REGENERATION OF OOCYTE-SURFACE FACTOR RESPONSIBLE FOR 1-METHYLADENINE-INDUCED OOCYTE MATURATION IN STARFISH

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ABSTRACT

Maturation of starfish oocytes is triggered by 1-methyladenine (1-MeAde) acting on the oocyte surface. However, the oocytes treated with 0.01 % Triton X-100 failed to respond to a physiological dose of 1-MeAde. On the other hand, the Triton X-100-treated oocytes once again acquired the capacity to respond to 1-MeAde when they were incubated with 'oocyte-surface factor (OSF)' obtained from the Triton X-100 extract, suggesting that OSF is responsible for 1-MeAde-induced oocyte maturation. OSF was found to regenerate spontaneously when the decapacitated oocytes were kept in seawater; they responded well to 1-MeAde after several hours. OSF regenerated repeatedly at least three times with the same oocytes. The hormone-dependent period (HDP), prolonged by the treatment with Triton X-100, was shortened to almost normal levels by keeping the treated oocytes in seawater for 20 h. The amount of OSF on the surface of intact oocyte appears to be much larger than that required for triggering oocyte maturation by 1-MeAde. Both the length of HDP and the rate of maturation appear to be determined as a function of the amount of OSF and the concentration of 1-MeAde when the amount of OSF is small.

Oocyte reinitiation of meiosis in starfish is triggered by the maturation-inducing hormone, 1-methyladenine (1-MeAde), which is produced in the follicle cells around the oocyte (6). Evidence has accumulated showing that the site of action of 1-MeAde in inducing oocyte maturation is the surface of the oocyte, *i.e.* the receptor of 1-MeAde is localized on or in the plasma membrane (1, 3, 5, 7, 13, 14, 17). Among these investigations, Dorée *et al.* (3) found that brief treatment of *Marthasterias* oocytes with 0.02% Triton X-100, a nonionic detergent, deprived the oocytes of their capacity to respond to 1-MeAde. This treatment with Triton X-100 was mild enough to maintain the normal resting potential of the oocyte surface. Morisawa and Kanatani (12) confirmed the results of these workers with the 'denuded oocytes' of *Asterina pectinifera* from which the vitelline coats were previously

removed. However, these 'decapacitated' oocytes obtained by Triton X-100 treatment once again acquired the capacity to respond to 1-MeAde when they were incubated in seawater containing the Triton X-100 extract of oocytes (the Triton X-100 was removed after the extraction). Recovery of the capacity was also observed even after washing such extract-treated oocytes with seawater. Therefore, some factor, extracted from the oocyte surface by Triton X-100, seems to be reconstituted on the oocyte surface so that the capacity of the oocytes to respond to 1-MeAde is recovered. This oocyte-surface factor (OSF) responsible for 1-MeAde-induced oocyte maturation seems to be the receptor of 1-MeAde or its fragment.

Recently we have found that the decapacitated oocytes recover the capacity to respond to 1-MeAde spontaneously one day after the treat-

ment with Triton X-100. The present paper reports the results of the detailed experiments carried out on the spontaneous regeneration of OSF.

MATERIALS AND METHODS

The material used was the starfish, *Asterina pectinifera*, collected during its breeding season from Hashirimizu (Kanagawa) and Asamushi (Aomori). The animals were kept in laboratory aquaria supplied with circulating cold seawater (14°C) at National Institute for Basic Biology.

Experiments were carried out at 20–22°C unless otherwise specified.

The seawater used was modified Van't Hoff's artificial seawater (ASW) (9), and calcium-free seawater (CaFSW) (15).

Preparation of Triton X-100-Treated Oocytes

After removal of follicular envelopes using CaFSW fully grown oocytes without follicular envelopes were isolated and treated with pronase-P (750,000 tyrosine units/g, Kaken Chemical Co.) to remove the jelly and vitelline coats according to the method of Morisawa and Kanatani (12). The oocytes were then suspended in 25 volumes of ASW containing Triton X-100 (0.01% except where specified) (Wako Pure Chem. Ind.) for 1 h with occasional swirling (every 10 min) and then washed with ASW.

Preparation of Triton X-100 Extract (TXE)

In the experiment shown in Fig. 1, Triton X-100-treated oocytes were prepared as above with 0.012% of the detergent. After the oocytes were sedimented, the supernatant was taken and centrifuged at 3,000 rpm for 5 min to remove some contaminating oocytes. The supernatant was applied to a column (1 × 10 cm) of Bio-Beads SM-2 (Bio-Rad Laboratories), equilibrated with ASW, to remove Triton X-100. The eluate without Triton X-100 was designated Triton X-100 Extract (TXE) (12). The factor contained in TXE responsible for 1-MeAde-induced oocyte maturation was designated Oocyte-Surface Factor (OSF).

Assay of Oocyte Maturation

Oocytes which had received various experimental procedures were treated with 1-MeAde at various concentrations for 40 min and the rate of germinal vesicle breakdown (GVBD) were

observed under a microscope.

