Zygote **22** (February), pp. 91–102. © Cambridge University Press 2012
doi:10.1017/S0967199412000457 First Published Online 16 November 2012

Perturbing microtubule integrity blocks AMP-activated protein kinase-induced meiotic resumption in cultured mouse oocytes

Ru Ya[2] *and Stephen M. Downs*[1]
Department of Biological Sciences, Marquette University, Milwaukee, Wisconsin, USA

Date submitted: 10.04.2012. Date revised: 15.06.2012. Date accepted: 19.06.2012

Summary

The oocyte meiotic spindle is comprised of microtubules (MT) that bind chromatin and regulate both metaphase plate formation and karyokinesis during meiotic maturation; however, little information is known about their role in meiosis reinitiation. This study was conducted to determine if microtubule integrity is required for meiotic induction and to ascertain how it affects activation of AMP-activated protein kinase (AMPK), an important participant in the meiotic induction process. Treatment with microtubule-disrupting agents nocodazole and vinblastine suppressed meiotic resumption in a dose-dependent manner in both arrested cumulus cell-enclosed oocytes (CEO) stimulated with follicle-stimulating hormone (FSH) and arrested denuded oocytes (DO) stimulated with the AMPK activator, 5-aminoimidazole-4-carboxamide-1-beta-4-ribofuranoside (AICAR). This effect coincided with suppression of AMPK activation as determined by western blotting and germinal vesicle immunostaining. Treatment with the MT stabilizer paclitaxel also suppressed meiotic induction. Targeting actin filament polymerization had only a marginal effect on meiotic induction. Immunolocalization experiments revealed that active AMPK colocalized with γ-tubulin during metaphase I and II stages, while it localized at the spindle midzone during anaphase. This discrete localization pattern was dependent on MT integrity. Treatment with nocodazole led to disruption of proper spindle pole localization of active AMPK, while paclitaxel induced excessive polymerization of spindle MT and formation of ectopic asters with accentuated AMPK colocalization. Although stimulation of AMPK increased the rate of germinal vesicle breakdown (GVB), spindle formation and polar body (PB) extrusion, the kinase had no effect on peripheral movement of the spindle. These data suggest that the meiosis-inducing action and localization of AMPK are regulated by MT spindle integrity during mouse oocyte maturation.

Keywords: AMPK, Meiotic maturation, Microtubules, Oocyte

Introduction

Mammalian oocyte maturation refers to the developmental period between GVB and the formation of the first polar body (PB) and involves dynamic changes within the cytoskeletal network. Initially, the meiotic spindle forms at a position slightly off-centre, where the germinal vesicle (GV) was located originally. After germinal vesicle breakdown (GVB), microtubules start to polymerize around the condensing chromosomes and, as the chromosomes congress, a bipolar metaphase I spindle is established with chromosomes aligned correctly at the metaphase plate. Meiotic spindle migration occurs after its formation and this process relies on a dynamic microfilament meshwork (Azoury *et al.*, 2008), actin filament nucleator formin-2 (Dumont *et al.*, 2007) and RhoGTPase (Na & Zernicka-Goetz, 2006). The peripheral spindle migration is a crucial process that assures conservation of ooplasm through asymmetrical cell division of the PB.

AMP-activated protein kinase (AMPK) is a heterotrimeric protein that contains an α catalytic subunit and β and γ regulatory subunits. It regulates the cellular energy level by sensing the ratio of AMP to ATP and responds to low ATP levels by shutting down the

[1]All correspondence to: Stephen M. Downs. Department of Biological Sciences, 530 N 15 St, Marquette University, Milwaukee, WI 5323, USA. Tel: +1 414 288 1698. Fax: +1 414 288 7351. e-mail: stephen.downs@marquette.edu
[2]Department of Biological Sciences, 530 N 15 St, Marquette University, Milwaukee, WI 5323, USA.

https://doi.org/10.1017/S0967199412000457 Published online by Cambridge University Press

energy-consuming pathways and upregulating ATP production. It is activated via phosphorylation by upstream kinases of threonine 172 on the catalytic α subunit (Hardie, 2003; Carling, 2004). Our laboratory has previously demonstrated a role for AMPK in the resumption of meiosis in mouse oocytes by showing that its activation by a variety of means induces GVB and that meiosis can be blocked by its inhibition (Chen *et al.*, 2006; Downs & Chen, 2006; LaRosa & Downs, 2007; Chen & Downs, 2008). Further, AMPK not only has a role in GVB, but participates throughout the entire maturation period, associating with chromosomes and the meiotic spindle and exerting positive and negative effects, respectively, on PB formation and activation (Downs *et al.*, 2010).

The meiotic spindle association of AMPK is intriguing, because numerous proteins that show a similar localization pattern are known to regulate meiotic spindle assembly and function. AMPK has also been shown to have a role in the maintenance of genome integrity and establishment of cell polarity in *Drosophila* embryos and other somatic cells (see Discussion). It was, therefore, of interest to test how microtubule and spindle integrity were related to AMPK function in mouse oocytes. Our data indicate that disruption of microtubule integrity blocks hormone-induced maturation in meiotically arrested oocytes while coincidentally blocking AMPK activity. Moreover, active AMPK colocalization with γ-tubulin at microtubule organization centres (MTOC) during metaphase I and metaphase II is dependent on intact microtubules. However, treatment of oocytes with AMPK inhibitor did not prevent spindle formation or migration, despite suppressing the completion of meiotic maturation.

Materials and methods

Oocyte isolation and culture condition

All procedures were approved by the Marquette University Institutional Animal Care and Use Committee. Immature, 19–23-day-old (C57B/ 6J × SJL/J) F_1 female mice were used for all experiments. Mice were primed with 5 IU pregnant mare serum gonadotropin (PMSG) 2 days before the experiments. Mice were killed by cervical dislocation and ovaries were removed and placed in a Petri dish containing culture medium. CEO were isolated by puncturing the follicles with sterile needles. DO were obtained by stripping cumulus cell by using a mouth-operated small-bore pipette. Both CEO and DO were washed twice and transferred to plastic culture tubes that contained 1 ml of the appropriate test medium. The culture medium used was Eagle's MEM (Sigma, St Louis, MO, USA) supplemented with penicillin, streptomycin sulphate, 0.23 mM sodium pyruvate and 3 mg/ml BSA (MP Biomedicals, Solon, OH, USA).

Immunofluorescence staining

Oocytes were fixed with microtubule-stabilizing buffer as described previously (Messinger & Albertini, 1991) at 4°C overnight. Oocytes were permeabilized for 30 min with 0.1% Triton-100 in blocking solution, which contained 10% donkey serum and 0.5 mg/ml saponin in phosphate-buffered saline (PBS), followed by 1 h in blocking solution minus Triton-100. Oocytes were incubated with primary antibody (1:100) overnight at 4°C, and washed four times at room temperature in blocking buffer. Oocytes were coincubated with FITC-conjugated anti-tubulin antibody (1:100) and Cy3-conjugated secondary antibody (1:100) 1 h at room temperature. After washing, oocytes were placed on slides and mounted with medium that contained 4′,6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA, USA).

Confocal microscopy

Oocytes were viewed on a laser scanning confocal microscope (Carl Zeiss Co., Thornwood, NY, USA) with a ×63 objective. During scanning, all settings were kept constant: i.e., laser power, detector gain, amplifier offset, amplifier gain, and pinhole size. Digitally recorded images were exported by LSM Examiner software (Carl Zeiss Co.).

Western blot analysis

Oocytes samples were washed with PBS/ polyvinylpyrrolidone (PVP), then twice with a protease inhibitor cocktail (Roche, Indianapolis, IN, USA). Samples were treated with Laemmli's Buffer with 20% beta-mercaptoethanol at 95°C for 5 min. Electrophoresis was carried out using NuPAGE 3–8% Tris–acetate gels (Invitrogen, Carlsbad, CA, USA), and proteins were transferred to nitrocellulose. Membranes were blocked with 5% non-fat milk, followed by incubation with pACC primary antibody (1:250, Cell Signaling) at 4°C overnight. Blots were rinsed with Tris-buffered saline (TBS) (pH 7.4) and TBS–Tween-20 (0.05%), and incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibody (1:2000 in 5% non-fat milk, Pierce) at room temperature for 60 min. After washing, protein signals were detected by Super Signal West Pico Chemiluminescent Substrate (Pierce). Blots were stripped with Restore Western Blot Stripping Buffer (Thermo Scientific; Rockford, IL, USA) and reprobed with anti-ACC1 antibody (1:250; Cell Signaling) as a loading control. The pACC/ACC1 ratios were quantified with ImageJ software based on protein band density.

https://doi.org/10.1017/S0967199412000457 Published online by Cambridge University Press

Chemicals

Saponin, dbcAMP, donkey serum, nocodazole, palmitoyl carnitine, and FITC-labelled mouse anti-α-tubulin were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Cy3-conjugated donkey anti-rabbit antibody was supplied by Jackson ImmunoResearch (West Grove, PA, USA). AICAR and compound C were obtained from Toronto Research Chemicals, Inc. (North York, Ontario, Canada). Anti-phospho-AMPK (PT172) antibody was purchased from Cell Signaling Technology (Beverly, MA, USA). Highly purified ovine FSH was from the National Hormone and Peptide Program (NHPP) and Dr A.F. Parlow.

