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AMPK regulation of mouse oocyte meiotic resumption in vitro

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Abstract

We have previously shown that the adenosine analog 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), an activator of AMP-activated protein kinase (AMPK), stimulates an increase in AMPK activity and induces meiotic resumption in mouse oocytes [Downs, S.M., Hudson, E.R., Hardie, D.G., 2002. A potential role for AMP-activated protein kinase in meiotic induction in mouse oocytes. Dev. Biol, 245, 200–212.]. The present study was carried out to better define a causative role for AMPK in oocyte meiotic maturation. When microinjected with a constitutively active AMPK, about 20% of mouse oocytes maintained in meiotic arrest with dibutyryl cAMP (dbcAMP) were stimulated to undergo germinal vesicle breakdown (GVB), while there was no effect of catalytically dead kinase. Western blot analysis revealed that germinal vesicle (GV)-stage oocytes cultured in dbcAMP-containing medium plus AICAR possessed elevated levels of active AMPK, and this was confirmed by AMPK assays using a peptide substrate of AMPK to directly measure AMPK activity. AICAR-induced meiotic resumption and AMPK activation were blocked by compound C or adenine 9-beta-D-arabinofuranoside (araA, a precursor of araATP), both inhibitors of AMPK. Compound C failed to suppress adenosine uptake and phosphorylation, indicating that it did not block AICAR action by preventing its metabolism to the AMP analog, ZMP. 2′-Deoxycoformycin (DCF), a potent adenosine deaminase inhibitor, reversed the inhibitory effect of adenosine on oocyte maturation by modulating intracellular AMP levels and activating AMPK. Rosiglitazone, an anti-diabetic agent, stimulated AMPK activation in oocytes and triggered meiotic resumption. In spontaneously maturing oocytes, GVB was preceded by AMPK activation and blocked by compound C. Collectively, these results support the proposition that active AMPK within mouse oocytes provides a potent meiosis-inducing signal in vitro.

Keywords: Mouse oocytes; AMP-activated protein kinase; Meiotic resumption; Compound C

Introduction

Mammalian oocytes are arrested in the diplotene stage of the first meiotic prophase soon after initiating meiosis. The meiotically arrested oocyte is characterized by a prominent nucleus, termed the germinal vesicle (GV), which is maintained throughout the period of oocyte growth. Fully grown oocytes within healthy, non-atretic secondary follicles are triggered to resume meiotic maturation in response to a preovulatory gonadotropin surge. This process is manifested by germinal vesicle breakdown (GVB), a morphological change commonly used to monitor meiosis initiation.

The mechanism by which oocytes overcome the prophase I meiotic arrest is not well understood. Oocytes cultured within their follicles in vitro are maintained in meiotic arrest unless stimulated by gonadotropin. When the oocyte is removed from the intrafollicular environment and cultured under permissible conditions, maturation occurs spontaneously in the absence of hormone stimulation, indicating a dependence on the follicular somatic compartment for maintenance of prophase I arrest. If cumulus cell-enclosed oocytes are isolated from follicles and maintained at the GV stage in vitro with exogenous inhibitor, meiotic resumption can be elicited by gonadotropin. This phenomenon is mediated by the cumulus cells that produce a signal driving the oocyte into meiotic maturation.

Cyclic adenosine monophosphate (cAMP) plays an important role in the regulation of meiotic resumption in oocytes.

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Agents that elevate cAMP levels, cAMP analogs or factors that prevent degradation of intracellular cAMP reversibly suppress oocyte maturation. cAMP prevents GVB through the activation of cAMP-dependent protein kinase (PKA). Indeed, mouse oocytes maintained in meiotic arrest by elevated cAMP can be induced to resume maturation by microinjection of PKA inhibitors (Bornslaeger et al., 1986; Eppig, 1989). In *Xenopus* oocytes, PKA phosphorylates and inactivates cdc25 phosphatase, thereby blocking activation of maturation promoting factor (cdc2 kinase) and contributing to meiotic arrest (Duckworth et al., 2002). Recently, protein tyrosine phosphatase nonreceptor type 13 (PTPN13), a substrate of PKA in both mouse and *Xenopus* oocytes, has been implicated in the regulation of oocyte meiotic resumption (Nedachi and Conti, 2004).

Oocyte cAMP synthesis and hydrolysis are regulated via adenylyl cyclase and cyclic nucleotide phosphodiesterase (PDE), respectively. PDE3A is the predominant PDE isoform expressed in rodent oocytes (Shitsukawa et al., 2001; Conti et al., 2002), and its activity is increased prior to oocyte maturation (Richard et al., 2001). PDE3A-null female mice are infertile as oocytes are ovulated in the immature GV stage (Masciarelli et al., 2004). PDE3A-null oocytes lack cAMP-specific PDE activity, contain increased cAMP levels, are persistently maintained in the GV stage in vivo and failed to undergo spontaneous maturation in vitro. These data indicate that PDE activity is required for oocyte maturation.

