Zygote **24** (June), pp. 338–345. © Cambridge University Press 2015
doi:10.1017/S0967199415000246 First Published Online 15 July 2015

PLK1 regulates spindle formation kinetics and APC/C activation in mouse zygote

Vladimir Baran[1], *Adela Brzakova*[3], *Pavol Rehak*[2], *Veronika Kovarikova*[2] *and Petr Solc*[3]
Institute of Animal Physiology, Slovak Academy of Sciences, Kosice, Slovakia; and Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Libechov, Czech Republic

Date submitted: 05.12.2014. Date revised: 23.04.2015. Date accepted: 26.04.2015

Summary

Polo-like kinase 1 (PLK1) is involved in essential events of cell cycle including mitosis in which it participates in centrosomal microtubule nucleation, spindle bipolarity establishment and cytokinesis. Although PLK1 function has been studied in cycling cancer cells, only limited data are known about its role in the first mitosis of mammalian zygotes. During the 1-cell stage of mouse embryo development, the acentriolar spindle is formed and the shift from acentriolar to centrosomal spindle formation progresses gradually throughout the preimplantation stage, thus providing a unique possibility to study acentriolar spindle formation. We have shown previously that PLK1 activity is not essential for entry into first mitosis, but is required for correct spindle formation and anaphase onset in 1-cell mouse embryos. In the present study, we extend this knowledge by employing quantitative confocal live cell imaging to determine spindle formation kinetics in the absence of PLK1 activity and answer the question whether metaphase arrest at PLK1-inhibited embryos is associated with low anaphase-promoting complex/cyclosome (APC/C) activity and consequently high securin level. We have shown that inhibition of PLK1 activity induces a delay in onset of acentriolar spindle formation during first mitosis. Although these PLK1-inhibited 1-cell embryos were finally able to form a bipolar spindle, not all chromosomes were aligned at the metaphase equator. PLK1-inhibited embryos were arrested in metaphase without any sign of APC/C activation with high securin levels. Our results document that PLK1 controls the onset of spindle assembly and spindle formation, and is essential for APC/C activation before anaphase onset in mouse zygotes.

Keywords APC/C, BI2536, Live cell imaging, Mouse zygote, PLK1, Securin, Spindle assembly

Introduction

Immediately after fertilization of the oocyte, male and female pronuclei are formed and approximate one another in the central region of the zygote. After completion of the first DNA replication, first mitosis occurs. An important feature of the oocyte is that it lacks centriole-containing centrosomes, which serve as predefined spindle poles in somatic cells. Oocyte centrioles degenerate before meiotic maturation. In oocytes, there are many acentriolar microtubule organizing centres (MTOCs) throughout the cytoplasm instead of centrosomes (Schatten *et al.*, 1985). In contrast with other mammalian species, sperm centrioles of rodents also degenerate during spermiogenesis. In this regard, the gradual shift from acentriolar spindle (MTOC-driven spindle) to centrosomal spindle formation is one of the most important transition features between meiosis and mitosis in rodents. Consequently, in the mouse embryo, the first mitotic bipolar spindle is formed in the absence of the centrioles (Manandhar *et al.*, 2005), which are produced *de novo* during the first three embryonic divisions when acentriolar MTOCs are replaced by true centrosomes. During meiotic resumption the MTOCs move towards nucleus and, after nuclear envelope breakdown, (NEBD) they gradually move toward the two poles and give rise

[1]All correspondence to: Vladimir Baran. Institute of Animal Physiology, Slovak Academy of Sciences, Soltesovej 4, 040 01 Kosice, Slovakia. E-mail: baran@saske.sk
[2]Institute of Animal Physiology, Slovak Academy of Sciences, Kosice, Slovakia.
[3]Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Libechov, Czech Republic.

https://doi.org/10.1017/S0967199415000246 Published online by Cambridge University Press

