Available online at www.sciencedirect.com

SCIENCE DIRECT

Developmental Biology 280 (2005) 38–47

DEVELOPMENTAL BIOLOGY

www.elsevier.com/locate/ydbio

PAR-3 defines a central subdomain of the cortical actin cap in mouse eggs

Francesca E. Duncan[a], Stuart B. Moss[a], Richard M. Schultz[a,b,1], Carmen J. Williams[a,*,1]

[a]*Center for Research on Reproduction and Women's Health and Department of Obstetrics and Gynecology, Philadelphia, PA 19104, USA*
[b]*Department of Biology, University of Pennsylvania Philadelphia, PA 19104, USA*

Received for publication 27 July 2004, revised 2 December 2004, accepted 23 December 2004
Available online 29 January 2005

Abstract

The evolutionarily conserved partitioning defective (PAR) protein PAR-3 is pivotal for establishing and maintaining cell polarity. During mammalian oocyte maturation, the radially symmetric oocyte is transformed into a highly polarized metaphase II (MII)-arrested egg. We therefore examined several aspects of PAR-3 expression during oocyte maturation. We cloned two novel PAR-3 transcripts from an oocyte library that likely encode proteins of M_r = 73 K and 133 K that are phosphorylated during maturation. PAR-3, which is found throughout the GV-intact oocyte, becomes asymmetrically localized during meiosis. Following germinal vesicle breakdown, PAR-3 surrounds the condensing chromosomes and associates with the meiotic spindles. Prior to emission of the first and second polar bodies, PAR-3 is located within a central subdomain of the polarized actin cap, which overlies the spindle. This cortical PAR-3 localization depends on intact microfilaments. These results suggest a role for PAR-3 in establishing asymmetry in the egg and in defining the future site of polar body emission.

Keywords: Mouse egg; PAR-3; Cortical actin; Meiosis

Introduction

During maturation of mouse oocytes, the radially symmetric oocyte is transformed into a highly polarized metaphase II (MII)-arrested egg, one hallmark of which is a cortical MII spindle and its overlying actin-rich domain. This polarization is necessary to produce two highly asymmetric cell divisions (emission of the polar bodies) that maximize retention of maternal components required for early development. This polarity has also been suggested to contribute to early axis determination in the preimplantation mouse embryo (Zernicka-Goetz, 2002), although this proposal is controversial (Hiiragi and Solter, 2004).

The molecular basis for how mammalian oocytes become polarized is poorly understood. Other than formin-2 (Leader et al., 2002) and MAP kinase (Verlhac et al., 2000) that are required for microfilament-based migration of the MI spindle to the cortex, there is a paucity of information regarding how other proteins are involved in establishing and/or maintaining mammalian egg polarity. In lower species, three evolutionarily conserved and interacting proteins, PAR-3, PAR-6, and aPKC (atypical PKC), are essential for development and maintenance of cell polarity (Henrique and Schweisguth, 2003; Knoblich, 2001; Macara, 2004; Ohno, 2001; Pellettieri and Seydoux, 2002). In *Caenorhabditis elegans*, these proteins are critical in establishing anterior–posterior polarity and regulating spindle orientation and asymmetric cell division in the 1-cell embryo. In *Drosophila,* PAR-3/PAR-6/aPKC homologs function to (1) regulate the anterior–posterior axis in the presumptive oocyte, (2) establish apical–basal polarity in embryonic epithelial cells, and (3) generate asymmetry in mitotic neuroblasts. In mammalian cells, this complex is critical for establishing

* Corresponding author. Center for Research on Reproduction and Women's Health, 1313 BRBII/III, 421 Curie Blvd., Philadelphia, PA 19104, USA. Fax: +1 215 537 7627.
E-mail address: cjwill@mail.med.upenn.edu (C.J. Williams).
[1] These authors contributed equally to this work.

0012-1606/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2004.12.034

and maintaining apical–basal polarity in epithelial cells via tight junctions. Because of their documented roles, these three proteins are logical candidates for participating in establishing and/or maintaining egg polarity in mammals.

In *Xenopus* and mouse, PAR proteins become asymmetrically distributed during meiotic maturation. In *Xenopus* oocytes, aPKC and the frog PAR-3 homolog, XASIP, become localized to the animal hemisphere during maturation (Nakaya et al., 2000). In mouse oocytes, two PAR-6 proteins, PAR-6a and PAR-6b, also become asymmetrically distributed towards the animal pole during meiotic maturation in vitro (Vinot et al., 2004). Changes in the localization during maturation of PAR-3 and aPKC, however, have not been reported in the mouse. We report here that mouse oocytes express at least two novel forms of PAR-3 that are post-translationally modified by phosphorylation. Following germinal vesicle breakdown, PAR-3 becomes enriched in the region of the chromosomes. At MI and MII, PAR-3 is associated with the spindle and enriched in the central subdomain of the actin cap overlying the meiotic spindles; this localization depends on intact actin microfilaments. The finding that PAR-3 occupies the central subdomain of the actin cap as early as MI and can interact with chromosomes suggests a role for PAR-3 in defining the future site of polar body emission.

