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Participation of MAPK, PKA and PP2A in the regulation of MPF activity in *Bufo arenarum* oocytes

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Summary

The objectives of the present paper were to study the involvement and possible interactions of both cAMP-PKA and protein phosphatases in *Bufo arenarum* oocyte maturation and to determine if these pathways are independent or not of the MAP kinase (MAPK) cascade. Our results indicated that the inhibition of PKA by treatment with H-89, an inhibitor of the catalytic subunit of PKA, was capable of inducing GVBD in a dose-dependent manner by a pathway in which Cdc25 phosphatase but not the MAPK cascade is involved. The injection of 50 nl of H-89 10 μM produced GVBD percentages similar to those obtained with treatment with progesterone. In addition, the assays with okadaic acid (OA), a PP2A inhibitor, significantly enhanced the percentage of oocytes that resumed meiosis by a signal transducing pathway in which the activation of the MEK–MAPK pathway is necessary, but in which Cdc25 phosphatase was not involved. Treatment with H-89, was able to overcome the inhibitory effect of PKA on GVBD; however, the inhibition of Cdc25 activity with $NaVO_3$ was able to overcome the induction of GVBD by H-89. Although the connections between PKA and other signalling molecules that regulate oocytes maturation are still unclear, our results suggest that phosphatase Cdc25 may be the direct substrate of PKA. In *Xenopus* oocytes it was proposed that PP2A, a major Ser/Thr phosphatase present, is a negative regulator of Cdc2 activation. However, in *Bufo arenarum* oocytes, inhibition of Cdc25 with $NaVO_3$ did not inhibit OA-induced maturation, suggesting that the target of PP2A was not the Cdc25 phosphatase. MAPK activation has been reported to be essential in *Xenopus* oocytes GVBD. In *B. arenarum* oocytes we demonstrated that the inhibition of MAPK by PD 98059 prevented the activation of MPF induced by OA, suggesting that the activation of the MAPK cascade produced an inhibition of Myt1 and, in consequence, the activation of MPF without participation of the Cdc25 phosphatase. Our results suggest that in incompetent oocytes of *B. arenarum* two signal transduction pathways may be involved in the control of MPF activation: (1) the inhibition of phosphatase 2A that through the MEK–MAPK pathway regulates the activity of the Myt1; and (2) the inhibition of AMPc–PKA, which affects the activity of the Cdc25 phosphatase.

Keywords: Amphibian, Oocyte maturation, Signalling pathway

Introduction

In almost all species studied, meiotic maturation is controlled by the maturation promoting factor (MPF), the key regulator of transition of G2/M in cell cycles. Although all species share an MPF complex that is required for oocytes meiotic maturation, some clear differences exist among the different species. This implies that the species-specific regulations are present even in the basic machinery for cell cycle regulation.

Significant progress has been made towards understanding the regulation of the meiotic cell cycle in *Bufo arenarum* oocytes although the pathway selected by progesterone, the physiological inducer of M-phase entry in this species, remains to be determined.

In immature oocytes of *Xenopus* (Gautier & Maller 1991; Minshull *et al*., 1991), *B. arenarum* (Sánchez Toranzo *et al*., 2006) and starfish (Kishimoto, 1998)

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there is an inactive complex (pre-MPF) that consists of cyclin B-bound Cdc2 phosphorylated on Thr-161 and on the inhibitory residues Thr-14/Tyr-15. In *B. arenarum* oocytes arrested in G2, the Thr14 and Tyr15 residues of pre-MPF are phosphorylated by the Myt1 kinase. To induce the MPF activation the inhibitory residues Thr-14/Tyr-15 must be dephosphorylated by the phosphatase Cdc25 (Duckworth *et al.*, 2002).

The activity of the Cdc25 phosphatase is regulated by multiple phosphorylation and dephosphorylation events. Fully grown oocytes preincubated with Cdc25 inhibitors such as sodium metavanadate ($NaVO_3$) are unable to activate MPF and cannot undergo meiotic resumption, highlighting the importance of this phosphatase in the process (Sánchez Toranzo *et al.*, 2006).

