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Expression and modification of PKA and AKAPs during meiosis in rat oocytes

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Abstract

Meiosis in oocytes is initiated during fetal life, arrested around birth and resumed after puberty. Meiotic arrest is controlled by a cAMP-dependent protein kinase (PKA)-mediated cAMP action. We examined oocytes for the presence and modulation of the regulatory (R) subunits of PKA and the A-kinase anchoring proteins (AKAPs) that target PKA to specific subcellular locations. We found that rat oocytes express the two regulatory subunit isoforms, RI and RII of PKA. Immunocytochemistry revealed that the regulatory subunits underwent cellular translocation upon resumption of meiosis. We also demonstrated the presence of a novel 140 kDa AKAP, AKAP140 that exhibited a retarded electrophoretic motility at reinitiation of meiosis. The mobility shift of AKAP140 was susceptible to alkaline phosphatase and prevented by inhibition of p34cdc2 kinase. We conclude that rat oocytes express AKAP140 that is phosphorylated during meiosis. AKAP140 phosphorylation is sensitive to p34cdc2 kinase inhibitors. We hypothesize that AKAP140 and its phosphorylation state may influence the translocation of the R subunits of PKA throughout resumption of meiosis.

Keywords: PKA; AKAP; Meiosis; Oocyte

1. Introduction

The entry of the female germ cell into meiosis takes place in the fetal ovary and is accompanied by DNA synthesis analogous to S phase in mitotic cells. Meiosis proceeds up to the diplotene stage of the first prophase and is arrested at diakinesis, just prior to or shortly after birth. This stage corresponds to the G2 phase of the cell cycle and is characterized by diffused chromosomes surrounded by an intact nuclear membrane termed 'germinal vesicle' (GV). Resumption of meiosis, also known as oocyte maturation, represents transition from G2 to M phase and involves condensation of the chromosomes, dissolution of the nuclear membrane referred to as 'germinal vesicle breakdown'(GVB) and formation of the first metaphase spindle (MI). The first meiotic division is completed by the emission of the first polar body (PBI) and is immediately followed by the second metaphase (MII, Reviewed by (Dekel, 1995).

A key regulator of the G2 to M transition is maturation promoting factor (MPF) that consists of the catalytic p34cdc2 kinase and the regulatory cyclin B. The activity of MPF is elevated in the oocytes upon reinitiation of meiosis, reaches a maximal level at MI and decreases prior to PBI emission. MPF is reactivated upon entry into the second meiotic division and remains highly active until fertilization (Choi et al., 1991; Zernicka-Goetz et al., 1997). Other regulators of meiotic maturation are ERK1 and ERK2, members of the mitogen-activated protein (MAP) kinase family. In the rat these serine/threonine kinases become active just prior to MI of meiosis and their elevated activity is sustained throughout oocyte maturation (Verlhac et al., 1993; Lazar et al., 2002).

Relatively high intraoocyte concentrations of cAMP prevent activation of MPF (Choi et al., 1991) and the two MAP kinases (Lazar et al., 2002), thereby inhibiting the resumption of meiosis. This negative regulation of

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meiosis by cAMP is mediated by the cAMP-dependent protein kinase (PKA) (Bornslaeger et al., 1988).

In the absence of cAMP, PKA exists as an inactive tetramer, comprised of a dimer of regulatory subunits (R), bound to two catalytic subunits (C). Binding of cAMP to R results in the release of two active C monomers that phosphorylate substrate proteins at serine and threonine residues. Two major isoenzymes of PKA, type I and type II containing RI and RII, respectively, have been described based on their elution position from DEAE cellulose columns (Corbin et al., 1975; Spaulding, 1993). The intracellular localization of the two isoenzymes is variable from cell to cell. RI is known to be mainly soluble, but has also been found close to membrane receptors such as antigen receptors on lymphoid cells and nicotinic acetylcholine receptors in neuromuscular junctions. In contrast, RII subunits are primarily particulate and associated with cytoskeletal elements and a number of organelles (Chen et al., 1997; Keryer et al., 1998). The regulatory subunits of PKA exert two main functions in the cAMP signal transduction pathway. The primary function is to bind and inactivate the catalytic subunit of the enzyme. A second function is to target different PKA isoenzymes to various subcellular loci and specific substrates through its interaction with A-kinase anchoring proteins (AKAPs) (Rubin et al., 1979).

