MATER protein as substrate of PKCε in human cumulus cells

T. Maraldi¹,4, M. Riccio¹, P. Sena¹, L. Marzona¹, A. Nicoli², A. La Marca³, S. Marmiroli¹, J. Bertacchini¹, G. La Sala², and A. De Pol¹

¹Department of Anatomy and Histology, University of Modena and Reggio Emilia, Via Del Pozzo 71, 41100 Modena, Italy ²Department of Obstetrics and Gynecology, Arcispedale Santa Maria Nuova, V.le Risorgimento 80, 42100 Reggio Emilia, Italy ³Mother-Infant Department, Unit of Obstetrics and Gynecology, University of Modena and Reggio Emilia, Via Del Pozzo 71, 41100 Modena, Italy ⁴Correspondence address. Fax: +39-059-4224861; E-mail: tullia.maraldi@unimo.it

ABSTRACT: High activity of the phosphoinositide 3-kinase/Akt pathway in cumulus cells plays an important role in FSH regulation of cell function and Protein Kinase C epsilon (PKCε) collaborates with these signalling pathways to regulate cell proliferation. Relevant roles in follicular development are played by Maternal Antigen That Embryos Require (MATER) that is a cumulus cell- and oocyte-specific protein dependent on the maternal genome. We recently demonstrated that human MATER localizes at specific domains of oocytes and, for the first time, also in cumulus cells. MATER contains a carboxy-terminal leucine-rich repeat domain involved in protein–protein interactions regulating different cellular functions. Here we investigated the functional role of MATER. Thus, we performed coimmunoprecipitation experiments using HEK293T cells expressing human MATER; a similar approach was then followed in human cumulus/follicular cells. In MATER⁺ HEK293T cells, we observed that this protein acts as a phosphorylation substrate of PKCε. Western blot experiments indicate that, unlike oocytes, human cumulus cells express PKCε. Immunoprecipitation and confocal analysis suggest for the first time that MATER protein interacts with this protein kinase in cumulus cells under physiological conditions. Since PKCε is known to collaborate with antiapoptotic signalling pathways, this suggests a novel mechanism for the function of MATER in follicular maturation.

Key words: cumulus cells / follicular development / human ovary / MATER / PKC

Introduction

Over the past few years a number of maternal effect genes, specifically expressed in the gonads, have been studied to understand the molecular mechanisms governing oocyte and early embryo development. Maternal effect genes encode for proteins and transcripts that accumulate during oogenesis and are necessary to support the development of both germ cell and somatic cells (Eppig, 2001; Cecconi et al., 2004). The pivotal role of these factors in folliculogenesis and oocyte development is revealed by the discovery of their involvement in repressing communication between the oocyte and its surrounding somatic cells. Interactions between the developing gametes and neighbouring somatic cells are crucial for fertility because this complex dialogue is essential for the coordinated development of both germ cell and somatic cells (Eppig, 2001; Cecconi et al., 2004).

Another maternal effect gene is Maternal Antigen That Embryos Require (MATER), supposed to be an oocyte specific protein. MATER (Nlrp5—Mouse Genome Informatics) was one of the earliest maternal effect genes molecularly characterized in mice (Tong et al., 2000a). The 125 kDa protein, encoded by a single-copy gene on Chromosome 7 (Tong et al., 2000b), has been more recently identified as a NALP protein (Tschopp et al., 2003), the largest clade in the Caterpillar family (Harton et al., 2002). NALP (NACHT, leucine rich repeat (LRR) and pyrin domain (PYD)-containing) proteins are characterized by a modular structure, with an N-terminal-based NACHT (NAIP; CIIA; HET-E; and TPI) domain with NTPase function which seems to mediate heterotypic interaction (Damiano et al., 2004). NALP proteins contain an amino-terminal PYD and carboxy-terminal LRR region. PYD domains are present in the members of the death superfamily (Martinon et al., 2001; Pawlowsk et al., 2001). Proteins carrying a PYD are mainly involved in inflammation, apoptosis and NF-kB transcription factor signalling (Koonin and Aravind, 2000; Hoffman et al., 2001; Gumucio et al., 2002). Moreover, MATER, also called NALP5, contains the LRR domain, known to be involved in protein–protein interactions that regulate different cellular functions (Lee and Vallee, 2006). The superfamily of LRR proteins can be subdivided into six subfamilies (Kajava, 1998). According to this classification, functional studies based on knock-out mouse models...
demonstrated that Mater-null females are fertilized normally, but the resulting embryos arrested the development at the 2-cell stage (Tong et al., 2006). Mater-null ovaries had a normal complement of all stages of developing follicles, corpora lutea were also present and the number of ovulated ova was similar to those from normal females (Tong et al., 2004).