to an acentriolar bipolar spindle (Schuh & Ellenberg, 2007). Although 1-cell embryos form a mitotic spindle by the same mechanism as the oocyte, with centripetal targeting of cytoplasmic MTOCs, some features of the mechanism are distinct from those in meiosis (Courtois *et al.*, 2012). The first mitotic spindle is substantially shorter than the spindle formed in 2-cell embryos, and spindle poles in first mitosis fail to extend to the plasmalemma even at anaphase. The factors that restrain spindle length in oocytes to allow two meiotic asymmetric divisions might continue to restrain spindle length in the 1-cell embryo (for review, see Howe & FitzHarris, 2013). Kinetochore–microtubule attachment followed by segregation of chromosomes into individual blastomeres of 2-cell embryos is monitored by the spindle-assembly checkpoint (SAC) machinery (Musacchio & Salmon, 2007). The SAC is an active signal produced by improperly attached kinetochores. and prevents premature anaphase entry and is active until proper kinetochore–microtubule attachment occurs. Silencing of the SAC machinery is mediated by correct kinetochore connection to microtubules and stabilization of forces within the spindle. Thus chromosome alignment in metaphase plate is formed and metaphase–anaphase transition is allowed (Foley & Kapoor, 2013). When components of the SAC regulatory mechanism have been satisfied, the anaphase-promoting complex/cyclosome (APC/C) becomes activated. APC/C activation mediates degradation of securin and cyclin B to trigger anaphase (Musacchio & Salmon, 2007). Previously it has been reported that Polo-like kinase 1 (PLK1) is involved in activatory phosphorylation of APC/C to facilitate metaphase–anaphase transition (Hansen *et al.*, 2004; Moshe *et al.*, 2004). In somatic cells PLK1 inhibition prevents SAC satisfaction and, as a consequence, the APC/C. In addition, Wei *et al.* (2011) suggested that the SAC is essential for mitosis regulation at the early cleavage stage. Previous studies have shown that PLK1 is employed in the regulation of mouse (Tong *et al.*, 2002) and rat (Fan *et al.*, 2003) oocyte maturation, fertilization and early embryonic mitosis, during which time this kinase regulates G2–M transition (Zhang *et al.*, 2007). The important role of PLK1 in the first cleavage of the fertilized egg has been thus confirmed (Zhao *et al.*, 2010). Our recent study showed that PLK1 activity is not essential for the entry of fertilized mouse oocytes into first mitosis, but that this kinase positively regulates the formation of the mitotic spindle and metaphase–anaphase transition in 1-cell embryos (Baran *et al.*, 2013).

In the present study we describe quantitative confocal live embryo imaging that shows that PLK1 supports the onset of spindle formation, regulates spindle length, and is essential for correct chromosome alignment; also PLK1 is needed for APC/C activation, which results in securin destruction.

Materials and methods

Animal use ethical statement

The use of mice in this study was approved by the Departmental Expert Committee for State Veterinary and Food Administration of the Slovak Republic and with the Approval of Projects of Experiments on Animals of Academy of Sciences of Czech Republic. Animal welfare was under the control of local committees. Mice were housed in a temperature-controlled room with proper 12 h/12 h darkness–light cycles, and fed with a regular *ad libitum* diet.

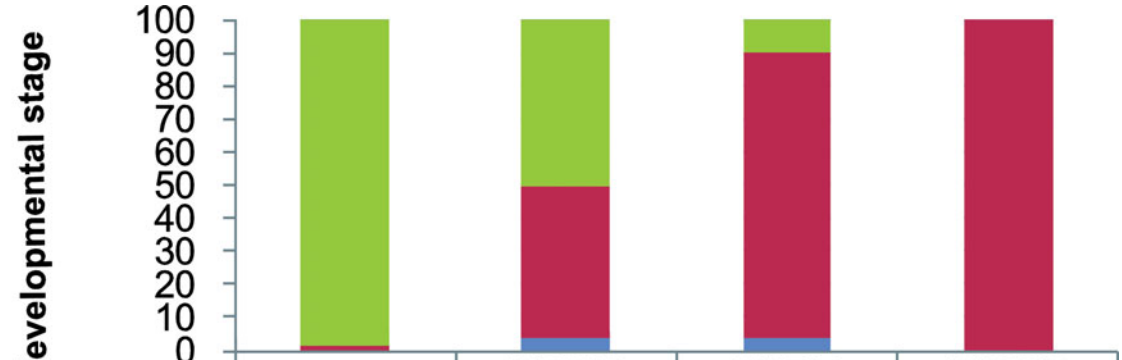
Embryo *in vitro* culture

CD1 or H2B–EGFP female mice aged 6 to 8 weeks old were superovulated with 5 IU of pregnant mares' serum gonadotropin (PMSG), followed 46–47 h later by 5 IU of human chorionic gonadotropin (hCG), then mated with males. Fertilized 1-cell embryos were obtained from the ampulla oviduct of plug-positive females 18 h post hCG, and cumulus cells were dispersed with 1 mg/ml hyaluronidase. Embryos were isolated into M2 medium (Sigma-Aldrich, St. Louis, MO) and cultured *in vitro* in potassium simplex optimized medium (KSOM) (Millipore Co., Billerica, MA, USA) at 37°C in air containing 5% CO_2 as we have described in more detail previously (Baran *et al.*, 2013). After 2 h culture in pure KSOM (20 h post hCG administration), collected embryos were microinjected with mRNA and cultured in pure KSOM for the next 3 h. After this 3-h culture (at time interval 23 h post hCG), the surviving microinjected embryos were divided into two groups: (i) control; and (ii) PLK1-inhibited embryos; and then cultured in DMSO-supplemented KSOM or medium supplemented with BI2536 (Axon Medchem, Groningen, Germany) diluted in DMSO. In three repeated pilot tests of cleavage ability, three concentrations of BI2536 (50, 100 or 500 nM) were used. The cleavage ability of the embryos was evaluated under an inverted microscope (Nikon-Eclipse-90i; Nikon Instruments Europe B.V.) at time interval 43 h post hCG administration. The concentration of 500 nM was chosen based on our pilot studies and published data (Lenart *et al.*, 2007; Steegmaier *et al.*, 2007). In the time-lapse experiments, a 500 nM concentration of BI2536 was applied.