AKAPs were initially identified as contaminating proteins that copurify with RII after affinity chromatography on cAMP-sepharose (Theurkauf and Vallee, 1982). More detailed studies of AKAPs were made possible by the observation that they retain their ability to bind RII after being transferred to nitrocellulose or polyvinyldifluoridene membranes (Lohmann et al., 1980, 1984). Numerous AKAPs have been identified to date. Biochemical analysis suggests that each AKAP contains at least two functional domains: a conserved acidic amphipathic helix region required for RII-binding and a targeting domain responsible for directing the complex to particular subcellular sites. Our knowledge of the subcellular targeting of RI via specific AKAPs is emerging, whereas the binding of many AKAPs to RII is well established (Carr et al., 1991; Carr and Scott, 1992; Reinitz et al., 1997; Colledge and Scott, 1999).

AKAPs appear to serve multiple roles in cells. One major role of AKAPs is to functionally compartmentalize PKA by tethering the regulatory subunits to specific subcellular sites. Some AKAPs bind not only PKA but also other kinases as well as phosphatases, suggesting their function as scaffolding proteins that coordinate the phosphorylation status of certain substrates (Coghlan et al., 1995; Klauck et al., 1996; Faux and Scott, 1997; Schillace and Scott, 1999). A more recent study shows that a cAMP-specific phsophodiesterase isoform compartmentalizes with PKA and that this signaling complex exists through their association with a certain AKAP (Dodge et al., 2001). Although many AKAPs have been cloned and biochemically characterized the physiological consequence of PKA/AKAP interaction remains unknown for most anchoring proteins.

Evidence that a key step of meiotic arrest in mammalian oocytes is dependent upon a phosphorylation event catalyzed by PKA has been established for more than 10 years (Bornslaeger et al., 1988). Modulation of intracellular concentrations of cAMP has been implicated as the major mechanism that regulates PKA action in oocytes (Dekel, 1988). We hypothesize that subcellular compartmentalization of PKA, through its interaction with AKAPs, would increase its efficiency in regulating meiosis. To test this hypothesis, the expression and phosphorylation levels of PKA subunits and AKAPs were monitored during meiosis in rat oocytes. We herein demonstrate for the first time that oocytes contain AKAPs, one of which is progressively phosphorylated during development. The phosphorylation of AKAP140 seems to be catalyzed by MPF but not by MAP kinase.

2. Materials and methods

2.1. Oocytes collection and culture

Twenty-four day-old Wistar female rats, treated with 10 IU of pregnant mare's serum gondotropin (PMSG, Sanofi Sante Nutrition Animale, France) for induction of follicular development were employed for our study. The rats were sacrificed 48 h later. Their ovaries were removed and placed in Leibovitz's L-15 tissue culture medium (Gibco) supplemented with 5% fetal bovine serum (Biolab, Israel), penicillin (100 IU/ml) and streptomycin (100 μg/ml Gibco) with or without isobutylmethylxantine (IBMX 0.2 mM, Sigma, USA), the phosphodiesterase inhibitor that prevents cAMP degradation and maintains the oocytes meiotically arrested.

The follicles were punctured under a stereoscopic microscope to release the cumulus-oocyte complexes. The cumulus cells were removed by repetitive pipeting after collagenase (Sigma, USA) treatment (50 IU/ml, 30 min, 37 °C). Denuded oocytes were incubated in the above medium, in a 37 °C humidified incubator. Oocytes were examined for maturation by differential interference contrast (DIC) microscopy. In the presence of GV the oocytes were classified as meiotically arrested. With IBMX (0.2 mM) in the medium the oocytes were maintained in meiotic arrest. Following approximately 4 h of incubation in IBMX-free medium, meiosis resumed, as indicated by GVB. Previous immunofluorescent staining revealed that the MI spindle is formed after 8 h of culture. This event is followed by the emission of PBI at 10–11 h of culture. Incubation for 12 h allowed the oocytes to progress to the second metaphase (MII) and arrest again at this stage of meiosis.

Another source for oocytes at MII of meiosis was the oviducts of the above-mentioned rats injected with an additional 10 IU of human chorionic gonadotrophin (hCG, Pregnyl, N.V.Organon Oss Holland, Holland) 48–52 h after PMSG to induce ovulation. These rats were sacrificed 20–24 h after hCG injection. These MII oocytes were released into phosphate buffered saline (PBS) and the cumulus cells were removed by repetitive pipeting after hyaluronidase (Sigma USA) treatment (1 mg/ml, 10 min 37 °C). Either prophase-arrested or MII oocytes were incubated with or without the following agents: okadaic acid (1 μM), roscovitine (100 μM, Calbiochem, Germany) and PD 098059 (50 μM Biomol Research Laboratories, Inc. USA). Where indicated, lysates of MII oocytes were treated with alkaline phosphatase (1 unit) in Tris–HCl 50 mM, EDTA 0.1 mM pH8.5 (Boehringer Mannheim) for 30 min at 37 °C.

