REPRODUCTION
RESEARCH

Spindle assembly checkpoint-related failure perturbs early embryonic divisions and reduces reproductive performance of LT/Sv mice

Zuzanna Maciejewska[1], Zbigniew Polanski[3,4], Katarzyna Kisiel[5], Jacek Z Kubiak[6] and Maria A Ciemerych[2]

Departments of [1]Embryology and [2]Cytology, Institute of Zoology, Faculty of Biology, University of Warsaw, Ilji Miecznikowa 1, 02-096 Warsaw, Poland, [3]Department of Genetics and Evolution, Institute of Zoology, Jagiellonian University, Ingardena 6, 30-060 Cracow, Poland, [4]Department of Developmental Biology, Max Planck Institute of Immunobiology, Freiburg D-79108, Germany, [5]Animal Facility, Faculty of Biology, University of Warsaw, Warsaw, Poland and [6]'Mitosis and Meiosis' Group, Institute of Genetics and Development, University of Rennes 1, CNRS-UMR 6061, IFR 140 GFAS, 35043 Rennes, France

*Correspondence should be addressed to M A Ciemerych; Email: ciemerych@biol.uw.edu.pl
J Z Kubiak; Email: jacek.kubiak@univ-rennes1.fr*

Z Maciejewska is now at Molecular Basis of Carcinogenesis, Institut de Recherche sur le Cancer de Montpellier, Montpellier, France

Abstract

The phenotype of the LT/Sv strain of mice is manifested by abnormalities in oocyte meiotic cell-cycle, spontaneous parthenogenetic activation, teratomas formation, and frequent occurrence of embryonic triploidy. These abnormalities lead to the low rate of reproductive success. Recently, metaphase I arrest of LT/Sv oocytes has been attributed to the inability to timely inactivate the spindle assembly checkpoint (SAC). As differences in meiotic and mitotic SAC functioning were described, it remains obscure whether this abnormality is limited to the meiosis or also impinges on the mitotic divisions of LT/Sv embryos. Here, we show that a failure to inactivate SAC affects mitoses during preimplantation development of LT/Sv embryos. This is manifested by the prolonged localization of MAD2L1 on kinetochores of mitotic chromosomes and abnormally lengthened early embryonic M-phases. Moreover, LT/Sv embryos exhibit elevated frequency of abnormal chromosome separation during the first mitotic division. These abnormalities participate in severe impairment of preimplantation development and significantly decrease the reproductive success of this strain of mice. Thus, the common meiosis and mitosis SAC-related failure participates in a complex LT/Sv phenotype.

Reproduction (2009) **137** 931–942

Introduction

The correct chromosome segregation during meiotic divisions of oocytes and spermatocytes, as well as during cleavage divisions of embryos, is one of the key factors determining successful development. Inaccuracies in these processes may result in aneuploid gametes or embryonic cells. This usually leads to pregnancy loss or, occasionally, birth of individuals suffering from disorders caused by chromosomal aberrations (e.g. Down syndrome).

The spindle assembly checkpoint (SAC) helps to prevent aneuploidy by blocking anaphase onset until correct kinetochore–microtubule (MT) attachments and tensions are attained, and thus facilitates correct chromosome segregation. SAC comprises a multicomponent pathway, in which the transitory metaphase arrest depends upon the lack of correct kinetochore–MT attachment at least at one kinetochore (Musacchio & Salmon 2007). Molecular mechanism of SAC activation engages localization of BUB and MAD proteins to unattached kinetochores of both mitotic and meiotic chromosomes (Li & Benezra 1996, Taylor & McKeon 1997), and sequestering CDC20 – an activator of the anaphase promoting complex/cyclosome (APC/C; Fang *et al*. 1998). Recently, it was shown that ubiquitination and degradation of CDC20 via APC/C activity is necessary to maintain SAC activity (Nilsson *et al*. 2008). As APC/C itself accumulates preferentially at kinetochores of unattached chromosomes when SAC is activated (Acquaviva *et al*. 2004) it seems that the kinetochore localization of all these components can be critical for