B. arenarum oocytes obtained during the reproductive period (spring–summer) resume meiosis with no need of an exogenous hormonal stimulus if deprived of their enveloping follicle cells, a phenomenon called spontaneous maturation. In this species it is possible to obtain oocytes competent and incompetent to undergo spontaneous maturation according to the seasonal period in which animals are captured (Zelarayán *et al.*, 1995). Interestingly, fully grown *B. arenarum* oocytes always respond to progesterone, independently of the time of the year in which they are obtained (Zelarayán *et al.*, 1996).

In *B. arenarum* oocytes, the classical adenylyl cyclase/cAMP/protein kinase A (PKA) signalling pathway is considered to be the primary signalling cascade through which progesterone regulates meiosis reinitiation. We previously showed that maintaining high cAMP levels by stimulation of oocytes adenylyl cyclase (AC) with forskolin, by including the analogous membrane-permeable dbcAMP, or by the pharmacological blockade of phosphodiesterase (PDE) with theophyline, prevented meiotic reinitiation (Zelarayán *et al.*, 2000; Sánchez Toranzo *et al.*, 2006). These results suggest that cAMP-dependent protein kinase (PKA) is active in G2-arrested oocytes and has an important negative role in oocytes maturation. In agreement with this idea, injection of PKA inhibitors should cause maturation in the absence of progesterone whereas overactivation of PKA potently inhibits oocyte maturation (Kwon & Lee, 1991).

Therefore, a simple hypothesis may explain the cAMP and PKA inhibitory effects on MPF activity. High cAMP levels within the oocytes result in the phosphorylation of Cdc2 on Thr14 and Tyr15, rendering it inactive (Duckworth *et al.*, 2002). A decrease in oocytes cAMP early in oocyte maturation leads to the dephosphorylation of Cdc2 on Thr14 and Tyr15, and the MPF complex becomes active so that oocytes can re-enter meiosis.

However, no evidence that MPF is a direct substrate of PKA has been reported, suggesting that intermediate steps are required. However, there is still no information on what the relevant PKA substrates in oocytes could be. The discrete sets of steps through which cAMP activates or inactivates MPF are still under investigation. It has been reported that PKA phosphorylates and inactivates Cdc25 in *Xenopus* oocytes (Duckworth *et al.*, 2002).

The decrease in cAMP concentration and the subsequent inactivation of PKA are the first early events known to be induced by progesterone (Maller & Krebs, 1977; Huchon *et al.*, 1981). Previous studies have shown that a transient increase in intracellular cAMP levels produced by inhibition of PDE activity or activation of adenyl cyclase (AC) is sufficient to inhibit GVBD induced by progesterone (Zelarayán *et al.*, 2000).

The identity of the protein phosphatase that catalyzes the dephosphorylation of the different inhibitory residues of Cdc25 is still uncertain. However, a role for high concentrations of PP2A in meiosis has long been suspected because MPF inactivation depends on an okadaic acid-sensitive phosphatase 2A.

In *Xenopus* oocytes *in vitro*, high concentrations of PP2A dephosphorylate the hyperphosphorylated form of Cdc25. Furthermore, okadaic acid (OA), a specific inhibitor of PP2A phosphatase, induces MPF autoamplification (Goris *et al.*, 1989; Felix *et al.*, 1990; Izumi *et al.*, 1992; Karaiskou *et al.*, 1999).

In addition, the inhibition of PP2A by okadaic acid (Rime *et al.*, 1990; Picard *et al.*, 1991) induces meiotic resumption in the oocytes of many species, including both competent and incompetent mouse oocytes (Rime & Ozon, 1990; Rime *et al.*, 1990).

Karaiskou *et al.* (1998), during the meiotic maturation of *Xenopus*, investigated the different molecular partners that are implicated in the network that allows Cdc2 activation in the mechanism of the MPF autocatalytic activation. This finding demonstrates that the initiation of the Cdc2/Cdc25 feedback loop, leading to an abrupt Cdc2 kinase activation, requires two sets of phosphorylation reactions on Cdc25: one is Cdc2 kinase-dependent and the second requires Plx1 activity and PP2A inhibition. Evidence for a crosstalk between cAMP–PKA and OA-sensitive protein phosphatases was presented (Schwartz & Schultz, 1991; Lu *et al.*, 2001); however, the direct physiological target of PP2A has not been unequivocally identified.

