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Original article

The germinal vesicle of the mouse oocyte contains elements of the phosphoinositide cycle: what is their role at meiosis resumption?

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Abstract — The role of the nuclear phosphoinositide (PI) cycle during meiotic resumption in mouse oocytes was examined. First, using indirect immunofluorescence staining with specific monoclonal antibodies (mAbs) against elements of this cycle, the presence of inositol trisphosphate receptors (IP_3Rs) (IP_3R-1 or IP_3R-3) or phosphoinositide-phospholipase (PLC) isoforms (PLCβ1 or PLCγ1) was monitored in the germinal vesicle (GV). Using confocal laser scanning microscopy, we analysed the effects of the nuclear microinjection of these antibodies on both spontaneous nuclear calcium oscillations and meiosis resumption. Immunostainings showed that IP_3R-1 and PLCβ1 isoforms were both present in the GV, whereas IP_3R-3 and PLCγ1 isoforms were not. The anti-IP_3R-1 mAbs or the anti-PLCβ1 mAbs microinjected into the GV, induced inhibition of both the nuclear Ca^{2+} oscillations and the meiotic process, whereas the anti-IP_3R-3 mAbs and the anti-PLCγ1 mAbs did not. We concluded that a specific nuclear PI cycle is present in the mouse oocyte and meiosis resumption requires a specific nuclear phosphoinositide-dependent Ca^{2+} signal. © Inra/Elsevier, Paris

oocyte / nucleus / calcium / IP_3 receptors / PLC

Résumé — La vésicule germinative de l'ovocyte de souris contient des éléments du cycle des phospho-inositides : quel est leur rôle dans la reprise de méiose ? Le rôle du cycle des phospho-inositides nucléaires (PI) dans la reprise de méiose a été étudié dans l'ovocyte de souris. Dans un premier temps, la présence et la localisation de certains éléments de ce cycle tels que deux types de récepteurs à IP_3 (IP_3R-1 et IP_3R-3), et deux isoformes de la phospholipase C (PLCβ1 et PLCγ1) ont été recherchées dans la vésicule germinative (GV) par immunofluorescence indirecte grâce à des anticorps monoclonaux spécifiques de chacun d'eux. De plus, nous avons analysé, en microscopie confocale, les effets de la micro-injection nucléaire de ces différents anticorps spécifiques sur les oscillations calciques nucléaires spontanées et la reprise de méiose. L'immunohistochimie a montré que seuls les récepteurs à IP_3 de type 1 et la PLCβ1 étaient présents dans la VG tandis que les récepteurs de type 3 et la PLCγ1 ne l'étaient pas. Microinjectés dans la VG, l'anticorps contre le récepteur à IP_3 de

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type 1 comme l'anticorps contre la PLCβ1 induisaient une inhibition des oscillations du Ca^{2+} nucléaire et du processus méiotique tandis que les anticorps contre les récepteurs à IP_3 de type 3 ou contre la PLCγ1 n'avaient aucun effet. En conclusion, un cycle des PI nucléaires est fonctionnel dans l'ovocyte de souris et la reprise de méiose nécessite un signal Ca^{2+} nucléaire dépendant des phosphoinositides. © Inra/Elsevier, Paris