Statistical analysis

All experiments were repeated at least three times and data presented as mean ± standard error of the mean (SEM). Percentages of GVB or nuclear stain underwent arcsin transformation and data were analyzed statistically by analysis of variance (ANOVA) followed by Duncan's multiple range test. A P-value <0.05 was considered significant.

Results

Effect of cytoskeletal disrupting agents on maturation and AMPK activity

We have shown that AMPK is involved in hormone-induced maturation and that its activation precedes GVB in mouse oocytes (Chen & Downs, 2008). We therefore determined how perturbation of microtubule integrity would affect AMPK activity and meiotic induction. dbcAMP-arrested CEO were treated with 0.1 μg/ml follicle-stimulating hormone (FSH) to induce meiotic resumption, exposed to increasing concentrations of nocodazole, and assessed for GVB after 17–18 h. As shown in Fig. 1*A*, FSH increased the percentage of GVB by 62%, while supplementation with nocodazole blocked this increase significantly in a dose-dependent manner, by 62% at the highest dose tested. Dimethyl sulphoxide (DMSO) alone, the nocodazole vehicle, had no effect on meiotic resumption (data not presented). To test for a direct effect of nocodazole on the oocyte, dbcAMP-arrested DO were treated with 250 μM AICAR, an AMPK activator, and scored for GVB. AICAR stimulated an increase in GVB from 23 to 89%. This stimulation was abolished by nocodazole, which decreased significantly the percentage of maturation to 8% (Fig. 1*B*). This inhibition was completely reversible, thereby demonstrating that the drug did not act through toxic means.

FSH-treated CEO were cultured for 4 h and AICAR-treated DO were cultured for 2.5 h before fixation and immunofluorescence staining for active AMPK, using an antibody against phospho-threonine 172 on the alpha catalytic subunit of AMPK. As shown previously, FSH and AICAR increased phospho-AMPK staining in the germinal vesicle prior to GVB. This staining pattern is characterized by homogeneous staining throughout the GV with absence of label within nucleoli (Chen & Downs, 2008). As nocodazole blocked AICAR- and FSH-induced GVB, it was important to determine if this GV-staining pattern was present after nocodazole treatment of these oocytes. As shown in Fig. 1*C*, 21% of control CEO arrested in dbcAMP showed nuclear staining, with a 20% increase following FSH treatment; exposure to 10 μg/ml nocodazole eliminated this increase. A similar pattern was evident in DO: GV staining was detected in 26% of control oocytes, with this number increasing to 57% after culture in AICAR; nocodazole again prevented the response (20% with active AMPK accumulation in the GV; Fig. 1*D*). These data support the idea that activity of AMPK is greatly influenced by microtubule integrity. This idea was confirmed by western blot analysis of nocodazole-treated oocytes that showed suppression of AICAR-induced AMPK activity, using phospho-acetyl coenzyme A (CoA) carboxylase (pACC) as a marker for AMPK activity (Fig. 1*E*, *F*).

When CEO were treated with another microtubule-destabilizing agent, vinblastine, meiotic induction was again suppressed, with a 31% decrease at the highest concentration tested (Fig. 2*A*). Inhibition was even more robust in AICAR-treated DO, with 20 μg/ml vinblastine reducing the maturation percentage to control levels (Fig. 2*B*).

The microtubule-stabilizing agent, paclitaxel, was utilized to test how stabilizing microtubules affects meiotic induction. CEO were cultured 17–18 h and treated with FSH plus increasing doses of paclitaxel. Similar to the finding with microtubule-disrupting agents, FSH-induced GVB was blocked in dose-dependent fashion by paclitaxel (Fig. 2*C*), and the agent was even more potent in AICAR-treated DO (Fig. 2*D*). No label was observed in the GV when FSH- and paclitaxel-treated oocytes were fixed after 4 h and stained with anti-PT172 antibody; however, punctate staining was present throughout the cytoplasm (data not shown). When paclitaxel was added to AICAR-treated DO, punctate staining of active AMPK was observed near or within the GV, but with no homogeneous staining within the GV. The increased punctate staining of active AMPK after paclitaxel treatment may correspond to excessive stabilization of microtubule polymers.

Cytochalasin D and latrunculin A were added to either FSH-stimulated CEO cultures or AICAR-stimulated DO cultures to determine whether

https://doi.org/10.1017/S0967199412000457 Published online by Cambridge University Press

