

# The role of cAMP in oocyte maturation and the role of the germinal vesicle contents in mediating maturation and subsequent developmental events in hydrozoans

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**Summary.** Externally applied membrane permeable cAMP derivatives and the injection of cAMP induce oocyte maturation in several species of hydrozoans. This technique for inducing oocyte maturation has been used to study ion permeability changes, maturation promoting factor activity and surface tension changes during maturation. Oocyte membrane potential remains constant during maturation. Cyclic AMP induced maturation proceeds in the absence of external  $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$  or  $\text{Na}^+$ . Cytoplasm from maturing oocytes that induces oocyte maturation when it is injected into untreated oocytes is produced during cAMP induced maturation. Surface tension, as measured by the application of a standardized force that mechanically deforms individual oocytes, declines during the first part of maturation. This is followed by a sharp rise and fall of surface tension at first and second polar body formation that accompanies a slow rise in the resistance of oocytes to deformation during the last part of maturation. The production of maturation promoting factor activity and some of the changes in surface tension during maturation can occur in the absence of germinal vesicle material. Two early developmental events that follow oocyte maturation are the production of sperm chemoattractant and calcium channel function. Neither of these events occurs in eggs that have undergone maturation in the absence of germinal vesicle material. The addition of germinal vesicle contents from oocytes to eggs that have undergone maturation in the absence of germinal vesicle material initiates calcium channel function. This experiment indicates that the germinal vesicle contains factors that are necessary for post-maturation developmental events.

**Key words:** cAMP – Oocyte maturation – Germinal vesicle – Calcium channels – Hydrozoa

## Introduction

Oocyte maturation is the transition that bridges oogenesis and embryogenesis. During this transition the germinal vesicle breaks down, meiosis is reinitiated and a number of changes occur that are necessary for later stages of embryogenesis. This process has been studied in some detail in

amphibians, asteroids and mammals (Masui and Clarke 1979; Meijer and Guerrier 1984; Schultz et al. 1983). In these animals a neuroendocrine system sends a signal to the ovarian follicle cells causing them to produce a hormone that initiates the process of maturation in oocytes that have reached an appropriate developmental stage. In both amphibians and asteroids, cAMP appears to function as a second messenger in oocytes at an early point in the hormone-induced maturation process (Meijer and Zarutskie 1987; Maller 1983).

In many hydrozoans oocyte maturation and spawning occur as a consequence of a light cue. In some species maturation and spawning occur at a predictable time after animals that have been in the dark are brought into the light, while in other species these events occur after animals that have been in the light are placed in the dark (Miller 1979). Experiments have been done on two species of hydrozoans, *Spirocodon* and *Hydractinia*, demonstrating that sea water in which gonads have spawned induces oocyte maturation in pieces of intact gonad or in isolated oocytes that are not under maturation-inducing light conditions (Ikegami et al. 1978; Freeman 1987). This indicates that there is a humoral factor which mediates this process. The active factor in the spawning water from *Spirocodon* has been partially purified; it appears to be a peptide. Nothing is known about how this hormone acts to bring about the intracellular events leading to maturation.

After the initiation of maturation, there are a number of changes that take place in the oocyte which are associated with the maturation process. In both amphibians and asteroids, there are changes in membrane potential during maturation. In amphibians, this involves a slow depolarization of the membrane (Kado et al. 1981; Wallace and Steinhardt 1976). In some asteroid species, there is also a slow depolarization of the membrane (Miyazaki et al. 1975), while in other species the membrane potential hyperpolarizes (Shen and Steinhardt 1976). These membrane potential changes probably reflect the maturation of the ionic mechanism that will be associated with the ability to generate a fertilization potential which serves as a block to polyspermy. At present a role for these changes in membrane potential in causing oocyte maturation is unclear (see Meijer and Guerrier 1984).

Work on amphibians and asteroids has demonstrated that the cytoplasm from an oocyte that is maturing as a

consequence of hormone treatment can induce maturation when injected into an untreated oocyte. Thus, the hormone induces the appearance of an intracellular factor which plays a causal role in maturation. Additional experiments with oocytes of these two classes of animals have also shown that the production of maturation promoting activity takes place in oocytes that have had their germinal vesicle removed prior to hormone treatment (Masui and Markert 1971; Schorderet-Slatkine and Drury 1973; Kishimoto et al. 1981; Picard and Dorée 1984). This experiment defines one aspect of maturation that can occur in the absence of a germinal vesicle.

Surface tension changes have been measured in oocytes of asteroids during maturation (Nemoto et al. 1980). Similar changes probably occur in amphibians. These changes involve a decline in surface tension preceding germinal vesicle breakdown followed by a transient rise and fall in surface tension at the time of first and second polar body formation. These changes probably reflect alterations in the cortical cytoskeleton (Schroeder and Otto 1984). The effect of removing the germinal vesicle on surface tension during oocyte maturation has also been studied (Yamamoto and Yoneda 1983). Without the germinal vesicle, the initial decline in surface tension occurs; however, the transient rise and fall in tension that would be associated with the times of first and second polar body formation does not take place. The absence of changes in tension during this period does not reflect the fact that the oocyte lacks a meiotic apparatus because oocyte halves that lack a meiotic apparatus, but have germinal vesicle contents, go through these changes in surface rigidity even though they do not produce polar bodies.

Experimental studies on amphibian and asteroid oocytes, that undergo maturation in the absence of a germinal vesicle, have demonstrated that some events that could not be induced prior to maturation can now be induced, for example, cortical granule breakdown (Masui and Markert 1971; Hirai et al. 1971). However, other events will not occur unless germinal vesicle contents are present. Amphibian oocyte germinal vesicles contain factors that are needed for the formation of asters (Heidemann and Kirschner 1978), the induction of DNA synthesis and male pronuclear development (Gurdon 1967; Katagiri and Moriya 1976) and development beyond the blastula stage (Briggs and Cassens 1966). Asteroid oocyte germinal vesicles also contain factors that are necessary for male pronuclear development (Yamada and Hirai 1984). These factors are stored in the germinal vesicle during oocyte growth and are released into the cytosol when the germinal vesicle breaks down during maturation. One developmental event that occurs after oocyte maturation in hydrozoans that depends on the presence of the germinal vesicle during maturation is fertilization (Freeman 1987). Hydrozoan eggs only have one fertilization site on their surface. This site is set-up by the meiotic apparatus during maturation. This case differs from the amphibian and asteroid examples because only a discrete structure that is assembled from the germinal vesicle, the meiotic apparatus, is necessary for the formation of the fertilization site.

Two early developmental events that occur after oocyte maturation in hydrozoans are: (1) the development of sperm chemotaxis, and (2) the appearance of voltage sensitive calcium channels. Sperm chemotaxis begins either during the time interval between first and second polar body

formation or as second polar body formation is taking place. If a mature egg is cut in half, both halves will attract sperm. Eggs continue to attract sperm until they have been fertilized or activated (Freeman 1987; Freeman and Miller 1982; Miller 1978). In the hydrozoan *Phialidium*, calcium channel function is first initiated about one hour after fertilization or egg activation (Freeman and Ridgway 1987) when first cleavage normally begins. If a mature egg is cut in half, and both halves are activated, calcium channel function will occur in each half.

This paper reports that membrane permeable cAMP derivatives, which presumably act as second messengers, induce oocyte maturation in several species of hydrozoans. Using this method for inducing maturation, we have examined membrane potential changes, maturation promoting activity, and tension at the surface of hydrozoan oocytes during the transition from oocyte to egg. These experiments have defined some of the intracellular events that occur during maturation and have established the extent to which these events depend on the germinal vesicle. The paper also examines the role the germinal vesicle contents play in the development of sperm chemotaxis and the appearance of voltage dependent calcium channels.

## Materials and methods

*The biological material.* All animals were collected in the vicinity of the Friday Harbor Laboratories, except for *Hydractinia* which was collected at Cape Cod, Mass, USA. The descriptions of Arai and Brinkmann-Voss (1980) were used to identify medusae. When eggs and sperm were needed, they were obtained through spawnings. The timing of spawning was manipulated by controlling the light cycle (Miller 1979). Eggs were fertilized by placing them in sea water from a bowl containing one or more male medusae that had just spawned. All of the medusae used have ovaries with oocytes at different developmental stages, except for *Hydractinia* where all of the oocytes in a gonophore are at a comparable stage. Oocytes were obtained by dissecting out ovaries, opening them and teasing out individual large oocytes with fine tungsten needles. None of the dissected oocytes had follicle cells around them. In most experiments, only large oocytes which are the size of spawned eggs were used. The diameter of living oocytes was measured using a compound microscope equipped with a screw type ocular micrometer. All experiments were done at 12–14° C, except for the experiments on *Hydractinia* which were done at 22° C.

