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Normal embryogenesis occurs in starfish eggs induced to mature by microinjection of cytoplasm containing maturation-promoting factor (MPF)

ANDRÉ PICARD[1,2], JEAN-CLAUDE LABBÉ[1] and MARCEL DORÉE[1]

[1]*CNRS LP 8402 and INSERM U. 249, BP 5051 34033 Montpellier CEDEX, France*
[2]*Laboratoire ARAGO, Université Paris VI, 66650, Banyuls-Sur-Mer, France*

Summary

Oocytes arrested at the first meiotic prophase reinitiate meiosis under the influence of a variety of signals, including hormones. These signals are thought to act indirectly to produce a maturation-promoting factor (MPF) which triggers transition to the metaphase. We show here that non-hormone-stimulated starfish oocytes, induced to mature by microinjection of MPF-containing cytosol taken from hormone-stimulated oocytes, produce normal *bipinnaria* larvae once fertilized.

The same result was obtained when donor oocytes were induced to mature by microinjecting heterologous MPF partially purified from amphibian oocytes. These results argue against the hypothesis that maturation events essential for normal embryogenesis occur independently of MPF, at least in starfish.

Key words: maturation-promoting factor, MPF, starfish oocytes, meiotic maturation, embryogenesis, microinjection.

Introduction

In the animal kingdom, oocytes enter meiosis, which is then arrested for a long period of time (from several weeks to several years) at the first meiotic prophase. At the end of vitellogenesis, the fully grown oocytes reinitiate meiosis under the influence of a variety of exogenous signals, including a change in daylight intensity in the Cnidaria *Hydractinia echinata* (Ballard, 1942), specific proteases in the polychaete annelid *Sabellaria alveolata* (Peaucellier, 1977), hormonal stimulation in starfish (Kanatani *et al.* 1969) as well as in many vertebrates (see Masui & Clarke, 1979 for review), or fertilization in many lamellibranchs (Allen, 1953; Dubé & Guerrier, 1982) or echiurians (Jaffe *et al.* 1979).

Most if not all of these signals are thought to act indirectly in inducing meiosis reinitiation. It is assumed that they first interact with components localized at the cell surface (Kanatani & Hiramoto, 1970; Dorée & Guerrier, 1975; Beaulieu *et al.* 1978) to induce structural change and/or to produce hypothetical second messengers. In a second step, this structural change and/or the second messenger is thought to trigger the activation (or production) of a cytosolic maturation-promoting factor (MPF), devoid of zoological specificity (Kishimoto *et al.* 1982), which ultimately induces, directly or indirectly, the transition to the metaphase, including the breakdown of the germinal vesicle (GVBD), chromosome condensation and spindle formation. The following observations have been reported:

(1) Cytoplasm taken from oocytes induced to mature under the influence of exogenous signals triggers all these maturational events when transferred to fully grown prophase-arrested oocytes of the same or other zoological species, even in the absence of protein synthesis (see Gerhart *et al.* 1985 for review).

(2) The active factor is recovered in the cytosolic fraction in stratified oocytes (Masui, 1972; Kishimoto & Kanatani, 1977) and in high-speed supernatants prepared from oocyte homogenates (Wu & Gerhart, 1980; Kishimoto & Kondo, 1986).

Once released from the prophase block, MPF-microinjected oocytes have been shown to complete

meiotic maturation in starfish (Kishimoto & Kanatani, 1976) or to reach metaphase II in amphibians (Masui & Markert, 1971). However, progesterone and 1-methyladenine, the hormones that act as primary messengers in inducing meiosis reinitiation in amphibians (Thibier-Fouchet *et al.* 1976; Smith & Ecker, 1971) and starfishes (Kanatani *et al.* 1969), respectively, have been shown to induce structural changes in the cortical region of the oocytes, which may not be related to the induction of meiotic maturation, but rather to subsequent developmental stages, such as the establishment of the polyspermy block or dorsoventral polarity (Hirai *et al.* 1971; Gautier & Beetschen, 1985). It has not been shown whether all developmental changes essential for normal embryogenesis in fertilized eggs are also triggered when hormonal stimulation (the physiological stimulus) is bypassed and meiotic maturation induced by direct microinjection of cytosol containing MPF activity. In the present work, we show that non-hormone-stimulated starfish oocytes induced to mature by microinjection of MPF-containing cytosol develop normally beyond gastrulation once fertilized. Our results argue against the hypothesis that maturation events essential for normal embryogenesis occur independently of MPF, at least in starfish.

Materials and methods

Handling of gametes

Immature follicle-free oocytes of the starfish *Marthasterias glacialis* were prepared as previously described (Dorée & Guerrier, 1975) by washing and centrifuging them in a large volume of artificial calcium-free sea water. Oocytes were then washed and resuspended in natural sea water. The oocytes without follicle cells were left in sea water for 1 h before cytoplasm microinjection, to confirm the absence of 'spontaneous' escape from the prophase block.

