Available online at www.sciencedirect.com
ScienceDirect
Theriogenology 68 (2007) 146–152
Theriogenology
www.theriojournal.com

Changes in MPF and MAPK activities in porcine oocytes activated by different methods

L. Nanassy [a], K. Lee [b], A. Javor [a], Z. Machaty [b,*]

[a] *Department of Animal Breeding Science, Center of Agricultural Sciences, University of Debrecen, Böszörményi Street 138, Debrecen, Hungary*

[b] *Department of Animal Sciences, Purdue University, 915 W. State Street, West Lafayette, IN 47907-2054, United States*

Received 2 February 2007; accepted 4 April 2007

Abstract

The effect of different oocyte activation methods on the dynamics of M-phase promoting factor (MPF) and mitogen-activated protein kinase (MAPK) activity in porcine oocytes were examined. Three activativation methods were tested: (1) electroporation (EP); (2) electroporation combined with butyrolactone I (BL), an inhibitor of cdc2 and cdk2 kinases; (3) electroporation followed by a treatment with cycloheximide (CHX), a protein synthesis blocker. The activity of cdc2 in MII oocytes was 0.067 ± 0.011 pmol/oocyte/min (mean ± S.E.M.), which by 1 h decreased in every treatment group ($P < 0.05$) and stayed at low levels until 6 h post-activation, approximately the time of pronuclear formation. The initial MAPK activity (0.123 ± 0.017 pmol/oocyte/min) also decreased 1 h after each type of activation treatment ($P < 0.005$). However, in the electroporation only group, activity reached its lowest level at 3 h; thereafter, it started to recover and at later time points, MAPK activity did not differ from that in non-treated oocytes ($P > 0.1$). In contrast, oocytes where electroporation was followed by protein kinase or protein synthesis inhibition had low MAPK activity by the time pronuclei were to be formed. Pronuclear formation in these groups (86.3 ± 3.3% for EP + BL and 87.6 ± 3.7% for EP + CHX) was higher compared to that found in the EP-only oocytes (69.4 ± 3.3%; $P < 0.05$). These findings demonstrated that electroporation alone efficiently triggered the inactivation of MPF but not that of MAPK. In order to achieve low MAPK activity to allow high frequency of pronuclear formation, electroporation should be followed by a treatment that inhibits protein synthesis or specific protein kinases. The combined activation methods provided stimuli that efficiently induced both MPF and MAPK inactivation and triggered pronuclear formation with high frequencies.

Keywords: Oocyte activation; MPF; MAPK; Pronuclear formation; Pig

1. Introduction

In vertebrate oocytes, meiosis consists of two successive cell divisions that are under the control of the M-phase promoting factor (MPF), a heterodimer of two subunits: a catalytic subunit, cyclin-dependent kinase 1 (cdk1) and a regulatory subunit, cyclin B [1,2]. The cdk1 subunit is homologous to the cell division control cdc2 gene product identified in the fission yeast, whereas cyclin B was demonstrated to belong to a family of proteins involved in the progression of cells through the cell cycle [3,4]. In immature oocytes, cdc2 binds to cyclin B; at this point, MPF is catalytically inactive due to inhibitory phosphorylation of cdc2 by the kinases CAK, Myt1 and Wee1 (reviewed by Ref. [5]). The complex gains kinase activity at germinal vesicle breakdown, when the phosphatase cdc25 dephosphorylates cdc2 [6]. Active MPF will then drive cell cycle progression into metaphase I. Exit from

* Corresponding author. Tel.: +1 765 494 8008; fax: +1 765 494 9346.
E-mail address: zmachaty@purdue.edu (Z. Machaty).

0093-691X/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2007.04.044

metaphase is controlled by the anaphase promoting complex (APC), a multi-subunit E3 ligase that ubiquitinates cyclin B, marking it for degradation by the 26S proteasome [7]. The degradation of cyclin B leads to a partial inactivation of MPF [8], which will then facilitate exit from meiosis I and extrusion of the first polar body. A subsequent increase in cyclin B synthesis will generate active MPF again, that will drive the cell cycle into meiosis II. Sustained MPF activity then keeps the chromatin in a condensed state and stabilizes the meiotic spindle [5].

