean that the large oocytes in the anuran species are in a different state of readiness from those in the newt species tested, a state which might be related to the different reproductive biology of the two groups. That is, in the newt species in which in vitro maturation experiments have been done, the eggs normally mature and are released a few at a time over an extended period, while in the anuran species a large number of eggs are released within a few hours.

Difference in responsiveness to progesterone in vitro seems to be correlated with the condition of the chromosomes within the germinal vesicle. Our cytological observations show that the chromosomes of full-grown oocytes from untreated newts, in the great majority, are in a distinctly different condition from those in animals treated with gonadotropin. Chromosomes similar in morphology to those observed in pretreated females have also been described in a few full-grown oocytes from newt females captured in nature shortly before egg deposition (Mancino & Barsacchi, 1966). Inspection of large oocytes of unstimulated Xenopus laevis in our laboratory has shown that the chromosomal condition in these is similar to that of oocytes taken from gonadotropin-treated newts. Müller (1974) has also found that in Xenopus the best-developed lampbrush chromosomes are obtained from oocytes with a diameter about half the size of a mature oocyte, since loops are already contracted in more advanced stages. Judging from the statement of Smith & Ecker (1969), the situation in Rana pipiens is similar to that in Xenopus, and in contrast to that in Notoph-

**Figure 2**
Photomicrographs of oocytes of Notophthalmus viridescens during induced in vitro maturation. The oocytes were incubated for 1 h in Ringer's solution containing progesterone (10 μg/ml), then transferred to Ringer's. Sections of 10 μm. Fast green stain.

(A) 12 h from the beginning of incubation. Early 1st meiotic metaphase. The bivalents show different degrees of condensation. × 685.

(B) 12 h from the beginning of incubation. The first meiotic spindle is forming. The alignment of the bivalents on the metaphase plate is not synchronous. × 685.

(C) 20 h from the beginning of incubation. 1st polar body. × 600.

(D) 20 h from the beginning of incubation. 2nd meiotic spindle. × 600.
In vitro maturation of oocytes in Notophthalmus. Similar observations have been made in other anurans (Duryee, 1950; Srivastava & Bhatnagar, 1962; Morescalchi, 1965; Morescalchi & Filosa, 1965; Giorgi & Galleni, 1972). At a speculative level one may hypothesize that the chromosomal condition of the oocytes is an expression or a consequence of different hormonal conditions in the examined anuran and urodele species, which in turn is related to a different biology of reproduction. Although an ‘advanced’ chromosomal condition seems to be correlated with readiness of oocytes to enter into the final stages of maturation, it would be premature to suggest a causal relationship, especially since the chromosomal condition is probably only one of several modifications of oocytes responsive to progesterone in vitro. There may well be changes in the cytoplasm and at the oocyte surface which are also associated with the attainment of responsiveness.

Except for the apparent need for prior treatment with gonadotropin, induction of in vitro maturation in Notophthalmus viridescens is similar to the process described for Rana pipiens and other anurans. Concentration of progesterone, time of exposure, general external and internal changes, and the usual lack of ovulation of matured oocytes agree largely with descriptions for anurans. In the newt ovary, however, there are far fewer large oocytes available, thus the total number of eggs matured per ovary is small, compared to the number of anurans. The percentage of large oocytes maturing, however, is similar.

The changes in the germinal vesicle as it moves toward the surface in maturation have been described and discussed previously by Brachet et al. (1970), particularly for Xenopus laevis. The work of these investigators, as well as our own, seems to show clearly that material from the germinal vesicle begins entering the cytoplasm early in the maturation process, and continues to pass into the cytoplasm during movement of the nucleus to the surface. We have found that even the small germinal vesicle at the egg surface seems to be surrounded by a membrane, but our observations, coupled with those of Brachet et al. (1970), make it appear unlikely that the nuclear envelope acts, during maturation, as a barrier to the passage of large portions of the nuclear content into the cytoplasm.

**Figure 3**

Phase-contrast photomicrographs of lampbrush chromosomes from Notophthalmus viridescens.

(A) Lampbrush bivalent 10 from a full-grown oocyte of a gonadotropin-treated female. \( S = \) sphere; \( gfl = \) giant fuzzy loops, distinctive of lampbrush chromosome 10. \( \times 400. \)

(B-D) Lampbrush chromosomes during in vitro oocyte maturation. The oocytes were incubated for 1 h in Ringer's solution containing progesterone (10 \( \mu \)g/ml), then transferred to Ringer’s. The chromosome morphology was analyzed respectively 2 h (B), 5 h (C) and 6 h (D) from the beginning of incubation. The chromosomes show a progressive condensation, while the lateral loops retract into the chromosome axes. The nucleoli become spheroidal and vacuolated. \( \times 505. \)
In vitro maturation of oocytes in Notophthalmus