In the oocytes of some species, MAP kinase is activated as a consequence of the activation of a cascade that involves the kinase Mos, including MAP kinase kinase (MEK) or through the Ras–Raf–MEK pathway (Palmer & Nebreda 2000; Lu *et al.*, 2001; Bodart *et al.*, 2002). MAPKs have thus been implicated in the cascade leading to MPF activation.

Active MAP kinase itself was found to be capable of inducing *Xenopus* oocytes maturation in the absence of hormone stimulation (Haccard *et al*., 1995). Activated MAPKs may directly or indirectly regulate some proteins that control MPF activity, such as Cdc25 phosphatase or Myt1 kinase. However, in starfish, no MAP kinase activation is required for Cdc2 kinase activation and GVBD induction (Picard *et al*., 1996) and, in rodents MAP kinase is only activated after GVBD and Cdc2 kinase activation (Verlhac *et al*., 1993).

It is well established that in *B. arenarum* oocytes progesterone-induced maturation depends on the synthesis of new proteins (Zelarayán *et al*., 1996). Several studies in different species have demonstrated that Mos synthesis is required for hormone induced GVBD (Wasserman & Masui, 1975; Takemoto & Horiuchi 1995). In *Xenopus*, the protein kinase Mos is the only newly synthesized protein that has been shown to be necessary for progesterone-induced maturation (Sagata *et al*., 1988). Inhibition of protein synthesis in porcine oocytes blocks MAP kinase activation and GVBD. However, in starfish, neither protein synthesis nor MAP kinase activation are required for Cdc2 kinase activation and GVBD induction. Mos is a MAP kinase kinase kinase that leads to the activation of MAPK through the activation of MEK (MAPKK) (Nebreda *et al*., 1993; Posada *et al*., 1993; Shibuya & Ruderman, 1993). Several studies have demonstrated that activation of MAP kinase is important or essential for MPF activation in *Xenopus* oocytes (Haccard *et al*., 1990; Ferrell *et al*., 1991), although Nebreda & Hunt (1993), Posada *et al.* (1993) and Shibuya & Ruderman (1993) showed that Mos leads to the activation of MAP kinase but not of Cdc2. However, in theory, the accumulation of a protein absolutely required for pre-MPF activation must depend on progesterone stimulation and be independent of Cdc2 activity.

The objectives of the present paper were to study the involvement and possible interactions of both cAMP–PKA and protein phosphatases in *B. arenarum* oocytes maturation and to determine if these pathways are independent or not of the MAPK cascade.

Materials and methods

Animals

Adult specimens of *B. arenarum* were collected in the northwestern area of Argentina from May to August (winter animals) and from September to December (summer animals) and kept at 15 °C until use.

Hormones and reagents

All hormones and reagents were purchased from Sigma. Progesterone was dissolved in ethanol and added directly to the culture medium to give a final concentration of 2.5 μM. Sodium metavanadate ($NaVO_3$) was dissolved in ddH_2O at 75 °C and various doses were added to the culture medium at a constant volume (5 μl). H-89 was dissolved in 4:6 ethanol/AR.

In vitro denuded oocytes culture

Experimental manipulation and culture were performed at room temperature (22–25 °C) in amphibian Ringer solution (AR) (6.6 g NaCl/l, 0.15 g $CaCl_2$/l and 0.15 g KCl/l) containing penicillin G–sodium (30 mg/l) and streptomycin sulphate (50 mg/l), pH 7.4.

Denuded fully grown oocytes were obtained according to Lin and Schuetz (1985). Follicle cells were removed by gentle shaking (100 oscillations/min) (Zelarayán *et al*., 1995).

Randomized samples of 20 oocytes were distributed into separate wells containing 2 ml of AR; the reagents were added (5 μl) directly to the culture medium.

Oocyte maturation was assessed 24 h after hormone or reagent addition. Meiosis reinitiation was scored both by the presence of a transient white spot in the animal pole and by the absence of GV after dissection of the oocytes fixed in trichloroacetic acid.

Microinjections

Denuded fully grown immature oocytes were microinjected with 50 nl of mature cytoplasm, with 50 nl of immature cytoplasm, or with 50 nl of the GV content. Following injection, oocytes were placed in AR and scored for GVBD after incubation for 24 h.

Microinjection was performed with a sharpened glass micropipette (outer diameter 40–50 μm) attached to a micromanipulator.