*Solutions.* In most experiments, oocytes were maintained in millipore filtered pasteurized sea water (PSW). This was prepared by passing sea water through a 0.45 µm filter, followed by heating at 80–90° C for 15 min. For some experiments, artificial sea waters that were either Ca<sup>2+</sup>, K<sup>+</sup>, Na<sup>+</sup>, or Mg<sup>2+</sup> free were used. Table 1 gives the ionic composition of these modified sea waters.

*Oocyte maturation.* Oocytes were placed in 1 ml wells in either PSW or PSW with a cAMP derivative and were examined at frequent intervals with a dissecting microscope for germinal vesicle breakdown and polar body formation. In some oocytes the germinal vesicle breaks down, but polar body formation does not occur. These cases are classified as false maturation. Both polar bodies must form in order

**Table 1.** The ionic concentration (mM) of the cation modified sea waters

Sea waters	Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	HCO <sub>3</sub> <sup>-</sup>	Other components
Ca free	425	9	0	49	472	30	2	1 mM EGTA
K free	425	0	9	49	481	30	2	
Mg free	425	9	9	0	452	0	2	
Na free	0	9	9	49	490	30	0	425 mM Tris HCl
Na free	0	9	9	49	490	30	0	425 mM Choline Cl

All salines were buffered with 20 mM TES at pH 7.8. NaOH was used to pH the sea water, except for the Na free sea waters where KOH was used

to classify an oocyte as mature. At this point, the oocyte has become an egg.

**Electrophysiology.** Standard recording equipment and electrophysiological techniques were used to make intracellular recordings during oocyte maturation. The microelectrodes had a resistance of 20–45 megaohms and were filled with 3 M potassium acetate. The oocyte was impaled with a single electrode. Membrane voltage was monitored with an oscilloscope, and recorded on a Gould pen recorder. The bath was surrounded by a cooling coil that kept the temperature at 12–13° C. The bottom of the bath was covered with Sylgard in which a small indentation was made to hold the oocyte.

**Operations on oocytes.** Hydrozoan oocytes are surrounded by a jelly layer. The jelly layer can be visualized by examining oocytes on a microscope slide in a thick particulate ink suspension in sea water. The ink suspension was prepared by rubbing a stick of Chinese ink in sea water. The jelly coat prevents the ink particles from making contact with the surface of the egg. In some experiments, the jelly layer was removed by treating oocytes with acidified sea water (pH 4) for 5 min followed by washing with PSW (pH 7.8–8.0).

Oocytes were cut in half, or the germinal vesicle was dissected out in a dish coated with 2% agar made up in sea water. Fine glass needles were used as knives for these operations.

Cytoplasm and nucleoplasm transfer experiments involved removing cytoplasm from an oocyte half, or nucleoplasm from a germinal vesicle in Ca<sup>2+</sup>-free sea water, and injection into either an intact oocyte or a half oocyte. The holder that contained the oocyte or oocyte half to be injected, the donor germinal vesicle, or oocyte half, and the injection system used for removing the nucleoplasm from the germinal vesicle or the cytoplasm from the oocyte half and transferring it into the oocyte, are all described in Kiehart (1982). The procedure used for determining the volume of injected material is given by Kishimoto (1986). A microscope with a stage that does not move when the microscope is focused was used for viewing the preparation during injection. A Leitz micromanipulator was used to position and move the injection needle. This same system was also used to inject cAMP into oocytes.

**Measurements of the surface force of maturing oocytes.** We have used changes in tension at the surface of intact oocytes and oocyte halves to monitor the process of maturation. This method involves the controlled compression of a single oocyte between two plates. One plate is attached to a cali-

brated bending balance. The other plate which is rigid is attached to a micromanipulator. The micromanipulator arm is lifted to put a measurable amount of force on the bending balance, and it simultaneously compresses the oocyte. The amount of oocyte compression is measured with an ocular micrometer using a horizontal microscope. From the force applied ( $F$ ), the initial diameter of the oocyte prior to compression ( $Z_0$ ), and the new diameter of the oocyte as a consequence of the force applied ( $Z$ ), the tension at the oocyte surface ( $T$ ) was calculated using the equation  $F = Z_0 \cdot (-ds/dz) \cdot T$ . In this study, the applied force ( $F$ ) varied between  $1-4 \times 10^{-7}$  Newtons for either the intact oocyte or its fragment.  $(-ds/dz)$  is the value of the relative surface area of the oocyte for different degrees of compression. Yoneda (1986) provides a table of  $(-ds/dz)$  values and a discussion of how they are calculated. A theoretical and practical discussion of this method can be found in Yoneda (1973, 1986). Since tension at the surface of eggs is temperature dependent (see Yoneda 1976), the observation chamber used in these experiments was cooled with a water jacket, and the temperature of the chamber was monitored with a thermistor.

**Assay for the production of sperm chemoattractant.** Siliconized slides were prepared. A solution of polyethylene glycol was prepared in distilled water (1 mg/5 ml). This was spread as a thin layer on the slide. The polyethylene glycol and siliconization treatment prevents sperm from sticking to the slide and allows sea water to form a large flat drop in which the egg to be tested is placed. The region around the egg was examined with dark-field illumination at a magnification of  $100\times$ . Water containing sperm was added some distance from the egg so that the behavior of sperm as they approached the egg could be observed. When an egg is producing sperm chemoattractant, sperm swimming in the vicinity of the egg change their direction of swimming, and swim toward the egg. After a short period of time, a large number of sperm accumulate around the egg. Miller (1978) and Freeman (1987) show chemotactic responses of hydrozoan sperm to eggs. An egg will continue to produce chemoattractant until it has been fertilized or activated.

**Activation of eggs and assay for calcium channel function.** Hydrozoan eggs can be activated with the calcium ionophore A23187 (Freeman and Miller 1982). A23187 is made up as a stock solution with 1 mg dissolved in 1 ml of ethanol. An egg in 1 ml of PSW is activated by treating it with 4  $\mu$ l of ionophore solution for 1–2 min, during which time the solution is agitated so that the ionophore which is precipitating will make contact with the egg. After ionophore

treatment, the egg half is washed in PSW to remove the ionophore.

The eggs of *Phialidium* contain a calcium-specific photoprotein in their cytosol (Freeman and Ridgway 1987). Functional calcium channels can be detected in activated or fertilized eggs beginning about 50 min post-fertilization or activation. In these experiments, calcium channel function was assayed 60–80 min post-activation. Calcium channel function is assayed by placing a single egg in 0.5 ml of PSW and measuring light production following the addition of 0.5 ml of 550 mM KCl. The KCl treatment depolarizes the cell membrane of the egg causing a calcium transient which is registered as light production. In the absence of calcium channels, light production does not occur. The integrating photomultiplier used to measure light production has been described by Freeman and Ridgway (1987).

## Results

### *The role of cAMP in oocyte maturation*

The visible events associated with hydrozoan oocyte maturation involve the breakdown of the germinal vesicle, the resumption of meiosis and first and second polar body formation. This process is shown for isolated oocytes of the hydrozoan *Phialidium* in Fig. 1. The effect of the cAMP derivatives bromoadenosine 3'5' cyclic monophosphate (Br-cAMP) and dibutyladenosine 3'5' cyclic monophosphate (dB-cAMP) on oocyte maturation was studied in nine species of hydrozoans from three orders (Table 2). These experiments involve the continuous treatment of isolated oocytes in PSW in the presence or absence of a given cAMP derivative. Our results show that Br-cAMP induces maturation in seven of these species. dB-cAMP only induces maturation in a significant percentage of cases in two species, and in both species, high concentrations of this cAMP derivative were needed. In *Mitrocoma*, neither cAMP derivative had an effect on oocyte maturation, while high extracellular concentrations of Br-cAMP only had a minimal effect in *Hydractinia*. The extracellular concentration of Br-cAMP that gives 50% oocyte maturation varies. In some species (e.g., *Sarsia*, *Mitrocomella*) the concentration is about 0.25 mM, while in other species (e.g., *Stomatoca*, *Aequorea*) it is about 5 mM. The predictability of the response of full grown oocytes to a given concentration of Br-cAMP is very good in some species (e.g., *Sarsia*, *Mitrocomella*) in that oocytes from different females undergo maturation in a comparable percentage of cases. However, the response of full grown *Phialidium* oocytes from different females to a given concentration of Br-cAMP can be rather variable. After oocytes of these different species have undergone cAMP induced maturation, the addition of sperm to these eggs fertilizes them and normal planula larvae develop.