© 2009 Society for Reproduction and Fertility
ISSN 1470–1626 (paper) 1741–7899 (online)
DOI: 10.1530/REP-09-0011
Online version via www.reproduction-online.org
Downloaded from Bioscientifica.com at 10/31/2020 03:43:11AM
via free access

SAC activity. The establishment of stable MT–kinetochore connections leads to the inactivation of SAC and removal of BUB and MAD proteins from kinetochores. This results in CDC20 release and APC/C activation. Active APC/C triggers ubiquitination and, in consequence, proteasome-dependent degradation of critical M-phase regulators. Among them are cyclin B – regulatory subunit of M-phase promoting factor (MPF; Draetta *et al.* 1989, Holloway *et al.* 1993) and PTTG1 (securin) responsible for cohesins stability necessary for chromatids cohesion (Marangos & Carroll 2008). Ubiquitination of cyclin B targets the MPF complex to the proteasome which dissociates cyclin B from CDC2A. This is a critical point in CDC2A inactivation (Chesnel *et al.* 2006, 2007). Following this step, free cyclin B is degraded within the proteasome (Chesnel *et al.* 2006, 2007). Degradation of PTTG1 results in a cascade of events enabling chromatid separation (Uhlmann 2003). As a result, initiation of anaphase movement of chromosomes is coordinated with MPF inactivation and the cell division can be completed.

Oocytes from the LT/Sv inbred strain represent an interesting example of heritable abnormal meiosis, reduced reproductive fitness and pathology of development expressed by teratoma formation. In more detail, this complex phenotype includes the elevated susceptibility to undergo parthenogenetic activation (Kaufman & Howlett 1986, Maleszewski & Yanagimachi 1995, Ciemerych & Kubiak 1998) that may result in ovarian teratomas (Stevens & Varnum 1974, Eppig 1978*b*, Artzt *et al.* 1987, Eppig *et al.* 1996, Lee *et al.* 1997). Also, defective calcium release, which may be linked to spontaneous parthenogenesis, was demonstrated recently at fertilization of LT/Sv oocytes (Archacka *et al.* 2008). Another prominent feature of LT/Sv oocytes is their inability to complete meiotic maturation. A high proportion of *in vivo* and *in vitro* maturing LT/Sv oocytes does not progress to metaphase II (MII) but arrests in the first meiotic metaphase I (MI; Kaufman & Howlett 1986, Hampl & Eppig 1995, Maleszewski & Yanagimachi 1995, Ciemerych & Kubiak 1998). Such MI-arrested oocytes are ovulated and upon fertilization give rise to non-viable triploid embryos (Kaufman & Speirs 1987, O'Neill & Kaufman 1987, Henery & Kaufman 1993*a*). The molecular basis of the MI arrest of LT/Sv oocytes is not fully known. However, a recent study revealed that the SAC proteins BUB1 and MAD2L1 localize for prolonged time to the kinetochores during first meiotic M-phase indicating involvement of SAC activity in MI arrest (Hupalowska *et al.* 2008). Consistent with this observation, inactivation of the SAC pathway through expression of dominant negative mutant BUB1 resulted in release of the majority of LT/Sv oocytes from MI arrest (Hupalowska *et al.* 2008). Thus, the MI arrest of LT/Sv oocytes appears to be related to the inability to timely inactivate SAC (Hupalowska *et al.* 2008). The precise cause of SAC-related failure in LT/Sv strain of mice remains unknown.

The cell-cycle regulation of mouse oocytes and one-cell embryos shares several similarities (Kubiak *et al.* 2008*c*). Both the meiotic maturation of the mouse oocyte and the first embryonic cell cycle occur in the absence of transcription, i.e., under the control of maternal factors accumulated during oocyte growth (Oh *et al.* 2000, Ma *et al.* 2001, Wang *et al.* 2004), with exception to certain rare transcripts appearing in the late zygote (Ram & Schultz 1993, Bouniol *et al.* 1995). Both in the maturing oocyte as well as in the one-cell embryo the cell-cycle progression is controlled in a similar way by yet unidentified factors located in the cell nucleus (Polanski *et al.* 2005, Greda *et al.* 2006, Hoffmann *et al.* 2006, Mohammed *et al.* 2008). Last but not the least, certain discrete similarities in regulation of the M-phases in oocytes (MPF activation/inactivation and related phenomena as M-phase duration or cytoskeleton activities) and one-cell and two-cell embryos were also reported (Ciemerych *et al.* 1999, Kubiak & Ciemerych 2001, Sikora-Polaczek *et al.* 2006). Therefore, the first mitotic cell cycle (and perhaps subsequent ones) is governed by specific mechanisms transitional between meiotic and mitotic type of cell-cycle regulation (Kubiak *et al.* 2008*a*, 2008*b*, 2008*c*).