Results

Effect of PKA inhibition on the maturation of fully grown oocytes

In order to evaluate whether in *B. arenarum* oocytes meiosis reinitiation is affected by the inhibition of PKA activity, we microinjected H-89, an inhibitor of the catalytic subunit of PKA, into the oocytes. Denuded oocytes incompetent to mature spontaneously were injected with 50 nl of different doses of H-89 (2.5–10 μM). GVBD was scored after 24 h of culture.

The result (Fig. 1) shows that the inhibition of PKA by treatment with H-89 is capable of inducing GVBD in a dose-dependent manner in the absence of progesterone. The maximum response (94%) was obtained with the dose of 10 μM of H-89.

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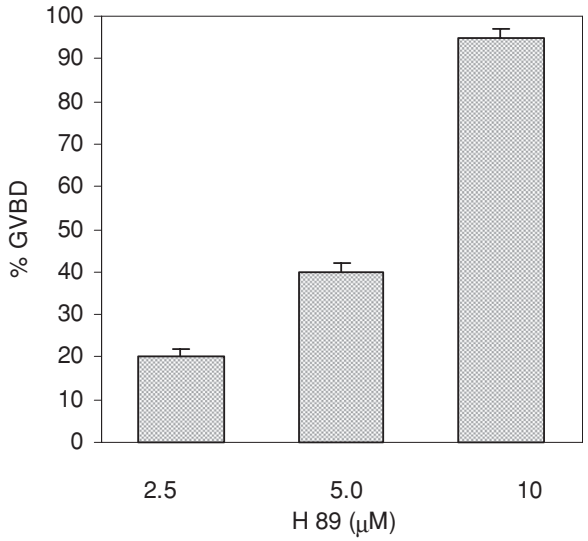

Figure 1 Effect of H89 on oocytes maturation. Fully grown denuded oocytes incompetent to mature spontaneously were injected with 50 nl of different doses of H-89 (2.5–10 μM). GVBD was scored after 24 h of culture. Values are the mean ± SEM ($n = 5$). Each experiment was performed on a different animal.

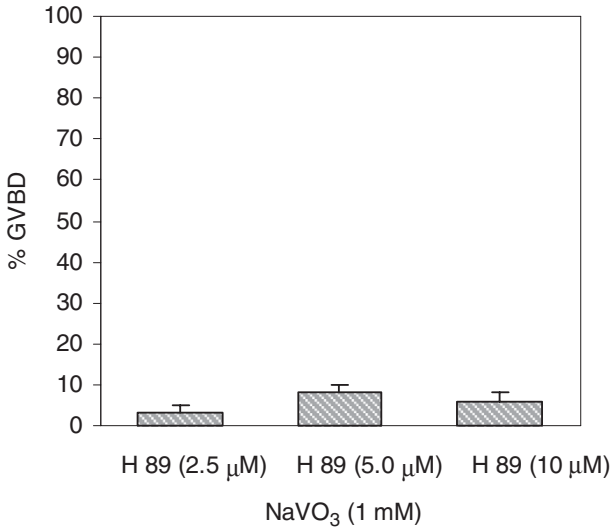

Figure 2 Effect of Cdc25 activity inhibition during oocytes maturation induced by H-89. Denuded fully grown oocytes incompetent to mature spontaneously were preincubated with $NaVO_3$ (1 MM) for 60 min and then injected with 50 nl of different doses of H-89 (2.5–10 μM). GVBD was scored after 24 h of culture. Values are the mean ± SEM ($n = 4$). Each experiment was performed on a different animal.

Effect of Cdc25 activity inhibition during oocytes maturation induced by H-89

Phosphatase Cdc25 is involved in the Cdc2 Thr-14/Tyr-15 dephosphorylation of pre-MPF during oocytes maturation.

The participation of Cdc25 in the transduction pathway of maturation induced by H-89 was assayed using $NaVO_3$, an inhibitor of Cdc25 activity.

Denuded fully grown oocytes incompetent to mature spontaneously were preincubated with $NaVO_3$ (1 MM) for 60 min and then injected with 50 nl of different doses of H-89 (2.5–10 μM).