*Phialidium* oocytes were also incubated in bromoguanosine 3'5' cyclic monophosphate (Br-cGMP) or cAMP in order to see if these cyclic nucleotides can induce maturation (Table 2). In both sets of experiments, control oocytes from the same female were incubated in Br-cAMP to demonstrate that these oocytes could mature. Br-cGMP and cAMP had no effect on maturation. The role of cAMP in inducing maturation was also tested by injecting it into oocytes. In these experiments, oocytes with diameters of approximately 190  $\mu$ m were injected with 30–50  $\mu$ l or about

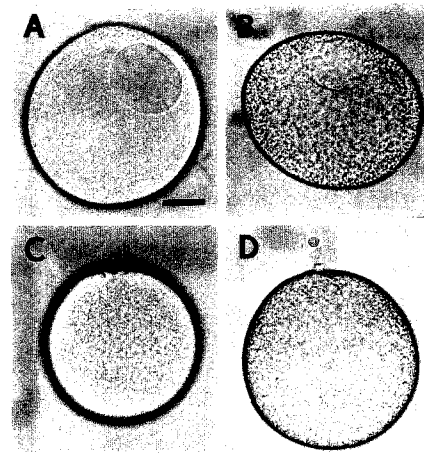


Fig. 1. The maturation response of *Phialidium* oocytes to Br-cAMP. (A) Oocyte with germinal vesicle prior to treatment with Br-cAMP. (B) The beginning of germinal vesicle breakdown. (C) Just after first polar body formation. (D) Just after second polar body formation. All photographs are at the same magnification. The bar instances 50  $\mu$ m

1% of their volume with a 70 mM solution of cAMP in 100 mM KCl. This is equivalent to raising the internal cAMP concentration by 0.8 mM if the volume of the oocyte is taken as the volume of the cytosol. Seven of the 13 cases that were injected underwent maturation. Control experiments in which KCl was injected had no effect (8 cases). We interpret this experiment to mean that the incubation of oocytes in cAMP does not induce maturation because their cell membranes are impermeable to cAMP.

Oocytes of each of the species that respond to Br-cAMP by undergoing maturation do so over a characteristic time course when optimal concentrations of Br-cAMP are used. The time elapsed between the addition of an optimal concentration of Br-cAMP until second polar body formation and the time between a change of light conditions needed to induce spawning and the onset of spawning are given for seven species in Fig. 2. Observations on oocytes dissected from these species at various time intervals prior to spawning indicate that the second polar body is normally given off within minutes before spawning takes place. Figure 2 shows that the time period of Br-cAMP induced maturation corresponds to or is shorter than the time period which elapses for natural oocyte maturation and spawning as a consequence of a light cue, in every species except *Stomatoca*. In *Aequorea* and *Sarsia* where the time for maturation is shorter than the time for the initiation of spawning, the stimulus for maturation is probably delayed for a characteristic time following the environmental light cue which initiates the process. These results suggest that the time course for cAMP initiated oocyte maturation is similar to the time course that is observed when a natural spawning occurs.

The time course of the process of oocyte maturation within a species depends on the kind of cAMP derivative employed, the concentration of the cAMP derivative used, and the developmental stage of the oocytes used. Figure 3 compares the effect of Br-cAMP and dB-cAMP on the time course of oocyte maturation for a batch of *Aequorea* oocytes from the same female. While both cAMP derivatives induce oocyte maturation in *Aequorea*, the first visible indi-

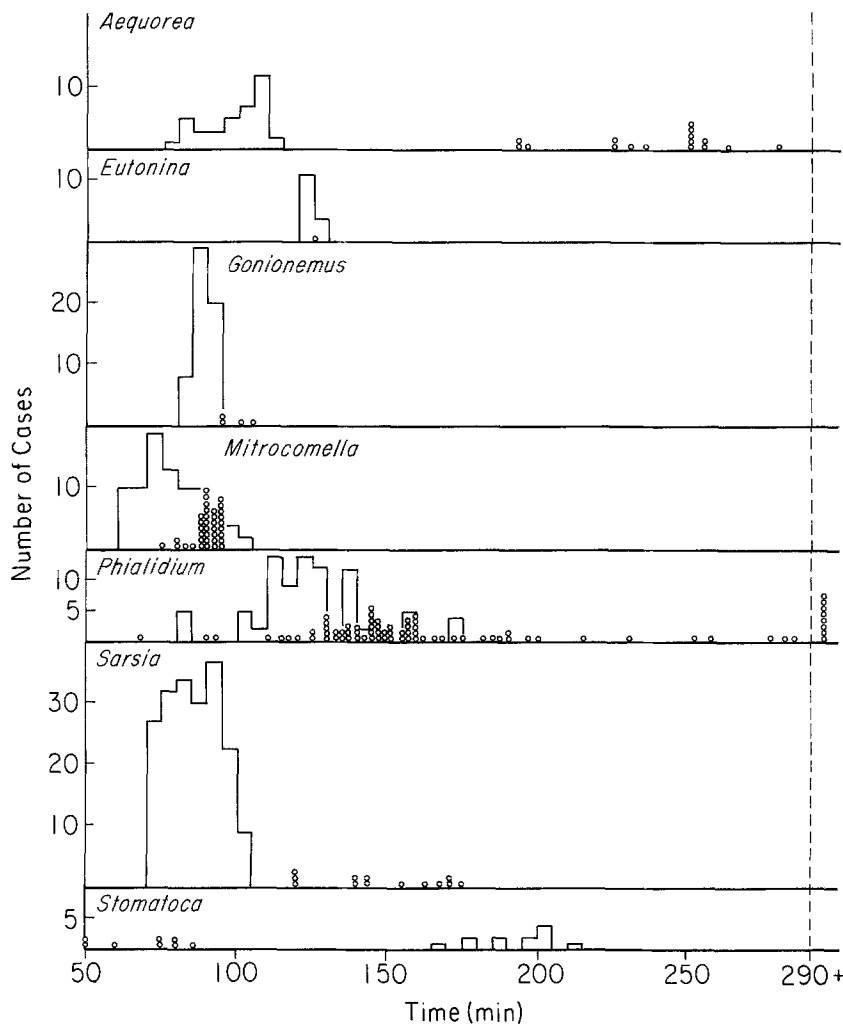
**Table 2.** The effect of cAMP derivatives on oocyte maturation

Order Species	Treatment	Conc. (mM)	# of cases	% matur.	% False matur. <sup>a</sup>
<b>Anthomedusae</b>					
<i>Hydractinia echinata</i>	sea water	—	29	0	10
	Br-cAMP	11.1	28	7	0
	Br-cAMP	2.3–4.7	13	0	8
	dB-cAMP	10.2	14	0	0
<i>Sarsia tubulosa</i>	sea water	—	101	0	0
	Br-cAMP	12–23	39	100	0
	Br-cAMP	1.2–2.3	104	98	1
	Br-cAMP	0.3–0.6	123	100	0
	Br-cAMP	0.23	15	47	0
	Br-cAMP	0.12	64	30	0
	dB-cAMP	1.0	16	0	0
<i>Stomatoca atra</i>	sea water	—	69	9	15
	Br-cAMP	23	15	67	13
	Br-cAMP	4.7	68	29	15
	Br-cAMP	2.3	20	15	0
	Br-cAMP	1.2	43	5	0
	dB-cAMP	4.1–20.4	48	15	13
<b>Leptomedusae</b>					
<i>Aequorea victoria</i>	sea water	—	28	0	25
	Br-cAMP	11.5–23	41	95	0
	Br-cAMP	4.7	21	57	0
	Br-cAMP	2.3	6	0	17
	dB-cAMP	20.4	15	53	0
	dB-cAMP	4.1	5	0	80
<i>Eutonina indicans</i>	sea water	—	13	0	31
	Br-cAMP	2.3	16	81	0
<i>Mitrocoma cellularia</i>	sea water	—	56	0	2
	Br-cAMP	23	23	0	0
	Br-cAMP	1.2–4.7	35	0	0
	dB-cAMP	20.4	24	0	0
	dB-cAMP	4.1	18	0	0
<i>Mitrocomella polydiademata</i>	sea water	—	78	0	0
	Br-cAMP	1.2	61	97	0
	Br-cAMP	0.3–0.6	123	95	0
	Br-cAMP	0.12	30	17	0
	dB-cAMP	1.0	9	0	0
<i>Phialidium gregarium</i>	sea water	—	64	0	2
	Br-cAMP	12	4	100	0
	Br-cAMP	1.2–2.3	135	79	7
	Br-cAMP	0.6	17	35	0
	Br-cAMP	0.12	12	0	8
	dB-cAMP	2–10	25	0	4
	dB-cAMP	0.1	10	0	0
	Br-cGMP	1.1	10	0	0
	cAMP	2.85–28.5	13	0	0
<b>Limnomedusae</b>					
<i>Gonionemus vertens</i>	sea water	—	66	20	3
	Br-cAMP	2.3	76	99	1
	Br-cAMP	1.2	21	72	0
	Br-cAMP	0.23–0.6	54	15	0
	dB-cAMP	20.4	15	40	0
	dB-cAMP	4.1–5.1	30	3	7