We have described a pattern of changes which occurs in the lampbrush chromosomes during their transformation into the bivalents of the first meiotic metaphase. The major features of the transformation are a progressive shortening and condensing of the chromosomes accompanied by the disappearance of the lateral loops, probably due to a retraction of the loop axis into the chromosome axis. While this pattern involves, generally, the entire lampbrush set, we observed a specific and peculiar deviation at the sphere loci, namely the development of loops not previously existing at these sites. Such loops were either coexistent with the spheres or inserted at the loci from which the spheres had detached; they persisted after most or all the other loops were withdrawn. These unusual loops have also been observed at the sphere loci in full-grown oocytes taken directly from gonadotropin-treated females (Barsacchi, unpublished observations; see Fig. 4D), thus they are not a peculiarity associated exclusively with in vitro maturation. The question as to the mechanism of the induction of these loops is, of course, part of the general problem of production and retraction of loops in these chromosomes, a problem which has yet to be solved (cf. Snow & Callan, 1969; Mancino et al. 1971; Fiume, Nardi, Bucci & Mancino, 1972; Muir & Whitley, 1972), but the stimulus for induction or expansion of these new loops during maturation would appear to be a specific one, since these loops emerge while most or all the

**Figure 4**

(A–C) Phase-contrast photomicrographs of lampbrush chromosomes from Notophthalmus viridescens.

(A) Lampbrush bivalent 5 from a full-grown oocyte of a gonadotropin-treated female. S = sphere. × 400.

(B, C) Lampbrush bivalents 5 from two oocytes taken respectively at 7 and 9 h from the beginning of incubation in progesterone solution. Experimental conditions as in Fig. 3. × 1265.

(B) Loops (L) emerge from the sites where the spheres (S) are inserted.

(C) Loops (L) expand from the sites usually occupied by the spheres. × 505.

(D) Radioautograph of a lampbrush preparation obtained from a gonadotropin-treated female. The slide was submitted to in situ hybridization with radioactive complementary RNA (cRNA) transcribed in vitro from N. viridescens DNA. The cRNA was used at a concentration of 1·3 × 10⁶ cpm/ml in 6 × SSC (SSC = 0·15 M-NaCl; 0·015 M-Na citrate, pH 7). The hybridization was carried out for 17 h at 66 °C. Exposure, 7 weeks. Giemsa stain. The photomicrograph represents part of lampbrush bivalent 5. In this bivalent loops expand from the sites where the spheres are usually inserted (brackets). Since the bivalent is partially condensed and most loops are retracted, the sites from which the new loops expand appear as gaps in the chromosome axes. Few autoradiographic grains are over the newly formed loops, indicating that they may contain repetitive DNA. S = a detached sphere. The arrows indicate the centromeres, which bind preferentially the cRNA. × 1520.

1 This experiment was carried out in the laboratory of Prof. J. G. Gall, Dept. of Biology, Yale University, New Haven, Conn. Details of the method are described elsewhere (Barsacchi & Gall, 1972).
other loops are withdrawing. One supposes that progesterone or some similar steroid may be involved directly or indirectly. There is extensive evidence suggesting the existence, in steroid hormone target cells, of an association between the cytoplasmic hormone-receptor complexes and chromatin (see Tomkins & Martin, 1970; O'Malley, Toft & Sherman, 1971; Steggles, Spelsberg, Glasser & O'Malley, 1971). Since in Notophthalmus viridescens the loops emerge from the sphere loci, one wonders whether the spheres, whose function is still unknown, may be involved in some sort of regulation in these portions of the chromosomes, and whether such regulation might be specifically associated with cellular response to the steroid stimulation.

However the new loops at the sphere loci may be produced, one wonders about the possible role(s) of their transcription products, if there are any. While experiments on in vitro maturation in Rana pipiens and Xenopus laevis appear to exclude involvement of the germinal vesicle, after hormone treatment, in the induction and completion of cytoplasmic maturation (Masui & Markert, 1971; Schorderet-Slatkine & Drury, 1973) there are several indications of other sorts of involvement. The work of Ziegler & Masui (1973) shows that the contents of the germinal vesicle of progesterone-stimulated oocytes are at least partly responsible for the chromosome-condensing influence exerted on brain nuclei transferred into oocyte cytoplasm; presumably the condensation of the chromosomes of the germinal vesicle itself is effected in a similar way. As for the importance of the germinal vesicle in the development of the embryo, there are indications that it contributes specifically to cleavage and gastrulation (Briggs & Cassens, 1966; Briggs & Justus, 1968; Smith & Ecker, 1969), although it should be pointed out that the cleavage factor is apparently not produced during the hormone-induced maturation period of Rana pipiens (Smith & Ecker, 1969). On the basis of nuclear transplant experiments it has been suggested that the germinal vesicle contributes either DNA polymerase or an activator of polymerase to the oocyte cytoplasm at the time of germinal vesicle dissolution (Gurdon, Birnstiel & Speight, 1969; Gurdon & Speight, 1969). During maturation in Xenopus, there appears a DNA polymerase activity different from that present earlier in the oocyte, but it has not been ascertained whether the activity is due to synthesis of a new enzyme or to an activation of a pre-existing inactive enzyme (Grippo & Lo Scavo, 1972). With regard to later development, it has been demonstrated in the axolotl that, during oogenesis, a component indispensable for gastrulation is produced, and this component is released into the cytoplasm at germinal vesicle breakdown (Briggs & Cassens, 1966; Briggs & Justus, 1968). The possibility that the loops at the sphere loci may be concerned with any of these matters is intriguing, but as yet is only a speculation worthy of further investigation.

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