Materials and methods

Antibodies and reagents

A rabbit polyclonal antibody made against the second and third PDZ domains of mPAR-3 was obtained from Upstate Biotechnology (Lake Placid, NY) and will be referred to as anti-PAR-3 (UBI) in the text. The anti-ASIP C2-2 antibody was a generous gift of S. Ohno (Yokohama City University School of Medicine, Yokohama, Japan) and was made against the aPKC binding region. The Cy5-conjugated anti-rabbit IgG was from Jackson Immunoresearch (West Grove, PA), Alexa Fluor 488-conjugated anti-rabbit IgG was from Molecular Probes (Eugene, OR), and FITC-conjugated phalloidin, Latrunculin A (Lat A), and nocodazole were from Sigma (St. Louis, MO).

Collection and culture of mouse oocytes and eggs

Female CF-1 mice (6–8 weeks old) were obtained from Harlan Sprague–Dawley (Indianopolis, IN). Fully-grown, germinal vesicle (GV)-intact oocytes and metaphase II-arrested (MII) eggs were collected from gonadotropin-treated females as previously described (Manejwala et al., 1986). The collection medium used was modified Whitten's medium (Whitten, 1971) containing 15 mM Hepes, pH 7.2, 7 mM $NaHCO_3$, 10 μg/ml gentamicin, and 0.01% polyvinyl alcohol. Unless otherwise stated, cells were cultured either in Whitten's medium supplemented with 10 μg/ml gentamicin and 0.01% polyvinyl alcohol or in CZB (Chatot et al., 1989) at 37°C in a humidified atmosphere of 5% CO_2 in air. For collecting and culturing germinal GV-intact oocytes, the medium was supplemented with 0.3 mM dibutyryl cAMP to inhibit resumption of meiosis (Cho et al., 1974). For maturation in vivo, mice were injected with 0.5 IU eCG and 48 h later with 0.5 IU hCG. Cells were then harvested from ovaries 3 h post-hCG for GVBD, 8 h post-hCG for MI, 10 h post-hCG for telophase I, and from oviducts 13–15 h post-hCG for MII. One-cell embryos were collected 14–15 h after hCG injection and mating to $B6D2F_1$/J males (Jackson Laboratory, Bar Harbor, ME).

Immunoblotting

Oocyte and egg protein extracts and control 3T3/A31 cell lysate were separated by SDS–PAGE using either 8% or 10% gels and then transferred electrophoretically onto nitrocellulose membranes. For use with the PAR-3 (UBI) antibody, membranes were immunoblotted according to the instructions included with the antibody, except for the following modifications: the membranes were incubated with 2 μg/ml of anti-PAR-3 (UBI) overnight at 4°C and phosphate-buffered saline (PBS) was used instead of Tris-buffered saline. As a control, a membrane containing identical samples was immunoblotted with 2 μg/ml of non-immune rabbit IgG. For use with the C2-2 antibody, membranes were blocked overnight at 4°C in PBS containing 5% nonfat milk and 0.1% Tween-20 (PBST). The membranes were then incubated in the C2-2 antibody diluted to a final of concentration of 4 μg/ml in PBST containing 3% BSA for 2 h at room temperature. A secondary anti-rabbit HRP-conjugated antibody was used to detect the primary antibody. Membranes probed with both antibodies were developed using ECL chemiluminescence (Amersham Biosciences, Piscataway, NJ) according to the manufacturer's instructions.

For phosphatase treatment, MII protein extracts were treated with 45.3 units of calf intestinal alkaline phosphatase (New England Biolabs, Beverly, MA) for 30 min at 37°C. As a control, the untreated MII extract was also incubated for 30 min under the same conditions. These samples were then separated on a 10% SDS–PAGE gel that was run such that the 47 kDa molecular weight marker reached the bottom of the gel, and were then processed for immunoblotting as described above.

Screening of an oocyte library with a PAR-3 probe and sequencing of complete transcripts

An mPAR-3 cDNA fragment (nt# 664–1393, Accession # NM_033620) was labeled with [$\alpha-^{32}$P]dCTP (3000 Ci/mmol) by random priming (Invitrogen, Piscataway, NJ). This probe was then used to screen a total of 600,000 plaques from an oocyte cDNA library (a gift of John J.