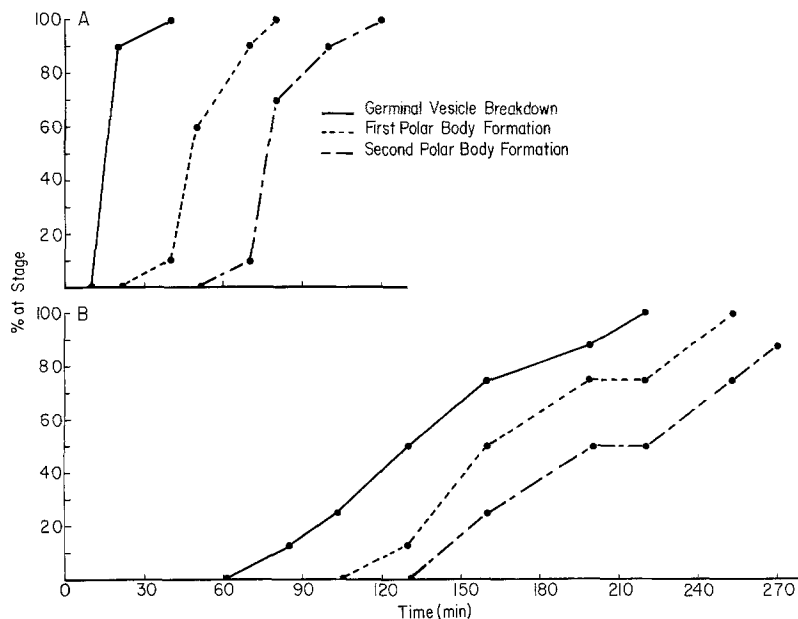
<sup>a</sup> False maturation refers to those oocytes in which the germinal vesicle breaks down, but the polar bodies do not form

cation of maturation (germinal vesicle breakdown) occurs earlier when Br-cAMP is used. The effect of different concentrations of Br-cAMP on the time course of oocyte maturation for a batch of oocytes from a *Gonionemus* medusa

is examined in Fig. 4. The results show that when a concentration of Br-cAMP is used which only allows a portion of the oocytes to mature, the time required for maturation is lengthened. A similar effect of Br-cAMP concentration



**Fig. 2.** Histograms showing the times for the completion of second polar body formation following Br-cAMP induced maturation (boxes) and light induced spawning (circles) of female medusae, for different species of hydrozoans. *Aequorea*, *Eutonina*, *Gonionemus*, *Mitrocomella*, *Phialidium*, *Sarsia* and *Stomatoca* spawn when medusae that have been in the dark are transferred to the light, while *Gonionemus* spawns when medusae that have been in the light are transferred to the dark for at least 45 min



**Fig. 3A.** The effect of 2.3 mM Br-cAMP (10 cases) and **B** 20.4 mM dB-cAMP (8 cases) on the time course of oocyte maturation in *Aequorea*. The time scale is the same in **A** and **B**

on the time course of oocyte maturation has been observed for *Mitrocomella* and *Sarsia*. Figure 5 examines the effect of oocyte developmental stage on the time course of oocyte maturation in *Mitrocomella*. In this experiment three size

classes of oocytes were dissected out of the gonads of one medusa: 18 oocytes had diameters of 102–110  $\mu\text{m}$  (the size of mature eggs), 8 oocytes had diameters of 90–98  $\mu\text{m}$ , and 14 oocytes had diameters of 70–88  $\mu\text{m}$ , the smaller

oocytes are presumably at earlier developmental stages than the full grown oocytes. Each oocyte size class was treated with 1.2 mM Br-cAMP. None of the small oocytes (70–88  $\mu\text{m}$ ) underwent maturation during the observation period. Five of the oocytes in the 90–98  $\mu\text{m}$  size class matured; however, these oocytes had to be incubated in Br-cAMP for a longer period than full grown oocytes in order to complete the process of maturation. In each of these experiments the time period until the first visible indication of maturation (germinal vesicle breakdown) is variable, while the time interval that elapses until subsequent maturation events (first and second polar formation) does not depend on the experimental manipulation. This suggests that once an appropriate intracellular level of Br-cAMP is achieved, the process of oocyte maturation is triggered and runs its

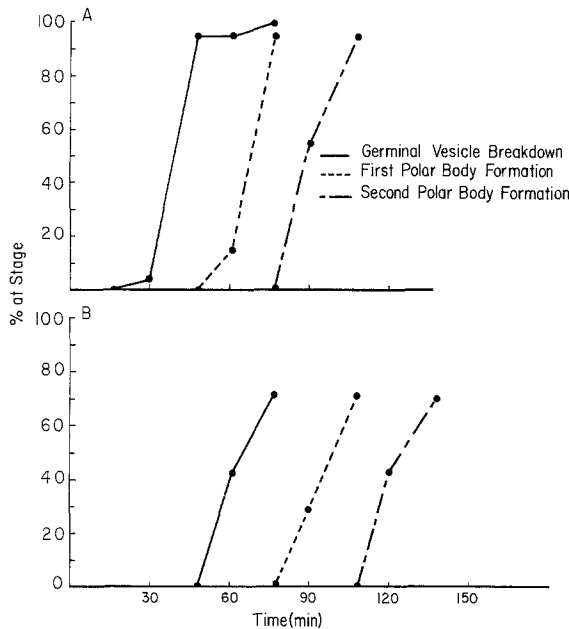
course over a characteristic time period. Figure 5 shows that smaller oocytes take longer to reach this trigger level. In small oocytes this critical level may be higher than in full sized oocytes. However, we cannot rule out other possible explanations (see discussion). Under normal conditions, only the largest oocytes are spawned.

In order to define the time period during which an elevated level of Br-cAMP is necessary for oocyte maturation, a cohort of oocytes was placed in 1.2 mM Br-cAMP and samples of oocytes were removed from the dish at various times, washed in two changes of PSW to remove the external Br-cAMP and set aside to see if they would mature. Figure 6A presents the results of a typical experiment on *Sarsia*. The results show that there is a transitional period prior to germinal vesicle breakdown after which oocytes no longer need to be incubated in Br-cAMP in order to mature. The timing of germinal vesicle breakdown and first and second polar body formation is the same for oocytes which are removed from Br-cAMP after the transitional period but prior to germinal vesicle breakdown, as it is for oocytes which have been incubated continuously in Br-cAMP (Fig. 6B). Similar results have been obtained in experiments on *Gonionemus*, *Mitrocomella* and *Phialidium*.

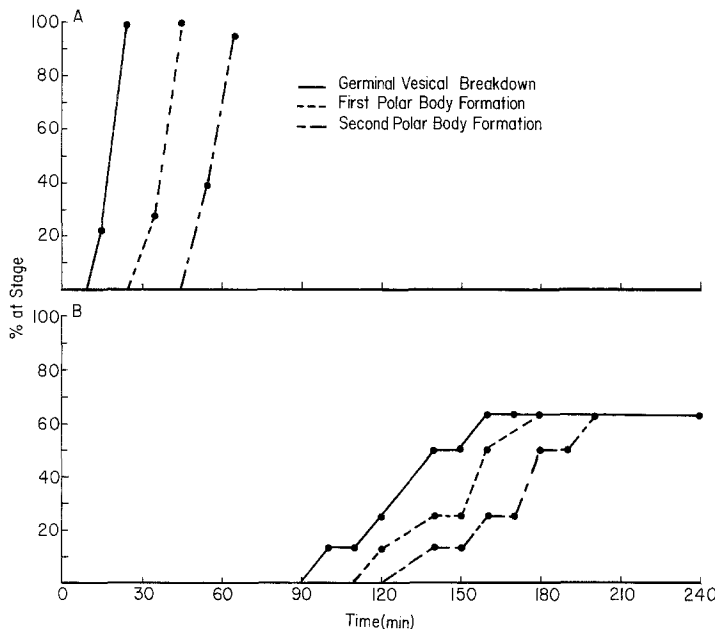
#### Subsequent events in the maturation process

**Ionic changes.** To find out if the membrane potential varies during oocyte maturation, four *Mitrocomella* and four *Phialidium* oocytes were impaled with a microelectrode, Br-cAMP was added to induce maturation and the resting potential of each oocyte was monitored through polar body formation. The initial resting potentials of the *Mitrocomella* oocytes were  $-42$  to  $-48$  mV, while the initial resting potentials of the *Phialidium* oocytes were  $-40$  to  $-60$  mV. There were no consistent changes in resting potential and no action potentials during the maturation process.