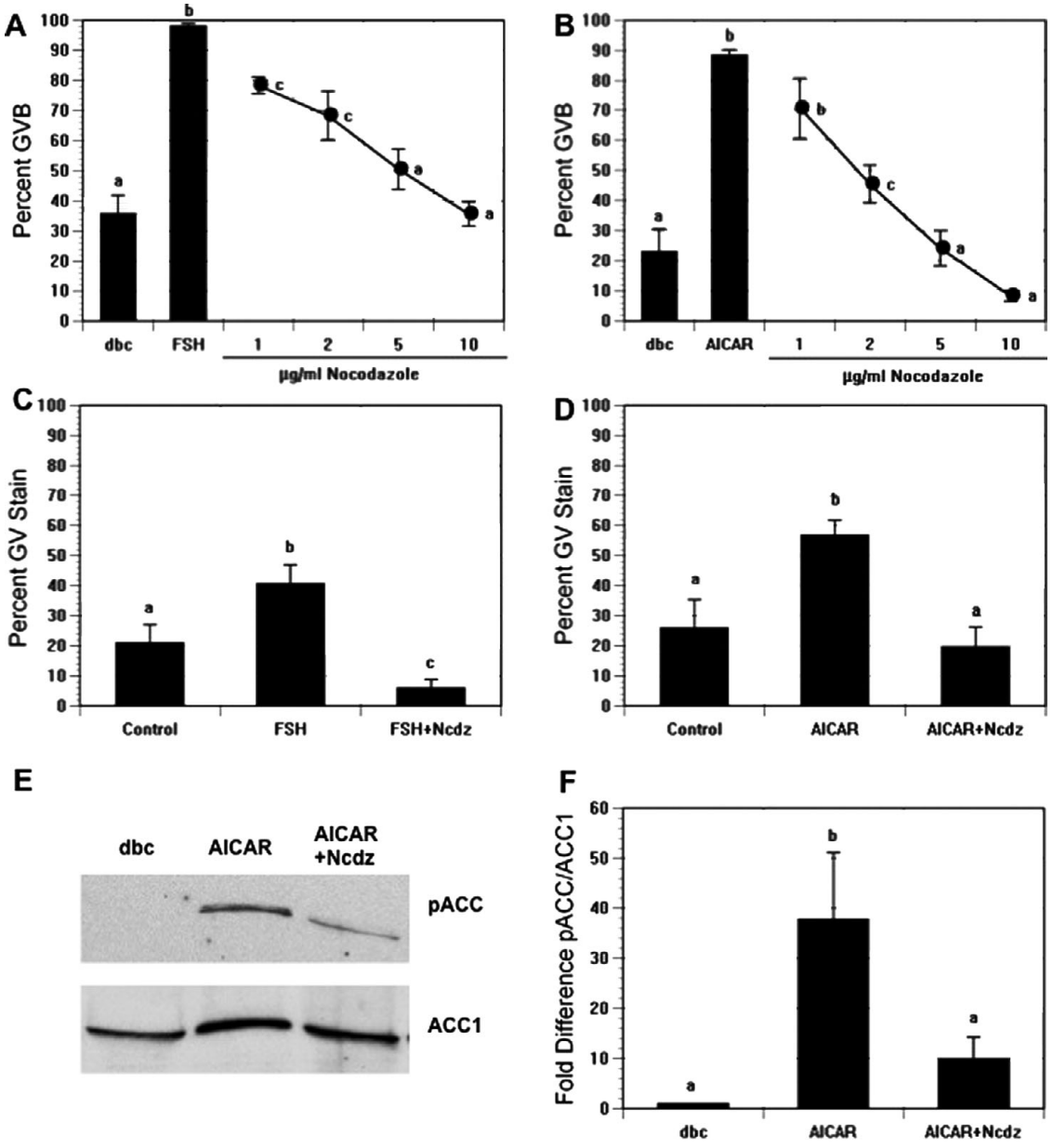

Figure 1 Effects of nocodazole treatment on meiotic resumption and AMP-activated protein kinase (AMPK) activation. (*A*) Cumulus cell-enclosed oocytes (CEO) were cultured in medium that contained 300 μM dbcAMP (dbc) plus follicle-stimulating hormone (FSH) in the presence of increasing concentrations of nocodazole (Ncdz). Germinal vesicle breakdown (GVB) was assessed 17–18 h later. (*B*) Denuded oocytes (DO) were cultured with 300 μM dbcAMP plus 250 μM 5-aminoimidazole-4-carboxamide-1-beta-4-ribofuranoside (AICAR) and treated with increasing doses of nocodazole. GVB was assessed after 4 h of culture. (*C*) CEO were cultured in 300 μM dbcAMP alone (control) or dbcAMP plus FSH with or without 10 μg/ml nocodazole; 4 h later oocytes were fixed and processed for PT172 staining. (*D*) DO were cultured for 2.5 h in the same medium as CEO but with FSH replaced by 250 μM AICAR; oocytes were then processed for PT172 staining. In both (*C*) and (*D*), nocodazole reduced the frequency of the GV stain to control levels. (*E*) Western blot analysis of pACC of oocytes that were treated as described in (*B*); acetyl coenzyme A carboxylase 1 (ACC1) was used as loading control. (*F*) Quantification of phospho-ACC (pACC) and ACC1 from western blot. pACC/ACC1 ratios were normalized to the control dbcAMP-treated group. [a,b,c]Groups with no common letter are significantly different.

inhibition of actin filament polymerization plays a role in meiotic induction. There was no effect of cytochalasin D on GVB. Latrunculin A showed a modest reduction (29%) of GVB in FSH-treated CEO (Fig. 5*E*), but it was toxic to AICAR-treated DO.

An experiment was carried out to determine if the nocodazole-sensitive step could be bypassed by stimulating a downstream meiosis-inducing pathway. To this end, we used the fatty acid derivative, palmitoyl carnitine, a compound shown previously

https://doi.org/10.1017/S0967199412000457 Published online by Cambridge University Press

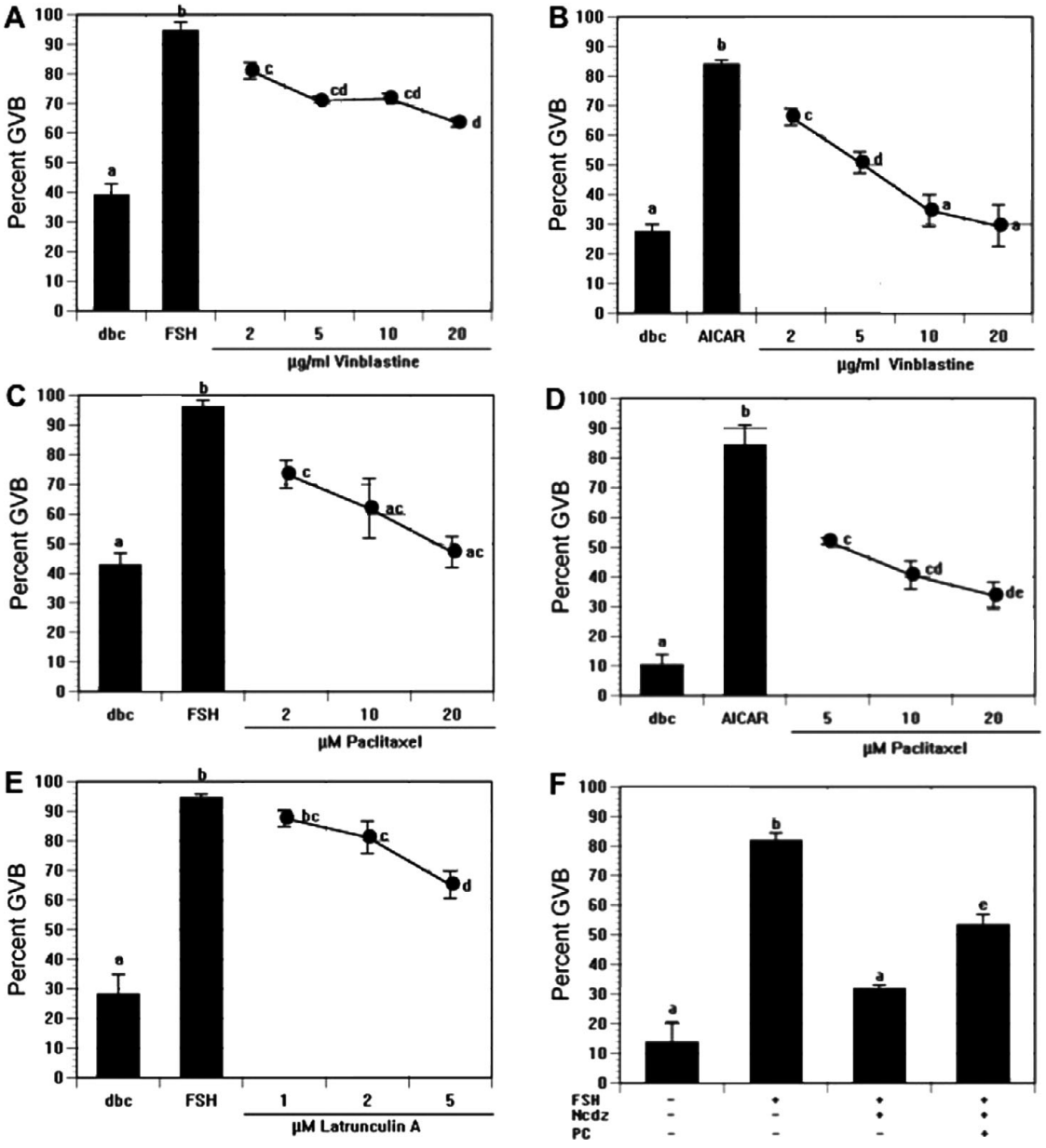

Figure 2 Effects of additional microtubule-targeted agents on follicle-stimulating hormone (FSH)-induced and 5-aminoimidazole-4-carboxamide-1-beta-4-ribofuranoside (AICAR)-induced maturation *in vitro*. (*A*, *C*) Cumulus cell-enclosed oocytes (CEO) were cultured 17–18 h in medium that contained 300 μM dbcAMP plus follicle-stimulating hormone (FSH) or (*B*, *D*) denuded oocytes (DO) were cultured 4 h in 300 μM dbcAMP plus 250 μM AICAR, in the presence of increasing concentrations of vinblastine (*A*, *B*) or paclitaxel (*C*, *D*). (*E*) CEO were cultured 17–18 h in 300 μM dbcAMP plus FSH and increasing concentrations of latrunculin A before germinal vesicle breakdown (GVB) assessment. (*F*) dbcAMP-arrested CEO were induced to undergo maturation with FSH, and this stimulation was suppressed by further treatment with nocodazole (Ncdz). Palmitoyl carnitine (PC) (50 μM) was added to the latter group to trigger GVB via activation of fatty acid oxidation. [a,b,c,d]Groups with no common letter are significantly different.

to drive meiotic resumption through an increase in fatty acid oxidation (Downs *et al.*, 2009). FSH treatment increased the frequency of GVB by 68% in dbcAMP-arrested CEO, and exposure to nocodazole suppressed this action by 50%. In the presence of nocodazole, palmitoyl carnitine restored the induction by 22% (Fig. 2*F*). These results demonstrate that the palmitoyl carnitine-stimulated pathway is still active in the presence of nocodazole, thereby indicating nocodazole treatment does not produce a generalized toxicity.