ovocyte / noyau / calcium / récepteurs à IP_3 / PLC

1. INTRODUCTION

The precise biochemical pathways that are responsible for inducing the phosphorylation/dephosphorylation cascade that results in meiosis resumption in mammals are still unknown. Many elements have been discovered, at least in rodents, revealing the clear implication of cyclic adenosine monophosphate (cAMP) as a 'negative' second messenger whose presence or disappearance in the oocyte plays a major role in meiosis arrest or resumption (for a review, see [10]). Despite the knowledge of these elements, nothing is clearly known concerning a factor of granulosa or cumulus cell origin that can override the inhibitory effects and promote meiotic resumption. For several years now, researchers have been focusing on the role of an intracellular calcium signal as the possible inducer of meiosis resumption. The debate, however, is still ongoing between the defenders of the calcium implication and those who claim that calcium is not essential at this time of meiosis (for review, see [10]). The major argument of the latter group is that buffering the intracytoplasmic calcium by BAPTA (1, 2-bis (o-aminophenoxy) ethane-N, N, N′, N′-tetraacetic acid) has no implication on meiosis resumption in the mouse [3, 29]. However, we verified this point and observed that the meiotic process slows down when the calcium sponge BAPTA 70 000 was microinjected into the mouse oocyte (unpublished data). The same observation was found for bovine oocytes [9]. Indeed, spontaneous calcium oscillations occurred in most of the fully grown oocytes in the minutes following their release from the follicles [3, 13, 29]. We also demonstrated a clear correlation between the acquisition of the meiotic competence during oocyte growth and the acquisition of the full functionality of the IP_3-dependent calcium release [14]. Moreover, we observed that the changes in the distribution of the chromatin in the germinal vesicle (GV) occurring before germinal vesicle breakdown (GVB) were related to the occurrence of these spontaneous oscillations [13], i.e. the oocytes that will resume meiosis were those that exhibited calcium oscillations. Furthermore, having observed that calcium was also oscillating in the nucleus during the first step of meiosis resumption [13], we continued our investigations to determine whether the nuclear Ca^{2+} could play a major role during the GVB. We were impelled in that direction by numerous recent studies on the nucleus of different cell types that demonstrated the presence of elements of the biochemical pathways playing a role in calcium homeostasis (*figure 1*) such as phosphoinositide-phospholipase C (PLC), inositol trisphosphate receptors (IP_3Rs), and protein kinase C (for reviews, see [16, 24]). Thus, we worked first on localising several isoforms of PLC or IP_3 receptors in the immature mouse oocyte and then on examining their respective importance in the propagation of nucleic Ca^{2+} nuclear and in the meiotic process.

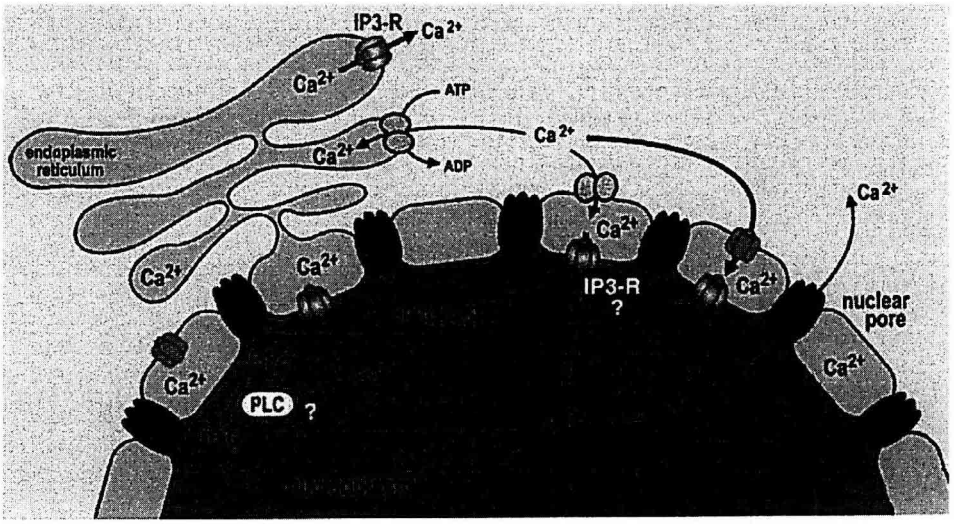

Figure 1. Phosphoinositide turnover in the nucleus. The nuclear envelope is constituted by two distinct membranes, the external and the internal membrane. The ATP-dependent calcium pump as well as the IP_4 receptors are present on the external nuclear membrane. This localisation permits calcium entry into the nuclear lumen. The IP_3Rs are localised on the external nuclear membrane. Phospholipase C (PLC) is located in the nucleoplasm and permits the formation of IP_3 which, in turn, induces the release of calcium when it binds to its receptor. This diagram has been realized from those proposed by Humbert et al. (Médecine/Sciences 11 (1995) 1437–1441).

2. MATERIALS AND METHODS

2.1. Media

M2 or M16 medium [7] supplemented, respectively, with 4 or 15 $mg \cdot mL^{-1}$ BSA (fraction V; Sigma, Saint-Quentin Fallavier, France) were used for the oocytes culture. The microinjection medium was $140 \cdot 10^{-3}$ $mol \cdot L^{-1}$ KCl, 10^{-3} $mol \cdot L^{-1}$ $MgCl_2$, $5 \cdot 10^{-3}$ $mol \cdot L^{-1}$ HEPES, pH 7.2.

2.2. Oocyte recovery

Oocyte-cumulus cell complexes were isolated from the antral ovarian follicles of 5-week-old Cd1 females (Charles River, Saint-Aubin-les-Elbœuf, France). Ovarian stimulation was induced by injection of 5 IU of a pregnant mare's gonadotropin serum (Chronogest, Intervet International, Boxmeer, Holland). The ovaries were removed 48 h later, placed in a Petri dish containing M2 medium and the antral follicles were disrupted with sterile needles. Then cumulus-enclosed oocytes were collected and freed from surrounding cells by repeated aspiration with a glass capillary in the M2 medium.