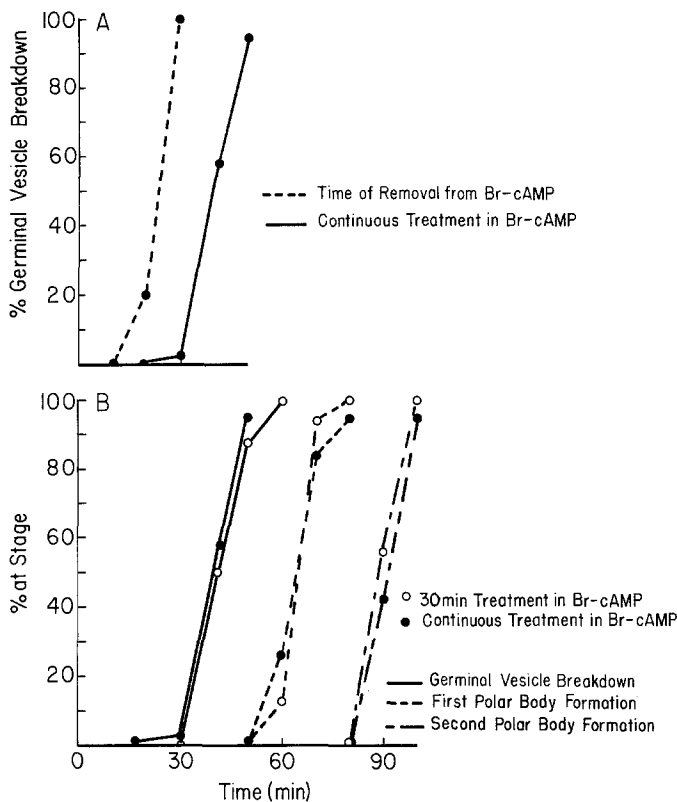
After the maturation of the *Phialidium* oocytes had been completed, the electrode was left in the egg and sperm were added. Three of these eggs were fertilized. The criterion used to score for fertilization was the initiation of first cleavage 1 h after sperm addition. The cleavage initiation time



**Fig. 4.** The effects of **A** 2.3 mM (27 cases) and **B** 1.2 mM (21 cases) Br-cAMP on the time course of oocyte maturation in *Gonionemus*. The time scale is the same in **A** and **B**



**Fig. 5.** The effect of oocyte size (developmental stage) on the time course of oocyte maturation in *Mitrocomella*. **(A)** Oocytes with a diameter of over 102  $\mu\text{m}$  (18 cases); **(B)** Oocytes with a diameters of 90–98  $\mu\text{m}$  (8 cases). The time scale is the same in **A** and **B**



**Fig. 6A.** Percent of *Sarsia* oocytes that eventually underwent germinal vesicle breakdown as a function of time of removal from 1.2 mM Br-cAMP (each point corresponds to 15–20 oocytes) compared with the times of germinal vesicle breakdown for oocytes incubated continuously in the same concentration of Br-cAMP. **(B)** The time course of oocyte maturation of oocytes incubated for 30 min (16 cases) versus those incubated continuously (19 cases) in Br-cAMP. The time scale is the same in A and B.

can be used to infer that fertilization had occurred within 1–2 min after sperm addition. No fertilization potential was observed. The membrane potential remained unchanged during this period.

The role of the ionic composition of the sea water on Br-cAMP mediated oocyte maturation was studied by incubating oocytes in  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  free sea water. Oocytes from various species were incubated for two hours in one of these cation free sea waters containing Br-cAMP in order to assess the effects of the lack of a single cation on oocyte maturation. The results of these experiments are summarized in Table 3. They show that  $\text{K}^+$  free sea water induces germinal vesicle breakdown, but not polar body formation in *Mitrocomella* and the absence of  $\text{Mg}^{2+}$  causes oocyte death in *Sarsia*. In those cases where oocytes remain viable, Br-cAMP induces maturation in a high proportion of cases. These results indicate that there is no specific external cation requirement for Br-cAMP-induced maturation.

**Maturation promoting factor.** In order to find out if maturation promoting factor is produced during maturation in hydrozoans, and to find out if the production of this factor depends on the germinal vesicle contents of the oocyte, the following experiment was done on *Phialidium* (Fig. 7). Large oocytes were dissected out of the gonads of a single female. Some of these oocytes were cut in half so that one half had the germinal vesicle and the other half lacked the

germinal vesicle. Each pair of halves was then treated with 2.3 mM Br-cAMP to induce maturation. Once maturation had been initiated, as monitored by germinal vesicle breakdown in the half with the germinal vesicle, each half was washed twice in PSW to dilute out the external Br-cAMP, and placed in a Kiehart chamber along with oocytes that had not been treated with Br-cAMP. Cytoplasm was then withdrawn from each of the Br-cAMP treated oocyte halves and injected into the untreated oocytes. The amount of cytoplasm injected was 250 pl or about 7% of the oocytes volume. The injections were done within 15 min of germinal vesicle breakdown.

Two kinds of controls were done in conjunction with this experiment. One control consisted of untreated oocytes that were placed in the Kiehart chamber with the bisected oocyte, but not injected. None of these control oocytes matured. The other control consisted of withdrawing cytoplasm from oocyte halves with a germinal vesicle that had not responded to Br-cAMP by undergoing germinal vesicle breakdown, and injecting it into untreated oocytes. The donor oocyte halves were treated with Br-cAMP for over 90 minutes; generally a 60 min treatment is sufficient to induce germinal vesicle breakdown. Five cases were injected; none of these matured.

The results of the injection experiments are presented in Table 4. They are derived from seven experiments in which the half of the oocyte that formerly contained the germinal vesicle and the half without the germinal vesicle are compared. In each experiment, the half in which the germinal vesicle broke down had maturation promoting activity. Each half lacking a germinal vesicle had maturation promoting activity, except one. The exceptional case (Experiment no. 5, Table 4) could reflect the fact that the oocyte half without a germinal vesicle had not matured, even though the other half of the oocyte did mature. If one assumes that the level of Br-cAMP in the cytosol of the donor oocyte matches the initial external concentration of Br-cAMP, the small amount of cytoplasm injected could not raise the level of Br-cAMP in the host oocytes to the level needed for inducing maturation (Table 2). The time between the injection of maturing cytoplasm into a recipient oocyte and germinal vesicle breakdown is 10–15 min faster than the time interval needed for germinal vesicle breakdown following incubation in Br-cAMP.

The results of these experiments show that maturation promoting factor appears during maturation and can take the place of cAMP in mediating the maturation process. The results also show that cytoplasm from an oocyte which has been induced to mature, but which lacks a germinal vesicle can produce this maturation promoting factor.

**Surface tension measurements.** Tension at the surface of intact *Phialidium* oocytes (4 cases) or oocytes that had their jelly coat removed (2 cases) was measured during oocyte maturation by placing an oocyte in the compression apparatus, making the measurements necessary to define the tension of the surface of the oocyte, and then adding Br-cAMP to the observation chamber to give a concentration of 2.3–4.6 mM, thereby inducing maturation. The visible events during maturation (germinal vesicle breakdown and polar body formation) were noted and tension at the surface of the oocyte was measured as a function of time. The results of a typical experiment are presented in Fig. 8. Following the addition of Br-cAMP, there is a variable period

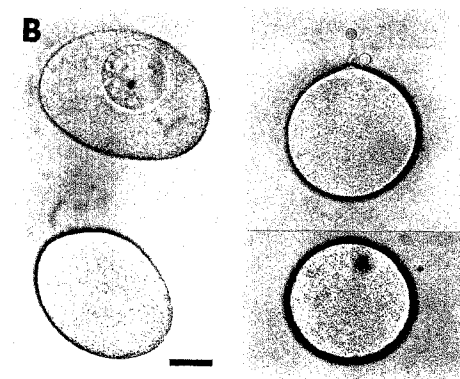
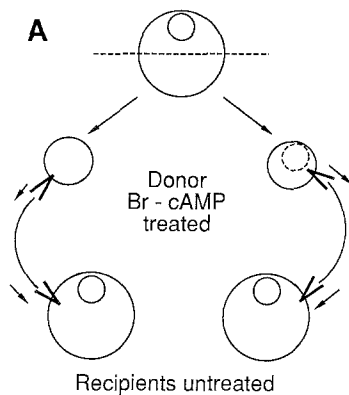


**Table 3.** The effect of cation substituted sea water on bromo cAMP mediated oocyte maturation

Species	Delet. ions	Effect of deleting specific ions				Effect of deleting specific ions & Adding Br-cAMP			
		# of cases	% dead	% False matur. <sup>a</sup>	% matur.	# of cases	% dead	% False matur. <sup>a</sup>	% matur.
<i>Mitrocomella</i>	Na	10	0	0	0	10	0	0	90
	K	14	0	100	0	14	0	0	100
	Ca	21	0	0	0	27	0	0	93
	Mg	10	0	0	0	10	0	0	100
<i>Phialidium</i>	Na	6	0	0	0	7	0	0	86
	K	16	0	0	0	20	5	5	65
	Ca	10	10	0	0	47	6	6	62
	Mg	7	0	0	0	7	0	0	43
<i>Sarsia</i>	Na	14	0	0	0	16	0	0	100
	K	21	0	0	0	21	0	0	100
	Ca	16	12	0	0	19	16	0	58
	Mg	42	93	5	0	79	82	5	0
<i>Gonionemus</i>	Ca	7	0	43	0	26	15	15	70

1.2–2.3 mM Br-cAMP used to induce maturation

<sup>a</sup> False maturation refers to those oocytes in which the germinal vesicle breaks down, but the polar bodies do not form