Association of active AMPK with microtubules and effect of microtubule perturbants on localization

As studies in other laboratories have demonstrated that the meiotic spindle-associated localization of

https://doi.org/10.1017/S0967199412000457 Published online by Cambridge University Press

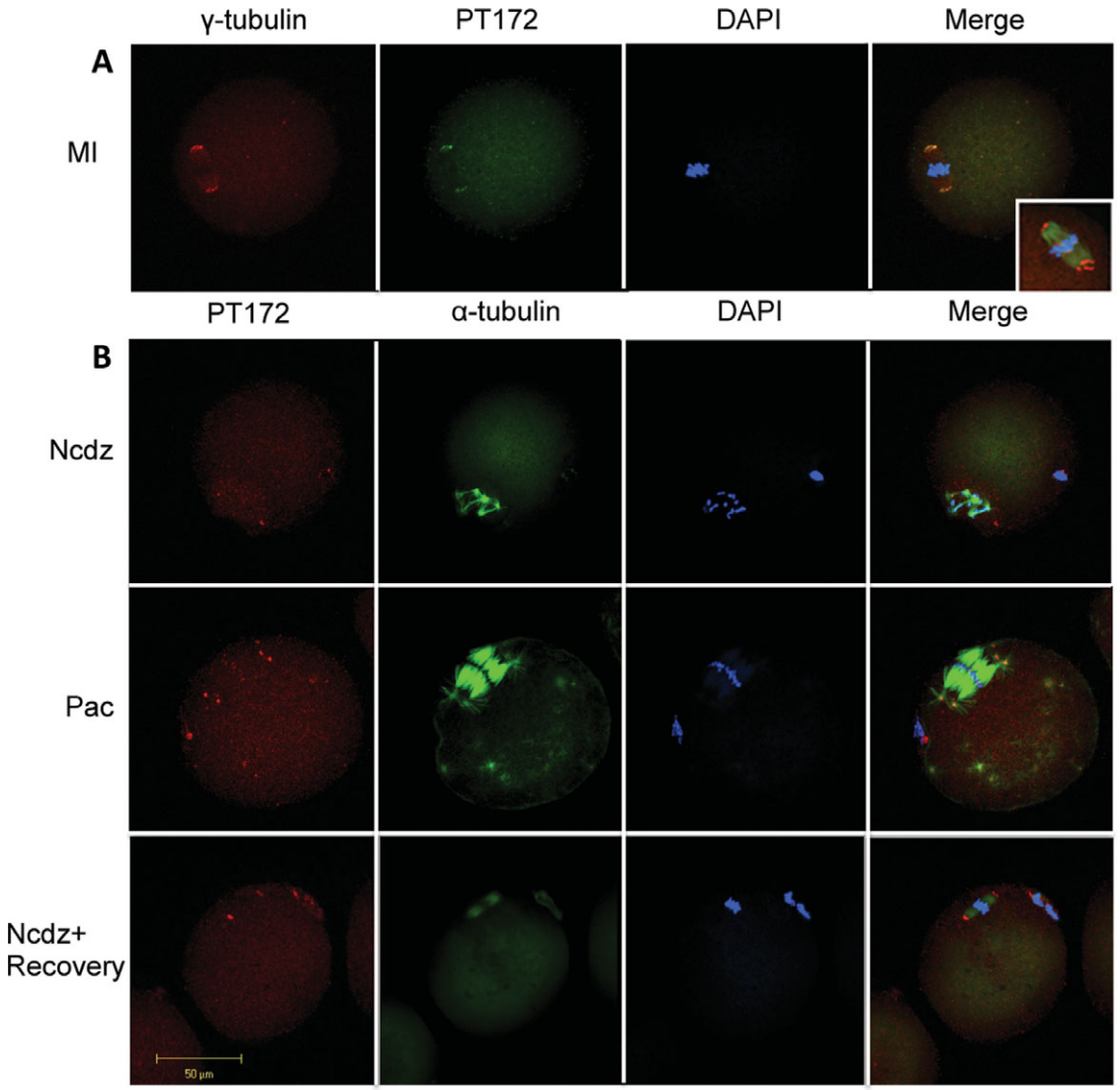

Figure 3 Immunofluorescence staining of active AMP-activated protein kinase (AMPK) and tubulin. (*A*) Oocytes were collected 8 h after administration of hCG to pregnant mare serum gonadotropin (PMSG)-primed mice to obtain oocytes at MI. Active AMPK (green) is colocalized at the spindle poles with γ-tubulin (red). Chromatin, blue. Inset: Metaphase I spindle from triple-stained oocyte showing α-tubulin, green; active AMPK, red; chromatin, blue. (*B*) Effect of microtubule-targeted agents on active AMPK localization. Cumulus cell-enclosed oocytes (CEO) were cultured 17 h in the presence of 300 μM dbcAMP plus follicle-stimulating hormone (FSH); then either the microtubule-destabilizing agent, nocodazole (Ncdz, 0.05 μg/ml), or microtubule-stabilizing agent, paclitaxel (Pac, 20 μM), was added and oocytes were fixed 1 h later and processed for immunostaining for active AMPK (PT172, red) and α-tubulin (green). Nocodazole treatment disrupted the normal spindle pole localization of active AMPK. When treated with paclitaxel, microtubules were excessively polymerized, and this was associated with increased AMPK staining. After CEO were cultured 17 h in the presence of 300 μM dbcAMP plus FSH, oocytes were exposed to nocodazole (5 μg/ml) for 10 min. The nocodazole was then washed out and oocytes were allowed to recover for 1 h before fixation and immunostaining. Upon microtubule repolymerization, active AMPK reestablished its normal spindle pole localization. Active AMPK, red; α-tubulin, green; chromatin, blue. Scale bar, 50 μm.

numerous proteins is dependent on MT integrity, the next series of experiments were conducted to confirm this relationship for AMPK. Oocytes undergoing *in vivo* maturation were fixed 8 h after human chorionic gonadotropin (hCG) injection to obtain metaphase I-stage oocytes and stained with antibodies to active AMPK and γ-tubulin. As expected, AMPK colocalized with γ-tubulin at the spindle poles during MI (Fig. 3A).

To test if active AMPK localization is dependent on spindle microtubule integrity, microtubule-targeted agents were tested on CEO during *in vitro* culture. Isolated CEO were arrested in 300 μM dibutyryl cAMP (dbcAMP) and then treated with 0.1 μg/ml FSH

https://doi.org/10.1017/S0967199412000457 Published online by Cambridge University Press

to induce maturation. CEO were cultured for 17 h before addition of the microtubule-depolymerization agent, nocodazole (0.05 μg/ml), or the microtubule-stabilizing agent, paclitaxel (20 μM), and oocytes were fixed 1 h later and stained for active AMPK and α-tubulin. Nocodazole depolymerized the spindle and, as a result, chromosomes became scattered and, not surprisingly, normal spindle pole localization of active AMPK was disrupted (Fig. 3*B*). When oocytes were treated with paclitaxel, microtubules were polymerized excessively and spindles were larger than normal; formation of a double meiotic spindle was occasionally observed, with two points of active AMPK localization at each pole. Many small asters also appeared in the cytoplasm with colocalized AMPK (Fig. 3*C*). *In vitro* matured MII-stage CEO were subjected to nocodazole (5 μg/ml) for 10 min to test whether re-establishment of a bipolar spindle could return active AMPK to its normal localization. The nocodazole was then washed out and oocytes were allowed to recover for 1 h to allow reformation of a bipolar MII spindle before fixation and processing for immunofluorescence staining. The MII spindle completely disappeared after 10 min treatment with nocodazole and active AMPK was dispersed randomly as before (data not shown). Upon spindle repolymerization, active AMPK localization returned to the spindle poles (Fig. 3*D*), thereby demonstrating reversibility of the nocodazole effect and a microtubule-dependent localization of active AMPK in mouse oocytes.

Effect of AMPK modulators on cytoskeleton dynamics

Our data suggest that microtubule integrity regulates localization and activity of AMPK. We next tested if the reverse regulation was true; that is, if AMPK regulates spindle dynamics. AMPK stimulators, AICAR, AMP and 8-Br-adenosine accelerate PB formation of spontaneously maturing CEO (Downs *et al.*, 2010), and it is possible that this effect is mediated by accelerated spindle formation. First, CEO were matured spontaneously in minimum essential medium (MEM)/bovine serum albumin (BSA) control medium in the presence or absence of 200 μM AICAR and the kinetics of GVB were determined. After 45 min of AICAR treatment, the percentage of GVB was increased from 35 to 52%; thereafter, maturation percentages were comparable up to 105 min of culture (Fig. 4*A*). CEO that were cultured in the presence or absence of AICAR were fixed at discrete time points and processed for α-tubulin immunofluorescence staining to examine the impact of AICAR on spindle formation. Polymerization of microtubules into a barrel-shaped spindle was the criterion used for assessment. AICAR increased spindle formation significantly at the early time point (an increase from 12 to 24% at 3 h), which is roughly 1 h after GVB (Fig. 4*B*). A trend towards increased spindle formation in AICAR-treated oocytes continued for the next 2 h, but differences were not significant.