mRNA *in vitro* transcription and microinjection

We used the described vectors for MAP4–EGFP (Schuh & Ellenberg, 2007), securin–EGFP and

https://doi.org/10.1017/S0967199415000246 Published online by Cambridge University Press



	Control	BI2536 50 nM	BI2536 100 nM	BI2536 500 nM
2 cell	98.2	50	9.6	0
Metaphase	1.8	46.2	86.6	100
PN	0	3.8	3.8	0

Figure 1 Effect of BI2536 on the cleavage of the zygote. Effect of 50, 100 or 500 nM PLK1 inhibitor BI2536 on zygote division (n = 106, 104, 97). Control embryos were cultured in DMSO-supplemented medium (n = 113). Pronucleus (PN), metaphase and 2-cell stages were distinguished. PN, zygote with both pronuclei that did not enter into the first mitosis.

H2B–mCHERRY (Kudo *et al.* 2009) for *in vitro* mRNA transcription as we have described previously (Solc *et al.*, 2008). Fertilized 1-cell embryos were microinjected with 4 pl of 125 ng/μl MAP4–EGFP, 50 ng/μl of securin–EGFP and 75 ng/μl of H2B–mCHERRY mRNAs in M2 medium at 20 h post hCG.

Time-lapse confocal microscopy and image analysis

Time-lapse image acquisitions were performed using a Leica TCS SP5 confocal system equipped with an acoustooptical beam splitter (AOBS) applying the following settings: lens Plan Neofluor ×40 1.25 oil objective, 1024 × 1024 px resolution, 16-bit depth, 12 stacks with 7.4 μm thickness, time frame 5 min; 488 nm and 566 nm lasers at 3% power were used for EGFP and mCHERRY excitation. The EGFP signal was detected at 500–560 nm, the mCHERRY signal was detected at 580–630 nm.

3D + t image datasets of individual oocytes were registered using the H2B–mCHERRY signal by the Correct 3D Drift plug-in in Fiji (Schindelin *et al.* 2012). The time of NEBD (time of mitosis resumption) was defined as the time when chromosomes began to be condensed (denoted '0:00' on Figs 2*A* and 3*A* and also Movies S1 and S2). Spindle volume was measured from 3D reconstructed images when the MAP4–EGFP raw data were processed by Gaussian Blur filter (r = 1) and then spindle structure was intensity-thresholded and individual Z-stacks of each embryo were arranged into single view.

Statistical analysis

Statistical analysis shown in Fig. 2 (see later) was performed using NCSS statistical software (NCSS, LLC, Utah, USA) by *t*-test. All experiments were repeated at least three times and the total numbers of observed embryos are indicated in the figure legends.

Results

Pharmacological inhibition of PLK1 by BI2536 prevents zygote cleavage

PLK1 inhibitor BI2536 was used to assess the involvement of PLK1 in the regulation of first mitosis of the fertilized embryo. To uncover a potential role for PLK1 during first embryonic mitosis we treated embryos with BI2536, a pharmacological inhibitor known to specifically inhibit PLK1 in somatic cells (Lenart *et al.*, 2007) and in oocytes (Vanderheyden *et al.*, 2009) as well as in chemical genetics studies (Scutt *et al.*, 2009; Burkard *et al.*, 2007). We tested differential concentrations of BI2536 and revealed that progression through the first mitosis was inhibited in a concentration-dependent manner (Fig. 1). In the control group, nearly all (98.2%) of the embryos completed first mitosis after a 19 h culture. Only a few control embryos (1.8%) were arrested in metaphase. However, treatment with increasing BI2536 concentrations (50, 100 or 500 nM) resulted in 46.2, 86.6 and 100% of embryos arrested permanently in metaphase.

https://doi.org/10.1017/S0967199415000246 Published online by Cambridge University Press