The study was conducted in accordance with the NIH Guide for Care and Use of Laboratory Animals (National research council, National Academy of Science, Bethesda, Maryland, USA).

2.2. Western immunoblotting

Oocytes were lysed in boiling sample buffer (125 mM Tris pH6.8, 4% SDS, 10% glycerol, 0.006% bromophenol blue, 2% β-mercaptoethanol). The samples were subjected to 10% SDS-PAGE, followed by transfer to a polyvinyliedenedifluoride (PVDF) membrane. After blocking (5% dried non-fat milk in 10 mM Tris pH 7.5, 100 mM NaCl, 0.1%Tween 20), the regulatory (RI and RII), and the catalytic (C) subunits were detected by incubating the membranes overnight at 4 °C with polyclonal anti-RI(1:1000), RIIα(1:1000) and C(1:2000) antibodies, previously characterized (Chestukhin et al., 1996; Morgenstern et al., 2001) and kindly provided by Professor Shaltiel (The Weizmann Institute of Science, Rehovot Israel), or with monoclonal anti-RI(1:250), RIIα(1:250) and C(1:1000, Transduction Laboratories) antibodies. Exposure of the PVDF membrane to the above mentioned antibodies was followed by TTBS washes and further incubation for 1 h with HRP conjugated secondary antibody (goat anti-mouse or goat anti-rabbit 1:2000, Jackson Immuno Research Laboratories Inc. USA). When indicated, the PVDF membrane was exposed to the first antibody that have been preincubated with the relevant recombinant peptide to confirm specificity. The immunoreactive proteins were detected by enhanced chemiluminescence (ECL, Amersham, Buckinghamshire, England).

2.3. Immunofluorescent staining and microscopy

At the end of the specified incubation time oocytes were fixed with 3% paraformaldehyde in PBS, permeabilized with 1% Triton X-100 and immunostained with the above mentioned monoclonal mouse anti-RI and anti-RII antibodies (1:150 dilution) followed by incubation with Cy3-conjugated AffiniPure donkey anti-mouse antibodies (1:300 dilution, Jackson Immunoresearch Lab, Inc. USA). The oocytes were visualized by a laser scanning confocal microscope (Bio-Rad, Radiance 2000/AGR-3 Confocal Imaging System connected to microscope Zeiss, Axiovert 100/TV).

2.4. MPF activity assay

Histone H1 is routinely used as a substrate to assess MPF activity (Fulka et al., 1992). MPF activity was determined in lysates of 25 oocytes frozen and then thawed in 10 μl kinase buffer (15 mM MOPS, 80 mM β-glycerophosphate, 10 mM EGTA, 15 mM $MgCl_2$, 0.1 mM PMSF, 10 μg/ml leupeptine, 10 μg/ml aprotenin, 10 μg/ml PKI, a cAMP-dependent protein kinase inhibitor peptide). Kinase reactions were initiated by adding 10 μl of substrate buffer, 2 mg/ml histone H1 (Sigma, USA), 2 mM dithiothreitol (DTT), 5 μCi [γ-^{32}P] ATP, and incubated at 30 °C for 30 min. Kinase reaction products were subjected to SDS-PAGE and autoradiography.

2.5. MAP kinase activity assay

MAP kinase activity was evaluated in lysates of 25 oocytes, frozen and then thawed in 10 μl of lysis buffer (50 mM β-glycerophosphate, 15 mM EGTA, 10 mM EDTA, 1 mM DTT, 1 mM sodium-ortho-vanadate, 1 mM benzamidine, 10 μg/ml leupeptine, 10 μg/ml aprotenin, 2 μg/ml pepstatine A, PMSF 1 mM). The samples were subjected to 10% SDS-PAGE, and transferred to nitrocellulose membrane. Following blocking with PBST containing 10% non-fat milk, the membranes were incubated with rabbit anti total-MAP (t-MAP) kinase (1:5000, immunoreacts with both the phosphorylated and non-phosphorylated forms of the enzyme) and mouse anti phospho-MAP kinase (p-MAP, 1:5000) antibodies, kindly provided by Prof. Roni Seger (Weizmann Institute of Science, Rehovot Israel, for characterization see (Yung et al., 1997). After an overnight incubation at 4 °C, the membranes were washed with PBST, and incubated for 1 h with HRP conjugated secondary antibody (goat anti-mouse or goat anti-rabbit 1:5000, Jackson Immuno Research Laboratories Inc. USA). The immunoreactive proteins were detected by enhanced chemiluminescence (ECL, Amersham, Buckinghamshire, England).