Thus, taking into consideration that meiotic factors may impinge on the embryonic mitoses it was possible that the hereditary defect affecting meiotic progression of LT/Sv oocytes influences also progression through the cell cycle of early LT/Sv embryos. We have previously shown that the meiotic defect of LT/Sv oocytes is related to SAC function (Hupalowska *et al.* 2008). Usually, SAC has a common mode of action in numerous eukaryotic cells studied so far. However, certain cell types may have modified checkpoint control depending on not yet well characterized factors. Mitotic versus meiotic differences are among the best known and were described for yeast, plant, and mammalian cells (LeMaire-Adkins *et al.* 1997, Yu *et al.* 1999, Kallio *et al.* 2000, LeMaire-Adkins & Hunt 2000, Jeganathan & van Deursen 2006, Lacefield & Murray 2007, Yamamoto *et al.* 2008). Therefore, the issue of whether the LT/Sv defect impinging on SAC function in oocytes affects also cell-cycles of the early embryos remained fully open.

We show here that the SAC-related LT/Sv phenotype is not exclusively meiotic, but also impairs the progression of the early mitotic divisions of LT/Sv embryos. We demonstrate prolonged localization of MAD2L1 on the kinetochores of the one-cell and two-cell LT/Sv embryos and dramatic prolongation of these embryonic mitoses. Moreover, this correlates with anomalies often associated with SAC malfunction, like lagging chromosomes or presence of micronuclei. These abnormalities seem closely related to compromised preimplantation development and participate in the reduction of reproductive success of this strain of mice.

Downloaded from Bioscientifica.com at 10/31/2020 03:43:11AM
via free access

Results

Variable duration of the first embryonic mitosis in inbred strains of mice

To determine whether SAC-related LT/Sv phenotype characterizing maturing oocytes (Hupalowska *et al.* 2008) also affects embryonic mitoses, we focused on the duration of the well characterized first mitosis (Sikora-Polaczek *et al.* 2006) in different strains of mice. First, we evaluated the range of the variability in duration of the first mitotic division in five different mouse strains: BALB/c, C3H, C57, DBA, and RI43. The latter one, i.e. RI43, is a recombinant inbred strain developed from the inbred strains CBA and KE (Golas *et al.* 2008). As in our previous studies concerning wild-type, either F1 (CBA/H×C57Bl/10) or outbred Swiss albino mice, the length of the mitosis was delineated by the nuclear-envelope breakdown (NEBD) and the onset of anaphase, correlated with the subtle change in the cell shape as it entered cleavage (Ciemerych *et al.* 1998, 1999, Sikora-Polaczek *et al.* 2006). The duration of the first-embryonic mitosis was on average (±S.D.) 116±14, 112±21, 100±22, 90±19 and 89±8 min in C57 (n=7), DBA (n=19), C3H (n=15), BALB/c (n=15) and RI43 (n=18) mice respectively. Statistical analysis (ANOVA) allowed us to distinguish the C57 and DBA strains as characterized by the 'long' first mitosis, differing significantly from BALB/c and RI43 strains, presenting the 'short' first-embryonic M-phase. One-cell embryos from C3H mice exhibited intermediate M-phase duration, and did not differ statistically from embryos of other four strains (Fig. 1). For comparison, the length of the first-embryonic mitosis of F1 (CBA/H ×C57Bl/6) hybrid mice, used in our previous studies on LT/Sv oocytes as wild-type control (Hupalowska *et al.* 2008), was 105±12 min (n=14), which is in the middle of the range delineated by the five inbred strains tested.