Spindle migration is important for asymmetric cell division in oocytes, which helps minimize loss of cytoplasm during PB extrusion. We determined whether blocking AMPK activity could influence the migration of the meiotic spindle. Spontaneously maturing CEO were supplemented with the AMPK inhibitor, compound C, and position of the spindle was checked at 8, 12 and 16 h after initiation of culture. Spindles were considered to have migrated to the periphery if they were located more than one-half the radius of the oocyte from its centre. As shown in Fig. 4*C*, by 8 h culture 79% of the control oocytes had a spindle located peripherally; this number was 68% in compound C-treated oocytes, which was not different significantly from controls. By 16 h, most CEO in control medium had released the first PB and 95% of the oocyte spindles were localized peripherally. Treatment with compound C significantly blocked PB formation, but did not interfere with the peripheral movement of the spindle.

Finally, we tested whether inhibition of AMPK activity would perturb actin microfilament dynamics, especially the polymerization status of the cortical region. Extensive actin polymerization occurs in the cortical area and facilitates attachment of the outermost spindle pole to the cortex (Azoury *et al.*, 2008). When CEO were cultured in control MEM or medium that contained 2.5 μM compound C for 9.5 h and 11.5 h and processed for phalloidin staining, normal F actin polymerization was observed in both groups (data not shown). Thus, compound C treatment did not interfere with PB formation by blocking actin microfilament polymerization.

Discussion

In this study, we showed that perturbation of microtubule integrity in mouse oocytes interferes reversibly with meiotic induction of CEO by FSH and DO by AICAR, as well as the localization of active AMPK during maturation, and was accompanied by loss of AMPK activity. Microfilament perturbants had little effect on maturation. While AMPK stimulation accelerated both GVB and spindle formation, blockage of its activity with compound C did not affect actin polymerization or interfere with peripheral spindle migration, although karyokinesis was suppressed.

Disruption of microtubules with nocodazole and vinblastine, as well as their stabilization with

https://doi.org/10.1017/S0967199412000457 Published online by Cambridge University Press

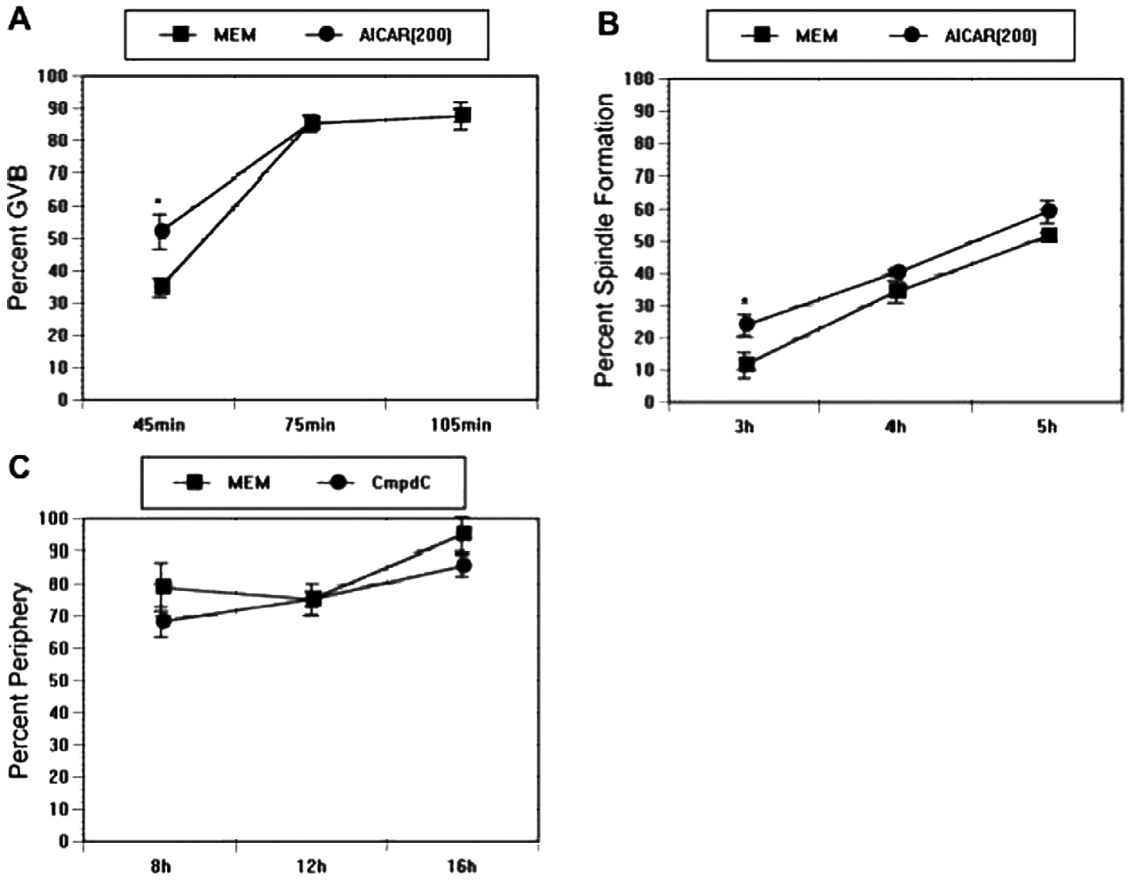

Figure 4 (*A*) Effect of AMP-activated protein kinase (AMPK) activator, 5-aminoimidazole-4-carboxamide-1-beta-4-ribofuranoside (AICAR), on spontaneous maturation. Cumulus cell-enclosed oocytes (CEO) were cultured in control MEM or medium supplemented with 250 μM AICAR, and percentage of germinal vesicle breakdown (GVB) was checked at 45, 75 and 105 min after initiation of the culture. (*B*) Effect of AICAR on early spindle formation. CEO were cultured in minimum essential medium (MEM) or MEM plus 250 μM AICAR for 3, 4, and 5 h before spindle formation was assessed by immunofluorescence staining. (*C*) Effect of the AMPK inhibitor, compound C, on spindle periphery movement. CEO were cultured in control medium or medium that contained 2.5 μM compound C. Spindle periphery movement was examined at the indicated time points. An asterisk denotes a significant difference from the corresponding control.

paclitaxel, blocked both AICAR-induced maturation in DO and FSH-induced maturation in CEO. This was associated with suppression of AMPK activation, as determined by western blotting, as well as loss of pAMPK staining in the GV prior to meiotic resumption (Chen & Downs, 2008). Compartmentalization of different cellular components is a crucial mechanism for controlling function in discrete microenvironments and influences cell cycle regulation (Pines, 1999). For instance, Cdc25B adopts nuclear localization shortly before GVB, where it regulates MPF activity (Oh *et al.*, 2010), and mitogen-activated protein kinase (MAPK) translocates to the GV just before GVB in pig oocytes (Inoue *et al.*, 1998). That nocodazole and paclitaxel suppressed GV staining of pAMPK in FSH- and AICAR-treated oocytes, coincident with a reduction in meiotic resumption, suggests a microtubule-dependent step within the oocyte in AMPK regulation of meiosis that involves nuclear localization. Nuclear transport is mediated by nuclear pore complexes that span the nuclear membrane and facilitate the exchange of proteins between the cytoplasm and nucleoplasm (Liu & Liu, 2007; Stewart, 2007). In some instances, successful nuclear import requires the active participation of the cytoskeleton (Campbell & Hope, 2003; Salman *et al.*, 2005; Wagstaff & Jans, 2009). This event has been demonstrated for p53 (Giannakakou *et al.*, 2000; Rathinasamy & Panda, 2008) and parathyroid hormone-related protein (Lam *et al.*, 2002), when microtubule-dependent transport of protein to the nucleus has been implicated. It has been proposed that microtubule-directed relocation of cellular components to a perinuclear location is instrumental in activation of maturation-promoting factor and driving meiotic resumption in oocytes (eg, Van Blerkom, 1991; Albertini, 1992). It is reasonable

https://doi.org/10.1017/S0967199412000457 Published online by Cambridge University Press

to propose that a similar mechanism exists for AMPK – i.e. that microtubules aid in the meiotic induction process by facilitating accumulation of AMPK at the nucleus periphery, where it is then imported by conventional means. It is also possible that disruption of microtubule dynamics perturbs the spatial organization of signalling pathway components other than, or in addition to, AMPK that help drive meiotic resumption in response to FSH and AICAR. Consistent with this possibility was the nocodazole-mediated suppression of AMPK activity as assessed by western analysis. It is unlikely that nocodazole acted via non-specific toxicity, because its inhibitory action could be bypassed by treatment with palmitoyl carnitine.