Determination of Hormone Dependent Period

In order to determine the hormone-dependent period, the denuded oocytes (about 31,000 in number) or the Triton X-100-treated oocytes were suspended in a petridish (7 cm in diameter) containing 5 ml of 10^{-5} M 1-MeAde-ASW; an aliquot (50 μ l) containing about 300 oocytes was taken every minute after suspension and diluted in 50 ml of ASW contained in a centrifuge tube. The oocytes were washed twice with ASW immediately after they sedimented on the bottom of the tube, and they were then transferred to an assay plate and the rates of GVBD were observed.

RESULTS

Spontaneous Recovery of the Capacity of Triton X-100-Treated Oocyte to Respond to 1-MeAde

When the denuded oocytes without vitelline coats were treated with Triton X-100 for 1 h and then transferred into ASW containing 1-MeAde at various concentrations, their capacity to respond to 1-MeAde decreased at lower concentrations (Fig. 1). If the decapacitated oocytes treated with Triton X-100 were kept in TXE for an additional 1 h before 1-MeAde treatment, their capacity to respond to 1-MeAde recovered considerably (Fig. 1), thus confirming our earlier result (12). However, it was found that the decapacitated oocytes spontaneously recovered their capacity to respond to 1-MeAde when assayed 20 h after the treatment with Triton X-100 (Fig. 1).

Effect of Triton X-100 Treatment on Hormone-Dependent Period

A high dose of 1-MeAde (10^{-5} M) induced oocyte maturation even in the Triton X-100-treated oocytes. However, in this case the hormone-dependent period (HDP), the period during which the presence of 1-MeAde in the medium is required for oocyte maturation to occur, was found to be longer in the denuded oocytes treated with Triton X-100 (Fig. 2). This prolonged HDP shortened to that of the control denuded oocytes when the decapacitated oocytes were kept for 20 h after Triton X-100 treatment (Fig. 2). HDP required for 50% oocyte maturation in the denuded oocytes and

Fig. 1 Spontaneous recovery of the capacity of Triton X-100-treated oocytes to respond to 1-MeAde. $\circ$, oocytes treated with pronase alone (control denuded oocytes); $\bullet$, denuded oocytes treated with Triton X-100; $\odot$, denuded oocytes treated with Triton X-100 and kept in seawater for 20 h; $\times$, denuded oocytes treated with Triton X-100 and then with TXE

Fig. 2 Effect of Triton X-100 treatment on the length of the hormone-dependent period when 1-MeAde is used at a high concentration (10^{-5} M). $\circ$, oocytes treated with pronase alone (control denuded oocytes); $\bullet$, denuded oocytes treated with Triton X-100; $\odot$, denuded oocytes treated with Triton X-100 and kept in seawater for 20 h

the Triton X-100-treated oocytes were 5.5 min and 11 min respectively. HDP of the decapitated oocytes shortened from 11 min to 6 min after 20 h.

Regeneration of OSF and Spontaneous Recovery of the Capacity of Triton X-100-Treated Oocytes to Respond to 1-MeAde

As shown in Fig. 1, even the decapitated oocytes matured at high rates in high concen-

trations of 1-MeAde. Five experiments were carried out in order to determine the concentration of 1-MeAde which gives the most conspicuous difference between the control and the Triton X-100-treated oocytes; the results showed that the concentration was 1.2×10^{-7} M, and this concentration was exclusively used in the following experiments.

The Triton X-100-treated oocytes were washed thoroughly with ASW and then kept in ASW. From this stock 20 μ l samples containing about

Fig. 3 Time course of spontaneous recovery of the capacity of Triton X-100-treated oocytes to respond to 1-MeAde. Arrows show the time of application of the first, second and third Triton X-100 treatments respectively. $\overleftarrow{\text{TX}}\overrightarrow{\text{ }}$, duration of Triton X-100 treatment

200 oocytes were taken every 30 min and mixed with 200 μl of ASW containing $1.2 \times 10^{-7}\text{M}$ 1-MeAde, and the rates of GVBD were observed 40 min after mixing. As shown in Fig. 3-I, the rates of GVBD increased gradually from about 3 h and reached maximal levels 7 to 8 h after the end of Triton X-100-treatment. It should be noted that the stock oocytes were also washed with ASW at every 30 min. Next, the stock Triton X-100-treated oocytes, which acquired the capacity to respond to 1-MeAde after 8 h, were once again treated with Triton X-100 and the same experiment was repeated. The rate of GVBD was found to increase in a similar fashion by keeping the oocytes in ASW (Fig. 3-II). Similar results were obtained when such experiments were repeated an additional two times (Fig. 3-III). Therefore, the effect of the treatment with a low concentration Triton X-100 is considered to be reversible and not toxic to the physiological activity of the oocytes.

The next experiment was undertaken to determine whether the recovery of the capacity of the oocytes to respond to 1-MeAde is due to spontaneous regeneration of OSF on the oocyte surface. The stock Triton X-100-treated oocytes, which acquired the capacity to respond to 1-

MeAde after 8 h, were once again treated with Triton X-100, and TXE was prepared from this Triton X-100 extract (the second TXE); the same oocytes, which had been thoroughly washed with ASW for 1 h after the Triton X-100 treatment, were then incubated with the second TXE. GVBD (58%) occurred in these decapitated oocytes treated with the second TXE in the presence of 1-MeAde, whereas the decapitated oocytes without TXE failed to undergo GVBD (Table 1).