Our previous work described that the distribution of mouse MATER is regulated in a manner typical of maternal effect genes: Mater is transcriptionally only during oogenesis, and the protein is stable until the morula stage and early blastocyst but disappears in late blastocyst (Tong et al., 2004).

Our recent article showed for the first time that during human folliculogenesis the simultaneous detection of MATER in follicular cells and oocytes starts in late primary follicles, when the maturation process is activated, persists for the entire process and also shows high levels after ovulation. MATER is expressed in cumulus oophorus cells, in intimate connection with the oocyte, but not in mural granulosa cells, spatially separated from the oocyte (Sena et al., 2009). Bidirectional signalling between the oocyte and the surrounding somatic cells is crucial for coordinated development of both germ and somatic cell compartments (Eppig et al., 1997, 2001; Hutt and Albertini, 2007).

Li et al. recently identified a subcortical maternal complex (SCMC) that assembles during oocyte growth and is essential for zygotes to progress beyond the first embryonic cell divisions. At least four maternally encoded proteins contribute to this complex: FLOPED, MATER and TLE6 interact with each other, although FILIA binds independently to MATER. The SCMC, located in the subcortex of oocytes, is excluded from regions of cell–cell contact in the cleavage-stage embryo and segregates to the outer cells of the morula and blastocyst. Floped<sup>pm/m</sup> and/or Mater<sup>pm/m</sup> oocytes lack the SCMC but can be fertilized. However, these embryos do not progress beyond cleavage stage development and female mice are sterile (Li et al., 2008).

The aim of this study was to obtain insights on MATER function during human folliculogenesis. We demonstrated that MATER is a physiological substrate of Protein Kinase C epsilon (PKC<sub>ε</sub>) during human folliculogenesis. We demonstrated that MATER is a physiological substrate of Protein Kinase C epsilon (PKC<sub>ε</sub>) during human folliculogenesis. We demonstrated that MATER is a physiological substrate of Protein Kinase C epsilon (PKC<sub>ε</sub>) during human folliculogenesis. We demonstrated that MATER is a physiological substrate of Protein Kinase C epsilon (PKC<sub>ε</sub>) during human folliculogenesis. We demonstrated that MATER is a physiological substrate of Protein Kinase C epsilon (PKC<sub>ε</sub>) during human folliculogenesis. We demonstrated that MATER is a physiological substrate of Protein Kinase C epsilon (PKC<sub>ε</sub>) during human folliculogenesis. We demonstrated that MATER is a physiological substrate of Protein Kinase C epsilon (PKC<sub>ε</sub>) during human folliculogenesis. We demonstrated that MATER is a physiological substrate of Protein Kinase C epsilon (PKC<sub>ε</sub>) during human folliculogenesis. We demonstrated that MATER is a physiological substrate of Protein Kinase C epsilon (PKC<sub>ε</sub>) during human folliculogenesis. We demonstrated that MATER is a physiological substrate of Protein Kinase C epsilon (PKC<sub>ε</sub>) during human folliculogenesis. We demonstrated that MATER is a physiological substrate of Protein Kinase C epsilon (PKC<sub>ε</sub>) during human folliculogenesis. We demonstrated that MATER is a physiological substrate of Protein Kinase C epsilon (PKC<sub>ε</sub>) during human folliculogenesis. We demonstrated that MATER is a physiological substrate of Protein Kinase C epsilon (PKC<sub>ε</sub>) during human folliculogenesis.}

**Materials and Methods**

**Human cumulus cells and oocytes collection**

Isolated oocytes and follicular cells were collected by pick-up technique during clinical in vitro fertilization procedures. These samples were collected by the U.O. Ginecologia e Ostetrica (Arcispedale S. Maria Nuova Reggio Emilia) with informed consent according to Italian law and ethical committee guideline.