2.6. RII overlay procedure

The overlay procedure is a modified Western blot analysis (Carr and Scott, 1992). Oocytes proteins were

separated on SDS-PAGE and transferred to Immobilon. After treatment with Blotto to prevent non-specific binding, the blot is probed with radiolabeled RIIα. Recombinant RIIα is radiolabeled by incubation with the catalytic subunit of PKA and ^{32}P-ATP. After separation from free ^{32}P-ATP, the ^{32}P-RII (500 000 cpm/10 ml Blotto) is incubated with the blot for 4 h followed by washing and autoradiography.

3. Result

3.1. RI, RII, and C subunits of PKA in rat oocytes resuming meiosis

Our knowledge of the subcellular targeting of RI via specific AKAPs is emerging, whereas the binding of many AKAPs to RII is well established (Colledge and Scott, 1999). However, using ^{32}P-azido-cAMP photoaffinity labeling, a previous report failed to detect RII in mouse oocytes (Downs and Hunzicker-Dun, 1995). Our first experiment was therefore designed to examine rat oocytes for the expression of this regulatory subunit of PKA. Western blot analysis using monoclonal antibodies to the different subunits of PKA revealed that, in addition to C and RI, rat oocytes, at different stages of meiosis, do express RIIα (Fig. 1a). Specificity of the anti RIIα antibodies was confirmed by a competition experiment using a recombinant RII peptide (Fig. 1b). Similar results were obtained with the polyclonal antibodies described in Materials and Methods (data not shown).

Interestingly, meiotically-arrested oocytes (GV) and oocytes at GVB showed a doublet of proteins that immunoreacted with the anti-RIIα antibodies, while MII oocytes exhibited only the slower migrating form. To examine the possibility that this RII isoform represents a product of phosphorylation, oocytes were incubated with the phosphatase inhibitor, okadaic acid. As illustrated in Fig. 1a, an inhibition of dephosphorylation eliminated the faster migrating form of RIIα. These results suggest that GV and GVB oocytes express both the phosphorylated and the non-phosphorylated RIIα while oocytes at MII express only its phosphorylated form of this PKA subunit.

Examination of immunofluorescently stained rat oocytes by confocal microscopy revealed distinct alterations in the subcellular localization of both RI and RII during oocyte maturation. We observed that RI, that was clearly associated with the membrane in meiotically arrested oocytes (Fig. 2a, a′), translocated to the spindle apparatus of the first meiotic division (Fig. 2b, b′). The RII subunit in meiotically arrested oocytes was mostly present at the perinuclear region (Fig. 2c, c′), but lost this distinct localization after the onset of meiosis, exhibiting an even distribution in the ooplasm (Fig. 2d, d′).

3.2. Presence and modification of AKAP140 in rat oocytes resuming meiosis

The previous experiment demonstrated that the R subunits of PKA are translocated in oocytes that resumed meiosis. As tethering of PKA to specific subcellular sites is attributed to the PKA anchoring proteins, we further employed the modified RII overlay procedure (Carr et al., 1991; Carr and Scott, 1992) to examine rat oocytes for the presence of AKAPs. As illustrated in Fig. 3, one AKAP of approximately 140 kDa (hereafter referred to as AKAP140) was detected in rat oocytes at all the developmental stages examined. This protein was not recognized by antibodies known to

