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**Morphological Changes of Somatic Cell Nuclei
Inserted into
Stage I and II Oocytes in *Xenopus laevis*¹⁾**

By

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(With 3 Text-figures and 2 Tables)

Recently, our knowledge on the nucleo-cytoplasmic interaction has been greatly advanced. A massive amount of investigation by means of cell fusion (Harris, 1967; Koprowski and Croce, 1973; Balakier, 1978; Tarkowski and Balakier, 1980), microinjection of somatic (Graham *et al.*, 1966; Gurdon, 1968, 1976; Gurdon *et al.*, 1976) and gametic (Katagiri and Moriya, 1976; Moriya and Katagiri, 1976) cell nuclei, and microinjection of foreign cytoplasm (Masui and Markert, 1971) into oocytes and eggs has clearly shown that the cytoplasm controls the activity of nucleus during the mitotic and meiotic cell cycles.

In amphibians, microinjection of nucleus or of foreign cytoplasm has mainly been performed into oocytes in later stages of oogenesis, maturing oocytes and eggs, so that some important problems associated with the maturation of oocytes or completion of the meiosis have been solved (Meyerhof and Masui, 1977, 1979; Katagiri, 1980; for a review, Masui and Clarke, 1979). However, at present, little information is available to know the cytoplasmic condition of the oocyte in very early stages of oogenesis (*cf.* Wakahara, 1980). In this respect, it is worth to note the experiments by Gurdon (1976) who has stated that the chromosomes of enlarged HeLa nuclei in the oocyte of later stages of oogenesis may possibly take up a lampbrush chromosome-like configuration. It seems, therefore, valid to inject the somatic cell nuclei into the small oocyte in early stages of oogenesis, in order to examine the cytoplasmic state of the oocyte during the early meiotic prophase.

The aim of this study is to find the smallest injectable stage of oocyte in *Xenopus*, and to describe the morphological changes of the somatic cell nuclei injected into very small oocytes.

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Materials and Methods

African clawed toads, *Xenopus laevis*, were used in this investigation: they were all obtained by induced matings with human chorionic gonadotropin or by artificial insemination following Moriya (1976). They were fed with boiled alfalfa powder during the tadpole stage and with an artificial diet of rainbow trout cultivation (Nissin Co., Tokyo) just after the metamorphosis. The artificial diet for rainbow trout was much better for *Xenopus* than chopped bovine liver, which had been customarily fed to the toads in our laboratory, from the view points of growth rate of animals and ease of handling the diet.

Ovaries were dissected out of toadlets 3 months after metamorphosis, and stage I and II (according to the oocyte developmental stages described by Dumont, 1972) were taken from ovarian walls. Isolated oocytes with their follicular envelopes were collected in modified Birth's solution (referred to as MBS-H after Gurdon, 1976). Stage I oocytes were divided into three size groups; early- (under 100 μm in diameter), mid- (100–200 μm) and late- (200–300 μm) stage I oocytes.

Just before microinjection, an oocyte was held in a suction glass with a little narrower diameter than the oocyte as just shown in Fig. 1. Then, the oocyte was injected with somatic cell nuclei by a glass micropipette with a sharpened tip. Since the envelope covering the oocyte was very thin, the oocyte was extremely sensitive to desiccation, the operation must be completed within 20–30 sec after the oocyte was sucked up even in a moist chamber. The oocytes injected were cultured in a small Petri dish lined with agar for few days at 23°C. Two culture media were employed; non-nutritive one, MBS-H, and nutritive one composed of Leibovitz L-15 (Flow Lab., Rockville), distilled water and fetal calf serum, mixed in the ratio 5:4:1, respectively (referred to as diluted L-15). All culture media contained penicillin G and streptomycin sulfate.

Nuclei for injection were taken from thymic cells of toadlets for the sake of convenient treatment. Four methods were tested for the preparation of nuclei, using lysolecithine, Triton X-100, mechanical disruption of cell membrane and

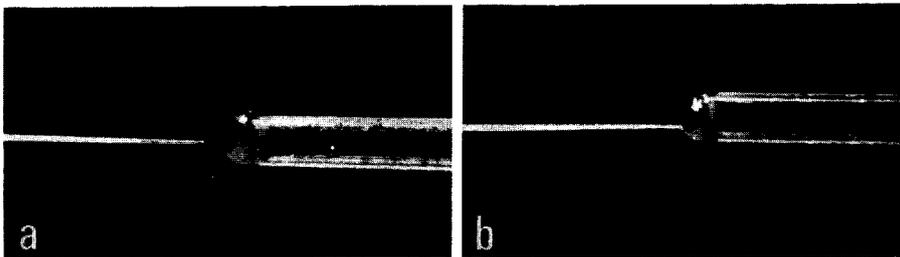


Fig. 1. The actual condition of the microinjection of thymic cell nuclei into the small oocyte of *Xenopus laevis*. The oocyte was held in a suction glass capillary with a little narrower diameter than the oocyte diameter in a moist chamber. a, Just before injection; b, after injection. $\times 30$.