The results in Figure 2 indicate that $NaVO_3$ effectively inhibited H-89-induced oocytes maturation, thus confirming that Cdc25 is involved in the PKA pathway of meiosis reinitiation.

Effect of the inhibition of PP2A activity on GVBD of oocytes incompetent to mature spontaneously

To determine if PP2A is involved in the negative regulation of MPF, we used the specific inhibitor of this phosphatase, OA.

Denuded fully grown oocytes incompetent to mature spontaneously were injected with 50 nl of different doses of OA (0.5–2.5 μM). GVBD was scored after 24 h of culture.

The result (Fig. 3) shows that the inhibition of PP2A by treatment with OA is sufficient to release the meiosis block and allow GVBD in a dose-dependent manner. Continuous exposure of *B. arenarum* immature oocytes to PP2A inhibitor OA (1 μM) for 24 h significantly enhanced the percentage of oocytes that resumed meiosis (84% GVBD).

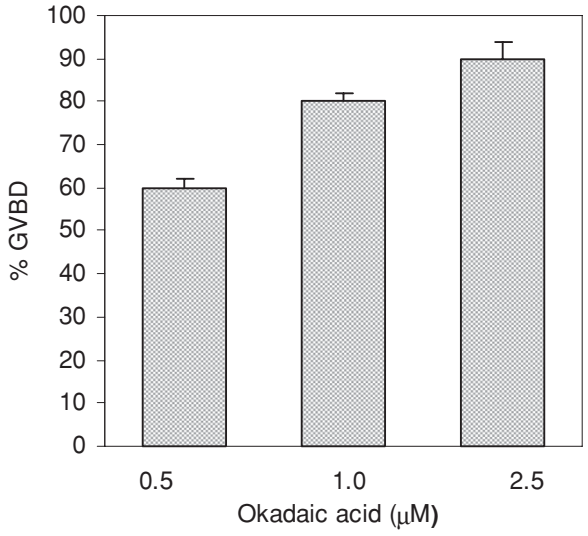

Figure 3 Effect of the inhibition of PP2A activity on the GVBD of oocytes incompetent to mature spontaneously. Denuded fully grown oocytes incompetent to mature spontaneously were injected with 50 nl of different doses of okadaic acid (0.5–2.5 μM). GVBD was scored after 24 h of culture. Values are the mean ± SEM ($n = 3$). Each experiment was performed on a different animal.

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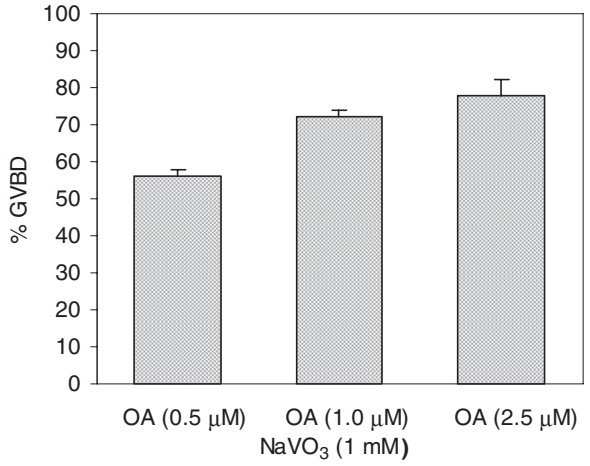

Figure 4 Effect of Cdc25 activity inhibition on MPF activation with OA. Denuded fully grown incompetent oocytes were cultured for 60 min with $NaVO_3$ (1 MM) and then injected with 50 nl of various doses of OA (0.5–2.5 μM). GVBD was scored after 24 h of culture. Values are the mean ± SEM (n = 3). Each experiment was performed on a different animal.

