

CYCLIC CYTOPLASMIC ACTIVITY OF NON-NUCLEATE EGG FRAGMENTS OF *XENOPUS* CONTROLS THE MORPHOLOGY OF INJECTED SPERMS

*MASAO SAKAI AND †ATSUNORI SHINAGAWA

Department of Zoology, Faculty of Science, Kyoto University, Kyoto 606, Japan

SUMMARY

Triton X-100-treated sperms were injected into non-nucleate egg fragments of *Xenopus laevis* to determine whether the structure of the injected sperm nucleus is affected by the cyclic activity of the cytoplasm.

Swollen vesicular nuclei were very frequently observed when the sperms were injected and incubated during the 'rounding-up' phase of the recipient fragment, whereas no such structures were found when they were incubated during the 'relaxing' phase.

INTRODUCTION

Non-nucleate egg fragments of wide-spread species, including sea-urchin (Yoneda, Ikeda & Washitani, 1978), newt (Sawai, 1979), *Xenopus laevis* (Hara, Tydeman & Kirschner, 1980; Sakai & Kubota, 1981) and *Tubifex* (Shimizu, 1981), exhibit cyclic surface changes in spite of the complete absence of cleavage. Since the interval of the cyclic cytoplasmic changes in the non-nucleate egg fragment is reported to be similar to or slightly longer than, but comparable to, the species-specific cleavage interval of the normal egg, it is plausible that these nucleus-independent cytoplasmic cycles play a definite role in the determination of the cleavage rate.

By measuring the cleavage rates of sand-dollar/sea-urchin hybrids, including those that resulted from the cross-inseminated merogones, Moore (1933) concluded that neither sperm nor egg nucleus has any effect on cleavage tempo, but that the reactions of the cytoplasm alone determine it. However, the nature of 'the reactions of the cytoplasm' was not inferred in his paper. Recent findings on non-nucleate egg fragments suggest that these reactions of the cytoplasm are closely linked to the reported changes in the cytoplasm. The first step to explore this possibility would be to examine whether or not the autonomous cytoplasmic rhythmicity could control the behaviour of the introduced nucleus, since normal cytokinesis is always accompanied by karyokinesis.

*Present address: Department of Biology, Faculty of Science, Kagoshima University, Kagoshima 890, Japan.

†Present address: Department of Biology, Faculty of Science, Yamagata University, Yamagata 990, Japan.

In the present study, Triton-treated sperms were injected into non-nucleate egg fragments of *X. laevis* to examine whether the autonomous cytoplasmic activity of the fragments can affect the morphology of the sperm nuclei.

MATERIALS AND METHODS

Preparation of materials

Non-nucleate egg fragments of *X. laevis* were obtained by the procedure described in a previous paper (Sakai & Kubota, 1981).

The sperm suspension for injection into non-nucleate egg fragments was prepared by a method similar to that of Moriya & Katagiri (1976) although the duration of the Triton X-100 treatment was lengthened to 30 min.

'Staging' of the non-nucleate fragment

One indication of the autonomous cyclic activities of non-nucleate egg fragments of *X. laevis* is a periodic rounding-up and relaxing movement (cf. Selman & Waddington, 1955), and we used this as a means of dividing the movement of the fragment into stages. Four to six fragments that settled in the solution were observed from above with a microscope and their major axes were measured every 3 min.

Fig. 1 illustrates the cyclic rounding-up (shortening of the diameter) and relaxation (high plateau of the diameter) of the fragments in one experimental series. The 'cytoplasmic cycle' consisted of some 20 min of a 'rounding-up' phase (from the onset of the rounding-up to the end of it) followed by 20–30 min of a 'relaxing' phase.