Sperm was prepared by tearing an isolated testis without sea water. Before use, 10 μl of semen were diluted in 500 μl of sea water. For fertilization, 10 μl of diluted sperm were added per ml of oocyte suspension.

Microinjections

The microinjections of cytoplasm or partially purified MPF were performed according to the method of Hiramoto (Hiramoto, 1974; Kishimoto, 1985). The volumes of microinjected material (contained between two oil droplets) did not exceed 70 pl (the volume of the full-grown oocytes used in this study was about 2 nl).

Cytological procedures

In some control experiments, cytoplasm-injected and fertilized eggs were treated for 15 min at room temperature with 0·5 mg ml^{-1} pronase (Calbiochem) in sea water to eliminate the fertilization membrane together with excess sperm; and were then extensively washed in natural sea water to remove the enzyme. After standing for 15 min in methanol at −20°C, the cells were washed once for 5 min in PBS, once again in PBS containing 1 μl ml^{-1} bis-benzimide (Hoechst dye 33258), and were mounted in Mowiol for microscopic examination.

Results

In a first set of experiments, fully grown oocytes of the starfish *Marthasterias glacialis* were treated with 10^{6} M-1-MeAde. 10 min after GVBD, cytoplasm (70 pl) was transferred from these oocytes to non-hormone-treated oocytes, which underwent GVBD 15 min later. 40 min after cytoplasm transfer, sperm was added to the recipient oocytes.

They readily formed a fertilization membrane, completed meiotic maturation and cleaved. Normal gastrulation was observed beginning about 18 h after cytoplasm transfer. The stage of young bipinnaria was attained after 3 days (Fig. 1). All embryos that formed a normal blastula reached the *bipinnaria* stage (Table 1). Failure of about 50 % of the embryos to form a hollow blastula was due to the microinjection itself, not to the lack of hormonal stimulation: the same result was obtained when cytoplasm-microinjected oocytes were simultaneously treated with 1-MeAde, whereas all control oocytes treated with 1-MeAde formed normal blastulae once fertilized. Since polyspermic eggs do not develop normally in starfish (Schuetz, 1975), the above results indicate that the polyspermy block was established following transfer of MPF-containing cytoplasm. To demonstrate this point more directly, some of the cytoplasm-injected eggs were treated with pronase 5 min after fertilization, to eliminate the fertilization membrane as well as adsorbed sperm, and then stained with Hoechst 33258, a DNA-specific dye. A single spot of bright fluorescence was observed, indicating that a single spermatozoon had penetrated the maturing oocyte (Fig. 2).

In the experiments described above, the MPF-containing cytoplasm used to induce meiotic maturation was taken from hormone-stimulated oocytes. Therefore the possibility still remained that, besides MPF, the transferred cytoplasm might have contained some MPF-independent material, produced or activated upon 1-MeAde stimulation and essential for normal development to occur. This was ruled out by the following experiments.

(1) Normal development beyond gastrulation was observed when cytoplasm taken from 1-MeAde-stimulated oocytes was serially transferred before fertilizing the third recipient. In this experiment, it was calculated that the third recipient received only about 0·0002 % of the material present in the 1-

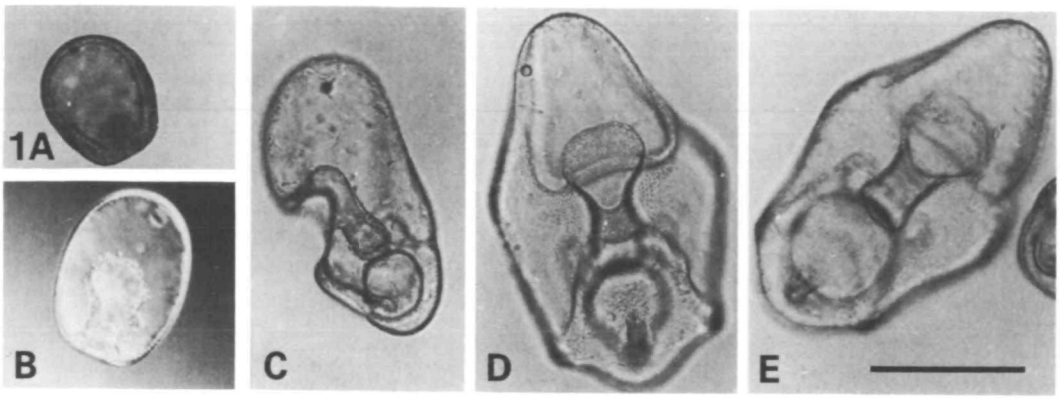

Fig. 1. Development of fertilized eggs of the starfish *Marthasterias glacialis* induced to mature by microinjection of MPF. (A) Onset of gastrulation 18 h after microinjection. (B) Late gastrula, 32 h. Mesenchymal cells are leaving the roof of the archenteron (Nomarski interferential contrast). (C,D) Bipinnariae 3 (C) and 5 (D) days after MPF microinjection: large mouth, oesophagus, round stomach and intestine are visible, (E) Control bipinnaria 5 days after induction of meiotic maturation by hormonal stimulation. The small sphere in each microinjected larva is an oil droplet accompanying the microinjected material. Bar, 200 μm. All micrographs are at the same magnification.