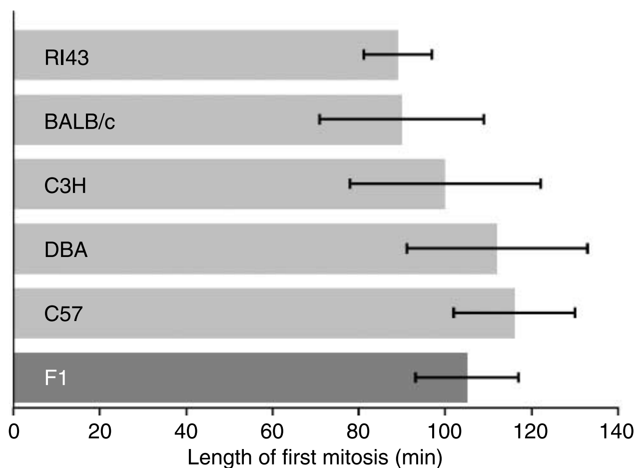

Figure 1 First-embryonic mitosis in different strains of mice. The length of mitoses was analyzed in embryos of RI43, BALB/c, C3H, DBA, and C57 strains of mice, as well as in F1 (CBA/H×C57Bl/6) embryos.

First mitosis of LT/Sv embryos is enormously prolonged

We analyzed the duration of the first mitosis in LT/Sv in parallel with the control, wild-type F1 (CBA/H×C57Bl/6) one-cell parthenogenetic embryos. We decided to use parthenogenetic one-cell embryos because, as shown previously, they undergo NEBD more synchronously than fertilized ones (Sikora-Polaczek *et al.* 2006). Moreover, we previously showed that the timing of MPF activation, spindle formation, mitosis duration, and the most importantly SAC inactivation do not differ between one-cell parthenogenotes and fertilized embryos (Ciemerych *et al.* 1999, Sikora-Polaczek *et al.* 2006). Other studies also confirmed the usefulness of one-cell parthenogenotes for the analyses of different aspects of the mouse preimplantation development (Waksmundzka *et al.* 1984, Borsuk & Tarkowski 1989, Ciemerych 1995, Winston & Maro 1995, Kalab *et al.* 1996). However, time-lapse recordings were performed using exclusively embryos obtained after *in vivo* fertilization, i.e., zygotes and two-cell embryos. Since parthenogenetic and fertilized one-cell embryos did not differ in the timing of first mitosis, we present the pooled data obtained from both types of embryos.

The first mitosis of wild-type embryos took on average 105±12 min (n=14, Figs 1 and 2A) consistent with the previous data (Ciemerych *et al.* 1999, Sikora-Polaczek *et al.* 2006). In LT/Sv embryos, however, the first mitosis took on average (±S.D.) 183±57 min (n=53; Fig. 3A). The length of the shortest of these mitoses was similar to those of one-cell F1 embryos, and was 115 min. However, the longest one took as long as 345 min (Fig. 3B). Thus, the length of the first mitosis in LT/Sv embryos seems to be extremely long beyond the length of the typical mitosis in the wild-type mice. Moreover, although we found that the length of the first mitosis can differ reasonably depending on the genetic background (Fig. 1), the duration of the process in the LT/Sv embryos exceeded greatly the range delineated by the six types of randomly chosen genotypes differing significantly both from F1 mice (Fig. 3A) and from all five inbred strains tested (P<0.0001 in all six cases, ANOVA).

It is important to remind that although the majority of LT/Sv oocytes are ovulated in the MI stage, some proceed to the MII before ovulation (Kaufman & Howlett 1986, O'Neill & Kaufman 1987). Thus, the great variability in the length of the first mitosis of LT/Sv embryos (Fig. 3B) may in fact reflect the heterogeneity of embryos tested, i.e. originating from MI and MII oocytes. To address this issue, in a separate experiment, we first classified ovulated oocytes into MI or MII group and measured duration of the first mitosis in parthenogenetic embryos originating from each group of oocytes. Interestingly, the duration of the first mitosis did not differ significantly regardless of the 'origin' of one-cell parthenogenetic