The other signal transduction pathway crucial for the regulation of meiosis is the mitogen-activated protein kinase (MAPK) pathway [9]. The enzyme MAPK is a serine/threonine protein kinase that, together with its downstream substrates, is believed to stabilize MPF and facilitate the MPF-driven process of progression into meiosis. Shortly after the signal for maturation is initiated, translation of maternal c-mos mRNA begins [10]. The product of the proto-oncogene c-mos is the MOS protein, a 39-kD germ cell-specific serine–threonine kinase [11]; it functions as a MAPK kinase kinase (MEKK) and activates MAPK kinase (MEK1 [12]) that in turn activates MAPK through phosphorylation [13]. Active MAPK phosphorylates and thus activates the serine/threonine kinase $p90^{Rsk}$, which then inhibits the inhibitory kinase Myt1, thus facilitating cdc25-mediated MPF activation [14]. Therefore, the MOS/MEK1/MAPK/$p90^{Rsk}$ cascade directly aids in the activation and stabilization of MPF during G2/M transition at the time of maturation [15].

Under physiological conditions, it is the fertilizing sperm that triggers the resumption of the second meiotic division by inducing an oscillation in the oocyte's intracellular free calcium concentration. The primary role of the fertilization calcium signal is to down-regulate cdc2 and cyclin B; the concomitant decrease in MPF activity then leads to dephosphorylation and subsequent degradation of MOS [15]. Increasing the intracellular free calcium concentration by artificial means can also stimulate the second meiotic resumption. As opposed to fertilization, most parthenogenetic activation methods are able to generate only a single calcium transient in the oocyte [16,17]. However, a single calcium transient may be effective only in activating aging oocytes. In freshly ovulated mouse oocytes, a single calcium transient was insufficient and led to the re-activation of MPF and MAPK [18]. The reduced activities of these crucial cell cycle-related protein kinases are used as an indicator for the success and efficacy of oocyte activation protocols [19]. Although several methods have been developed that trigger the resumption of second meiotic division in pig oocytes, it is not entirely clear whether a single calcium transient is sufficient to downregulate the activity of these proteins or if there is a requirement for additional stimuli for effective oocyte activation. The objective of this study was to determine the dynamics of these cell cycle-related regulatory proteins in pig oocytes activated by electroporation and compare it to those measured in oocytes where activation was induced by electroporation followed by protein kinase or protein synthesis inhibition. The ability of the treatments to induce pronuclear formation in the oocytes was also evaluated.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA), unless stated otherwise.

2.2. In vitro maturation of oocytes

Ovaries of prepubertal gilts were collected at a commercial abattoir. The follicular fluid was aspirated from 3 to 6 mm antral follicles with a 10 mL disposable syringe and 20 gauge needle and collected in a 50 mL centrifuge tube. Cumulus–oocyte complexes (COCs) were recovered under a stereomicroscope; those with several compact layers of cumulus cells were selected for in vitro maturation. The COCs were transferred into 500 μL of maturation medium (TCM-199 supplemented with 26 mM sodium bicarbonate, 3.05 mM glucose, 0.91 mM sodium pyruvate, 0.57 mM cysteine, 10 ng/mL epidermal growth factor, 0.5 IU/mL porcine LH, 0.5 IU/mL porcine FSH, 0.1% (w/v) polyvinyl alcohol (PVA), 75 μg/mL penicillin G and 50 μg/mL streptomycin) covered with 400 μL of mineral oil. Maturation took place in a four-well Nunc dish (Nunc, Roskilde, Denmark) for 42–44 h at 39 °C, under 5% CO_2 in air.

2.3. Activation of oocytes

After maturation, cumulus cells were removed by vortexing of the COCs for 8 min in Tyrode's lactate (TL)-Hepes medium supplemented with 0.1% hyaluronidase. Oocytes with evenly dark ooplasm and visible first polar bodies were selected and activated. They were transferred into electroporation medium (300 mM mannitol, 0.1 mM $CaCl_2$, 0.1 mM $MgSO_4$, 0.5 mM Hepes, 0.01 mg/mL BSA) and washed twice. They were