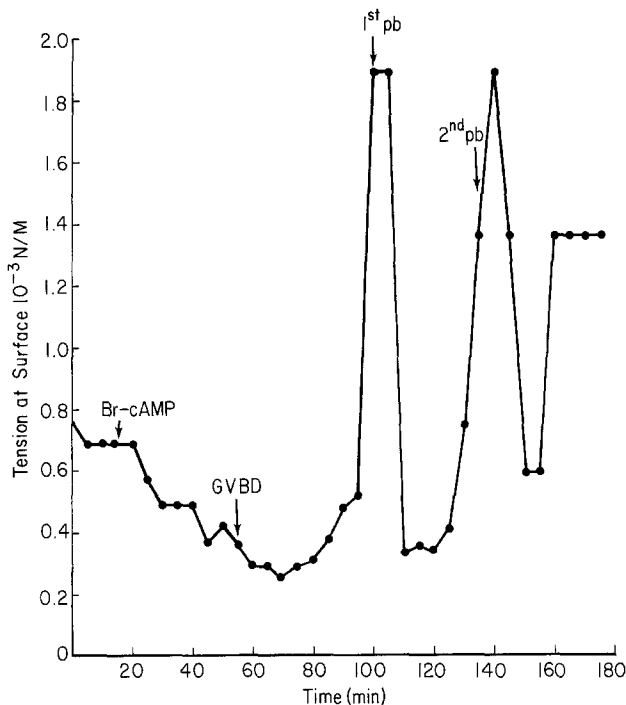
**Fig. 7A.** Diagram of experiment used to assay for maturation promoting factor activity in *Phialidium* oocyte halves with and without a germinal vesicle. An oocyte was cut in half to create a half with a germinal vesicle and a half that lacks a germinal vesicle. Both halves were treated with Br-cAMP to induce maturation. At the time of germinal vesicle breakdown cytoplasm was withdrawn from each oocyte half in separate experiments and injected into intact oocytes that had not been treated with Br-cAMP. **(B)** (Left) Oocyte cut to give a half with a germinal vesicle and a half that lacks a germinal vesicle. (Right) Both halves following oocyte maturation. All photographs are at the same magnifications; the bar indicates 50  $\mu$ m

**Table 4.** The production of MPF in Br-cAMP treated oocyte halves with or without a germinal vesicle (GV)

Exper. No.	Donor oocyte half with GV		Donor oocyte half without GV	
	# of cases	# matur.	# of cases	# matur.
1	5	3	3	2
2	3	2	6	4
3	4	4	4	2
4	4	3	5	3
5	3	2	5	0
6	3	2	4	3
7	2	2	3	1
% Matur.		75%		50%

(5–20 min) during which the surface tension of the oocyte remains unchanged, followed by a gradual decline in surface tension. During this period the germinal vesicle breaks down. Prior to first polar body formation, there is a gradual rise in surface tension followed by a sharp rise and fall in tension at first polar body formation, followed by a similar sharp rise and fall of tension at second polar body formation. After second polar body formation, tension at the egg surface is about the same or higher than the tension at the oocyte surface prior to the initiation of maturation. All six cases behaved in a comparable manner, the presence or absence of a jelly coat did not affect the outcome of the experiment.

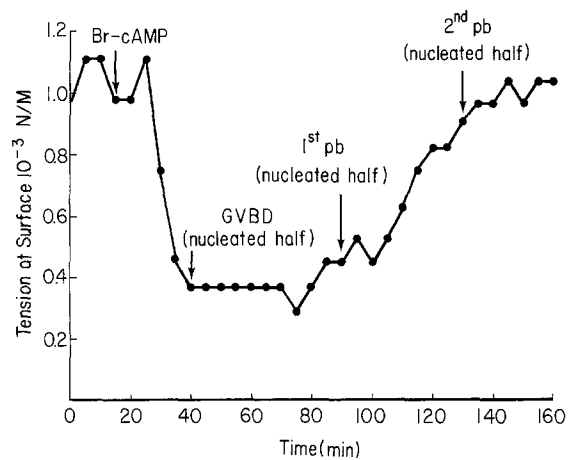
The initial decline in the surface tension of an oocyte following the addition of Br-cAMP may reflect either a generalized response of a cell to an intracellular increase in cAMP or a specific response of an oocyte that is related to maturation. In order to distinguish between these possibilities, an unfertilized egg was placed in the compression apparatus (4 eggs with a jelly coat and 2 eggs with the jelly coat removed were studied), Br-cAMP was added to



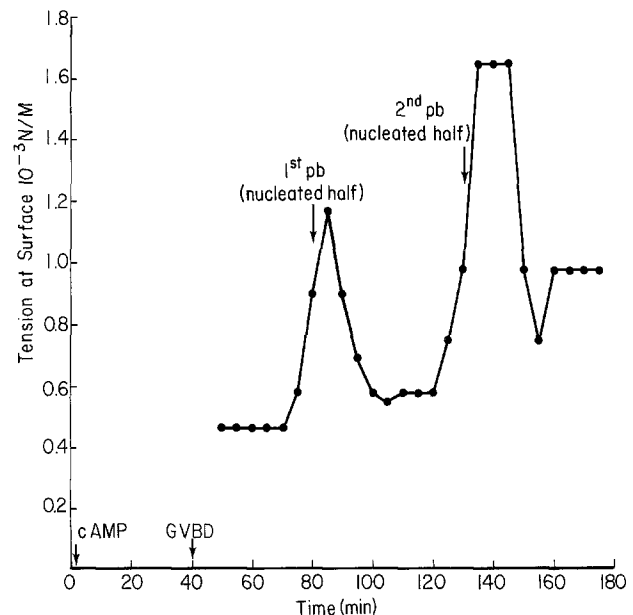
**Fig. 8.** Tension at the surface of an oocyte as a function of time during Br-cAMP induced maturation (GVBD, germinal vesicle breakdown; Pb, polar body)

give a final concentration of 2.3–4.6 mM, and tension at the surface of the egg was measured as a function of time. None of these eggs showed significant tension changes. We also measured changes in the tension at the surface of eggs during the process of fertilization by adding sea water containing sperm to individual unfertilized eggs in the compression apparatus. Fertilization was assayed by noting whether or not the eggs were cleaving 1 h after sperm addition. Four eggs were successfully fertilized. There was no change in the tension at the surface of the egg during the time period when fertilization occurred. In sea urchin eggs, there is a transient increase in surface rigidity at fertilization (Hiramoto 1974).

The effect of the germinal vesicle contents on the pattern of surface tension changes was studied in the following experiment. An oocyte was cut in half to give a half with the germinal vesicle and a half that lacked the germinal vesicle. The half without the germinal vesicle was allowed to round up and was placed in the compression apparatus. Tension measurements were made on this oocyte half, then Br-cAMP was added to the chamber (final concentration 2.3–4.6 mM) to induce maturation and the tension measurements were continued. At the same time Br-cAMP was also added to the oocyte half with the germinal vesicle. The visible events of oocyte maturation were monitored in the oocyte half with the germinal vesicle, while tension changes were simultaneously monitored in the oocyte half that lacked the germinal vesicle. Eleven of these experiments were carried out. Figure 9 presents the results of a typical experiment. Following the addition of Br-cAMP, there is a gradual decline in surface tension just as there is in intact oocytes. However, the sharp rise and fall of surface tension that accompanies first and second polar body formation was not observed in these germinal vesicle free oocytes. During the period when these events occur in intact oocytes,



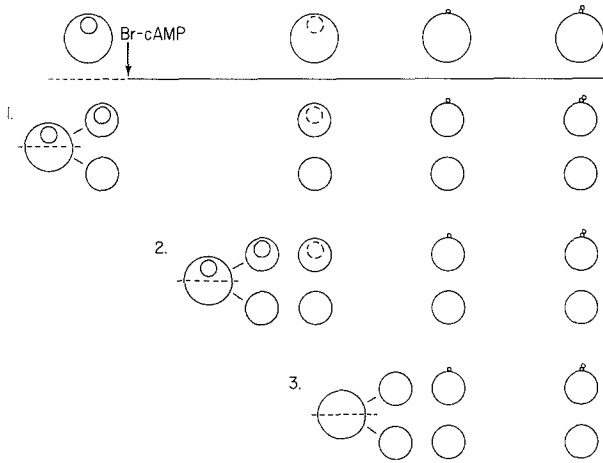
**Fig. 9.** Tension at the surface of an oocyte half that lacks a germinal vesicle, as a function of time during Br-cAMP-induced maturation. The times of germinal vesicle breakdown (GVBD) and polar body formation (Pb) were taken from this half oocyte's sister half which had a germinal vesicle



**Fig. 10.** Tension at the surface of an oocyte half that has inherited germinal vesicle contents but lacks a meiotic apparatus, as a function of time during the last half of Br-cAMP induced maturation. The times of polar body formation (Pb) were taken from this half oocyte's sister half that inherited the meiotic apparatus

there is a gradual rise in surface tension so that at the end of the maturation period, the half that lacked the germinal vesicle had the same or higher tension values at its surface than it did prior to the initiation of maturation. Nine of the cases behaved in this way. In two cases the initial decline in surface tension occurred. However, local bleb formation began to occur on the surface of the oocyte approximately half-way through the maturation process, making it impossible to measure surface tension.