Downloaded from Bioscientifica.com at 10/31/2020 03:43:11AM
via free access

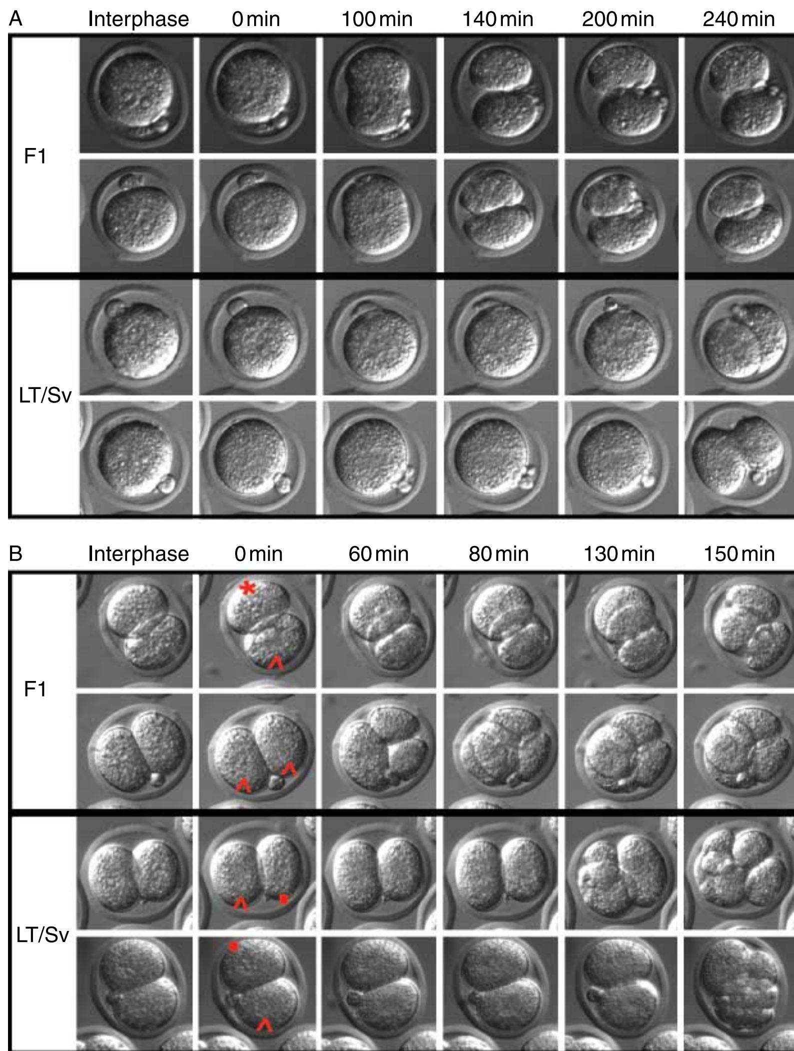

Figure 2 Time-lapse recording of the first and the second division of F1 (CBA/H×C57Bl/6) and LT/Sv embryos. (A) Examples of one-cell embryos (zygotes) progressing through the first mitosis. (B) Examples of two-cell embryos progressing through mitosis. Blastomeres undergoing NEBD exactly at time 0 min are marked with red arrowheads. The F1 blastomere marked with a red asterisk underwent NEBD at time 80 min. LT/Sv blastomeres marked with red boxes underwent NEBD at time 5 min. Due to space limitations, instead of the whole recording only the selected images are shown.

embryos (Fig. 3C). Thus, 'escaping' MI during oocyte meiosis does not guarantee the normal progression of LT/Sv embryos throughout the first mitotic division.

Second mitosis of LT/Sv embryos is also prolonged in comparison with the wild-type mice

The second mitosis was investigated in blastomeres of two-cell embryos obtained by *in vivo* fertilization, similarly as described previously (Ciemerych *et al*. 1999, Sikora-Polaczek *et al*. 2006). In control, F1 embryos this cleavage division took on average 77 ±10 min (n=19) consistently with the data presented previously (Ciemerych *et al*. 1999, Sikora-Polaczek *et al*. 2006)), while in LT/Sv embryos it took 140±40 min (n=37). Similarly, as it was observed for the first mitosis, the length of second mitoses varied enormously, i.e. from 78 to 235 min (Figs 2B and 3B). Thus, the average first or second mitotic division was significantly prolonged in LT/Sv embryos, as compared with F1 embryos. Notably, although the prolonged M-phase significantly delayed the exit from the first and the second mitosis, embryos have never arrested permanently during these divisions. These data indicate that the prolonged M-phases are specific feature of at least two first-embryonic mitoses of the LT/Sv mice.