2.3. Immunolocalisation of IP_3-receptors and PLC isoforms

For localisation in whole oocytes, the zona pellucidae were removed in a solution of α-chymotrypsin (0.1 ‰) in PBS supplemented with BSA (3 %). The oocytes were then treated as already described [21]. Finally, the immunostained oocytes were observed by confocal microscopy using a 40 × objective (NPL Fluotar 40/0.70) at a single optical plane through the GV. Images were produced by an average of six consecutive frames using the on-line Kalman algorithm.

For immunolocalisation in isolated nuclei, suction was applied with a very thin micropipette on the cytoplasmic membrane of the oocytes, to obtain its mechanical disrupter. Then, the isolated nuclei were treated as already described and observed with a 60 × objective (PlanApo, 60/0.95) [21].

2.4. Experimental protocols

The experimental protocols are summarised in *figure 2*. As soon as the oocytes were recovered, either the agonist or the antagonists of the phosphoinositide turnover, diluted in the microinjection medium, were microinjected into the GV. The microinjections were performed either after Ca^{2+} indicator loading to record the immediate calcium events or without loading to study the GVB kinetics for 4 h in culture. The agonist used was the inositol 1,4,5-trisphosphate (IP_3, 10^{-6} mol·L^{-1}; Calbiochem, San Diego, CA, USA); the antagonists were the low molecular weight heparin ($2 \cdot 10^{-6}$ mol·L^{-1}), which binds non-specifically to the IP_3Rs (Calbiochem), two monoclonal antibodies (mAbs) against the IP_3Rs (the anti-IP_3 type 1 isoform, 1:20, anti-IP_3R-1 mAbs; Calbiochem, and the anti-IP_3 type 3 isoform, 1:20, anti-IP_3 R-3 mAbs; Transduction Laboratories, Lexington, KY, USA) and two mAbs against PLC isoforms (the anti-PLCβ1, 1:20, and the anti-PLCγ1, 1:20; UBI, Santa Cruz, CA, USA). *Table I* gives the specificity of all the antibodies used.

2.5. Fluorescent Ca^{2+} indicator loading of the oocytes

The cell-impermeant oregon green 488 BAPTA-1 dextran (Oregon green dextran; Molecular Probes Inc., Eugene, OR, USA) was used to load the nucleus since its high molecular weight (70 000 MW) circumvents it from passing through any membrane. It was diluted extemporarily in the microinjection medium, then injected into the GV of the oocytes.

2.6. Confocal microscopy of living oocytes

All measurements of fluorescence emission were performed using a Bio-Rad MRC600 confocal laser scanning station interfaced with a Nikon Diaphot inverted microscope as described previously [14]. In brief, to perform time-lapse calcium imaging studies, dye-loaded oocytes were viewed through a 20 × or a 40 × objective (Fluor 20/0.75; NPL Fluotar 40/0.70) at a single optical plane through the GV. Calcium kinetic images were resolved by monitoring the oocytes with a 2-s interval between two consecutive acquisitions. Sequential digitized images were recorded for later analysis. Fluorescence intensity was measured in a rectangular area positioned in the nucleoplasm excluding the nucleolus. On the kinetic curves, data are expressed in arbitrary units uncalibrated in terms of calcium concentration.