Injection and incubation

Injection of the sperm suspension was done into the upper central region of the fragment at two

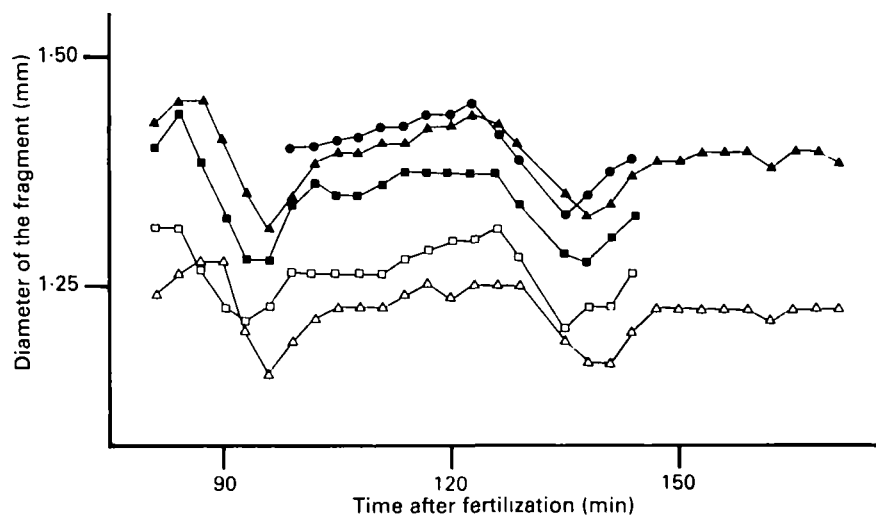


Fig. 1. Cyclic changes in the diameter of non-nucleate egg fragments, which indicate the cyclic rounding-up and relaxing movement of the fragment. Five fragments were obtained from one batch of eggs. They showed good synchrony in the timing of the rounding-up and relaxing movements. In each fragment, one cytoplasmic cycle consisted of some 20 min of a rounding-up phase followed by 20–30 min of a relaxing phase. These fragments were used for one series of experiment, shown in Fig. 3.

stages of the cytoplasmic cycle: (1) just before the rounding-up (the end of the relaxing phase); and (2) just after the rounding-up (the time of the initiation of the relaxing phase). For the injection, a glass micropipette (about $40\ \mu\text{m}$ in diameter) attached to a micromanipulator (Narishige Co., Ltd) was used. The volume injected into each fragment was 20–50 nl, which included some 10–100 sperms, as counted on serial sections of the injected fragment. Following the injection, the fragment was incubated for a certain time before fixation for cytological examination. Preliminary experiments showed that this time of incubation was critical in the present study: if the time was less than half a cytoplasmic cycle, most of the injected sperm retained their needle-shaped