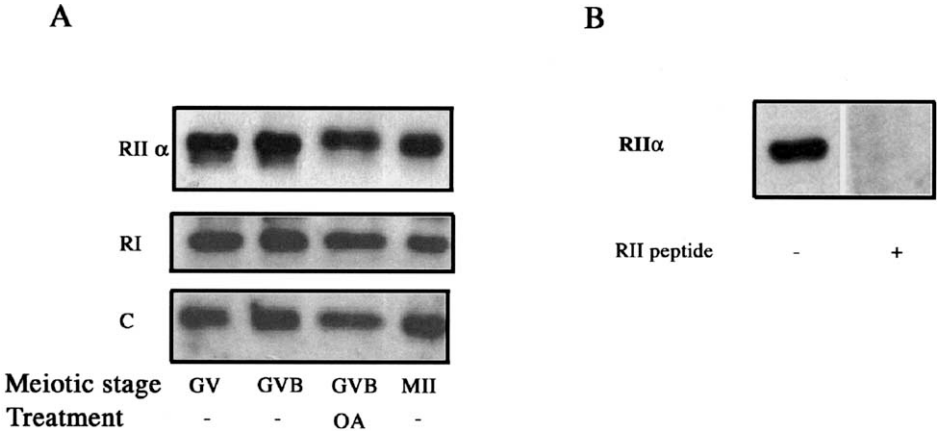

Fig. 1. Expression of C, RI and RII subunits of PKA in rat oocytes. (a) Oocytes (150 per sample) incubated in either 0.2 mM IBMX (GV) or IBMX-free medium with or without okadaic acid (OA, 1 μM) for 4 h (GVB) and 12 h (MII) were extracted, and subjected to 10% SDS-PAGE. The proteins were then transferred to PVDF membranes and immunobloted with monoclonal anti-C (1:1000), anti-RI (1:250) and anti-RIIα antibodies. One representative experiment out of three repetitions is presented. (b) Depletion of the anti-RIIα antibodies by its preincubation with an RIIα recombinant peptide completely eliminated the signal.

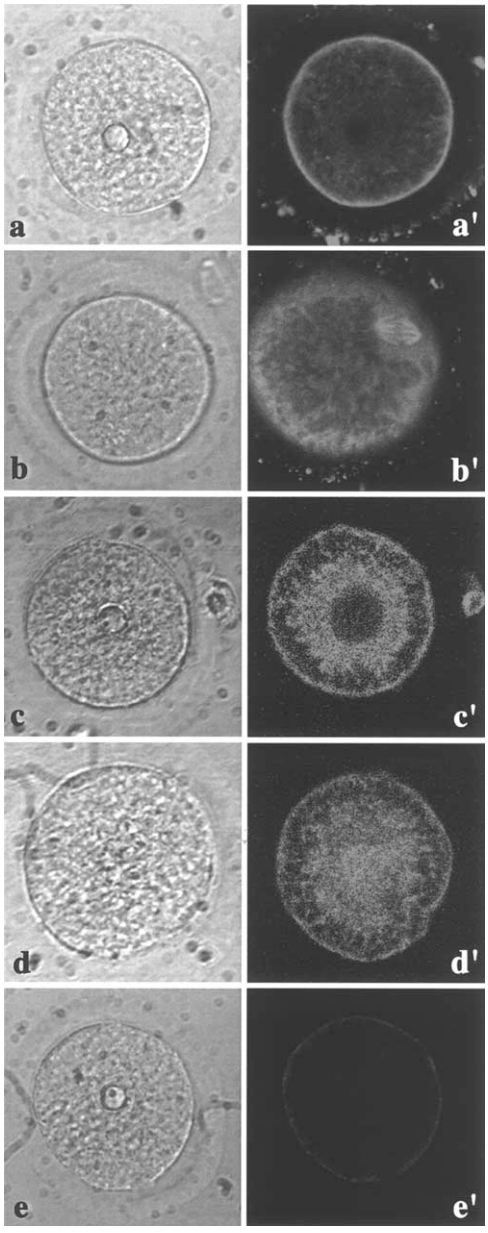

Fig. 2. Translocation of RI and RII in rat oocytes resuming meiosis Oocytes at GV (a, a′, c, c′, e, e′) and MI (b, b′, d, d′) stages of development were collected and fixed as described in Materials and Methods. Localization of RI (a′, b′) and RII (c′, d′) subunits of PKA was detected using monoclonal primary antibodies and a donkey anti-mouse Cy-3 secondary antibody. Secondary antibody alone was used as control (e′). Phase contrast images (a, b, c, d, e), along with immunofluorescence (a′, b′, c′, d′, e′) are presented. One representative experiment out of two repetitions is presented.

react with other AKAPs (AKAP150, AKAP149 and AKAP KL) previously identified in rat ovaries (Carr et al., 1999) suggesting that AKAP140 is a novel, hitherto unidentified RII-binding protein. This experiment also revealed that the mobility of AKAP140 in GV oocytes is faster than in oocytes at MII (Fig. 3a). To determine if the slower migrating form of the protein appearing at MII of meiosis represents a phosphorylated form of