**Cell culture and transfections**

Human embryonic kidney (HEK) epithelial 293T cells were grown in Dulbecco’s Modified Eagle’s Medium High Glucose (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% Foetal Calf Serum (EuroClone Ltd, Wetherby, Yorkshire, UK).

Transient transfections of 293T cells were performed by the calcium-phosphate method (Cenni et al., 2003).

**Plasmids**

The human MATER expression vector (pcDNA/His/HuMater-26ORF) was kindly provided by Dr Tong Z.B (NIH) and was constructed by subcloning a BamHI–Apall fragment of human MATER cDNA (encoding human MATER residue 25–1199) into BamHI–Apall sites of pcDNA3.1/Hisb vector (Invitrogen, Carlsbad, CA, USA).

The mouse cDNA of PKCe with a BglII site in the 5’ and 3’ termini was obtained by PCR (sense primer 5’-GGAAGATCTTCTCATGGTAGTG TTCAATGGCCTTC-3’, antisense primer 5’-GGAAGATCTTCTCCAT CAGGCTACAGGTCTTCSCC-3’) and subcloned into the BglII site of the pEGFPC1 eukaryotic expression vector, in frame with the green fluorescent protein (GFP)-tag.

**Preparation of cell extracts**

Subconfluent cells were extracted by addition of RIPA buffer (20 mM Tris-Cl, pH 7.0, 1% NP-40, 150 mM NaCl, 10% Glycerol, 10 mM EDTA, 20 mM NaF, 5 mM Sodium Pyrophosphate, 1 mM Na<sub>3</sub>VO<sub>4</sub>, and freshly added Sigma-Aldrich Protease Inhibitor Cocktail) at 4°C for 10 min. Lysates were cleared by centrifugation and used for immunoprecipitation experiments, as described below. A total of 50 µl of the total lysate was immediately boiled in SDS sample buffer, resolved, and immunoblotted with the indicated antibodies. The following antibodies were used: anti-PKCe from Sigma-Aldrich (St. Louis, MO, USA); anti-actin, anti-NALP5 (MATER), anti-Akt, from Santa Cruz Biotechnology (Santa Cruz, CA, USA); anti-pPDK1, anti-Akt phosphoserine 473 (pAkt S473), anti-PKC<sub>ε</sub>, phosphosubstrate and anti-Akt phosphosubstrate, from Cell Signaling Technology, Inc. (Beverly, MA, USA).

**Immunoprecipitation and electrophoresis**

Equal amounts of precleared lysates, whose protein concentration was determined by the Bradford method, were incubated overnight with anti-Nalp5 (Santa Cruz Biotech., Santa Cruz, CA, USA), anti-PKCe (Sigma-Aldrich, St. Louis, MO, USA) (3 µg each). Then the two samples were treated with 30 µl of anti-goat IgG-Agarose (Sigma-Aldrich, St. Louis, MO, USA) and 50% (v/v) of protein A/G agarose slurry (Santa Cruz Biotech, Santa Cruz, CA, USA) respectively at 4°C with gentle rocking for 1 h. Pellets were washed twice with PBS plus 1% NP-40, twice in TNE (10 mM Tris-Cl, pH 7.5, 100 mM NaCl, 1 mM EDTA), once with 10 mM Tris-Cl, pH 7.4, boiled in Laemmli sample buffer, and centrifuged. Supernatants were loaded onto SDS-polyacrylamide gel, blotted on Immobilon-P membranes (Millipore, Billerica, MA, USA), and processed by western blot with the indicated antibody, detected by Supersignal substrate chemiluminescence detection kit (Pierce, Rockford, IL, USA). Quantification of the signal was obtained by chemiluminescence detection on a Kodak Image Station 440CF and analysis with the Kodak 1D Image software.