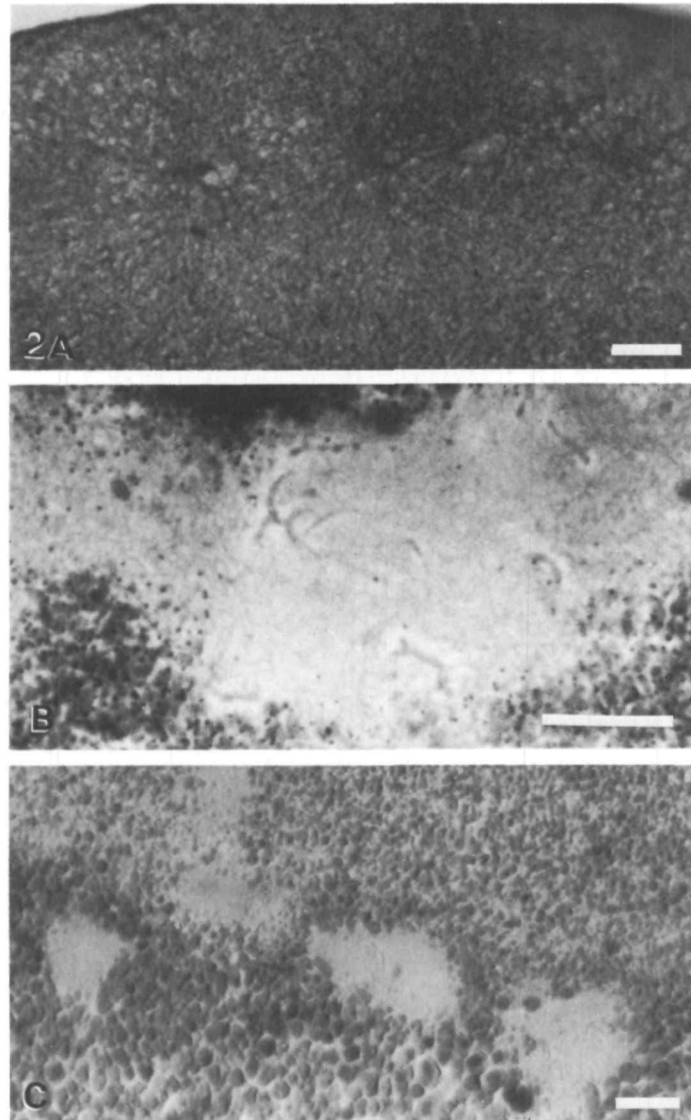


Fig. 2. Injected sperms in the non-nucleate egg fragment. *a*. Swollen nuclear envelopes in one fragment incubated through the rounding-up phase. *b*. A cluster of non-reacted sperms. *c*. Several spherical yolk-free areas. This fragment was incubated through the relaxing phase and no SNE was observed throughout the serial sections of this fragment. Bars, $20\ \mu\text{m}$.

structure regardless of the cytoplasmic state at the time of injection. This lack of response was perhaps due to insufficient exposure of the injected sperm to the recipient cytoplasm. Incubation for half a cytoplasmic cycle provided sufficient exposure to enable us to detect the *cyclic* activity of the cytoplasm that influenced the structure of the injected sperm nuclei. On the other hand, incubation for much longer (more than one cytoplasmic cycle) usually induced abortive cleavage furrows in the recipient cytoplasm, which caused technical difficulty in determining the stage of the fragment, because the measurement of the diameter of the fragment could not be continued.

For these reasons, in the main experiments sperm-injected fragments were usually incubated for half a cytoplasmic cycle, using the graph drawn as a monitor to determine the time required for fixation (cf. Figs 2, 3).

Cytology

For observation of injected sperms, the fragments were fixed in Bouin's solution, dehydrated in ethanol, and embedded in paraffin. The 8 μm thick serial sections were stained with Orange-G, Anilin Blue and Water Blue.

RESULTS

Sperm nuclei in the non-nucleate fragments

Three types of structures derived from the injected sperm were found in serial sections of the sperm-injected fragments: (1) swollen nuclear envelopes (SNEs, Fig. 2A); (2) non-reacted sperms (Fig. 2B); and (3) spherical yolk-free areas (Fig. 2C).