The absence of the surface tension changes associated with polar body formation may reflect the fact that the oocyte half which lacks a germinal vesicle also lacks a meiotic apparatus. However, it may also reflect the fact that this oocyte half lacks other germinal vesicle components. To distinguish between these possibilities, an experi-



**Fig. 11.** Diagram of experiment designed to examine the effect of the germinal vesicle contents on the production of sperm chemoattractant and calcium channel function. The top part of the figure shows an immature oocyte and the visible events (germinal vesicle breakdown and polar body formation) of maturation that occur as a function of time after treatment with 2.3 mM Br-cAMP. (1) The operation of cutting an oocyte to give a half with a germinal vesicle and a half that lacks a germinal vesicle prior to Br-cAMP treatment. (2) The operation of cutting an oocyte to give a half with a germinal vesicle and a half that lacks a germinal vesicle after Br-cAMP treatment, but prior to germinal vesicle breakdown. (3) The operation of cutting an oocyte in half under conditions where both halves inherit germinal contents, after germinal vesicle breakdown, but prior to first polar body formation

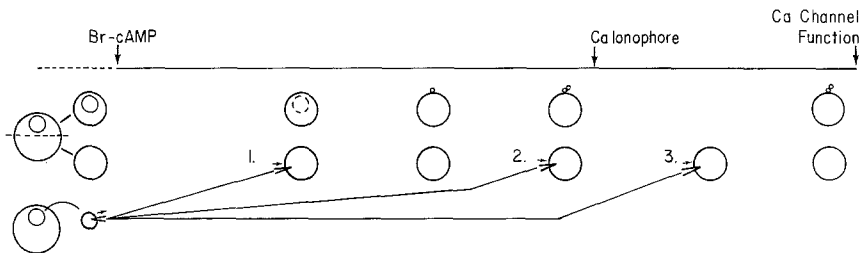
ment was done in which an oocyte was treated with 2.3 mM Br-cAMP in order to induce maturation. As soon as the germinal vesicle broke down, the oocyte was cut in half so that one half contained the region formerly occupied by the germinal vesicle. This half subsequently formed polar bodies; the timing of these events was recorded. The half which did not contain the meiotic apparatus was placed in the compression apparatus, and tension measurements were made on this oocyte half until the oocyte half with the meiotic apparatus completed maturation. The tension measurements for a typical experiment are presented in Fig. 10. These halves did not produce polar bodies. However, there was a sharp rise and fall in surface tension of these oocyte halves at times which approximated the times of first and second polar body formation in the other half. This experiment was done three times, and similar results were obtained in each case. These experiments show that certain elements of the pattern of surface tension changes during oocyte maturation, such as the gradual decline in surface tension at the beginning of the maturation process and the slow increase in tension at the end of the maturation process, do not depend on the germinal vesicle contents. Other elements of the pattern, such as the rapid rise and fall of surface tension that occurs at the times of polar body formation, depend on germinal vesicle contents.

#### *The role of the oocyte germinal vesicle in post-maturation events*

Two early developmental events that occur after oocyte maturation has been completed in *Phialidium* are: (1) the production of sperm chemoattractant by the egg, which begins at second polar body formation, and (2) voltage dependent calcium channel function which begins about

**Table 5.** The effect of the germinal vesicle on the development of calcium channels and the ability to attract sperm

Time of oocyte bissection operation	Sperm attraction (%)				Calcium channel function (%)			
	# of cases		Pb half + non Pb half +		# of cases		Pb half + non Pb half +	
	Pb half + non Pb half +	Pb half + non Pb half +	Pb half + non Pb half +	Pb half + non Pb half +	Pb half + non Pb half +	Pb half + non Pb half +	Pb half + non Pb half +	Pb half + non Pb half +
Oocyte cut to give a non-GV half prior treatment with cyclic nucleotide	11	0	100	0	18	6	88	6
Oocyte cut to give a non-GV half after start of cyclic nucleotide treatment but prior to GV breakdown	22	5	95	0	18	11	83	6
Oocyte cut in half after GV breakdown but prior to polar body formation	9	89	0	11	13	85	0	15



**Fig. 12.** Diagram of experiment designed to examine the effect of the addition of germinal vesicle contents to oocyte halves that have undergone part or all of maturation in the absence of a germinal vesicle, on the subsequent development of calcium channel function. Intact oocytes were cut to give a half with a germinal vesicle and a half that lacks a germinal vesicle. Both halves were treated with 2.3 mM Br-cAMP to induce maturation. The germinal vesicle was dissected out of oocytes that had not been Br-cAMP treated and nucleoplasm was injected into the oocyte halves that lacked a germinal vesicle, (1) after germinal vesicle breakdown but prior to first polar body formation in the oocyte's sister half, (2) after second polar body formation in the oocyte's sister half, but prior to ionophore induced activation, and (3) after ionophore induced activation but prior to testing for calcium channel function

1 h after fertilization or egg activation. The role of the germinal vesicle contents in the initiation of these events was studied in an experiment outlined in Fig. 11. Oocytes were cut to give a half with a germinal vesicle and a half that lacked the germinal vesicle: (1) prior to the beginning of Br-cAMP treatment; (2) during the period between the initiation of Br-cAMP treatment and germinal vesicle breakdown; or (3) after germinal vesicle breakdown but prior to first polar body formation. The consequences of the third operation differs from the previous cuts because both oocyte halves inherit germinal vesicle contents. However, only one half inherits the meiotic apparatus and goes on to produce polar bodies. After the process of oocyte maturation was judged to be complete (by noting second polar body formation in the oocyte half that inherited the meiotic apparatus), the two halves were tested for their ability to attract sperm, or they were activated and tested 60–80 min later for calcium channel function. The results are presented in Table 5. When the contents of the germinal vesicle are inherited by only one oocyte half, only that half has the ability to attract sperm or to form functional calcium channels (i.e., to give a light flash on treatment with KCl, see Materials and methods). However, when oocyte halves are created after germinal vesicle breakdown, then both oocyte halves have the ability to attract sperm and form functional calcium channels. There were three cases in which both oocyte halves attracted sperm or formed calcium channels when oocytes were cut in half after Br-cAMP addition, but prior to germinal vesicle breakdown. In each of these cases, the oocytes were cut in half 1 or 2 min before germinal vesicle breakdown. We suspect that the germinal vesicle contents may have already been mixing with the cytoplasm prior to the cut. There were three cases in which oocyte halves that lacked germinal vesicle contents produced light. These cases are best interpreted as false positives. Oocyte and unfertilized eggs, both of which are known to lack functional calcium channels will occasionally produce light following KCl treatment. We suspect that under these conditions the addition of KCl causes the egg or oocyte to bump the side of the container or make contact with the air-water interface. We have independent evidence that mechanical stimulation will cause light production in unfertilized eggs or oocytes (Freeman and Ridgway, unpublished work). As a control, 25 oocytes, that showed no sign of maturation, that were dissected from the same females used to provide experimental oocytes, and that had been exposed to PSW for a comparable time, were treated with KCl. Twelve per-

**Table 6.** The effect of injecting germinal vesicle contents on calcium channel function in oocyte halves that have undergone all or part of maturation in the absence of the germinal vesicle

Time of germinal vesicle injections	# of cases	% calcium channel function
Germinal vesicle breakdown to first polar body	3	100
Second polar body to egg activation	2	100
After egg activation	27	70

cent of these cases produced light, defining the level of false positives.

The role of the germinal vesicle contents in calcium channel function has also been examined in another experiment outlined in Fig. 12. An oocyte half that lacked a germinal vesicle was induced to undergo maturation. Then germinal vesicle contents of oocytes that were not maturing were injected into this oocyte half, either during maturation or after it had been activated following maturation. Approximately 45 pl of nucleoplasm (this volume of nucleoplasm is about 2% of the half oocyte volume) was injected in these experiments. The results of the experiment are presented in Table 6. They show that the germinal vesicle contents from an oocyte that is not undergoing maturation will bring about calcium channel function in an oocyte half which has been matured and activated in the absence of the germinal vesicle. This experiment also demonstrates that the germinal vesicle contents are not necessary for the events that lead to egg activation. When germinal vesicle contents are injected into activated eggs from half oocytes without a germinal vesicle a few minutes before these eggs are assayed for calcium channel function, the eggs exhibit functional calcium channels. This indicates that the germinal vesicle contents do not play a role in determining the time period which must elapse before calcium channels become functional.