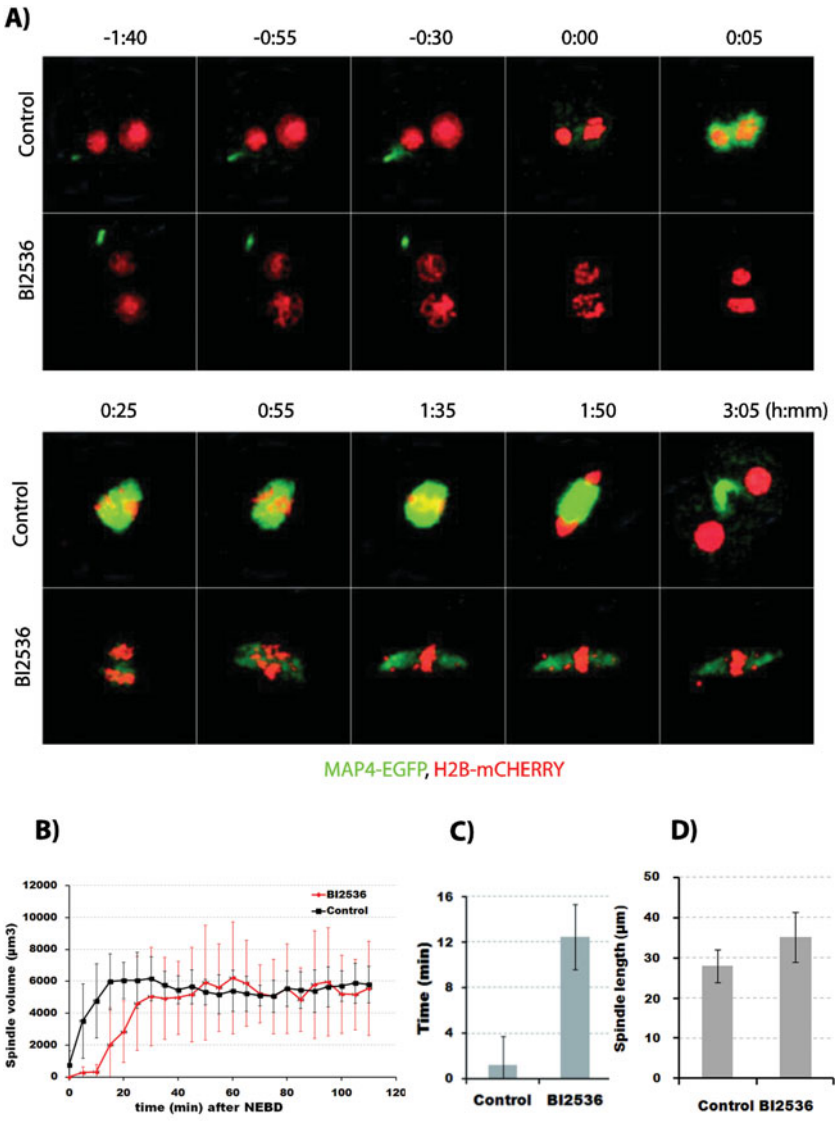


Figure 2 Spindle formation in PLK1-inhibited embryos. (*A*) Imaging of spindle formation during mitosis in live embryos expressing microtubule marker MAP4–EGFP and chromosome marker H2B–mCHERRY in the presence of DMSO (control) or BI2536. A snapshot of selected time points is shown. Time is relative to the NEBD (h:mm). EGFP and mCHERRY channels are presented as maximum intensity z-projections, and bright field as a single confocal section. See also Movie S1. (*B*) The volume of the spindle during mitosis progression was measured after 3D reconstruction of the MAP4–EGFP signals. Mean and standard deviation (s.d.) are shown ($n = 11, 11$). (*C*) Time of spindle formation onset was determined from individual spindle volume curves. Time after NEBD (min). Mean and s.d. are shown ($n = 11, 11$). (*D*) The length of the spindle at 100 min after NEBD was measured after 3D reconstruction of MAP4–EGFP signals. Mean and s.d. are shown ($n = 11, 11$).

These data suggest that PLK1 is essential for completion of first embryonic mitosis and raises the questions how spindle formation dynamics and, mainly, securin destruction are affected by PLK1 inhibition.

PLK1 controls spindle formation onset as well as spindle length

To precisely monitor the dynamics of spindle formation during the first mitosis, we expressed the well established spindle marker MAP4–EGFP together with chromosome marker H2B–mCHERRY from microinjected mRNAs and monitored spindle formation using confocal live cell imaging (Fig. 2*A*). The incorporation of MAP4–EGFP into the spindle was clearly lower in BI2536 embryos, indicating that there was lower microtubule polymerization activity during mitosis. For quantitative analysis of spindle formation we defined the time when embryos went through nuclear envelope breakdown (NEBD) arbitrarily as time 0 (Fig. 2*B*). Although embryos both in the control

https://doi.org/10.1017/S0967199415000246 Published online by Cambridge University Press