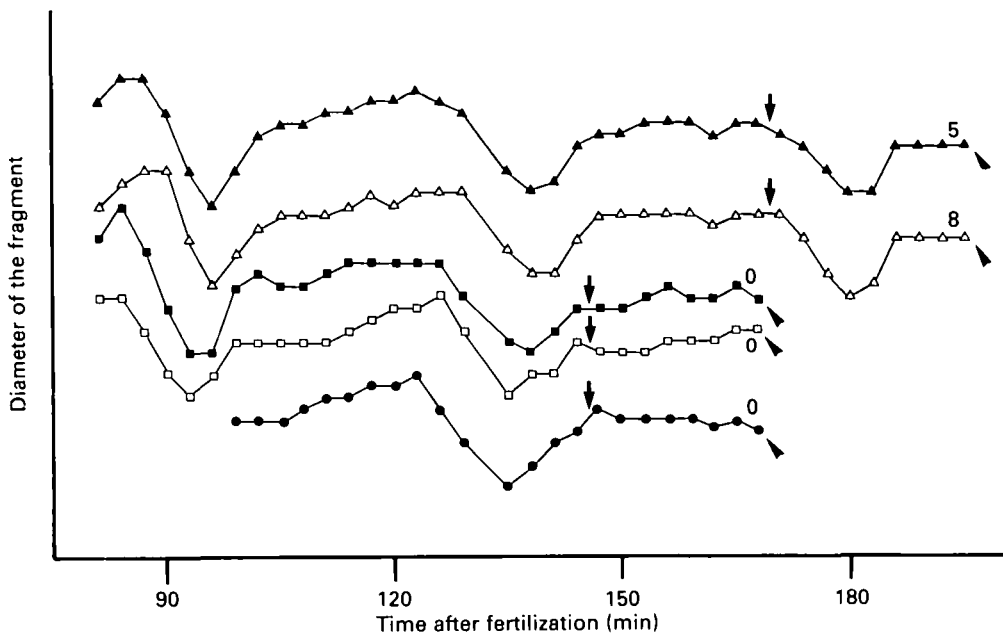


Fig. 3. One series of experiment that includes five half-cycle-incubated fragments. Serial sections of these fragments were examined and the numbers of SNEs were scored. Note that the regular cytoplasmic changes (e.g., rounding-up and relaxing movements) persist after the injection (at arrows). Each curve of the diameter of the egg fragment (see Fig. 1) has been shifted to avoid overlapping of lines. 5, 8, 0, 0, 0, denote the number of SNEs per fragment. Arrowheads show the time of fixation.

Table 1. Number of SNEs formed in the sperm-injected fragments incubated for half a cycle

Series	Period of incubation	
	Rounding-up phase	Relaxing phase
A	1,3	0
B	0,4	
C	7	0,0
D	8,9,16,43	0
E	0,0	0,0
F	0,1	0
G	2,4,7,23	0,1
H	0,0	0,0,0
I	2,5,8,10	0,0,0,0
J	1,1,3,5	0,0,0,0
K	6,16	0,0,0,0

Numerals show the number of SNEs formed in one fragment. Note that most (23/29) of the fragments incubated in the rounding-up phase have at least one SNE whereas only one out of 24 relaxing fragments have one SNE.

Spherical yolk-free areas were not nuclear structures, but they must be derived from the injected sperm because they were not found in the non-injected fragments and one or several sperms usually resided at the centre of each area.

Non-reacted sperms and yolk-free areas were usually found in both series (rounding-up phase, incubated and relaxing phase, incubated) of fragments. These two structures were not used for the quantitative analysis because: (1) non-reacted sperms usually constituted clusters so that we could not count their exact number; and (2) yolk-free areas were quite variable in size and shape. So the most conspicuous nuclear structures, swollen nuclear envelopes (SNEs; Fig. 2A) only, were used for the quantitative analysis.

Serial sections of each experimental fragment were examined and the numbers of residing SNEs were scored. One experimental series is shown in Fig. 3. Two fragments incubated throughout the rounding-up phase had five and eight SNEs, respectively.

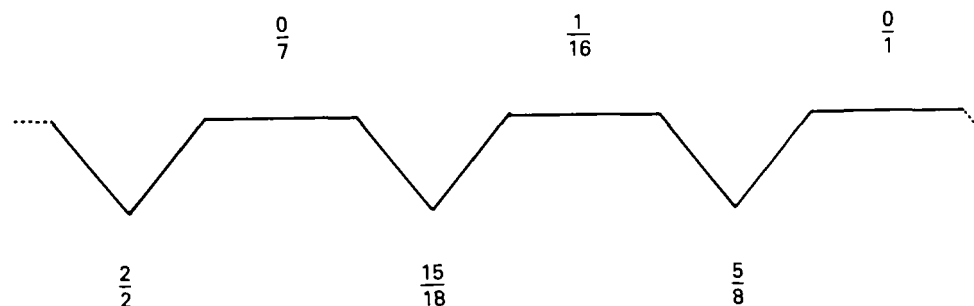


Fig. 4. Diagrammatic representation of the cyclic SNE-forming activity of the non-nucleate egg fragments. Denominator: number of fragments injected with sperms and incubated in that phase. Numerator: number of fragments that contained SNEs.

The other three fragments incubated in the relaxing phase had no such SNEs. Eleven series of experiments are summarized in Table 1, which shows that most (23/29) fragments incubated in the rounding-up phase had SNEs whereas only one out of 24 fragments incubated in the relaxing phase had SNEs.