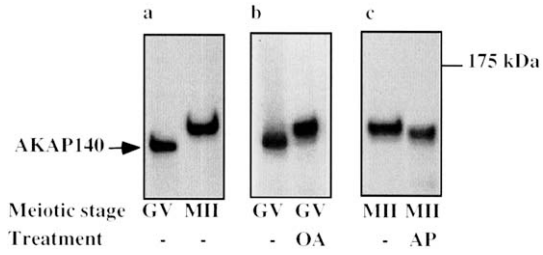

Fig. 3. Changes in electrophoretic mobility of AKAP140 (a) Oocytes (250 per sample) at GV and MII stages of development were collected and their protein extracts were separated on SDS-PAGE, transferred to Immobilon and probed with ^{32}P-RIIα for RII overlay assay as described in Materials and Methods. Results of one representative experiment of at least four repetitions are presented. (b) GV oocytes were incubated with okadaic acid (OA, 1 μM) prior to their extraction. (c) Extracts of MII oocytes were treated with alkaline phosphates (AP, 1 unit) as described in Materials and Methods.

AKAP140, GV oocytes were incubated with okadaic acid. This treatment resulted in retarded electrophoretic mobility of AKAP140 similar to that seen in MII oocytes (Fig. 3b). Conversely, treatment of extracts of MII oocytes with alkaline phosphatase increased the mobility of this protein (Fig. 3c). Taken together, these experiments suggest that oocytes at MII of meiosis express a phosphorylated form of AKAP140.

Kinetic analysis further revealed that the mobility shift that probably represents phosphorylation of AKAP140, takes place prior to GVB (2 h of incubation), increasing gradually after GVB (4 h) and reaching its maximum prior to the completion of the first meiotic division and first PB emission (6 and 8 h of incubation, Fig. 4). The level of phosphorylation slightly decreases after 10 h with no further change in oocytes that have reached MII (12 h of incubation, Fig. 4). Taken together, these data illustrate that AKAP140 is expressed during all stages of oocyte development and that the phosphorylation state of this protein increases upon reinitiation of meiosis.

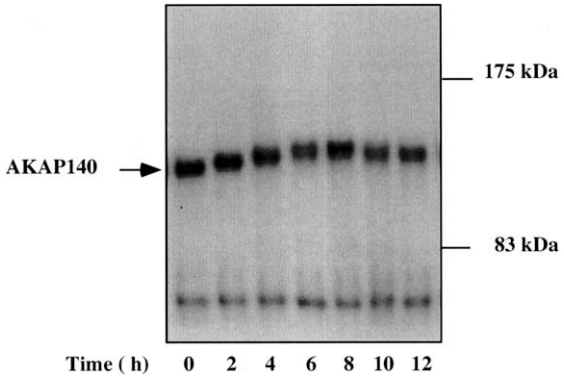

Fig. 4. Kinetics of AKAP140 phosphorylation during oocyte maturation Oocytes (100 per sample) were extracted at the indicated time points of incubation. The proteins were separated by SDS-PAGE, transferred to Immobilon and probed with ^{32}P-RIIα for RII overlay assay. One representative experiment of two repetitions is presented.

3.3. AKAP140 is phosphorylated by MPF

As mentioned in the Introduction, resumption of meiosis is accompanied by elevated activity of regulatory kinases such as p34cdc2 and the MAP kinases. AKAP140 phosphorylation during meiosis could be catalyzed by either or both of these enzymes. If these enzymes are involved in AKAP phosphorylation, then inhibition of their kinase activity should prevent the electrophoretic shift of AKAP140 observed during oocyte maturation. To test this hypothesis, specific inhibitors of either p34cdc2 or MAP kinase have been employed. One such compound is PD 098059, a selective inhibitor of the MAP kinase kinase, MEK, which is the direct upstream regulator of MAP kinase (Alessi et al., 1995). The addition of PD 098059 (50 μM) effectively prevented MAP kinase activation in rat oocytes (Fig. 5a), whereas a similar concentration of this inhibitor failed to prevent phosphorylation of AKAP140 (Fig. 5c). On the other hand, treatment of oocytes with roscovitine (Meijer et al., 1997), a purine analog that successfully inhibited oocytes p34cdc2 kinase activity (Fig. 5b) interfered with AKAP140 phosphorylation (Fig. 5d). These data suggest that the elevated activity of p34cdc2 may be responsible for the developmentally regulated phosphorylation of AKAP140.