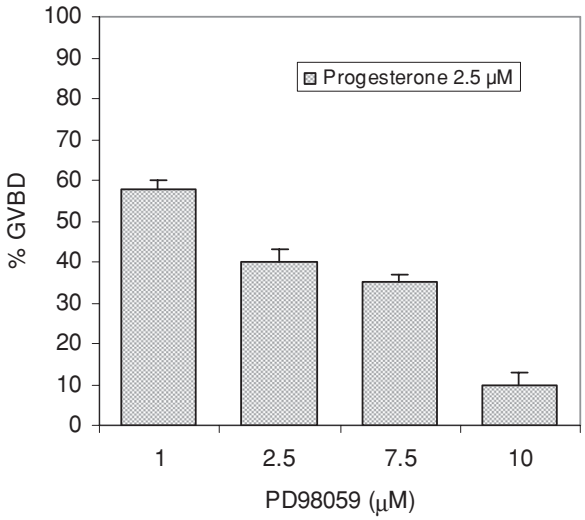

Figure 5 Inhibition of MAPK activity on meiotic resumption in fully grown incompetent oocytes: fully grown incompetent oocytes were preincubated for 60 min in the presence of PD98059 at various doses (1–10 μM) and then exposed to progesterone (2.5 μM). GVBD was scored after 24 h of culture. Values are the mean ± SEM (n = 4). Each experiment was performed on a different animal.

Effect of Cdc25 activity inhibition on MPF activation with OA

The direct physiological target of PP2A has not been unequivocally identified. It has been suggest that PP2A could be the phosphatase responsible for the dephosphorylation of the Cdc25 activator residues.

Our aim was to elucidate whether OA-induced meiotic resumption of incompetent oocytes was mediated through PP2A effect on Cdc25 activity.

Denuded fully grown incompetent oocytes were cultured for 60 min with $NaVO_3$ (1 MM) and then injected with 50 nl of various doses of OA (0.5–2.5 μM).

The percentage of GVBD obtained (Fig. 4) showed that the inhibition of Cdc25 activity with $NaVO_3$ reached values similar to those when oocytes were injected with OA alone, suggesting that OA induces GVBD by a transduction pathway in which Cdc25 phosphatase is not involved.

Inhibition of MAPK activity on meiotic resumption in fully grown incompetent oocytes

We used an inhibitor of MEK, PD98059, as a pharmacological tool to study the involvement of MAPK in the induction of meiotic resumption of incompetent oocytes of *B. arenarum*. Different groups of fully grown incompetent oocytes were preincubated for 60 min in the presence of PD98059 at various doses (1–10 μM). Oocytes were subsequently exposed to progesterone (2.5 μM) for 24 h and GVBD was scored.

As shown in Figure 5, treatment with PD98059 consistently inhibited progesterone-induced meiotic maturation in a dose-dependent manner. These results indicate that MAPK activity was necessary for progesterone-induced maturation in denuded fully grown incompetent oocytes of this species.

Effect of MAPK inhibition on OA-induced maturation in fully grown ovarian oocytes

To determine whether the OA-induced meiotic resumption of incompetent oocytes was mediated through the MEK–MAPK pathway, fully grown denuded oocytes were preincubated for 60 min in the presence of PD98059 at various doses (1–10 μM) and then injected with 50 nl of OA (2.5 μM). Oocytes were cultured for 24 h before GVBD was scored.

The results in Figure 6 show that the inhibition of MAPK activity by PD98058 inhibited the meiosis reinitiation induced by OA in a dose-dependent manner.

We also tested the effect of cycloheximide (10 μg/ml) on OA-induced meiotic resumption of incompetent oocytes because activation of MAP kinases requires protein synthesis. No oocytes underwent GVBD (0%), indirectly confirming the implication of MAP kinases in OA-induced meiotic resumption.

Together, these results indicate that OA induces meiotic resumption of incompetent oocytes through the activation of the MEK–MAPK pathway.

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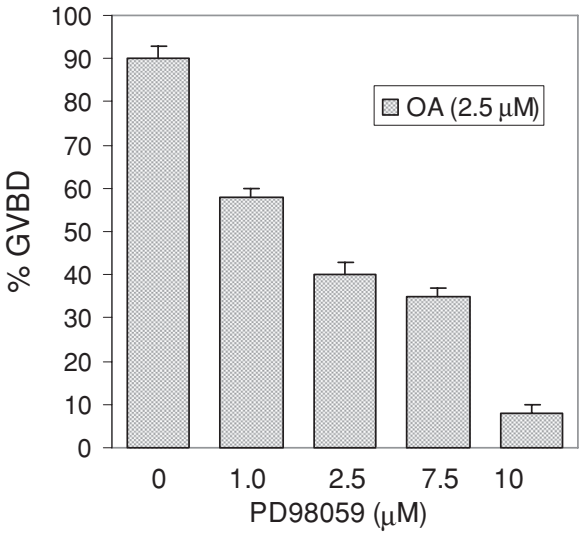

Figure 6 Effect of MAPK inhibition on OA-induced maturation in fully grown ovarian oocytes. Fully grown denuded oocytes were preincubated for 60 min in the presence of PD98059 at various doses (1–10 μM) and then injected with 50 nl of OA (2.5 μM). Oocytes were cultured for 24 h before GVBD was scored. Values are the mean ± SEM (n = 3). Each experiment was performed on a different animal.