In these experimental series, the injection-incubation was done in either the first, second or third rounding-up phase or the corresponding intermediate relaxing phase. Thus the observed change in the activity required to form SNEs is undoubtedly cyclic, since the activity appeared in every rounding-up phase but not during each relaxing phase (see Fig. 4).

As described before, structures other than SNEs, such as non-reacted sperms and yolk-free areas, were usually found in both experimental fragments, although SNEs were usually found only in fragments incubated in the rounding-up phase. Thus there was some variation in morphology among injected sperm nuclei in a single fragment. This point will be discussed below.

DISCUSSION

Several studies have shown that some cytoplasmic states affect the morphology and activity (e.g. DNA synthesis etc.) of the nucleus. In *Amoeba proteus*, transplantation of a nucleus engaged in DNA synthesis into a G_2 phase (after DNA synthesis) cell results in inhibition of such synthesis and, conversely, when the nucleus of a G_2 cell is transplanted into an S phase (period of DNA synthesis) cell, such a nucleus may begin to synthesize DNA (Prescott & Goldstein, 1967). When sperm nuclei or somatic cell nuclei were transplanted into eggs or oocytes of *X. laevis*, they behaved in synchrony with the resident nuclei (Graham, 1966; Graham, Arms & Gurdon, 1966; Gurdon, 1967). Cytoplasmic states that regulate nuclear behaviour were also demonstrated in Japanese toad oocytes undergoing maturation division (Moriya & Katagiri, 1976). Usui & Yanagimachi (1976) showed that the ability of the egg cytoplasm to decondense sperm chromatin appears during the time of the breakdown of the germinal vesicle and reappears during the first cleavage division. However, in all these reports, the nucleus of the recipient cell resided in the cytoplasm, so that the possibility of the control of cytoplasm by the resident nucleus has not been ruled out.

In the present study, *non-nucleate* cytoplasmic fragments were used as recipients, whose cyclic activity is manifest as the rounding-up and relaxing movement. The present results therefore prove that the factor that affects the structure of the injected nuclei is the *autonomous* cyclic activity of the cytoplasm itself.

It is worthwhile to compare the cyclical change in the structure of nuclei during normal cleavage with that of sperm nuclei injected into non-nucleate fragments. In the normal cleavage process, swollen nuclear envelopes are formed just after the time of furrow formation and disappear at some stage during cleavage (data not shown). Since the cleavage furrow in the normal egg first appears when the egg is nearly maximally rounded-up (Selman & Waddington, 1955), we can say that swollen nuclear envelopes are formed just after the rounding-up in the normal egg. This is consistent with the present observation of injected sperm nuclei; i.e., swollen nuclear

envelopes were formed only in those fragments that were injected just before rounding-up and fixed just after rounding-up (see Figs 3, 4; Table 1). These results strongly suggest that the autonomous activity of the cytoplasm that causes the injected sperm to form swollen nuclear envelopes is exerting its effect also on the normal cleavage process, by controlling nuclear behaviour.

In connection with the present experiments, the influence of the donor nuclei on the recipient egg fragments will be discussed. *Irregular furrows* formed after a long incubation time must be a consequence of the transplantation of *many sperms* (10–100 per fragment, see Materials and Methods) because transplantation of *a single nucleus* caused *normal cleavages* in the recipient fragment (Sakai, 1982). The irregular furrows did not appear cyclically as in the normal cleavage process but sporadically, eventually forming an irregular fragmented mass of cytoplasm. Thus, after a long incubation time, the original cytoplasmic cycle must have been disturbed by the injected sperms. For this reason the period of incubation was reduced to less (half a cytoplasmic cycle = 20–30 min) than that in the previous nuclear transplantation studies (see Moriya & Katagiri, more than 1 h; see Usui & Yanagimachi, 1–3 h), so that the sperm might not be over-exposed to the cytoplasm. The observed variety in the structure of injected nuclei in each fragment incubated for half a cycle may be due to the short incubation time. However, in spite of this difficulty, the present experimental system has demonstrated that the non-nucleate egg fragment shows cyclic activity in the formation of swollen nuclear envelopes.

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