Table 1. *Oocytes microinjected with MPF-containing cytoplasm produce normal* bipinnaria *larvae once fertilized*

Induction of maturation in donor oocytes	Induction of maturation in recipient oocytes	Proportion of embryos reaching the specified stage			
		cleavage	blastula	gastrula	bipinnaria
1-MeAde	Cytoplasm transfer*	15/15†	8/15	8/15	8/15
	Cytoplasm transfer*+1-MeAde	15/15	7/15	7/15	7/15
Xenopus MPF	Cytoplasm transfer*	10/10	5/10	5/10	5/10

* 70 pl of cytoplasm were transferred to recipient oocytes (internal volume: 2 nl).
† Number of embryos reaching the specified stage/total number of microinjected oocytes.

MeAde-stimulated first donor oocyte (data not shown).

(2) Normal development beyond gastrulation was observed (Table 1) when cytoplasm was taken from oocytes induced to mature by microinjection of partially purified MPF prepared from *Xenopus* oocytes (Wu & Gerhart, 1980). Oocytes directly injected with partially purified MPF were fertilized under polyspermic conditions, probably due to the high EGTA content of the active extract, which prevents formation of the fertilization membrane. These polyspermic eggs cleaved but did not blastulate.

Discussion

1-MeAde has been shown to trigger a transient increase in intracellular Ca^{2+} (Moreau *et al.* 1978*a*,*b*), a decrease in intracellular cAMP (Meijer & Zarutskie, 1987) an increased permeability to Na^+ (Dorée, 1981) and activation of the Na^+ pump (Guerrier *et al.* 1979; Moreau *et al.* 1978; Dorée, 1981*a*,*b*) in starfish oocytes. However, (1) oocytes clamped at less than 0·1 μM-free Ca^{2+} by microinjecting EGTA still undergo meiotic maturation following hormonal stimulation (Picard & Dorée, 1983); (2) forskolin (100 μM), which causes a 37-fold increase of the resting cAMP, does not inhibit meiotic maturation triggered by 1-MeAde (Meijer & Zarutskie, 1987); (3) oocytes readily undergo meiotic maturation in Na^+-free sea water. Suppression of external Na^+ even induces maturation in the absence of hormonal stimulation (Peaucellier *et al.* 1988) and (4) ouabain, which inactivates the Na^+ pump, does not prevent 1-MeAde from inducing meiotic maturation (Guerrier *et al.* 1979).

The above results show that, besides its role in MPF activation, the hormone triggers events not related to meiotic maturation, whose significance for further development is unknown. Nor has it been shown that the whole series of changes which take place in hormone-treated oocytes can be completed in MPF-treated oocytes. Therefore it was possible that, independently of its role in MPF activation, the hormone could trigger some event(s) unnecessary for meiotic maturation but required for subsequent embryogenesis.

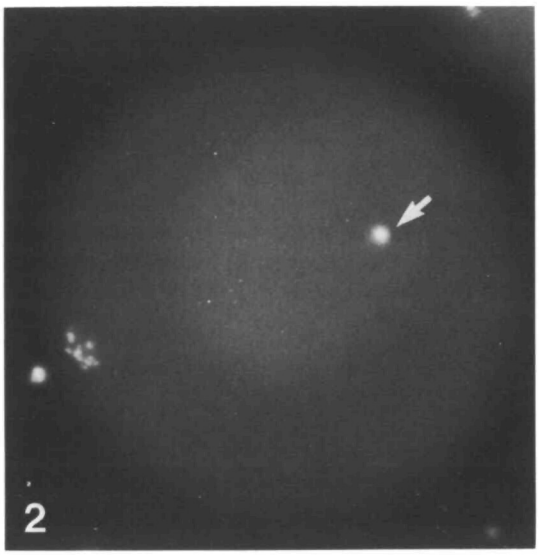

Fig. 2. Polyspermy block established under the influence of MPF microinjection. 5 min after fertilization, an oocyte induced to mature by microinjection of MPF was stained with Hoechst 33258, as described in Materials and Methods. Besides maternal chromosomes, a single spot of bright fluorescence can be seen (arrow) indicating that a single sperm atozoom has penetrated the maturing oocyte.

However, in the present work, we found that microinjection of cytoplasm taken either directly from hormone-stimulated oocytes or from oocytes induced to mature by sequential transfer of cytoplasm initially taken from hormone-stimulated oocytes, allowed the recipient oocytes not only to complete meiotic maturation, but also to develop normally, at least to the *bipinnaria* stage, once fertilized. Identical results were obtained when cytoplasm was taken from oocytes induced to mature by the transfer of heterologous MPF, partially purified from *Xenopus* eggs. Taken together, these results demonstrate that in the whole series of events that take place in hormone-treated oocytes, only MPF activation is required for both meiotic maturation and further development. Spawning also has been shown to be directly triggered by MPF produced under the influence of 1-MeAde (Kishimoto *et al.* 1984).

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