hypotonic osmotic shock. The first three methods were described in details by Gurdon (1976). The last method using hypotonic osmosis was employed for *Xenopus* red blood cells (Graham *et al.*, 1966). Nuclei from 10^6 thymic cells were suspended in 50 μ l of injection medium. Volume of microinjection should be varied in relation to the size of oocytes. About 5% of whole volume of oocytes was injected; for example, approximately 0.7 nl of nuclear suspension was applied to oocytes of 300 μ m in diameter and 0.2 nl for 200 μ m oocytes.

Oocytes injected with thymic cell nuclei were fixed in Bouin's fixative, 1 hr, 1 day and 3 days after the injection. They were embedded in paraffin, sectioned serially at a 5 μ m thickness, and stained with Delafield's hematoxylin and eosin.

Results

Survival of Oocytes Injected.

Table 1 shows the survival rates of oocytes injected with thymic cells nuclei prepared by using Triton X-100. The survival rates of oocytes were determined histologically: the oocytes with an entire basophilic cytoplasm were judged as alive and those with acidophilic and chromophobic cytoplasm as dead.

Table 1. Survival of *Xenopus* oocytes *in vitro* after injected with thymic cell nuclei.

Oocytes injected		Number of oocytes		
Stage	Diameter (μ m)	Injected	Survival	With intact GV
I	<100	100	0	0
I	100-200	100	5	0
I	200-300	100	42	12
II	300-450	100	76	52

The number of oocytes with intact germinal vesicles (GVs) increased in proportion to the size of oocytes injected. No oocytes with intact GVs were observed in the survivals of early- and mid-stage I. But, a small number of oocytes in the late-stage I oocytes survived (12 oocytes out of 100 injected) with intact GVs. When the injection was performed in stage II, approximately half of the oocytes were alive for 3 days in the culture with intact GVs. The survival rates of oocytes were raised with the increased size of oocytes. When the injection of nuclei was performed into early-stage I oocyte, no oocytes injected survived for the 3 days culture in this experiment. A very small number of the mid-stage I oocytes (only 5 oocytes out of 100 injected) and approximately half of the late-stage I oocytes (42 out of 100 injected) were alive for 3 days culture after the injection of thymic cell nuclei.

The suitability of culture media used, which were the non-nutritive one, MBS-H, and the nutritive, diluted L-15, varied according to the size of oocytes. Larger

oocytes, over 300 μm in diameter, were satisfactorily viable even in the non-nutritive medium, whether they had been injected with nuclei or not. Smaller oocytes could not survive for 3 days culture in MBS-H after the injection, while they were sufficiently healthy in MBS-H without injection. For the culture of smaller oocytes injected with nuclei, diluted L-15 seemed more suitable than MBS-H, though Gurdon (1976) had pointed out that the diluted L-15 was slightly less satisfactory for the culture of oocytes injected than MBS-H in larger oocytes of *Xenopus*.

Among the methods for preparation of nuclei employed in this study, relatively good results were obtained using Triton X-100 and hypotonic osmosis. Lysolecithine, which was recommended by Gurdon (1976) for mammalian culture cells such as HeLa brought about less satisfactory results.

Fate and Morphology of Injected Nuclei.

Fate of the injected nuclei into stage I and II oocytes is summarized in Table 2. These results were obtained with nuclei prepared by hypotonic osmotic shock. The number of nuclei scored on serial histological sections varied one oocyte to another. There was, however, a clear correlation between the size of oocytes injected and the number of nuclei scored: the number of nuclei per oocyte increased proportionally to the size of oocyte.

Table 2. Fate of thymic cell nuclei after injected into *Xenopus* oocytes.

Oocytes examined		Injected nuclei	
Diameter (μm)	No.	No. of nuclei/oocyte	Diameter
100-200	8	0-2 (0.4)*	4-8 (5.3)*
200-300	32	2-31 (6.5)	4-22 (9.6)
300-450	48	5-45 (11.2)	4-26 (13.0)

* Minimal-maximal (mean).