4. Discussion

It has been well established that relatively high concentrations of cAMP maintain fully-grown oocytes in meiotic arrest and that reinitiation of meiosis is subsequent to a drop in intraoocyte concentrations of this cyclic nucleotide (Reviewed by Dekel, 1995). It was also commonly accepted that similar to other cellular systems, in the oocyte, modulations in cAMP concentrations regulate the activity of PKA. Revealing the

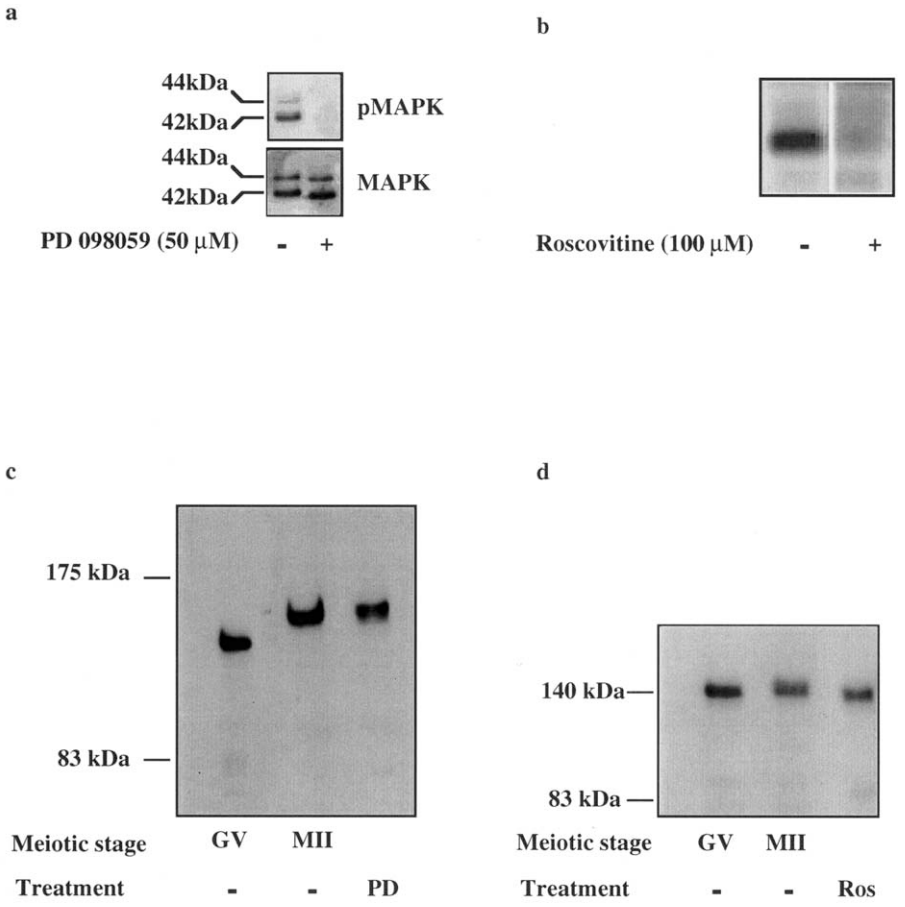

Fig. 5. Effect of MAP kinase and MPF inhibitors on AKAP140 phosphorylation (a) Oocytes (25 per sample) were incubated for 8 h in the presence and absence of PD 098059 (50 μM). Their extracts were electrophoretically separated and immunobloted with anti phosphorylated (P)-MAP kinase and anti-total MAP kinase, as described in Materials and Methods. PD 098059 eliminated the presence of the phosphorylated active MAP kinase. (b) Oocytes (100 per sample) were incubated for 8 h in the presence or absence of roscovitine (100 μM) and examined for p34cdc2 kinase activity using histone H1 as substrate as described in Materials and Methods. Roscovitine effectively inhibited p34cdc2 kinase activity. (c–d) Oocytes (250 per sample), incubated with or without PD 098059 (50 μM) (c) or roscovitine (Ros, 100 μM) (d), for 10 h (as described in Materials and Methods) were extracted, and the proteins were separated on SDS-PAGE and transferred to Immobilon. The membranes were probed with ^{32}P-RIIα for RII overlay assay. Roscovitine but not PD 098059-treatment prevented the mobility shift of AKAP140.

expression of a novel 140 kDa AKAP our study suggests for the first time that an additional mechanism may be involved in regulation of PKA activity in oocytes. This idea is strongly supported by the translocation of the R subunits of PKA that is associated with resumption of meiosis. The phosphorylation of AKAP140 that takes place in oocytes upon reinitiation of meiosis also agrees with this notion.