**PKC kinase assay and phosphosubstrate analysis**

All proteins (MATER expressed in 293T and PKCe, wild type or PKCe/GFP overexpressed in 293T) were immunoprecipitated as described above. PKC Kinase in vitro Assay were performed adding PKCe samples to MATER immunoprecipitated in a buffer comprising 20 mM Tris-Cl, pH 7.5, 25 mM MgCl<sub>2</sub>, 1 mM DTT, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 80 mM ATP and carried out for 15 min at 37°C. Pellets were then immediately boiled in SDS sample buffer and immunoblotted with the indicated antibodies.
MATER bands were localized among the proteins recognized by Phospho-(Ser) PKC substrate antibody (Cell Signaling Technology Inc., Beverly, MA, USA).

**Immunofluorescence confocal microscopy**

Isolated oocytes and cumulus cells were fixed in toto in 4% paraformaldehyde in PBS and permeabilized with 1% Triton X-100 in PBS for 10 min.

After a treatment with 3% BSA in PBS for 30 min at room temperature, the oocytes and cumulus cells were incubated with the primary antibodies diluted in PBS containing 3% BSA [goat anti-NALP5 diluted 1:20, rabbit anti-PKCδ diluted 1:50 and mouse anti-phosphatidylinositol (3,4,5)-triphoshate (PIP3) (Echelon, Salt Lake City, UT, USA) diluted 1:50 overnight at 4°C]. After washing in PBS containing 3% BSA, the samples were incubated 1 h at room temperature with the secondary Abs diluted 1:200 in PBS containing 3% BSA (bovine anti-goat FITC, sheep anti-rabbit Cy3 and Sheep anti-Mouse Cy3). After washing in PBS the samples were then mounted with anti-fading medium (0.21 M DABCO and 90% glycerol in 0.02 M Tris, pH 8.0). Negative controls consisted of samples not incubated with the primary antibody. To confirm antibody specificity a control was performed pre-incubating anti-NALP5 antibody with an excess of the blocking peptide (20 μg of peptide and 2 μg of antibody) for 1 h at 37°C. The multi-labeling immunofluorescence experiments were carried out avoiding cross-reactions between primary and secondary antibodies.

The confocal imaging was performed on a Leica TCS SP2 AOBS confocal laser scanning microscope. For FITC and Cy3 double detection, the samples were sequentially excited with the 488 nm lines of the argon laser and the 543 nm line of a HeNe laser. The excitation and the detection of the samples were carried out in sequential mode to avoid overlapping of the two signals. Optical sections were obtained at increments of 0.3 μm in the z-axis and were digitized with a scanning mode format of 512 x 512 pixels and 256 gray levels. The confocal serial sections were processed with the Leica LCS software to obtain three-dimensional projections. The image rendering was performed by adobe Photoshop software. Co-localization was evaluated on medial optical sections using Leica LCS software. Briefly, the two-dimensional scatter plot diagram of each image was analyzed to evaluate the spatial co-localization of the fluorochromes. For each scatter plot diagram, areas with the strongest co-localizing green and red fluorescence, i.e. pixels with intensity values greater than 150 gray levels (on a scale from 0 to 255) for both detectors, were selected to calculate the co-localization maps and create a binary image (Riccio et al., 2004).

**Results**

**PKCδ interacts with MATER in MATER⁺-HEK293T**

Scansite is a web-accessible programme that predicts interactions between proteins using experimental binding data from peptide library and phage display experiments (Obenauer and Yaffe, 2004). Scansite predicts PKCδ as a binding partner of human MATER protein. This prompted us to investigate whether MATER and PKCδ interact in vitro and in vivo.

In order to obtain a large expression of MATER for coimmunoprecipitation assay, MATER was transiently expressed in HEK293T cells, as reported in Materials and Methods section. Figure 1A shows that the exogenous MATER was localized by anti-NALP5 antibody at the same molecular weight of MATER expressed in oocytes and cumulus cells. In fact the MATER expression vector encodes human MATER residue 25–1199, therefore the almost full length protein.

The optimal transfection time is 24 h, therefore all the experiments below will be carried out in this condition.

Immunofluorescence analysis shows the high efficiency of MATER transfection that localizes mostly in the cytoplasm of 293T cells (Fig. 1B).