Nocodazole blocks GVB in spontaneously maturing rat oocytes (Albertini, 1987), but we did not observe a similar inhibitory action on spontaneously maturing mouse oocytes (data not shown), results consistent with those of others (Wassarman *et al.*, 1976; Van Blerkom & Bell, 1986). Interestingly, Rime & Ozon (1988) suppressed GVB in mouse oocytes with estramustine, an anti-mitotic compound that can block microtubule polymerization through its interaction with microtubule-associated proteins. Supplementation with taxol also did not affect the GVB of spontaneously maturing mouse oocytes (data not shown) and this effect has been shown by others for both rat and mouse oocytes; however, like the effects with nocodazole, taxol impairs later meiotic progression, resulting in failure of karyokinesis in mouse oocytes (Albertini, 1987; Combelles & Albertini, 2001). These effects of microtubule perturbants imply differences between the two species regarding meiotic regulation (c.f., Downs, 2011).

In a previous study (Downs *et al.*, 2010), we demonstrated that active AMPK associates with chromosomes after GVB, localizes to the spindle pole during metaphase I and II, and moves to the spindle midzone during anaphase. Here we show that active AMPK colocalizes with γ-tubulin, indicating an association with spindle organizing centres during formation of metaphase I spindles. This distribution pattern of pAMPK during oocyte maturation is similar to that occurring during mitosis of somatic cells (Vazquez-Martin *et al.*, 2009) and is consistent with a reported role for AMPK in cell cycle progression (Bettencourt-Dias *et al.*, 2004; Alessi *et al.*, 2006; Koh & Chung, 2007; Williams & Brenman, 2008). Indeed, previous work from this laboratory has demonstrated that AMPK not only induces meiotic resumption in mouse oocytes (Chen *et al.*, 2006; Chen & Downs, 2008) but also promotes the completion of maturation and prevents premature activation (Downs *et al.*, 2010). The changes of AMPK localization throughout maturation suggest a dynamic cellular transport process at work. The poleward transport of active AMPK might be dependent on the minus-end directed motor protein, dynein, which has been shown to be critical for spindle pole transport of proteins such as NuMA and Eg5 (Merdes *et al.*, 2000; Uteng *et al.*, 2008).

As AMPK shows a close association with microtubule-containing structures, it is not surprising that perturbation of microtubules dramatically affected the localization of AMPK within the oocyte. Nocodazole destruction of spindle structure led to dispersal of active AMPK in a punctate staining pattern that coincided with staining of microtubule asters, which indicated that the localization pattern of active AMPK is dependent on the integrity of the meiotic spindle and presence of MTOCs. Mouse oocytes have multiple acentriolar MTOCs that can be grouped into two subsets: one subset is associated with the meiotic spindle and the other is cortical (Maro *et al.*, 1985; Schatten *et al.*, 1986; Messinger & Albertini, 1991). Active AMPK was associated with both types of MTOCs. It is possible that active AMPK is more concentrated around MTOCs with nucleating capacity, as stabilization of microtubules with paclitaxel treatment induced further accumulation of AMPK at MTOCs and enlarged spindles.

In many cells, AMPK is regulated by LKB1 through phosphorylation of the threonine 172 activation site on the alpha subunit (Alessi *et al.*, 2006). LKB1 is a tumour suppressor that has been implicated in establishment of cell polarity through its involvement in AMPK activation (Alessi *et al.*, 2006; Williams & Brenman, 2008; Jansen *et al.*, 2009). For example, loss of LKB1 led to polarity defects in lung cancer cells (Zhang *et al.*, 2008) and epithelial cells (Mirouse *et al.*, 2007), neuroblasts (Bonaccorsi *et al.*, 2007) and developing embryos (Lee *et al.*, 2007) of *Drosophila*. Moreover, Szczepańska & Maleszewski (2005) reported that LKB1 associates with the meiotic spindle in mouse oocytes and proposed that it has a role in oocyte polarity; thus, it was of interest to test whether AMPK is also involved in a similar capacity in mouse oocytes. In oocytes, the spindle forms at the centre of the oocyte after GVB, and then migrates toward the oocyte periphery where the PB is extruded, a process that is crucial to asymmetric cell division and differentiation of cortex into a cortical granule-free domain (Sun & Schatten, 2006).

Inhibition of AMPK activity in spontaneously maturing CEO with compound C did not perturb the peripheral movement of the spindle. Although some recent studies have also suggested the participation of microtubules (Ai *et al.*, 2009), spindle movement in the oocyte is known to be highly dependent on a dense actin microfilament meshwork (Azoury *et al.*, 2008). Our results suggest that interfering with AMPK activity does not affect the integrity of the cortical actin

https://doi.org/10.1017/S0967199412000457 Published online by Cambridge University Press

network, because the extensive F actin polymerization in the cortex near the spindle appeared unperturbed by compound C treatment. Myosin regulatory light chain (MLC) is an important component of myosin II that participates in actomyosin-mediated cytokinesis at the cleavage furrow (Dumont *et al.*, 2007) and becomes phosphorylated in somatic cells in response to AMPK activation (Lee *et al.*, 2007). However, it is unclear whether MLC is a physiological substrate for AMPK under energy-depleted conditions (Bultot *et al.*, 2009). It will be interesting to determine if AMPK influences the interaction of actin and myosin at the time of cytokinesis.

Acceleration of the kinetics of PB extrusion by AMPK-stimulating agents (Downs *et al.*, 2010) indicates an active role for AMPK throughout the entire maturation process, and this role might be accomplished by action on earlier meiotic events. Indeed, we show herein that the rate of GVB and spindle formation is increased by AICAR treatment. These data indicate that AMPK manifests an early effect during maturation that shortens the time required to complete maturation. In support of this indication, the increase in PB formation is eliminated when AICAR treatment is delayed, while delaying compound C treatment decreases its inhibitory action (Downs *et al.*, 2010). The early increase in spindle formation is likely due to the accelerated kinetics of GVB, but it should be pointed out that, in addition to faster maturation kinetics, AMPK activation also leads to increased PB formation.

The close association of AMPK with microtubules suggests a functional role with the meiotic apparatus. Numerous proteins exhibit a similar localization pattern and have important roles in spindle organization and function. For instance, aurora-A kinase induces GVB in *Xenopus* oocyte and has to be dephosphorylated for the MI to MII transition (Ma *et al.*, 2003). In mouse oocytes, aurora-A is critical for spindle assembly, centrosome maturation and chromosome segregation. Furthermore, its role is not limited to oocyte maturation, as it also contributes to early embryo spindle organization (Yao *et al.*, 2004; Ding *et al.*, 2011). Another aurora kinase family member, aurora-B, is one of the chromosome passenger proteins and localizes specifically to the metaphase chromosomes, where it mediates chromosome segregation and spindle kinetochore attachment (Ruchaud *et al.*, 2007; Uzbekova *et al.*, 2008). Aurora-C kinase localization is similar to that of active AMPK; moreover, injection of deficient kinase-induced failure of meiosis I (MI) and disrupted proper localization of kinetochore proteins, BubR1 and Bub (Yang *et al.*, 2010). Astrin is associated with meiotic spindle microtubules with particular concentration at the spindle poles, and perturbation of its function greatly compromises normal spindle integrity and chromosome segregation (Yuan *et al.*, 2009). The apoptosis inhibitor protein, survivin, mimics AMPK localization in mouse oocytes, and its depletion adversely affects chromosome alignment and alters normal PB extrusion (Sun *et al.*, 2009). Another similarly localized kinase, Polo-like kinase-1 (Plk1), was implicated in spindle assembly in oocytes and later mitosis in the embryo after fertilization (Wianny *et al.*, 1998; Tong *et al.*, 2002). In addition to regulation of spindle organization, Plk1 also promotes M phase entry by participating in the MPF amplification loop (Karaiskou *et al.*, 2004), and blocking its activity with antibody reduces the GVB rate (Tong *et al.*, 2002). Of particular relevance to the present study is the recent finding that pharmacological inhibition of Plk1 blocks AMPK activation and cytokinesis in HeLa cells (Vazquez-Martin *et al.*, 2011). Another kinase, protein kinase C delta (PKCδ), associates with γ-tubulin and pericentrin, and disruption of its function leads to spindle disorganization and misalignment of chromosomes (Ma *et al.*, 2008). PKC has also been shown to be involved in the MI to MII transition (Viveiros *et al.*, 2001). Finally, phospho-MEK1/2, the upstream kinase for MAPK activation, displays a prominent presence at the spindle poles in mouse oocytes, and blocking MAPK leads to meiotic spindle abnormalities and poor chromatin condensation (Lu *et al.*, 2002; Yu *et al.*, 2007; Sun *et al.*, 2008). It may be that AMPK interacts with one or more of these proteins to influence meiotic progression. An alternate scenario is that the kinase docks at MTOCs without contributing to spindle assembly or function.