The injected nuclei underwent some morphological changes in many cases, but also pycnotic and unchanged nuclei were sometimes encountered in the histological sections of oocytes. Table 2 shows the maximal and minimal diameter in nuclei except pycnotic ones. In late-stage I and stage II oocytes, injected nuclei were significantly increased in size. But, the enlargement of nuclei in oocyte could not conclusively affirmed in early- and mid-stage I oocytes, since absolute number of nuclei observed were very small in these size groups. Fig. 2 demonstrates the time course of changes in nuclear diameter in late-stage I oocytes. These data were combined from several experiments which were performed using different methods for the nuclear preparation, because the nuclei encountered on the histological sections were relatively few in single experiments. Almost all nuclei were 4-6 μm in diameter just after the injection into the oocytes. But, the nucleus population shifted to the large size group day by day in the oocyte cytoplasm.

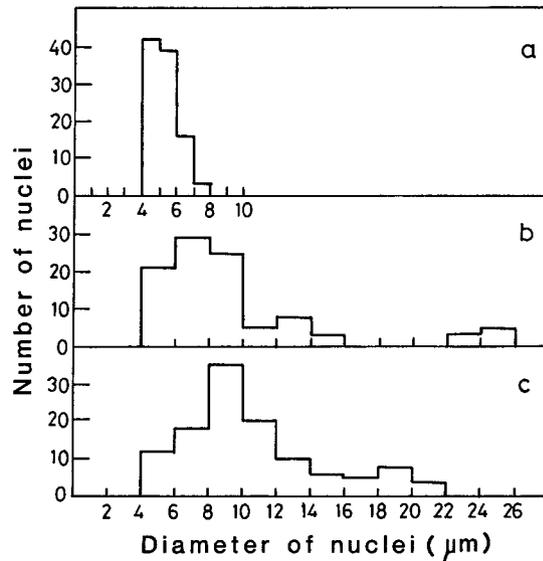


Fig. 2. The time course of swelling of the injected nuclei into late-stage I oocytes (200–300 μm in diameter) of *Xenopus laevis*. One hr (a), 1 day (b) and 3 days (c) after the injection the oocytes were fixed and processed for histological examination.

Although the survival of oocytes with intact GVs was relatively few, several instances permitted a comparison of eventual size of nuclei in the oocytes with intact GV and dispersed GV. In general, the nuclei in the oocytes without GV were much larger than in the oocytes with intact GV. The largest nucleus was 26 μm in diameter in the region of dispersed GV (Fig. 3, c), but only 10 μm in diameter in the oocyte cytoplasm with intact GV.

Morphology of nuclei in oocytes was very diverse mainly in accordance with the stage of oocytes injected. The nuclei in stage II oocyte were characterized by a swell in size, chromatin dispersion and disappearance of nucleoli (Fig. 3, b), which resembled the morphology described previously on HeLa nuclei inserted into later stages of oocytes (Gurdon, 1976). Two different responses of the injected nuclei were noted in late-stage I oocyte; completely dispersed chromatin in swelled nuclei (Fig. 3, c) as like as the swelled nuclei in stage II oocyte, or condensed chromatin similar to the lampbrush chromosome (Figs. 3, d and e). In almost all cases of swelled nuclei, chromatin was completely dispersed into finely granulous or fibrous structure. In fewer cases, however, clearly thick condensed chromatin fibers were observed in swelled nuclei in late-stage I oocyte. These condensed chromatin fibers or chromosome-like structure had fuzzy lateral element along their long axis (Fig. 3, d), which were similar to the lampbrush chromosomes in GV of oocyte. Nuclei in mid-stage I oocyte were characterized by thickened granulous

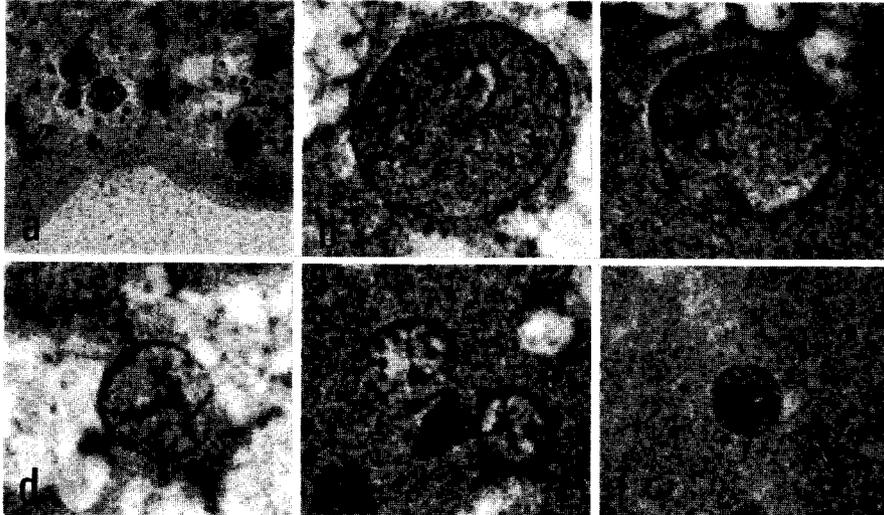


Fig. 3. Sections through the injected nuclei in oocytes stained with hematoxylin and eosin. a, Nuclei just after the injection in late-stage I oocyte; b-f, nuclei 3 days after the injection. Swelled nuclei in stage II (b) and late-stage I (c), whose chromatin is completely dispersed; nuclei with lampbrush chromosome-like structure in late-stage I (d and e); and nucleus with granulous chromatin in mid-stage I (f). $\times 1200$.