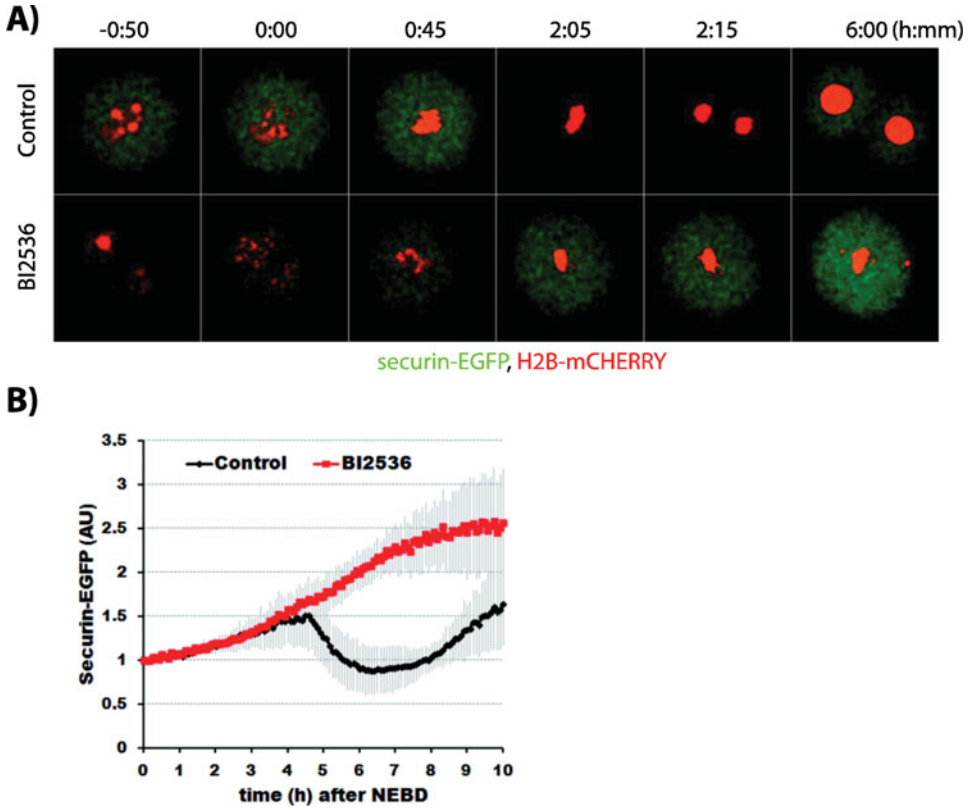


Figure 3 APC/C activity in PLK1-inhibited embryos. (*A*) Imaging of live embryos expressing securin–EGFP and H2B–mCHERRY in the presence of DMSO (control) or BI2536. A snapshot of selected time points is shown. Time is relative to the NEBD (h:mm). Maximum intensity z-projection images are shown. See also Movie S2. (*B*) Intensities of securin–EGFP signals normalized to 1 at NEBD (t = 0). Mean and s.d. are shown. Time after NEBD (h).

and BI2536 groups finally reached a comparable spindle volume, the onset of spindle formation was significantly delayed in BI2536-treated embryos (Fig. 2C). In the control, embryo spindle formation began concomitantly or very shortly after NEBD (0.00–5.22 min, 95% confidence interval) but in BI2536 embryos it started significantly later (7.80–17.09 min, 95% confidence interval). Both control and BI2536 embryos formed bipolar spindles, but spindles in the BI2536-treated embryos were significantly longer (27.92 ± 3.75 μm in control versus 35.14 ± 9.78 μm in BI2536). After bipolar spindle establishment and correct chromosome alignment in metaphase, plate control embryos triggered anaphase and divided into 2-cell stage embryos. However, as we have shown previously (Baran *et al.* 2013), BI25266 embryos were unable to precisely align all chromosomes in the metaphase plate and permanently arrested at metaphase with one or a few chromosomes oscillating around the spindle poles of more elongated spindles (Fig. 2*A*).

In conclusion, these data suggest that although PLK1 is not absolutely essential for spindle formation, it affects the onset of spindle formation, regulates spindle elongation and is critical for correct chromosome alignment during first embryonic mitosis.

PLK1 is essential for APC/C activation and anaphase onset

Because PLK1 inhibition results in strong metaphase arrest, we addressed the question whether or not APC/C is activated in PLK1-inhibited embryos. We monitored APC/C activity using the well established marker securin–EGFP (Herbert *et al.*, 2003) expressed together with H2B–mCHERRY from microinjected mRNAs (Fig. 3*A*). As expected, in the control embryos after successful alignment of all chromosomes to metaphase plate, securin–EGFP destruction (Fig. 3*B*) was initiated and chromosome segregation occurred normally. Securin–EGFP re-accumulated in the control (BI2536-free) embryos after the first division. In BI2536-treated embryos, however, neither securin–EGFP destruction nor chromosome segregation was initiated. Chromosome alignment was never correct. Securin–EGFP levels accumulated constantly as a result of continuing translation from microinjected

https://doi.org/10.1017/S0967199415000246 Published online by Cambridge University Press

mRNA. These data prove that PLK1 activity is essential for APC/C activation, which results in destruction of the enzyme for anaphase onset.

Discussion

Here we present 4D analysis (3D + time) of spindle formation and securin degradation after pharmacological inhibition of PLK1 activity during the first mitotic cycle of mouse preimplantation embryos.

In somatic cycling cells, PLK1 activity is required for centrosome maturation (Meraldi & Nigg, 2002), bipolar spindle formation (Sumara *et al.*, 2004) and also for initiation of cytokinesis (Brennan *et al.*, 2007; Santamaria *et al.* 2007). Plk1-deficient somatic cells fail to form a bipolar mitotic spindle because they are unable to separate their centrosomes (Hanisch *et al.*, 2006). PLK1 activity leading to phosphorylation of pericentrin facilitates the recruitment of γ-tubulin to centrosomal structures (Lee & Rhee, 2011) and results in the promotion of microtubule nucleation to establish a proper size of bipolar spindle.