In conclusion, both AMPK localization and activity in the mouse oocyte are dependent on intact microtubules. Further, while perturbation of microtubule integrity blocks meiotic induction at the level of the oocyte, it has no effect on spontaneous maturation. This situation demonstrates that additional microtubule-sensitive modalities are required to overcome induction of maturation in meiotically arrested oocytes and lends further support to the idea that these are distinct processes.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Acknowledgements

This work was funded by a grant from the NIH (R15D061858) to SMD.

https://doi.org/10.1017/S0967199412000457 Published online by Cambridge University Press

References

Ai, J.-S., Li, M., Schatten, H. & Sun, Q.-Y. (2009). Regulatory mechanism of spindle movements during oocyte meiotic division. *Asian–Aust J. Anim. Sci.* **22**, 1477–86.

Albertini, D.F. (1987). Cytoplasmic reorganization during the resumption of meiosis in cultured preovulatory rat oocytes. *Dev. Biol.* **120**, 121–31.

Albertini, D.F. (1992). Regulation of meiotic maturation in the mammalian oocyte: interplay between exogenous cues and the microtubule cytoskeleton. *BioEssays* **14**, 97–103.

Alessi, D.R., Sakamoto, K. & Bayascas, J.R. (2006). LKB1-dependent signaling pathways. *Ann. Rev. Biochem.* **75**, 137–63.

Azoury, J., Lee, K. W., Georget, V., Rassinier, P., Leader, B. & Verlhac, M.-H. (2008). Spindle positioning in mouse oocytes relies on a dynamic meshwork of actin filaments. *Curr. Biol.* **18**, 1514–19.

Bettencourt-Dias, M., Glet, R., Sinka, R., Mazumdar, A., Lock, W.G., Balloux, F., Zafiropoulos, P.J., Yamaguchi, S., Winter, S., Carthew, R.W., Cooper, M., Jones, D., Frenze, L. & Glover, D.M. (2004). Genome-wide survey of protein kinases required for cell cycle progression. *Nature* **432**, 980–7.

Bonaccorsi, S., Mottier, V., Giansanti, M.G., Bolkan, B.J., Williams, B., Goldberg, M.L. & Gatti, M. (2007). The *Drosophila* Lkb1 kinase is required for spindle formation and asymmetric neuroblast division. *Development* **134**, 2183–93.

Bultot, L., Horman, S., Neumann, D., Walsh, M. & Hue, L. (2009). Myosin light chains are not a physiological substrate of AMPK in the control of cell structure changes. *FEBS Lett.* **583**, 25–8.

Campbell, E.M. & Hope, T.J. (2003). Role of the cytoskeleton in nuclear import. *Adv. Drug. Deliv. Rev.* **55**, 761–71.

Carling, D. (2004). The AMP-activated protein kinase cascade—a unifying system for energy control. *Trends Biochem. Sci.* **29**, 18–24.

Chen, J. & Downs, S. M. (2008). AMP-activated protein kinase is involved in hormone-induced mouse oocyte meiotic maturation *in vitro*. *Dev. Biol.* **313**, 47–57.

Chen, J., Hudson, E., Chi, M. M., Chang, A. S., Moley, K. H., Hardie, D. G. & Downs, S. M. (2006). AMPK regulation of mouse oocyte meiotic resumption in vitro. *Dev. Biol.* **291**, 227–38.

Combelles, C.M.H. & Albertini, D.F. (2001). Microtubule patterning during meiotic maturation in mouse oocytes is determined by cell cycle-specific sorting and redistribution of γ-tubulin. *Dev. Biol.* **239**, 281–94.

Ding, J., Swain, J.E. & Smith, G.D. (2011). Aurora kinase-A regulates microtubule organizing center (MTOC) localization, chromosome dynamics, and histone-H3 phosphorylation in mouse oocytes. *Mol. Reprod. Dev.* **78**, 80–90.

Downs, S.M. (2011). Mouse versus rat: profound differences in meiotic regulation at the level of the isolated oocyte. *Mol. Reprod. Dev.* **78**, 778–94.

Downs, S.M. & Chen, J. (2006). Induction of meiotic maturation in mouse oocytes by adenosine analogs. *Mol. Reprod. Dev.* **73**, 1159–68.

Downs, S.M., Mosey, J.L. & Klinger, J. (2009). Fatty acid oxidation and meiotic resumption in mouse oocytes. *Mol Reprod. Dev.* **76**, 844–53.

Downs, S.M., Ya, R. & Davis, C.C. (2010). Role of AMPK throughout meiotic maturation in the mouse oocyte: evidence for promotion of polar body formation and suppression of premature activation. *Mol. Reprod. Dev.* **77**, 888–99.

Dumont, J., Million, K., Sunderland, K., Rassinier, P., Lim, H., Leader, B. & Verlhac, M.-H. (2007). Formin-2 is required for spindle migration and for the late steps of cytokinesis in mouse oocytes. *Dev. Biol.* **301**, 254–65.

Giannakakou, P., Sackett, D.L., Ward, Y., Webster, K.R., Blagosklonnyu, M.V. & Fojo, T. (2000). p53 is associated with cellular microtubules and is transported to the nucleus by dynein. *Nature Cell Biol.* **2**, 709–17.

Hardie, D.G. (2003). Minireview: the AMP-activated protein kinase cascade: the key sensor of cellular energy status. *Endocrinology* **144**, 5179–83.

Inoue, M., Naito, K., Nakayama, T. & Sato, E. (1998). Mitogen-activated protein kinase translocates into the germinal vesicle and induces germinal vesicle breakdown in porcine oocytes. *Biol. Reprod.* **58**, 130–6.

Jansen, M., Ten Klooster, J.P., Offerhaus, G.J. & Clevers, H. (2009). LKB1 and AMPK family signaling: the intimate link between cell polarity and energy metabolism. *Physiol. Rev.* **89**, 777–98.

Karaiskou, A., Leprêtre, A.-C., Pahlavan, G., Pasquier, Du, D., Ozon, R. & Jessus, C. (2004). Polo-like kinase confers MPF autoamplification competence to growing *Xenopus* oocytes. *Development* **131**, 1543–52.

Koh, H. & Chung, J. (2007). AMPK links energy status to cell structure and mitosis. *Biochem. Biophys. Res. Commun.* **362**, 789–92.

Lam, M.H.C., Thomas, R.J., Loveland, L.K., Schilders, S., Gu, M., Martin, T.J., Gillespie, M.T. & Jans, D.A. (2002). Nuclear transport of parathyroid hormone (PTH)-related protein is dependent on microtubules. *Mol. Endocrinol.* **16**, 390–401.

LaRosa, C. & Downs, S.M. (2007). Meiotic induction by heat stress in mouse oocytes: involvement of AMP-activated protein kinase and MAPK family members. *Biol. Reprod.* **76**, 476–86.

Lee, J. H., Koh, H., Kim, M., Kim, Y., Lee, S. Y., Karess, R.E., Lee, S.-H., Shong, M., Kim, J.-M., Kim, J. & Chung, J. (2007). Energy-dependent regulation of cell structure by AMP-activated protein kinase. *Nature* **447**, 1017–20.

Liu, S.M. and Liu, W.M. (2007). Recent developments in the understanding of nuclear protein import. *Protein Peptide Lett.* **14**, 723–33.

Lu, Z., Dunn, R.L., Angeles, R. & Smith, G.D. (2002). Regulation of spindle formation by active mitogen-activated protein kinase and protein phosphatase 2A during mouse oocyte meiosis. *Biol. Reprod.* **66**, 29–37.

Ma, C., Cummings, C. & Liu, X. J. (2003). Biphasic activation of aurora-A kinase during the meiosis I–meiosis II transition in *Xenopus* oocytes. *Mol. Cell. Biol.* **23**, 1703–16.

Ma, W., Koch, J. & Vivieros, M.M. (2008). Protein kinase C delta (PKC δ) interacts with microtubule organizing center (MTOC)-associated proteins and participates in meiotic spindle organization. *Dev. Biol.* **320**, 414–25.