SAC activity is dramatically prolonged during the first mitotic division of LT/Sv embryos

The prolongation of SAC activity during meiotic maturation of LT/Sv oocytes led to the MI arrest (Hupalowska *et al*. 2008). Thus, we investigated whether the sustained SAC activity plays a role also in the abnormal regulation of the first two embryonic mitoses of this strain of mice. To this point, we compared the presence or absence of MAD2L1 at kinetochores in F1 and LT/Sv embryos. Previously, we documented that in F1 embryos MAD2L1 was lost from kinetochores ∼20 min after NEBD during the first

Downloaded from Bioscientifica.com at 10/31/2020 03:43:11AM
via free access

mitosis, and ~30 min after NEBD during the second one (Sikora-Polaczek *et al.* 2006). Accordingly, 60 min after NEBD none of the F1 one-cell embryos ($n=14$) contained MAD2L1 at kinetochores (Fig. 4A). However, in all one-cell LT/Sv embryos analyzed at 30 min ($n=13$) and 60 min after NEBD ($n=15$) MAD2L1 was detectable at kinetochores (Fig. 4B). At 90 min after NEBD two out of eight embryos still had MAD2L1 localized at kinetochores. At 120 min after NEBD in all analyzed LT/Sv embryos ($n=8$) MAD2L1 was absent from kinetochores, indicating that they entered metaphase after prolonged prometaphase. Thus, the delay of SAC inactivation is not limited exclusively to meiosis, as revealed recently (Hupalowska *et al.* 2008), but also to the early mitotic divisions of LT/Sv embryos.

Early LT/Sv embryos exhibit chromosome segregation errors and impaired development

Next, we investigated whether the problems with timely SAC inactivation and delayed mitotic anaphase onset in embryos correlate with the anomalies in chromosome

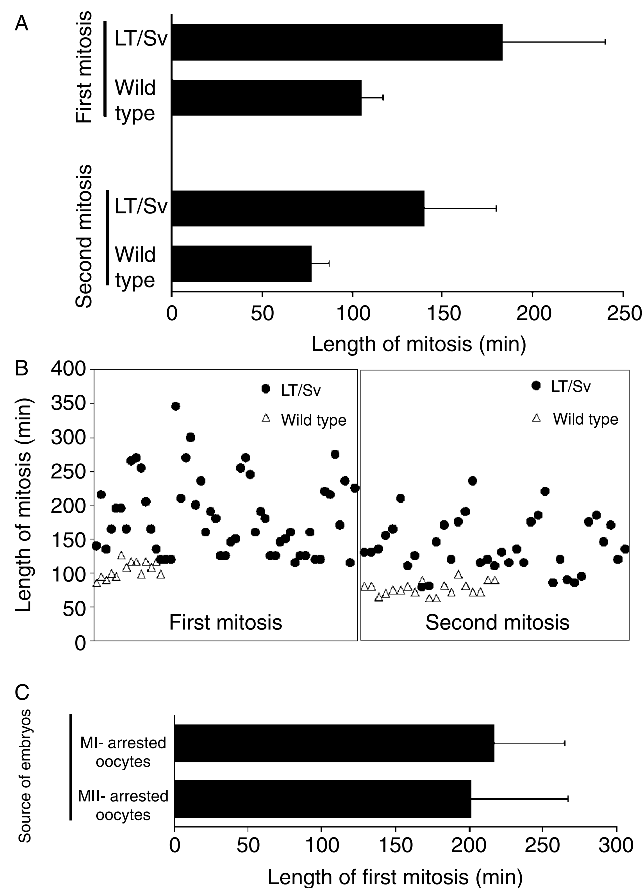

Figure 3 First and second mitosis is prolonged in LT/Sv embryos. (A) Average length of mitosis in wild-type and LT/Sv embryos. For both mitoses, the differences between LT/Sv and F1 embryos are statistically significant (*t*-test). (B) Scatters showing variability in the duration of mitoses. (C) Average length of first mitosis in LT/Sv embryos originated from MI and MII oocytes.