Our attempts to identify the kinase responsible for the phosphorylation of AKAP140 suggest that this event is catalyzed by p34cdc2, the catalytic subunit of MPF, which is the key regulator of entry into meiotic M-phase. This conclusion is based on the use of a highly specific inhibitor of this kinase and supported by the close correlation between the kinetics of AKAP140 phosphorylation and p34cdc2 kinase activation (Zernicka-Goetz et al., 1997), that are both elevated in oocytes immediately following reinitiation of meiosis.

The first observation of AKAP phosphorylation was reported for AKAP79. Phosphorylation of this neuronal anchoring protein was shown to be associated with its release from membrane particulate fractions (Dell'Acqua et al., 1998). Another example of phosphorylation of AKAPs that elicits translocation of these anchor protein/PKAII complex from the plasma membrane to the cytoplasm was reported in Drosophila (Rossi et al., 1999). The translocation of the PKA regulatory subunits in oocytes resuming meiosis demonstrated in our study could possibly represent the release of the AKAP/PKA complex from its site of action and may be also achieved by AKAP140 phosphorylation. This phosphorylation–dependent targeting of AKAP/PKA complexes shown in the above mentioned system and possibly present in oocytes could constitute a reversible mode of routing signals carried by cAMP.

Compartmentalization of PKA to subcellular loci is mediated through binding of the R subunit of this enzyme to AKAPs. RII subunits bind to AKAPs with nanomolar affinity and many AKAP-RII complexes have been isolated from cell extracts. By contrast, the binding of RI to AKAPs requires micromolar concentrations, and it remains to be determined whether AKAP-RI complexes do form in-vivo (Carr et al., 1992; Burton et al., 1997; Colledge and Scott, 1999). Using ^{32}P-azido-cAMP photoaffinity labeling, a previous study failed to detect the expression of RII in mouse oocytes (Downs and Hunzicker-Dun, 1995). Since the binding of many AKAPs to RII is well established while evidence for subcellular targeting of RI via specific AKAPs is limited we thought that it would be worthwhile to analyze rat oocytes for the presence of RII. Using both polyclonal and monoclonal anti-RIIα antibodies we provide the first evidence for the presence of RIIα subunit in oocytes. Furthermore, we demonstrate that the phosphorylation-state of RII changes during progression through the meiotic cell cycle.

Our findings that RIIα is phosphorylated during oocyte maturation is consistent with previous findings in dividing cells, demonstrating different phosphorylation patterns of this subunit of PKA during the mitotic cell cycle. Specifically, it has been suggested that RIIα phosphorylation during mitosis is catalyzed by the cyclin B/p34cdc2 kinase (CDK1) and takes place concomitantly with its dissociation from the centrosome (Keryer et al., 1998). Phosphorylation of RII was also suggested to be involved in regulation of its association with the centrosomal AKAPs (Carlson et al., 2001). An intriguing hypothesis is that in rat oocytes, during meiosis, phosphorylation of RIIα by p34cdc2 regulates its interaction with AKAP140 potentially affecting the cellular redistribution of type II PKA.

Collectively, the information generated in our present and previous studies are compatible with the following working model: Meiotic arrest is maintained by PKA, which is activated by relatively high intraoocytes concentrations of cAMP. This inhibitory cAMP is not generated by the oocyte but rather transmitted from the follicular cells through gap junctions (Dekel, 1988). This mode of cAMP supply will apparently create a centripetal concentration -gradient of the nucleotide within the oocyte (the diameter of a fully-grown oocyte is 80–90 μm). Under these unique conditions, targeting of PKA by AKAP to the perinuclear region, which is its site of action, may be essential for an efficient control of meiotic arrest. Alternatively, either hormonally- induced or mechanical termination of cell-to-cell communication in this system, that results in cessation of cAMP transfer, leads to an initial drop in its intra-oocyte concentrations (Dekel, 1988) and allows a partial elevation of MPF activity (Choi et al., 1991). MPF in turn may phosphorylate AKAP140 followed by the release of the AKAP/PKA complex from its subcellular location. The drop in cAMP within the oocyte when combined with PKA translocation from its site of action may provide an efficient mechanism for down regulation of this enzyme resulting in a further elevation of MPF activity. This mechanism may comprise part of the well-established autocatalytic loop of MPF action (Karaiskou et al., 1999).

Further studies will determine whether sequestration of PKA indeed provides a mechanism by which PKA/AKAP interaction participates in regulation of oocyte maturation.

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