Centrioles are completely degraded in mammalian oocytes and partially in spermatozoa, except in rodents, in which both spermatid centrioles are lost during spermiogenesis. Because rodent fertilized oocytes do not receive centrioles from the spermatozoon (Manandhar *et al.*, 2005), spindle assembly is fully controlled by oocyte-inherent microtubule-organizing centres (MTOCs) that are distributed randomly throughout the cytoplasm. In non-rodent species, sperm centrioles duplicate before resumption of first mitosis, followed by migration of duplicated centrosomes to opposite poles of apposed pronuclei (Schatten & Sun, 2009). Acentriolar mouse zygotes accomplish early embryonic cleavage using MTOCs until late preimplantation development (Courtois *et al.*, 2012). Thus, the shift from acentriolar to centrosomal spindle formation is the most remarkable feature of the transition from meiosis to mitosis. The MTOCs of mouse oocyte origin converge around the apposed pronuclei and migrate to the opposite poles during prometaphase.

In the PLK1-inhibited mouse zygote (Baran *et al.* 2013) and oocytes (Solc *et al.* 2015) targeting of γ-tubulin to MTOCs is disturbed, but a bipolar spindle is formed during first mitosis (Fig. 2*A*) and similarly also in meiosis I. However, the onset of the spindle formation is delayed (Fig. 2*B, C*) when PLK1 is inhibited. These results suggest that, in contrast with somatic cells, PLK1 activity is not required for spindle bipolarization in mouse zygote but it facilitates spindle formation at least partly by promoting γ-tubulin loading to MTOCs. Because formation of the bipolar spindle was also observed at even higher concentrations of BI2536 (1–2μM), it is possible to exclude the idea that the bipolar phenotype observed in BI2536-treated zygotes was due to partial inhibition of PLK1 activity (data not shown). Although bipolar but elongated spindles formed after BI2536 treatment, chromosome alignment was defective showing one or few chromosomes around the spindle poles at the time when control embryos already had chromosomes in the metaphase plate. These defects can be at least partly due to the recently discovered role of PLK1 in MCAK stability (Sanhaji *et al.*, 2014).

We have shown recently that in mouse oocytes PLK1 is not absolutely essential for spindle bipolarization although bipolarization is delayed and a smaller spindle is formed when PLK1 is inhibited. Importantly, PLK1 promotes the recruitment of γ-tubulin and pericentrin to MTOCs and also PLK1 is essential for proper kinetochore–microtubule attachment in mouse oocytes (Solc *et al.*, 2015).

In somatic cells, PLK1 inhibition leads to prometaphase/metaphase arrest accompanied by the absence of APC/C. This arrest can be reverted by concomitant SAC abrogation (Sumara *et al.*, 2004; van Vugt *et al.*, 2004). Here we have shown that PLK1 inhibition prevented APC/C activation and securin destruction (Fig. 3). In somatic cells it was shown that PLK phosphorylates and activates APC/C (Kotani *et al.*, 1998; Golan *et al.*, 2002; Kraft *et al.*, 2003) although this PLK1 function is dispensable. (Lenart *et al.*, 2007). We have shown previously that metaphase arrest caused by PLK1 inhibition cannot be rescued by concomitant SAC silencing by acute inhibition of MPS1, a critical SAC component (Baran *et al.*, 2013). This result suggests that, in mouse zygotes, PLK1 is essential for APC/C activation and anaphase onset independently of SAC satisfaction.

A similar situation to that in the mouse zygote occurs in oocytes, in which PLK1 is required for full APC/C activation independently of SAC satisfaction. Metaphase I arrest in PLK1-inhibited oocytes cannot be reversed by SAC inhibition (Solc *et al.*, 2015).

Taken together, our results in mouse zygotes show that PLK1 supports efficient spindle formation and it is required for chromosome alignment. PLK1 is also essential for full APC/C activation and anaphase onset in mouse zygotes. Thus the role of PLK1 in the zygote is more similar to the PLK1 function in oocytes than that in somatic cells.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0967199415000246

https://doi.org/10.1017/S0967199415000246 Published online by Cambridge University Press

Acknowledgements

This work was supported by the Slovak Research and Development Agency (APVV-0237–10). Work at the Institute of Animal Physiology and Genetics (IAPG) was supported by ExAM no. CZ.1.05/2.1.00/03.0124. PS and AB were also supported by grant no. LH12057.

Conflicts of interest

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

References

Baran, V., Solc, P., Kovarikova, V., Rehak, P. & Sutovsky, P. (2013). Polo-like kinase 1 is essential for the first mitotic division in the mouse embryo. *Mol. Reprod. Dev.* **80**, 522–34.

Brennan, I.M., Peters, U., Kapoor, T.M. & Straight, A.F. (2007). Polo-like kinase controls vertebrate spindle elongation and cytokinesis. *PLoS One* **2**, e409.