## Discussion

### *The role of cAMP in oocyte maturation*

We have shown that the direct injection of cAMP into oocytes, or the addition of membrane permeable cyclic AMP

derivatives to their surrounding medium, induces maturation in a number of hydrozoan species. Recently, Yamashta (1988) has demonstrated that an increase in cAMP induces oocyte maturation in an ophiuroid. The differences observed (Table 2) in the effectiveness of dB-cAMP and Br-cAMP in bringing about maturation in different species of hydrozoans can be attributed to several factors. There may be permeability differences in the cell membranes of oocytes from different species. The fact that dB-cAMP acts more slowly than Br-cAMP may reflect the fact that it has to be hydrolyzed to monobutyl-cAMP before it can function. We have no reason to suppose that these cAMP analogues would have differential effects once inside an oocyte. The membrane permeable cyclic AMP derivatives presumably increase the effective concentration of cAMP inside the oocyte. At this point we do not know if an increase in cAMP is a normal event in hydrozoan oocyte maturation. It should be possible to measure cAMP levels during maturation in oocytes from hydrozoans like *Hydractinia* and *Spirocodon* where hormone preparations that induce maturation are available. In amphibians, asteroid and mammalian oocytes where changes in cAMP levels have been implicated in the maturation process, there is a decline in cAMP levels shortly after the induction of maturation (Meijer and Zarutskie 1987; Cicirelli and Smith 1985; Schultz et al. 1983). The increase in cAMP which we infer from our experiments could come about either by activation of adenylate cyclase or the inhibition of phosphodiesterase. Cyclic AMP appears to work exclusively by activating cAMP dependent protein kinase. The relative roles of cAMP dependent protein kinase in the metabolic pathways used to bring about maturation probably differ in hydrozoans and ophiuroids, versus amphibians asteroids and mammals.

#### *Events during the maturation process*

Unlike the situation in amphibians and asteroids, there is no change in membrane potential during oocyte maturation in hydrozoans. This observation suggests that there has not been a major change in ion channel function during maturation. However, it does not rule out this possibility. There is no fertilization potential in *Phialidium*, and only a very small response in *Hydractinia* (Berg et al. 1986). The absence of a change in ion channel function during maturation may be because there is no need to establish channel mechanisms for an electrical block to polyspermy. *Phialidium* oocytes and unfertilized eggs do not give an active membrane response on depolarization (Freeman and Ridgway 1987), while oocytes and eggs of asteroids and amphibians give an active membrane response on depolarization and produce a fertilization potential (Jaffe and Cross 1986).

Our work also shows that there are no cations in the external medium that are necessary for the maturation process (Table 3). In asteroids,  $Mg^{2+}$  is needed in the external medium in order for maturation to proceed (Rosenberg and Lee 1981), while  $Ca^{2+}$  or  $Mg^{2+}$  are needed in order for oocyte maturation to proceed in amphibians (Merriam 1971).

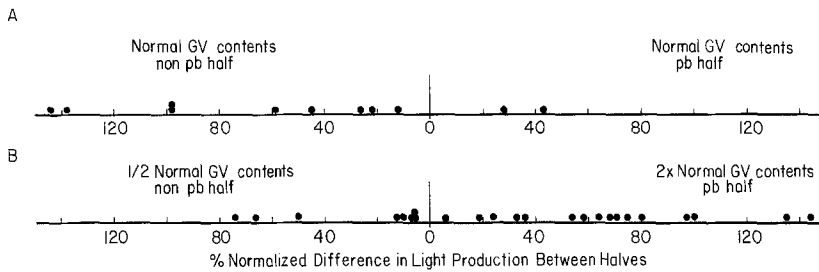
Hydrozoan oocytes produce maturation promoting factor activity and go through a set of surface rigidity changes during maturation. In these respects, they are typical maturing oocytes. Maturation promoting factor is produced in the absence of a germinal vesicle in hydrozoans. There have

been two reports in asteroids that maturation promoting factor is produced, but at a lower level, in the absence of the germinal vesicle (Kishimoto et al. 1981; Picard and Doree 1984). This is also reported in amphibians (Gautier 1987). The experiments that we have done on *Phialidium* were not designed to address this question. Some of the surface tension changes that occur during oocyte maturation in hydrozoans will also take place in the absence of the germinal vesicle. These include the drop in surface tension after the addition of Br-cAMP, and the slow increase in surface tension that would normally begin about the time that the polar bodies are formed (Fig. 8). The initial drop in surface tension, and the absence of a transient rise and fall in surface tension at what would be the time of polar body formation reported here for a hydrozoan (Fig. 9), have also been observed in asteroid oocytes which undergo maturation in the absence of a germinal vesicle. However, the slow increase in surface tension during the last part of the maturation process, that is responsible for the rise of tension at the surface to the level where it was before the initiation of maturation, occurs in hydrozoan oocyte halves with and without a germinal vesicle. Only asteroid oocytes that lack germinal vesicle contents tend to show an increase in tension at their surface during the last part of the maturation process (Yamamoto and Yoneda 1983). These results clearly show that some aspects of oocyte maturation can occur in the absence of the germinal vesicle.

#### *Events after maturation*

Our results show that two post-maturation differentiations, production of sperm chemoattractant and appearance of calcium channel function, depend on the germinal vesicle (see Table 5 and Fig. 11). The germinal vesicle contents may be necessary for these post-maturation events because they are needed in order to complete some aspect of maturation which is a necessary prerequisite for these events. However, this explanation appears to be unlikely because the injection of germinal vesicle contents into an egg that has matured in the absence of a germinal vesicle, and then been activated, causes the formation of calcium channels. Our previous work has demonstrated that egg activation is necessary for calcium channel formation, and that egg activation will not occur until oocyte maturation has occurred (Freeman and Ridgway 1987). Oocytes treated with the Ca-ionophore A23187 do not subsequently form calcium channels. Unfortunately, it has not yet been possible to inject germinal vesicle contents, and test for subsequent sperm chemoattractant production, in eggs that have undergone maturation in the absence of a germinal vesicle, because the injection process itself activates these eggs, causing a turnoff of chemoattractant production.

The germinal vesicle may contain sperm chemoattractant or calcium channel components, and/or the vesicle may contain factors which are necessary for the expression of these activities. For example, both the release of chemoattractant and the establishment of calcium channel function may depend on some aspect of cytoskeletal function that is mediated by a factor stored in the germinal vesicle. At present, we cannot distinguish between these possibilities. Neither of these activities appears at the time of germinal vesicle breakdown. This implies that other events must occur before these activities are expressed. One observation



**Fig. 13.** Differences in the amount of calcium channel function by sister oocyte halves during the time period just after calcium channels become functional. The amount of light produced by each half was measured and the average was determined. The differences between the two halves was then determined and expressed as a percent of the average. Each point represents one of these comparisons. A point on the left side of the center indicates that the oocyte half that lacks a polar body has produced a certain percent more light than its sister half with a polar body, while a point on the right side of center indicates that the oocyte half that has a polar body has produced a certain percentage more light than its sister half that lacks a polar body. (A) Comparison of oocyte halves that have similar amounts of germinal vesicle material (from Part 3 of the experiment outlined in Fig. 11). (B) Comparison of oocyte halves that have different amounts of germinal vesicle material (from Part 3 of the experiment outlined in Fig. 12)

that may help to explain how the germinal vesicle contents bring about calcium channel function is provided by an analysis of the relationship between the amount of germinal vesicle material an oocyte inherits and the amount of calcium channel function, as measured by the size of the calcium transients (light production) when channel function is beginning. In one part of the experiment outlined in Fig. 11, oocytes were cut in half after germinal vesicle breakdown. Under these conditions, each half presumably inherits the same amount of germinal vesicle material. When one compares the amount of light produced by each oocyte half for those pairs in which both halves produce light, the half that does not produce polar bodies tends to produce more light (Fig. 13A). This result defines the background conditions for the observations that follow. In the experiments outlined in Fig. 12, oocytes were cut in half so that one half inherited the germinal vesicle contents and the other half did not, and both halves were treated with Br-cAMP so that they would mature. The oocyte half with the germinal vesicle contained twice the amount of germinal vesicle material that it would have had under normal conditions following germinal vesicle breakdown. The oocyte half without germinal vesicle material was subsequently injected with the equivalent of 25% of the contents of a germinal vesicle, and, as a consequence, it had half the amount of germinal vesicle material as it would normally have had. This means that the oocyte half which produces polar bodies ends up with four times as much germinal vesicle material as the half that does not produce polar bodies. Subsequently, both of these oocyte halves were activated and assayed for calcium channel function. In those cases in which both halves of an oocyte pair produced light, in most cases the half with the contents of one entire germinal vesicle (the half with polar bodies) produced more light (Fig. 13B). This suggests that the amount of germinal vesicle material present in a standard sized oocyte controls the number of calcium channels formed.

The germinal vesicle appears to contain factors that are necessary for a number of developmental functions following maturation. Some of these developmental functions occur at early stages of development such as those described in this paper. These events can occur in the absence of a nucleus. Other functions occur at later developmental stages and probably play a role in modulating gene activity (Brothers 1976; Dyer et al. 1982). These factors in the ger-

mal vesicle with diverse developmental functions deserve to be better characterized.

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