chromatin distributed evenly over the whole nucleoplasm (Fig. 3, f). This chromatin structure was resembling the leptotene chromosomes in meiotic prophase, but the precise morphology was unclear since the nuclei observed on sections were very few.

Discussion

The smallest oocyte that was conveniently injectable and sufficiently viable for several days *in vitro* was of late-stage I (200–300 μm in diameter) in *Xenopus laevis* so far as examined. Although a few oocytes at mid-stage I (100–200 μm in diameter) survived, no oocytes at early-stage I (under 100 μm) survived for 3 days culture after receiving the donor nuclei. It is conceivable that the tip of microinjection pipette employed in this experiment was too thick for the small oocytes, resulting in a leak of cytoplasm and ultimately in the death of oocytes.

A swelling is the most prominent change noted in the nuclei inserted into growing oocytes (Gurdon, 1968, 1976; Gurdon *et al.*, 1976). The swelling is presumably maintained by an inflow of pre-formed proteins in the oocyte cytoplasm (Ficq, 1972) into the donor nuclei (Merriam, 1969), and is paralleled by stimulation of RNA synthesis (Merriam, 1969; Gurdon *et al.*, 1976). Substantial enlargement of donor nuclei in late-stage I oocyte observed in this study indicates that such

cytoplasmic proteins which make the nuclei swell and synthesize RNA are already accumulated in the cytoplasm of late-stage I oocyte. The lampbrush chromosome-like configuration of chromatin observed in the foreign nuclei in late-stage I should be an indication of certain physiological changes exerted from the oocyte cytoplasm, even if such structures were observable in fewer cases. It could not be determined, however, when the factor(s) bringing about the lampbrush chromosome-like structure appear in the cytoplasm and how the factor(s) affect the donor nuclei, since the observation on such structures was insufficient.

The development of oocyte in *Xenopus* has been divided into six stages according to the size and morphological features (Dumont, 1972). So far as examined morphologically, stage I oocyte is pre-vitellogenic and has evenly basophilic cytoplasm regardless of the size of oocytes. But, the physiological condition of cytoplasm of stage I oocyte is probably varied according to the size; larger oocytes in stage I contain cytoplasmic factor(s) which allow the nuclei to swell, and somewhat smaller oocyte in stage I may have factor(s) to induce the lampbrush chromosome in the nuclei. In this respect, an attempt to induce the cell fusion between very small oocytes and their follicle cells has been carried out in *Xenopus* using polyethylene glycol as a fusogen (Wakahara, 1980): the follicle cell nuclei underwent some morphological changes similar to pachytene chromosomes after fusing with very small oocyte. It seems, therefore, reasonable to assume the stage specific cytoplasmic factor(s) are concealed in the developing oocytes.

Some important biochemical and physiological events other than meiotic prophase happen in very small oocyte in *Xenopus*, such as an amplification of ribosomal DNA in nucleus (Brown and Dawid, 1968; Gall, 1968) and an accumulation of poly(A)-containing RNAs in cytoplasm (Rosbash and Ford, 1974). Especially, the poly(A)-containing RNA, at least, a part of which is believed to act as maternal messenger RNA after the fertilization, is very long-lived and begin to accumulate in oocyte cytoplasm before vitellogenesis (Ford *et al.*, 1977). Then, knowing the cytoplasmic conditions of very small oocyte is absolutely necessary to understand the integrated mechanism regulating the initial phase of oogenesis. At present, however, it is difficult to inject somatic cell nuclei into the small oocyte under 100 μm in diameter, according to the microinjection procedure employed in this study. Thus it is inevitable to develop other technique such as the cell fusion (*cf.* Wakahara, 1980) to examine the cytoplasmic state of oocyte at the early phase of meiosis.

Summary

A procedure to inject somatic cell nuclei into small oocytes of *Xenopus laevis* was described. The smallest oocyte that was conveniently injectable and sufficiently viable was of late-stage I (200–300 μm in diameter). Several morphological changes in the injected nuclei were demonstrated. The results suggest that certain cytoplasmic factor(s) in the developing oocytes induce lampbrush chromosome structure.

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