Burkard, M.E., Randall, C.L., Larochelle, S., Zhang, C., Shokat, K.M., Fisher, R.P. & Jallepalli, P.V. (2007). Chemical genetics reveals the requirement for Polo-like kinase 1 activity in positioning RhoA and triggering cytokinesis in human cells. *Proc. Natl. Acad. Sci. USA* **104**, 4383–8.

Courtois, A., Schuh, M., Ellenberg, J. & Hiiragi, T. (2012). The transition from meiotic to mitotic spindle assembly is gradual during early mammalian development. *J. Cell. Biol.* **198**, 357–70.

Fan, H.Y., Tong, C., Teng, C.B., Lian, L., Li, S.W., Yang, Z.M., Chen, D.Y., Schatten, H. & Sun, Q.Y. (2003). Characterization of polo-like kinase-1 in rat oocytes and early embryos implies its functional roles in the regulation of meiotic maturation, fertilization, and cleavage. *Mol. Reprod. Dev.* **65**, 318–29.

Foley, E.A. & Kapoor, T.M. (2013). Microtubule attachment and spindle assembly checkpoint signalling at the kinetochore. *Nat. Rev. Mol. Cell Biol.* **14**, 25–37.

Golan, A., Yudkovsky, Y. & Hershko, A. (2002). The cyclin-ubiquitin ligase activity of cyclosome/APC is jointly activated by protein kinases Cdk1-cyclin B and Plk. *J. Biol. Chem.* **277**, 15552–7.

Hanisch, A., Wehner, A., Nigg, E.A. & Sillje, H.H.W. (2006). Different PLK1 function show distinct dependencies on polo-box domain-mediated targeting. *Mol. Biol. Cell* **17**, 448–59.

Hansen, D.V., Loktev, A.V., Ban, K.H. & Jackson, P.K. (2004). Plk1 regulates activation of the anaphase promoting complex by phosphorylating and triggering CFbetaTrCP-dependent destruction of the APC Inhibitor Emi1. *Mol. Biol. Cell* **15**, 5623–34.

Herbert, M., Levasseur, M., Homer, H., Yallop, K., Murdoch, A. & McDougall, A. (2003). Homologue disjunction in mouse oocytes requires proteolysis of securin and cyclin B1. *Nat. Cell Biol.* **5**, 1023–5.

Howe, K. & FitzHarris, G. (2013). Recent insights into spindle function in mammalian oocytes and early embryos. *Biol. Reprod.* **89**, 1–9.

Kotani, S., Tugendreich, S., Fuji, M., Jorgensen, P.M., Watanabe, N., et al. (1998). PKA and MPF-activated polo-like kinase regulate anaphase-promoting complex activity and mitosis progression. *Mol. Cell* **1**, 371–80.

Kraft, C., Herzog, F., Gieffers, C., Mechler, K., Hagting, A. et al. (2003). Mitotic regulation of the human anaphase-promoting complex by phosphorylation. *EMBO J.* **22**, 6598–609.

Kudo, N.R., Anger, M., Peters, A.H., Stemmann, O., Theussl, H.C., Helmhart, W., Kudo, H., Heyting, C. & Nasmyth, K. (2009). Role of cleavage by separase of the Rec8 kleisin subunit of cohesin during mammalian meiosis I. *J. Cell Sci.* **122**, 2686–98.

Lee, K. & Rhee, K. (2011). Plk1 phosphorylation of pericentrin initiates centrosome maturation at the onset of mitosis. *J. Cell Biol.* **195**, 1093–101.

Lenart, P., Petronczki, M., Steegmaier, M., Di Fiore, B., Lipp, J.J., Hoffmann, M., Rettig, W.J., Kraut, N. & Peters, J.M. (2007). The small-molecule inhibitor BI 2536 reveals novel insights into mitotic roles of polo-like kinase 1. *Curr. Biol.* **17**, 304–15.

Manandhar, G., Schatten, H. & Sutovsky, P. (2005). Centrosome reduction during gametogenesis and its significance. *Biol. Reprod.* **72**, 2–13.

Meraldi, P. & Nigg, E.A. (2002). The centrosome cycle. *FEBS Lett.* **521**, 9–13.

Moshe, Y., Boulaire, J., Pagano, M. & Hershko, A. (2004). Role of Polo-like kinase in the degradation of early mitotic inhibitor 1, a regulator of the anaphase promoting complex/cyclosome. *Proc. Natl. Acad. Sci. USA* **101**, 7937–42.

Musacchio, A. & Salmon, E.D. (2007). The spindle-assembly checkpoint in space and time. *Nat. Rev. Mol. Cell Biol.* **8**, 379–93.

Sanhaji, M., Ritter, A., Belsham, H.R., Friel, C.T., Roth, S., Louwen, F. & Yuan, J. (2014). Polo-like kinase 1 regulates the stability of the mitotic centromere-associated kinesin in mitosis. *Oncotarget* **5**, 3130–44.