The product of PDE activity and cAMP degradation, 5′-AMP, is a potent stimulator of the stress response kinase, AMP-activated protein kinase (AMPK), and has been suggested to have an important meiosis induction function via activating AMPK (Downs et al., 2002). AMPK, a serine/threonine kinase, composed of an α catalytic subunit and β and γ regulatory subunits, is a pivotal enzyme in the regulation of cellular energy charge (Hardie and Hawley, 2001; Hardie, 2004; Kahn et al., 2005). AMPK is activated by an upstream kinase, recently identified as the tumor repressor, LKB1 (Hawley et al., 2003; Woods et al., 2003), via phosphorylation of Thr-172 on the α subunit (Hawley et al., 1996). Binding of AMP or ATP to CBS domains on the γ subunit (Scott et al., 2004) regulates the activation state of the kinase (reviewed by Hardie and Hawley, 2001; Hardie, 2004). AMP promotes phosphorylation, and thereby activation, of AMPK by directly binding to the γ subunit of AMPK and changing the conformation of the kinase, making it a better substrate for the upstream kinase (Hawley et al., 2002) and a worse substrate for the phosphatase (Davies et al., 1990). By controlling the activity and expression of important rate-limiting enzymes of carbohydrate, fat and protein metabolism, AMPK regulates the cell energy status through conservation of ATP levels.

5-Aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), an analog of adenosine, is widely used to activate AMPK in vitro. After entering cells, it is phosphorylated to form AICA ribotide (ZMP), an analog of AMP. ZMP, though less effective than AMP, mimics all the activating effects of AMP in the AMPK cascade (Corton et al., 1995). Thus, AICAR provides a means of activating AMPK without affecting AMP or ATP levels. In a previous study, AICAR was shown to rapidly stimulate GVB in mouse oocytes maintained in meiotic arrest with a variety of inhibitors (Downs et al., 2002). AICAR was more potent in denuded oocytes (DO) than cumulus cell-enclosed oocytes (CEO), identifying the oocyte as the target of AICAR action. Meiotic resumption was also induced by AMP in dbcAMP-arrested oocytes. Therefore, these data were consistent with the possibility that activation of AMPK provides a potent meiosis-inducing signal.

In the present study, we determined if AMPK has a causative role in GVB in mouse oocytes by modifying AMPK activity and relating it to meiotic status. Using four different strategies to expose oocytes to active AMPK and using two putative inhibitors to block AMPK activity, we show that stimulation of AMPK in prophase-I-arrested oocytes leads to meiotic resumption and that preventing AMPK activation abolishes the meiotic response.

Materials and methods

Oocyte isolation and culture conditions

C57BL/6JxSJL/J F1 mice, 19–23 days old, were used for all experiments. Mice were primed with 5 IU equine choronic gonadotropin and killed 48 h later by cervical dislocation. Ovaries were removed and placed in the culture medium, and cumulus cell-enclosed oocytes (CEO) were obtained by puncturing large antral follicles with sterile needles. Denuded oocytes were prepared by repeated pipetting with a Pasteur pipette. Oocytes were cultured in glass tubes for long-term culture (17–18 h) or in plastic tubes for short-term culture (≤4 h). Tubes were gassed with a humidified mixture of 5% CO_2, 5% O_2 and 90% N_2 and placed in a water bath at 37°C for the duration of culture. The culture medium used for most experiments was Eagle's minimum essential medium with Earle's salts (GIBCO), supplemented with 0.23 mM pyruvate, penicillin, streptomycin sulfate and 3 mg/ml crystallized lyophilized bovine serum albumin (ICN ImmunoBiologicals, Lisle, IL) and buffered with 26 mM bicarbonate. For oocyte microinjection, medium was buffered with 25 mM HEPES, pH 7.2, with bicarbonate reduced to 6 mM.

Microinjection of active/dead AMPK

To test the effects of microinjected AMPK on oocyte maturation, denuded oocytes were maintained in meiotic arrest with 300 μM dbcAMP and microinjected with a preparation of either constitutively active AMPK (T172D) or dead (inactive) kinase (D139A), each at a concentration of 7.5 mg/ml. Oocytes were placed into a micro-culture dish at room temperature, and approximately 3–10 pl of solution containing active or inactivate kinase was injected into the cytoplasm of germinal vesicle-stage oocytes using femtotip II needles with an Eppendorf Transjector 5246 system (Eppendorf, Madsion, WI) under a Nikon Diaphot 200 inverted microscope equipped with Nomarski optics. Following microinjection, oocytes were transferred to 500 μl of dbcAMP-containing medium under mineral oil and placed in a 37°C incubator for 22 h. A positive control was included in which non-injected oocytes were cultured in medium containing dbcAMP plus 250 μM AICAR. At the conclusion of the incubation period, oocytes were assessed for GVB.

Construction and expression of GST-AMPK α1 catalytic domain proteins (T172D, D139A)

Plasmid DNA encoding GST-tagged AMPK α1 catalytic domain has been previously described (Scott et al., 2002). This DNA was used in site-directed mutagenesis (QuickChange, Stratagene) to generate constitutively active (T172D) and kinase dead (D139A) GST-tagged AMPK α1 catalytic domain