Next, the amounts of OSF obtained from the oocytes received the first, second and third treatments with Triton X-100 were compared. For this purpose, TXE was serially diluted and assayed to find the concentration of OSF required for inducing 50% GVBD. The relative amount of extracted OSF was determined by estimating the total volume of TXE containing OSF at the concentration required for 50% GVBD in each case. Table 2 shows relative amount of OSF in each case when the relative amount of OSF obtained from the first Triton X-100-treated oocytes was assumed as 100. The amount of OSF extracted from the same number of oocytes decreased considerably with repeated treatments with Triton X-100.

Table 1 Effect of the second TXE on Recovery of the Capacity of *Asterina* Oocytes to Respond to 1-MeAde after Triton X-100-Treatment

	Control oocytes (Pronase-treatment alone)	Triton X-100-treated oocytes	
		Without TXE	With TXE
GVBD (%)	95 ± 2^a	2 ± 1	58 ± 14

^a Mean $\pm$ SE of 5 experiments

Table 2 Comparison of the Amount of OSF Obtained from the First, Second and Third Treatment with Triton X-100

	First	Second	Third
Relative amount	100 ± 7 ^a	75 ± 10	45 ± 6

^a Mean ± SE of 5 experiments

DISCUSSION

Morisawa and Kanatani (12) have found that the decapacitated oocytes treated with Triton X-100 acquire their capacity to respond to 1-MeAde when they are incubated with TXE.

The present study confirms their results and further demonstrates that the decapacitated oocytes gradually become responsive to 1-MeAde when they are kept for several hours in ASW without TXE. The rate of this spontaneous recovery reaches to maximal level 7 to 8 h after Triton X-100-treatment, and this recovery can be repeated at least 3 times. There are some interesting similarities between the phenomenon and the binding capacity of the macrophage surface for Concanavalin A (11). Concanavalin A fails to bind to the surface of macrophages when they are treated with trypsin. However, this plant lectin binds to the cells spontaneously once again at a normal rate 8 to 10 h after treatment with trypsin.

The fact that the oocyte-surface factor (OSF), responsible for 1-MeAde-induced oocyte maturation, can be extracted again to a considerable degree by Triton X-100 from the oocytes which have spontaneously recovered 8 h after the first Triton X-100 treatment (Table 2) indicates that the recovery of the capacity of the oocytes to respond to 1-MeAde is due to the spontaneous regeneration of OSF on the surface of the decapacitated oocytes. Furthermore, that a considerable amount of OSF is once again extracted by the third treatment with Triton X-100 supports this idea of the spontaneous regeneration of OSF.

When a high dose of 1-MeAde such as 10^{-5} M was applied to the oocytes treated with Triton X-100 a high rate oocyte maturation occurred, although at physiologically active concentrations of 1-MeAde (around 10^{-7} M) they failed to mature (Fig. 1). However, even in the case of the application of a high dose of 1-MeAde (10^{-5} M) the present study has demonstrated that the hormone-dependent period (HDP) is approximately doubled in the oocytes treated with Triton X-100. The prolonged HDP of

these Triton X-100-treated oocytes is shortened, or recovered, to the normal length of HDP when these oocytes are kept in ASW for 20 h and then treated with 1-MeAde. This indicates that the length of HDP is dependent on the amount of OSF on the oocyte surface, although if the amount of OSF is sufficient, as in the intact oocytes, HDP is not decreased even when the concentration of 1-MeAde is greatly increased (2, 4).

That a high dose of 1-MeAde was effective in inducing oocyte maturation even in the oocytes treated with Triton X-100 is understandable if we consider that the extraction of OSF with dilute Triton X-100 carried out in the present study was not sufficient to remove all OSF present on the oocyte surface. Further, in intact oocytes the amount of OSF on the oocyte surface seems to be much larger than that required for triggering oocyte maturation by 1-MeAde at a low concentration (see Fig. 3 and Table 2). Shortridge *et al.* (17) also suggest that the oocyte surface has multiple 1-MeAde reactive sites and that the reaction of all the sites with 1-MeAde is not necessary to bring about GVBD. Supporting evidence for this notion is that a local application of 1-MeAde on the oocyte surface can induce oocyte maturation (16). However, when the amount of OSF is decreased by treatment with Triton X-100, the length of HDP and the rate of GVBD appear to be determined as a function of the amount of OSF remaining and the concentration of 1-MeAde.

The role of OSF in producing a cytoplasmic maturation-promoting factor which directly induces GVBD (8, 10) remains to be elucidated for further understanding of the hormonal mechanism of starfish oocyte maturation.

We thank Dr Graham Young for reading the manuscript. This work has been supported in part by a grant-in-aid from the Ministry of Education, Science and Culture, Japan to H. K. and a postdoctoral fellowship in special areas from the Japan Society for the Promotion of Science to H. I.

Received for publication 24 May 1982

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