Santamaria, A., Neef, R., Eberspacher, U., Eis, K., Husemann, M., Mumberg, D., Prechtl, S., Schulze, V., Siemeister, G., Wortmann, L., Barr, F.A. & Nigg, E.A. (2007). Use of the novel Plk1 inhibitor ZK thiazolidinone to elucidate functions of Plk1 in early and late stages of mitosis. *Mol. Biol. Cell* **18**, 4024–36.

Scutt, P.J., Chu, M.L., Sloane, D.A., Cherry, M., Bignell, C.R., Williams, D.H. & Eyers, P.A. (2009). Discovery and exploitation of inhibitor-resistant aurora and polo kinase mutants for the analysis of mitotic networks. *J. Biol. Chem.* **284**, 15880–93.

Schatten, G., Simerly, C. & Schatten, H. (1985). Microtubule configuration during fertilization, mitosis, and early development in the mouse and the requirement for egg microtubule-mediated mobility during mammalian fertilization. *Proc. Natl. Acad. Sci. USA* **82**, 4152–6.

Schatten, H. & Sun, Q.Y. (2009). The role of centrosomes in mammalian fertilization and its significance for ICSI. *Mol. Hum. Reprod.* **15**, 531–6.

https://doi.org/10.1017/S0967199415000246 Published online by Cambridge University Press

Schindelin, J., Arganda-Carreras, I., Frise, E, Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C. Saalfeld, S., Schmid, B., Tinevez, J.Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P. & Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nat. Methods* **9**, 676–82.

Schuh, M. & Ellenberg, J. (2007). Self-organization of MTOCs replaces centrosome function during acentrosomal spindle assembly in live mouse oocytes. *Cell* **130**, 484–98.

Solc, P., Saskova, A., Baran, V., Kubelka, M., Schultz, R.M. & Motlik, J. (2008). CDC25A phosphatase control meiosis I progression in mouse oocytes. *Dev. Biol.* **317**, 260–9.

Solc, P., Kitajima, T.S., Yoshida, S., Brzakova, A., Kaido, M., Baran, V., Mayer, A., Samalova, P., Motlik, J. & Ellenberg, J. (2015). Multiple requirements of PLK1 during mouse oocyte maturation. *PLoS One* **10**, e0116783.

Steegmaier, M., Hoffmann, M., Baum, A., Lenart, P., Petronczki, M., Krssak, M., Gurtler, U., Garin-Chesa, P., Lieb, S., Quant, J., Grauert, M., Adolf, G.R., Kraut, N., Peters, J.M. & Rettig, W. (2007). BI 2536, a potent and selective inhibitor of polo-like kinase 1, inhibits tumor growth *in vivo*. *Curr. Biol.* **17**, 316–22.

Sumara, I., Gimenez-Abian, J.F., Gerlich, D., Hirota, T., Kraft, C., de la Torre, C., Ellenberg, J. & Peters, J.M. (2004). Roles of polo-like kinase 1 in the assembly of functional mitotic spindles. *Curr. Biol.* **14**, 1712–22.

Tong, C., Fan, H.Y., 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.

Wei, Y., Multi, S., Yang, C.R., Ma, J., Zhang, Q.H., Wang, Z.B., Li, M., Wei, L., Ge, Z.J., Zhang, C.H., Ouyang, Y.C., Hou, Y, Shatten, H. & Yuang, Q. (2011). Spindle assembly checkpoint regulates mitotic cell cycle progression during preimplantation embryo development. *PLoS One* **6**, e21557.

Vanderheyden, V., Wakai, T., Bultynck, G., De Smedt, H., Parys, J.B. & Fissiore, R.A. (2009). Regulation of inositol 1,4,5-trisphosphate receptor type 1 function during oocyte maturation by MPM-2 phosphorylation. *Cell Calcium* **46**, 56–64.

van Vugt, M.A., van de Weerdt, B.C., Vaderm, G., Janssen, H., Calafat, J., Klompmaker, R., Wolthuis, R.M. & Medema, R.H. (2004). Polo-like kinase-1 is required for bipolar spindle formation but is dispensable for anaphase promoting complex/Cdc20 activation and initiation of cytokinesis. *J. Biol. Chem.* **279**, 36841–54.

Zhang, Z., Su, W.H., Feng, C., Yu, D.H., Cui, C., Xu, X.Y. & Yu, B.Z. (2007). Polo like kinase 1 may regulate G2/M transition of mouse fertilized eggs by means of inhibiting the phosphorylation of Tyr 15 of Cdc2. *Mol. Reprod. Dev.* **74**, 1247–54.

Zhao, Y., Ai, J., Zhang, H. & Zhu, G. (2010). Polo-like kinase-1 regulates first cleavage of 1-cell embryos in culture during assisted reproduction. *Saudi Med. J.* **31**, 247–52.

https://doi.org/10.1017/S0967199415000246 Published online by Cambridge University Press