3. RESULTS

3.1. Immunolocalisation of IP_3 receptors and PLC isoforms

3.1.1. Whole oocytes

Immunostaining with anti-IP_3R-1 mAbs revealed not only fluorescence located on

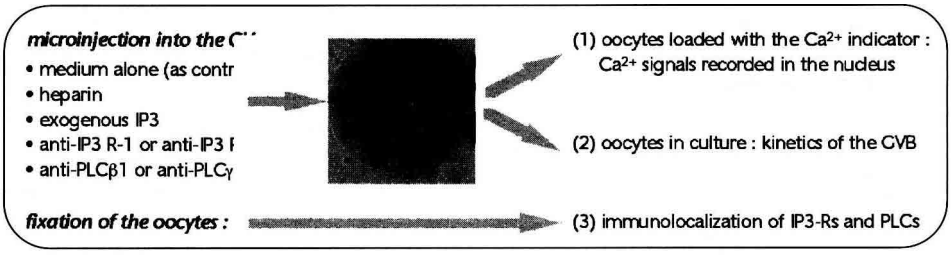

Figure 2. Experimental protocols. After recovery from antral follicles, the oocytes were denuded and placed in a microdrop of M2 medium covered with mineral oil. (**1**) The oocytes were immediately loaded with the fluorescent calcium probe and then the different agonist or antagonists of the phosphoinositide cycle were microinjected into the germinal vesicle for intranuclear calcium signal recording; (**2**) the different agonist or antagonists of the nuclear phosphoinositide (PI) cycle were immediately microinjected directly into the germinal vesicles (GV) and the oocytes were further cultured in M16 for germinal vesicle breakdown (GVB) kinetics study; (**3**) the whole oocytes or their isolated nuclei were immediately fixed in paraformaldehyde for immunolocalisation of the different IP_3 receptors or phosphoinositide-phospholipase C (PLC) isoforms.

Table I. Origin and specificity of the monoclonal antibodies (mAbs).

mAbs	Laboratory	Specificity	Cross-reactivity
Anti-IP_3 R-1	Calbiochem	C terminus domain	Human, rabbit, pig, mouse and bovine
Anti-IP_3 R-3	Transduction Laboratories	N terminal domain	
Anti-PLC β1	UBI	PLCβ1 (150 kDa) also 100 kDa and 41 kDa fragments of PLCβ1	Bovine, rat, mouse and human
Anti-PLC γ1	UBI	PLCγ1 and with some non-PLCγ1 SH-3 containing proteins.	Rabbit, rat, mouse and human

the cytoplasmic network but also a high fluorescence intensity associated with the nuclear membrane (*figure 3A*). Using anti-IP_3R-3 mAbs, immunohistochemistry also evoked a localisation of IP_3R-3 on the cytoplasmic network, with higher fluorescence in the vicinity of the plasma membrane, but the nucleus area was not stained (*figure 3B*). The examination under confocal microscopy of oocytes immunostained with the anti-PLCβ1 mAbs showed an important nuclear staining. The cytoplasm was slightly fluorescent whereas a positive signal was also detected at the plasma membrane level (*figure 4A*). When the anti-PLCγ1 antibody was applied, the presence of this PLC isoform was always revealed both in the cytoplasm and in the nucleus, with a higher fluorescence intensity in the cytoplasm (*figure 4B*).

3.1.2. Isolated nuclei

The observations of the immunostained isolated nuclei confirmed the previous data obtained in the whole oocytes. IP_3 receptors of type 1 were localised at the nuclear membrane level (*figure 3C*). No IP_3R-3 were found in the nucleus, and even if a slight signal appeared in the vicinity of the nuclear membrane, it was not significant compared to the signal obtained with IP_3R-1 (*figure 3D*). The PLCβ1 isoform was present mainly on the nuclear membrane and around the nucleolus and slightly in the nucleoplasm (*figure 4C*). Even though the PLCγ1 isoform was slightly detectable on the nuclear membrane and around the nucleolus, the fluorescence intensity was very weak and almost insignificant compared to that obtained with the other PLC isoform (*figure 4D*).

3.2. Effects of the PI turnover agonist or antagonists on spontaneous calcium oscillations

The microinjection of the calcium indicator, oregon green dextran, into the GV induced first, an important release of calcium and then, the spontaneous nuclear Ca^{2+} oscillations progressively became firmly established (*figure 5A*). In *figure 5B*, the images show a nuclear calcium wave, the dextran-conjugated probe excluding the nucleolus.

Low molecular weight heparin induced a very fast decrease in nuclear Ca^{2+} oscillations into typical small fluctuations of basal fluorescence. Only some spikes of reduced amplitude appeared with irregular frequency for the following 30 min (*figure 6A*).

A low percentage of oocytes does not exhibit calcium oscillations after their release from the follicle. The microinjection of exogenous IP_3 into the GV of these non-oscillating oocytes induced first irregular, then regular, nuclear Ca^{2+} oscillations (*figure 6B*).