Maro, B., Howlett, S.K. & Webb, M. (1985). Non-spindle microtubule organizing centers in metaphase II-arrested mouse oocytes. *J. Cell Biol.* **101**, 1665–72.

Merdes, A., Heald, R., Samejima, K., Earnshaw, W.C. & Cleveland, D.W. (2000). Formation of spindle poles by

https://doi.org/10.1017/S0967199412000457 Published online by Cambridge University Press

dynein/dynactin-dependent transport of NuMA. *J. Cell Biol.* **149**, 851–62.
Messinger, S.M. & Albertini, D.F. (1991). Centrosome and microtubule dynamics during meiotic progression in mouse oocyte. *J. Cell Sci.* **100**, 289–98.
Mirouse, V., Swick, L.L., Kazgan, N., St Johnston, D. & Brenman, J.E. (2007). LKB1 and AMPK maintain epithelial cell polarity under energetic stress. *J. Cell Biol.* **177**, 387–92.
Na, J. & Zernicka-Goetz, M. (2006). Asymmetric positioning and organization of the meiotic spindle of mouse oocytes requires CDC42 function. *Curr. Biol.* **16**, 1249–54.
Oh, J.S., Han, S.J. & Conti, M. (2010). Wee1B, Myt1, and Cdc25 function in distinct compartments of the mouse oocyte to control meiotic resumption. *J. Cell Biol.* **188**, 199–207.
Pines, J. (1999). Four-dimensional control of the cell cycle. *Nature Cell Biol.* **1**, E73–9.
Rathinasamy, K. & Panda, D. (2008). Kinetic stabilization of microtubule dynamic instability by benomyl increases the nuclear transport of p53. *Biochem. Pharmacol.* **76**, 1669–80.
Rime, H., Jessus, C.& R.Ozon. (1988). Estramustine phosphate inhibits germinal vesicle breakdown and induces depolymerization of microtubules in mouse oocyte. *Reprod. Nutr. Dev.* **28** (**2A**), 319–34.
Ruchaud, S., Carmena, M. & Earnshaw, W.C. (2007). Chromosomal passengers: conducting cell division. *Nat. Reviews. Mol. Cell Biol.* **8**, 798–812.
Salman, H., Abu-Arish, A., Oliel, S., Loyter, A., Klafter, J., Granek, R. & Elbaum, M. (2005). Nuclear localization signal peptides induce molecular delivery along microtubules. *Biophys. J.* **89**, 2134–45.
Schatten, H., Schatten, G., Mazia, D., Balczon, R. & Simerly, C. (1986). Behavior of centrosomes during fertilization and cell division in mouse oocytes and sea urchin eggs. *Proc. Natl. Acad. Sci. USA* **83**, 105–9.
Stewart, M. (2007). Molecular mechanism of the nuclear protein import cycle. *Nature Rev.* **8**, 195–208.
Sun, Q.-Y. & Schatten, H. (2006). Regulation of dynamic events by microfilaments during oocyte maturation and fertilization. *Reproduction* **131**, 193–205.
Sun, S.-C., Xiong, B., Lu, S.-S. & Sun, Q.-Y. (2008). MEK1/2 is a critical regulator of microtubule assembly and spindle organization during rat oocyte meiotic maturation. *Mol. Reprod. Dev.* **75**, 1542–8.
Sun, S.-C., Wei, L., Li, M., Lin, S.-L., Xu, B.-Z., Liang, X.-W., Kim, N.-H., Schatten, H., Lu, S.-S. & Sun, Q.-Y. (2009). Perturbation of survivin expression affects chromosome alignment and spindle checkpoint in mouse oocyte meiotic maturation. *Cell Cycle* **20**, 3365–72.
Szczepańska, K. & Maleszewski, M. (2005). LKB1/PAR4 protein is asymmetrically localized in mouse oocytes and associates with meiotic spindle. *Gene Expr. Patterns* **6**, 86–93.
Tong, C., Fan, H.-Y., Lian, L., Li, S.-W., Chen, D.-Y., Schatten, H. & Sun, Q.-Y. (2002). Polo-like kinase-1 is a pivotal regulator of microtubule assembly during mouse oocyte meiotic maturation, fertilization, and early embryonic mitosis. *Biol. Reprod.* **67**, 546–54.
Uteng, M., Hentrich, C., Miura, K., Bieling, P. & Surrey, T. (2008). Poleward transport of Eg5 by dynein-dynactin in *Xenopus laevis* egg extract spindles. *J. Cell Biol*. **182**, 715–26.
Uzbekova, S., Arlot-Bonnemains, Y., Dupont, J., Dalbies-Tran, R., Papillier, P., Pennetier, S., Thélie, A., T., Perreau, C., Mermillod, P., Prigent, C. & Uzbekov, R. (2008). Spatio-temporal expression patterns of aurora kinases A, B, and C and cytoplasmic polyadenylation-element-binding protein in bovine oocytes during meiotic maturation. *Biol. Reprod.* **78**, 218–33.
Van Blerkom, J. (1991). Microtubule mediation of cytoplasmic and nuclear maturation during the early stages of resumed meiosis in cultured mouse oocytes. *Proc. Natl. Acad. Sci. USA* **88**, 5031–5.
Van Blerkom, K. & Bell, H. (1986). Regulation of development in the fully grown mouse oocyte: chromosome-mediated temporal and spatial differentiation of the cytoplasm and plasma membrane. *J. Embryol. Exp. Morph.* **93**, 213–38.
Vazquez-Martin, A., Oliveras-Ferraros, C. & Menendez, J.A. (2009). The active form of the metabolic sensor: AMP-activated protein kinase (AMPK) directly binds the mitotic apparatus and travels from centrosomes to the spindle midzone during mitosis and cytokinesis. *Cell Cycle* **8**, 2385–98.
Vazquez-Martin, A., Oliveras-Ferraros, C., Cufi, S. & Menendez, J.A. (2011). Polo-like kinase 1 regulates activation of AMP-activated protein kinase (AMPK) at the mitotic apparatus. *Cell Cycle* **10**, 1295–302.
Viveiros, M.M., Hirao, Y. & Eppig, J.J. (2001). Evidence that protein kinase C (PKC) participates in the meiosis I to meiosis II transition in mouse oocytes. *Dev. Biol.* **235**, 330–42.
Wagstaff, K.M. & Jans, D.A. (2009). Importins and beyond: non-conventional nuclear transport mechanisms. *Traffic* **10**, 1188–98.
Wassarman, P.M., Josefowicz, W.J. & Letourneau, G.E. (1976). Meiotic maturation of mouse oocytes in vitro: inhibition of maturation at specific stages of nuclear progression. *J. Cell Sci.* **22**, 531–45.
Wianny, F., Tayares, A., Evans, M.J., Glover, D.M. & Zernicka-Goetz, M. (1998). Mouse Polo-like kinase 1 associates with the acentriolar spindle poles, meiotic chromosomes and spindle midzone during oocyte maturation. *Chromosoma* **107**, 430–9.
Williams, T. & Brenman, J.E. (2008). LKB1 and AMPK in cell polarity and division. *Trends Cell Biol.* **18**, 193–8.
Yang, K.T., Li, S.K., Chang, C.C., Tang, C.J.C., Lin, Y.N., Lee, S.C. & Tang, T.K. (2010). Aurora-C kinase deficiency causes cytokinesis failure in meiosis I and production of large polyploid oocytes in mice. *Mol. Biol. Cell* **21**, 2371–83.
Yao, L., Zhong, Z., Zhang, L., Chen, D., Schatten, H. & Sun, Q. (2004). Aurora-A is a critical regulator of microtubule assembly and nuclear activity in mouse oocytes, fertilized eggs, and early embryos. *Biol. Reprod.* **70**, 1392–9.
Yuan, J., Li, M., Wei, L., Yin, S., Xiong, B., Li, S., Lin, S.-L., Schatten, H. & Sun, Q.-Y. (2009). Astrin regulates meiotic spindle organization, spindle pole tethering and cell cycle progression in mouse oocytes. *Cell Cycle* **8**, 3384–95.
Zhang, S., Schafer-Hales, K., Khuri, F.R., Zhou, W., Vertino, P.M. & Marcus, A.I. (2008). The tumor suppressor LKB1 regulates lung cancer cell polarity by mediating Cdc42 recruitment and activity. *Cancer Res.* **68**, 740–8.

https://doi.org/10.1017/S0967199412000457 Published online by Cambridge University Press