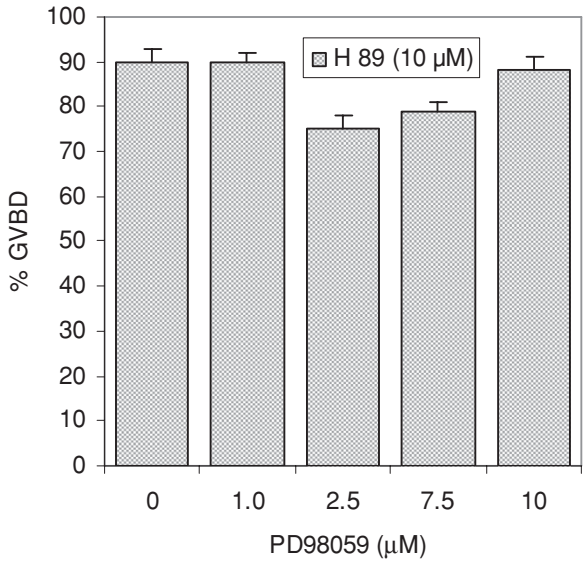

Figure 7 Effect of MAPK inhibition on the resumption of meiosis induced by PKA inhibition. Fully grown denuded oocytes were preincubated for 60 min in the presence of PD98059 (1–10 μM) and subsequently treated with increasing concentrations of H-89. Oocytes were cultured for 24 h before GVBD was scored. Values are the mean ± SEM (n = 3). Each experiment was performed on a different animal.

Effect of MAPK inhibition on the resumption of meiosis induced by PKA inhibition

To analyze the participation of MAPK in the GVBD induced by inhibition of PKA we pre-treated ovarian oocytes with PD98059 for 60 min and subsequently treated them with increasing concentrations of H-89, an inhibitor of PKA activity.

Figure 7 shows that the inhibition of MAPK with PD98059 did not significantly modify GVBD percentages induced by H-89. These results suggest that the MAPK pathway was not involved in the GVBD induced by the inhibition of PKA activity.

Discussion

Our results indicated that the inhibition of PKA by treatment with H-89 was capable of inducing GVBD in a dose-dependent manner by a pathway in which Cdc25 phosphatase but not the MAPK cascade is involved. In addition, the assays with OA, a PP2A inhibitor, significantly enhanced the percentage of oocytes that resumed meiosis by a signal transducing pathway in which the activation of the MEK–MAPK pathway is necessary, but in which Cdc25 phosphatase was not involved.

It has been reported that, in *B. arenarum* oocytes, progesterone decreases cAMP intracellular levels by inhibiting adenylyl cyclase or cAMP-dependent PDE activity (Zelarayán *et al.*, 2000; Sánchez Toranzo *et al.*, 2006). This progesterone effect is believed to be sufficient to reduce the activity of the cAMP-dependent PKA, which prevents the activation of the maturation-promoting factor (MPF) in the oocytes. The involvement of PKA in oocytes maturation has been demonstrated in *Fundulus heteroclitus* (Cerdà *et al.*, 1997) and *C. batrachus* (Haider & Baqri, 2002). In both species, the PKA inhibitor H-8 or H-89 stimulated GVBD, bypassing the MIS. In the Atlantic croaker, PKA inhibitors (Rp-cAMP and KT5720) did not stimulate GVBD in the absence of the MIS (20β-S) nor did they enhance the efficacy of the MIS (Pace & Thomas, 2005). In the present study we demonstrated that the injection of 50 nl of H-89 10 μM produced GVBD percentages similar to those obtained with treatment with progesterone.