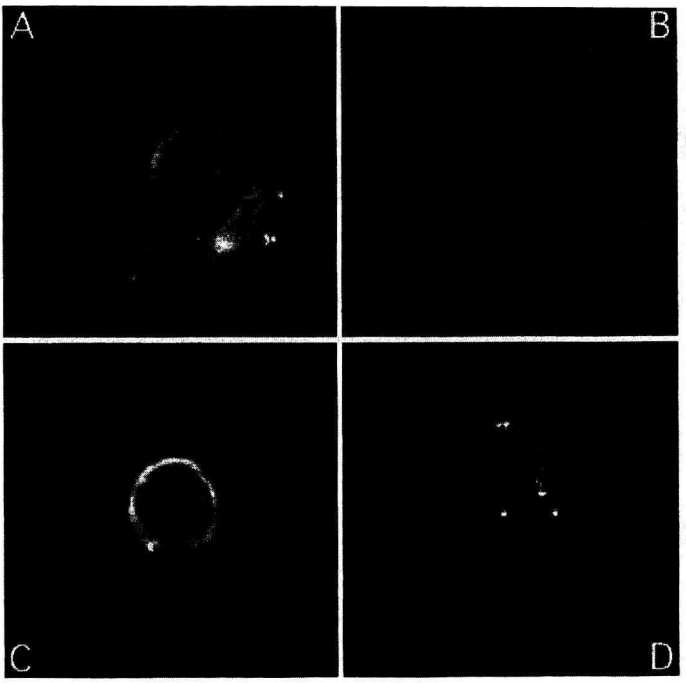

Figure 3. Immunolocalisation of different isoforms of IP_3 receptors in whole immature mouse oocytes and in isolated nuclei. After overnight incubation in the presence of the anti-IP_3R-1 mAbs (1:4) or anti-IP_3R-3 mAbs (1:10), the oocytes were immunostained first with a biotin-SP-conjugated goat anti-mouse antibody (1:50), then with a streptavidin-fluorescein conjugate (1:1000). Anti-IP_3R-1 double immunostaining of the whole oocyte (**A**) or the isolated nuclei (**C**) revealed a high density of fluorescence associated with the nuclear membrane, whereas anti-IP_3R-3 double immunostaining of the whole oocytes showed fluorescence mainly spread through the cytoplasmic network (**B**), and no significant fluorescent signal appeared in the isolated nuclei (**D**), compared to those obtained

The microinjection of anti-IP_3R-1 mAbs into the GV induced an important release of nuclear calcium which was not uptaken again. The fluorescence level remained high and nuclear Ca^{2+} oscillations did not reappear in half of the studied oocytes, whereas the other half exhibited only a transitory disorganisation of the signal (*figure 7A*). The microinjection of anti-IP_3R-3 mAbs, however, was without effect on nuclear calcium oscillations (*figure 7B*).

In most of the cases, the microinjection of the anti-PLCβ1 mAbs seemed to inhibit the nuclear Ca^{2+} oscillations, whereas the nuclear microinjection of anti-PLCγ1 mAbs did not affect the spontaneous nuclear calcium events.

3.3. Implication of the nuclear phosphoinositide cycle in meiosis resumption

When the anti-IP_3R-1 mAbs was microinjected into the GV, the meiotic process was strongly inhibited: the GVB rate remained low and constant during the 4 h in culture, compared to the oocytes in the control group ($P < 0.05$) (*figure 8A*).