To determine if the two proteins physically interact, lysates from 293T cells expressing MATER were immunoprecipitated with anti-NALP5. The immunoprecipitated proteins were separated by SDS-PAGE and analyzed by immunoblot using antibodies specific to PKCδ (Fig. 2A). PKCδ was detected as a 90 kDa protein in whole lysate of 293T expressing MATER or MATER/PKCδ (Fig. 2A, lines 5–6) and was immunoprecipitated by anti-MATER antibody from 293T expressing MATER or MATER/PKCδ (Fig. 2A, lines 2–3). The overexpression of PKCδ in MATER⁺-HEK-293T cells improves the amount of MATER–PKCδ complex (Fig. 2A, line 3). PKCδ was not immunoprecipitated from 293T wild type (Fig. 2A, line 1). Moreover, reciprocal experiments using monospecific antibody to PKCδ to immunoprecipitate MATER in whole cell lysates from 293T expressing MATER or MATER/PKCδ were successful (Fig. 2B). MATER is clearly detectable in anti-PKCδ immunoprecipitates (Fig. 2B, lines 2–3). The presence of endogenous PKCδ in the IP with anti-MATER and vice versa suggested that the two proteins interact in vivo.

For confocal analysis 293T transiently expressing MATER were permeabilized and stained over-night with specific antibody to PKCδ and to MATER. Each antibody was raised in a different species and did not cross-react with the other target protein. Merging of FITC and Cy3 signals from MATER⁺-HEK-293T cells double labelled with anti-MATER and anti-PKCδ Abs, demonstrated the localization of both proteins in the cytoplasm of 293T cells. Colocalization binary map shows the pixels of the image in which both green and red signals are present.
thus indicating around the 40% of the signal from MATER colocalize with the signal from endogenous PKC<sub>1</sub> (Fig. 2C).

**MATER is phosphorylated by PKC<sub>1</sub> in vitro**

Scansite programme indicates the Serine 331 as MATER’s site of interaction (phosphorylation site) with this basophilic serine/threonine kinase, PKC<sub>1</sub>. Furthermore, immunoblot experiments by Ab directed against the substrates phosphorylated by PKC (Phospho-(Ser) PKC<sub>1</sub> substrate antibody) show a 125 kDa band corresponding to MATER protein (Fig. 3A). This data suggests that MATER could be a phospho-substrate of PKC<sub>1</sub>, as was then confirmed by in vitro phosphorylation experiments below.

In vitro phosphorylation assay was performed as reported in Materials and Methods section. In particular, MATER-IP, obtained from 293T cells transiently transfected only with MATER, was treated for 15 min with the phosphorylation buffer in presence (M<sup>+</sup>P) or not (M) of PKC<sub>1</sub>-IP. Samples were run on SDS-polyacrylamide gel and stained with anti-MATER and anti-Phospho-(Ser) PKC<sub>1</sub> Substrate antibody. Figure 3B shows that MATER is clearly detectable in both the IP samples and that, after anti-Phospho-(Ser) PKC<sub>1</sub> Substrate incubation, a weak phosphorylation of MATER is detected in the M sample, most probably due to endogenous PKC<sub>1</sub> which, as shown above, co-immunoprecipitates with MATER. In addition, the band corresponding to 125 kDa becomes more intense in the sample (M<sup>+</sup>P) in which MATER IP have been exposed to phosphorylation buffer with PKC<sub>1</sub>-IP (Fig. 3B line 4).

**Effect of MATER expression on phosphorylation pathways in HEK293T cells**

Further experiments on HEK-293T cells expressing MATER show that the presence of MATER protein induces an increase of PKC<sub>1</sub> amount, confirming the cross-talk between the expression of these two proteins (Fig. 4A left panel).

PKC has served as the archetypal lipid-regulated kinase, providing a prototype for lipid-controlled kinase activation that is followed by
kinases throughout the kinome, including its close cousin, Akt (protein kinase B). PKC and Akt transduce signals propagated by the two major lipid second messenger pathways in cells, those of diacylglycerol signalling and phosphatidylinositol-3,4,5-trisphosphate (PIP3) signalling, respectively.

In fact, PIP3 is another interesting lipid signalling messenger suggested to interact with MATER with high stringency by Scansite programme. PIP3-binding Pleckstrin homology (PH) domain could associate with MATER. Colocalization immunofluorescence experiments shown in Fig. 4B suggest that association indeed occurs.