Although the connections between PKA and other signalling molecules that regulate oocytes maturation are still unclear, our results suggest that phosphatase Cdc25 may be the direct substrate of PKA. In agreement with this hypothesis, recent findings demonstrate that in *Xenopus* and mouse oocytes Cdc2 is a direct substrate of PKA (Qian *et al.*, 2001; Han *et al.*, 2005; Han & Conti, 2006).

Treatment with H-89 was able to overcome the inhibitory effect of PKA on GVBD; however, the

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inhibition of Cdc25 activity with $NaVO_3$ was able to overcome the induction of GVBD by H-89.

During the past few years, important information has been acquired on how the natural G2 arrest is maintained in oocytes with Myt1 activity probably playing a critical role. In this sense, a link between MAPK and Cdc2–cyclin B activation has been identified, via the p90-induced phosphorylation and inactivation of the Cdc2 inhibitory kinase Myt1. Mishra & Joy, (2006) have shown a positive involvement of MAPK in catfish (*Heteropneustes fossilis*) oocytes maturation.

A role of PP2A in oocytes maturation has long been suspected because MPF inactivation depends on an OA-sensitive phosphatase (Forester *et al*., 2007). It is a generally accepted view that PP2A negatively regulates Cdc2-induced Cdc25 phosphorylation (Izumi *et al*., 1992; Clarke *et al*., 1993), and it has been suggested that PP2A acts downstream of cAMP-dependent protein kinase (PKA) yet prior to, or at the point of, MPF activation (Rime *et al*., 1990), but it is unclear whether PP2A is regulated early or late during oocytes maturation. In *Xenopus* oocytes it was proposed that PP2A, a major Ser/Thr phosphatase present, is a negative regulator of Cdc2 activation (Hermann *et al*., 1988; Lee *et al*., 1994). However, in *B. arenarum* oocytes, inhibition of Cdc25 with $NaVO_3$ did not inhibit OA-induced maturation, suggesting that the target of PP2A was not the Cdc25 phosphatase.

OA stimulates oocytes maturation in starfish, *Xenopus* and mammals (Goris *et al*., 1989; Rime & Ozon, 1990; Gavin *et al*., 1991; Schwartz & Schultz, 1991; Sun *et al*., 2002), an effect that has been attributed to MPF (Goris *et al*., 1989) or MAPK (Zernika-Goetz *et al*., 1997; Sun *et al*., 2002) activation. MAP kinase activation has been reported to be essential in *Xenopus* oocytes GVBD (Ferrell & Machleder, 1998).

In order to confirm the implication of the MAPK pathway in the meiotic resumption of *B. arenarum* incompetent oocytes treated with progesterone, we used an inhibitor of MEK, PD98059, which selectively blocks the activity of MEK by inhibiting the activation of MAPK and subsequent phosphorylation of MAPK substrates. Our results showed that this inhibitor prevented activation of MAPKs and meiotic resumption, suggesting that MAP kinase activation is an early event in response to progesterone in this species.

It has recently been proposed (Palmer *et al*., 1998) that kinase acting downstream of MAP kinase p90 inactivates the Cdc2 inhibitory kinase Myt1 by phosphorylation and thereby links MAP kinase activation to progesterone-induced MPF activation. However, Fisher *et al*., (1999) showed that *Xenopus* oocytes can undergo GVBD in the absence of MAP kinase activity.

In *B. arenarum* oocytes we demonstrated that the inhibition of MAP kinase by PD 98059 prevented the activation of MPF induced by OA, suggesting that the activation of the MAP kinase cascade produced an inhibition of Myt1 and, in consequence, the activation of MPF without participation of the Cdc25 phosphatase.

In many species, including starfish, *Spisula* and mice, protein synthesis is not required for fully grown oocytes to undergo GVBD in response to the natural maturation-inducing signals. In *B. arenarum*, however, protein synthesis is required for GVBD. It is possible that this requirement is for Mos synthesis and thus MAP kinase activation, and no further cyclin B synthesis is required before GVBD.

It has been suggested that imbalanced coordination between protein kinases and protein phosphatases determines cellular responses. Our results suggest that in incompetent oocytes of *B. arenarum* two signal transduction pathways may be involved in the control of MPF activation: (1) the inhibition of phosphatase 2A that through the MEK–MAPK pathway regulate the activity of the Myt1; and (2) the inhibition of AMPc–PKA, which affects the activity of the Cdc25 phosphatase.

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