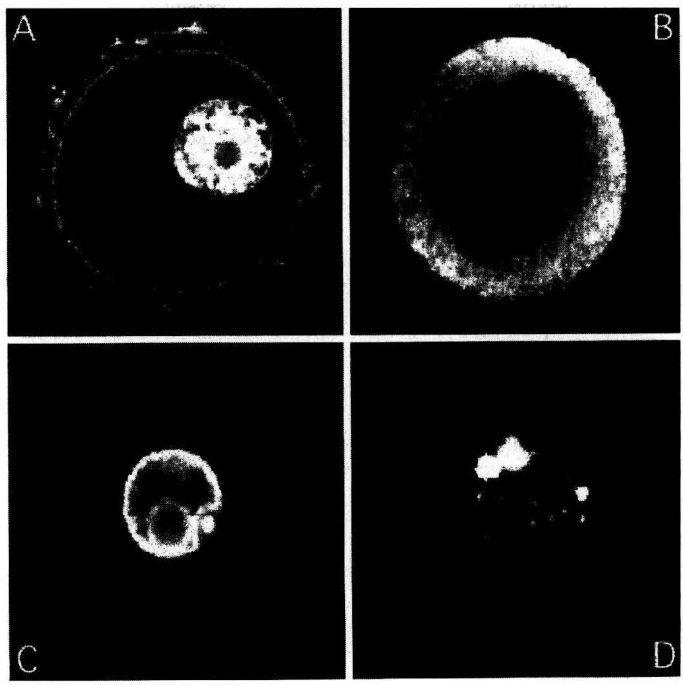

Figure 4. Immunolocalisation of different isoforms of phosphoinositide-phospholipase C (PLC) in whole immature mouse oocytes and in isolated nuclei. After overnight incubation in the presence of the anti-PLCβ1 mAbs (1:20) or anti-PLCγ1 mAbs (1:20), the oocytes were immunostained with a fluorescein-conjugated goat anti-mouse antibody (1:50). Anti-PLCβ1 double immunostaining of the whole oocyte (**A**) or the isolated nuclei (**C**) revealed a high density of fluorescence associated with the nuclear membrane, whereas anti-PLCγ1 immunostaining of the whole oocytes showed fluorescence that was spread mainly through the cytoplasmic network (**B**), and no significant fluorescent signal appeared in the isolated nuclei (**D**), compared to those obtained with anti-PLCβ1 mAbs.

Oocytes receiving a microinjection of anti-IP_3R-3 mAbs into the nucleus resumed meiosis, as did the control group oocytes (*figure 8B*).

When the anti-PLCβ1 mAbs was microinjected into the nucleus, the GVB kinetics slowed down compared to control group oocytes (*figure 8C*) with a GVB rate at 4 h still significantly lower.

Anti-PLCγ1 mAbs microinjected into the GV had no effect on the GVB process (*figure 8D*).

4. DISCUSSION

Our results demonstrate for the first time that, in the mouse oocyte, a specific nuclear IP_3-dependent Ca^{2+} signal does exist, and that the spontaneous calcium events observed in the GV immediately after follicular release play a role in the meiotic process since its inhibition induces a dramatic decrease in the GVB rate. Indeed, IP_3 directly microinjected into the GV induces nuclear Ca^{2+} oscillations in the oocytes that did not previously exhibit spontaneous Ca^{2+}

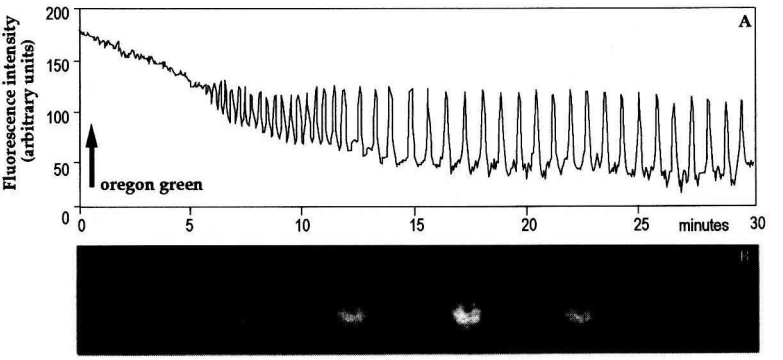

Figure 5. Control nuclear calcium oscillations exhibited by oocytes loaded with oregon green dextran. To perform time-lapse calcium imaging studies, dye-loaded oocytes were viewed through a 40 × objective at a single optical plane through the germinal vesicle (GV) and nucleolus. Calcium kinetic images were resolved by monitoring the oocytes continuously with a 2-s interval between two consecutive acquisitions. Fluorescence intensity was measured in rectangles of similar areas positioned in the cytoplasm and the nucleoplasm excluding the nucleolus. On the kinetic curves, data are expressed in arbitrary units uncalibrated in terms of calcium concentration. (**A**) The microinjection of the calcium indicator, oregon green dextran, into the GV induced first an important release of calcium and then, the spontaneous nuclear Ca^{2+} oscillations progressively became firmly established. (**B**) The images showed a single nuclear calcium wave, the dextran-conjugated probe excluding the nucleolus.