MATER expression could also modulate Akt pathways. Notably, members of the Akt family of kinases contain a PH domain that selectively recognizes PIP3, thus serving as one of the major mechanisms to transduce signals that activate PI3 kinase. In fact, a phosphorylation increase of Akt and PDK1, the upstream kinase for Akt containing a PH domain, occurs in MATER†HEK-293T cells (Fig. 4A left panel).

Indeed, whole lysates of cells expressing MATER present higher intensity level of Akt phospho-substrates than control cells (Fig. 4A right panel).

**PKCε – MATER interaction in human cumulus cells**

To determine if the two proteins can interact in cells physiologically expressing MATER, at first we performed immunoblot analysis of lysates obtained from human oocytes and cumulus cells, in order to check the expression of PKC isoforms. As reported in the literature, some of the conventional PKC isoforms are present in both the...
cells types (data not shown), but the band corresponding to PKC1, member of the novel PKC family, is clearly visible only in the cumulus sample, although in human oocyte line is not detectable (Fig. 5A).

On the basis of this result and of data recently published by our group demonstrating the MATER is also expressed in cumulus cells (Sena et al., 2009), we investigated the possible interaction between MATER and PKC1 only in human isolated cumulus cells.

Cumulus cells collected by pick-up technique during clinical in vitro fertilization procedures, were isolated, permeabilized and stained with specific antibodies to PKC1 and to MATER. Confocal analysis demonstrated the co-localization of the two proteins in the cytoplasm of this follicular cell population (Fig. 5B).

To determine if the two proteins physically interact, immunoprecipitation assay was performed as described above. The specific antibodies to MATER and PKC1 were used to immunoprecipitate whole cell lysates from cumulus cells. The immunoprecipitated material was separated by SDS-PAGE and analysed by immunoblot (Fig. 5C). A band recognized by PKC1 antibody is visible in cumulus cells lysate and in anti-MATER IP (Fig. 5C, lines 1–2). MATER was detected as a 125 kDa protein in cumulus lysate (Fig. 5C, line 4) and was immunoprecipitated by anti-PKC1 antibody (Fig. 5C, line 3).

All the data together confirm the suggestion that the interaction, observed in HEK293T cells expressing MATER, occurs similarly in cumulus cells under physiological conditions.

**Discussion**

Mammalian oocytes mature in a stepwise manner through ovarian folliculogenesis, ultimately reaching competence to complete oocyte maturation at the final stage of Graafian follicle development. The fully-grown oocyte is tightly surrounded by compact layers of specialized granulosa cells (cumulus cells) to form a cumulus–oocyte complex (COC). Cellular events that immediately occur in COCs in the ovari- tory phase are strictly regulated by pituitary hormones, steroids, growth factors and so on (Kimura et al., 2007). Knowledge of the efficient mechanisms and the downstream cascades of the key molecules controlling follicular maturation may gradually lead to improve- ment of the present oocyte/embryo culture systems and gamete biotechnology.

There is now increasingly ample molecular evidence that maternal effect genes are crucial in preimplantation (Christians et al., 2000; Gurtu et al., 2002; Wu et al., 2003; Payer et al., 2003; Burns et al., 2003; Bortvin et al., 2004; Ma et al., 2006; Bulman et al., 2006; Nakamura et al., 2007) and post-implantation (Bourchis et al., 2001; Howell et al., 2001; Leader et al., 2002; Ye et al., 2005) development.

The human MATER gene has been well characterized and the protein is clearly detectable in oocytes (Tong et al., 2002) and in cumulus cells (Sena et al., 2009) but the physiological role during human folliculogenesis has yet to be characterized. The 1111 amino acid cytoplasmic protein contains a NACHT (NTPase) domain (Koonin and Aravind, 2000) and two repeat motifs near its termini. A five tandem hydrophilic repeat (18 amino acids) at the amino terminus of MATER has homology with dentin matrix protein 1 (George et al., 1993), and a 14 tandem LRR (28–29 amino acids) near the carboxyl terminus (Tong et al., 2000b) is a motif implicated in protein–protein interactions (Kobe and Kajava, 2001).