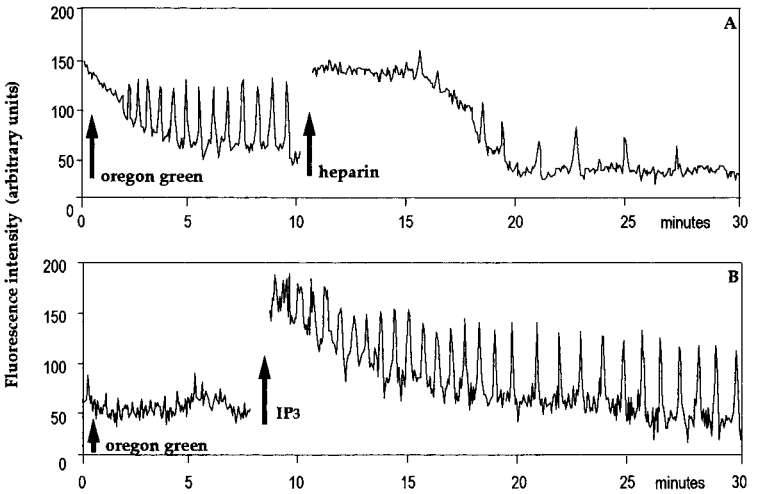

Figure 6. Microinjection of IP_3 or low molecular weight heparin into oocytes loaded with oregon green dextran. The oocytes were maintained in a 30 µL drop of M2 medium under paraffin oil. Oregon green dextran was first microinjected into the nucleus: (**A**) When the oocytes exhibited regular spontaneous calcium oscillations, heparin, diluted in the microinjection medium ($2 \cdot 10^{-3}$ mol·L^{-1}), was injected into the germinal vesicle (GV). The regular nucleoplasmic calcium oscillations disappeared towards the baseline with some irregular spikes. (**B**) When the oocytes did not exhibit spontaneous calcium oscillations, IP_3, diluted in microinjection medium (10^{-6} mol·L^{-1}), was injected into the GV. Spontaneous nuclear calcium signals recorded in a single optical section showed irregular spikes at first and then increasingly regular oscillations similar to those usually observed in fully grown oocytes.

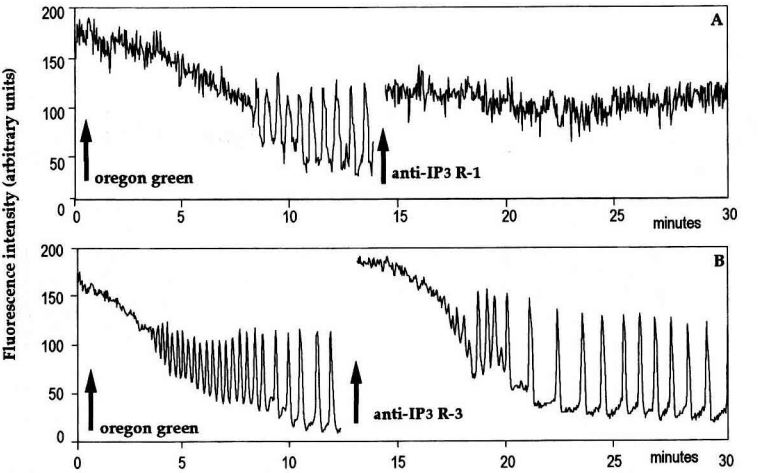

Figure 7. Blockage of the activation of type 1 or type 3 nuclear IP_3 receptors on the calcium signal. Oregon green dextran was first microinjected into the nucleus of the oocytes maintained in an M2 medium drop and the fluorescence intensity was recorded. When spontaneous calcium oscillations occurred, either anti-IP_3R-1 or R-3 mAbs diluted in microinjection medium (1:20) was microinjected into the germinal vesicle (GV): (**A**) The nuclear calcium concentration remained high and spontaneous oscillations did not appear again in most of the oocytes when anti-IP_3R-1 mAbs was injected. (**B**) The nuclear oscillations remained regular after an anti-IP_3R-3 mAbs nuclear microinjection.

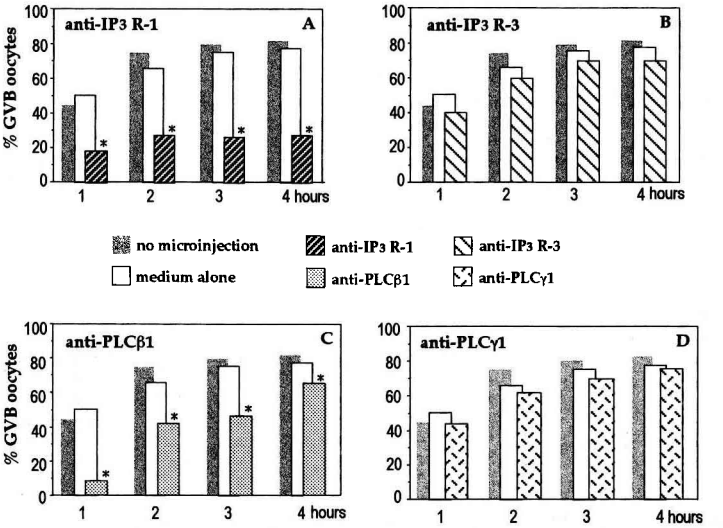

Figure 8. Effects of the nuclear phosphoinositide (PI) turnover antagonists on the germinal vesicle breakdown (GVB) kinetics. The frequencies of oocytes resuming meiosis were calculated on the total number of studied oocytes (n) from at least three experiments (* $P < 0.05$). Microinjection of medium alone into the germinal vesicle (GV) ($n = 54$) did not affect the GVB rate as compared to untreated oocytes ($n = 92$). The GVB process was inhibited when anti-IP_3R-1 (**A**) was microinjected into the GV ($n = 22$); it remained almost unaffected by microinjection of anti-IP_3R-3 (**B**) into the GV ($n = 20$). The GVB process was inhibited when anti-PLCβ1 (**C**) was microinjected into the GV ($n = 72$), whereas it remained almost unaffected when anti-PLCγ1 (**D**) was microinjected into the GV ($n = 72$).

oscillations, while the injection of heparin, which is known to bind non-specifically to IP_3 receptors, into the nucleus of spontaneously oscillating oocytes inhibits the Ca^{2+} oscillations.

As for IP_3R isoforms [19, 22, 30, 31], the presence of β1 and γ1 isoforms of PI-PLC has been demonstrated in the nucleus of several cell types [1, 5, 12]. Thus, we verified the presence of these different isoforms in our cellular model. In the mouse oocyte, we found that the nuclear IP_3-dependent Ca^{2+} release occurs through Ca^{2+} channels associated with type 1 IP_3 receptors. Indeed, both nuclear Ca^{2+} oscillations and GVB are inhibited when a monoclonal antibody, which blocks the activation of type 1 receptors, is injected into the nucleus. These observations indicate that IP_3 receptors are present in the GV of the mouse oocyte as already demonstrated in the *Xenopus* oocyte [2, 15, 26] and that, at least, type 1 is functional and type 3 is either absent or has a lower affinity for IP_3 than type 1. These data also establish that the nuclear IP_3-dependent Ca^{2+} oscillations observed in the preliminary stages of meiosis resumption are necessary for that process. Moreover, nuclear Ca^{2+} oscillations are regulated by at least one PLC isoform present in the nucleus of fully grown immature mice oocytes, the PLCβ1. Indeed, the nuclear microinjection of anti-PLCβ1 mAbs inhibits the GVB process, whereas the nuclear microinjection of anti-PLCγ1 mAbs has no effect.

Furthermore, our immunofluorescent images provide complementary evidence for the existence of a specific nuclear PI turnover. The staining of whole oocytes and isolated nuclei with anti-IP_3R-1 mAbs reveals a perinuclear repartition of type 1 receptors, as for the *Xenopus* oocyte [20]. Moreover, this study allows us to specify that IP_3R of type 3 are absent from the GV since no staining of the nuclear area is noticeable on the immunofluorescent images of whole oocytes and the staining of the isolated nuclei is almost insignificant. The presence of PLCβ1 is revealed both in the cytoplasm and in the nucleus, with a higher fluorescence intensity in this last cellular compartment as already observed in rat liver nuclei [1]. The tight association of the PLCβ1 isoform with the nuclear envelope revealed by the immunostained isolated nuclei is consistent with the fact that this isoform possesses a long COOH-terminal sequence which is responsible for its association with the nucleus [12, 18]. When whole oocytes are immunostained with the anti-PLCγ1 mAbs, the nuclear area is not stained. On isolated nuclei, the PLCγ1 specific fluorescence is mainly around the nucleus and the nuclear membrane, but no staining is detected in the nucleoplasm. The intensity, however, is very weak compared to that obtained with anti-PLCβ1 antibodies.

To conclude, in the fully grown mouse oocyte, the free nuclear Ca^{2+} level appears to be regulated by a specific nuclear pathway since the nucleus has the key enzyme (PLCβ1) and the IP_3Rs [4, 11, 15, 16, 21] is necessary for Ca^{2+} release. The implication of nuclear calcium in the meiotic process has already been demonstrated in non-mammalian species. In starfish oocytes, an increase in nuclear Ca^{2+} is hormonally regulated and is required for reinitiating meiosis [25]. In the sea urchin, fertilisation provokes a transient increase in free calcium in the egg nucleus [8, 27]. What could be the role of these nuclear calcium oscillations? The fact that calcium mobilisation is required for vesicle fusion during nuclear reconstruction in *Xenopus* eggs [28] permits us to hypothesise a role in the breakdown of the nuclear envelope. It could also be responsible for regulating gene expression as previously demonstrated in other cells, the specificity being encoded by the oscillation frequency [6]. Moreover, both cytoplasmic and nuclear calcium are probably implicated in the cascade of phosphorylation/dephosphorylation of the cell cycle proteins since the role of calcium during mitosis has begun to be well documented (for review, see [23]).

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