Therefore, we set out to investigate the role of MATER protein in human follicle. The localization and the interaction of MATER was ana- lysed by indirect immunofluorescence, immunoblot and coimmunoprecipitation experiments in both MATER+/HEK293T and isolated oocytes and cumulus cells after ovarian stimulation for in vitro fertilization.

Here we show that in vitro MATER expression leads to phosphoryl- ation pathways activation. In particular, we observed that MATER interacts with phosphatidylinositol-3,4,5-trisphosphate (PIP3) and Akt signalling is stimulated in MATER+/HEK293T.

Protein kinases transduce signals emanating from the plasma mem- brane, thus lipid second-messenger regulated kinases contain one or more membrane-targeting modules whose membrane engagement results in protein kinase activation. As reported in review by Newton, notably members of the Akt family of kinases contain a PH domain that selectively recognizes PIP3, thus serving as one of the major mechanisms to transduce signals that activate PI3 kinase. PKD1, the upstream kinase for most of the AGC kinases (protein kinase A/protein kinase G/PKC) also has a PH domain; although it binds phosphoinositides with high affinity, this kinase is constitutively active in cells, with substrate conformation controlling down-stream signalling (Newton, 2009). Figure 5 shows that when MATER is expressed PIP3 co-localizes with MATER and an increase in PKD1 and Akt phosphorylation occurs, suggesting that MATER, that is expressed specifically in oocytes and cumulus cells (Sena et al., 2009), could be involved in coordinating PI3-K/Akt pathway activation.

These effects can be explained by recent studies implying that the PI3-K/Akt pathway and the mitogen activated protein kinase (MAPK) pathway contribute to the progress of follicle stimulating hormone (FSH)-induced meiosis in mice (Kimura et al., 2007). Thus, high activity of the PI3-kinase/Akt pathway in cumulus cells plays an important role in FSH regulation of cell function. It is estimated that PI3-kinase in cumulus cells is required for both the suppression of spontaneous meiotic resumption and the induction of gonadotrophin-stimulated meiotic resumption (Shimada et al., 2003).

Furthermore, here we have demonstrated for the first time that MATER can be also a phosphorylation substrate of the isoform ε of PKC, indicating that MATER can play an active role in signal transduc- tion from the plasma membrane to the cytoplasm. This interaction occurs both in MATER+/HEK293T cells and in cumulus cells. In con- trast PKCε is not expressed in human oocytes.

PKC, a family of serine/threonine kinases, plays an important role in the growth factor signal transduction pathway. PKC1, a member of the novel PKC group, is believed to function as an antiapoptotic protein. PKC1 collaborates with other signalling pathways, such as Ras/Raf/ ERK and Akt, to regulate cell survival and cell death. Conventional and novel PKC isoforms, lipid second messenger-sensing Ser/Thr protein kinases, contain diacylglycerol-binding C1 domains and trans- duce signals that trigger diacylglycerol production. Moreover, the PH domain of Akt binds agonist-produced PIP3, an event that exposes the activation loop for phosphorylation by PKD1, the same upstream kinase that modifies PKC; this phosphorylation is rapidly followed by phosphorylation of the hydrophobic motif. Once fully-phosphorylated, Akt is locked in an active conformation and diffuses throughout the cell phosphorylating down-stream substrates (see review by Newton, 2009). There is considerable evidence for PKC-mediated increase
in Akt phosphorylation and activity (Basu and Svaprasad, 2007). Increased Akt activity may be required to mediate the antiapoptotic effects of PKCs. Interestingly, signalling via both proteins was required for efficient MAPK activation, suggesting that the PKCs – Akt complex can cross-talk with a third pathway to mediate its antiapoptotic effects. PKCs may also enhance Akt activity indirectly (Newton, 2009). The indirect regulation of Akt by PKCs can involve integrins or secretion of growth factors that can elicit signalling through the pro-survival PI3-K pathway (Basu and Svaprasad, 2007). Notably, the rate of proliferation of cumulus cells has been suggested to correlate with implantation potential in human assisted reproduction (Gregory, 1998). Therefore a proper MATER expression during follicular maturation could be crucial for pro-survival signal transduction, such as PI3-K/Akt/PKC pathways.

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