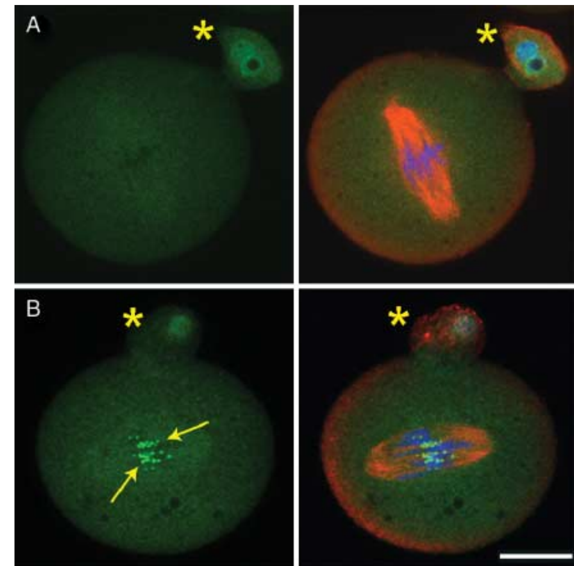

Figure 4 Localization of MAD2L1 in wild-type and LT/Sv one-cell embryos during the first mitotic division. (A) Wild-type one-cell embryo fixed 60 min after NEBD, no MAD2L1 visible at kinetochores. (B) LT/Sv oocyte fixed 60 min after NEBD, MAD2L1 localized at kinetochores. Left panel: MAD2L1-green, right panel: overlay of MAD2L1, tubulin – red and chromatin labeled with chromomycine – blue. Asterisks indicate polar body, arrows indicate MAD2L1 localization in kinetochore region. Bar 20 μm.

segregation as observed in oocytes (Hupalowska *et al.* 2008). All but one of the control F1 embryos fixed in telophase of the first mitotic division ($n=19$) and all fixed in the two-cell stage ($n=40$) contained either correctly separating chromosomes (Table 1 and Fig. 5A) or morphologically normal interphase nuclei (Table 1 and Fig. 5B). However, this was not the case in LT/Sv embryos. Similarly, as during induced meiotic anaphase I/telophase I in LT/Sv oocytes (Hupalowska *et al.* 2008), anomalous chromosome separation was also detectable in embryos of this strain (Table 1). One out of five dividing one-cell embryos had abnormally positioned chromosomes (Fig. 5C). Moreover, 3 out of 24 analyzed two-cell embryos contained micronuclei documenting aberrant chromosome separation (Fig. 5D). Statistical analysis using Fisher's exact probability test, which is an excellent choice for small samples, revealed that the frequency of abnormal chromosome arrangements related to errors during the first embryonic division is clearly higher and statistically important in LT/Sv embryos when compared with wild-type ones (Table 1).

Further comparisons of control F1 and LT/Sv embryos obtained after *in vivo* fertilization revealed that not only the first but also subsequent cleavages proceed incorrectly. Analysis of ten wild-type embryos fixed at 80 h after hCG, i.e. at the morula stage, showed that they contained ~14 blastomeres per embryo (Fig. 5E and F). By contrast, such analysis of 12 simultaneously cultured LT/Sv embryos revealed severely decreased cell number,

Downloaded from Bioscientifica.com at 10/31/2020 03:43:11AM
via free access

Table 1 Frequency of chromosomal abnormalities related to the errors during first-embryonic division in the embryos from LT/Sv and wild-type mice.

	Type of abnormalities		
Embryos	Aberrant chromosome separation	Micronuclei at 2-cell stage	Total abnormalities
Wild-type	1/19 (5%)	0/40 (0%)*	1/59 (2%)*
LT/Sv	1/5 (20%)	3/24 (12.5%)*	4/29 (14%)*

*Difference between LT/Sv and wild-type embryos statistically significant ($P<0.05$, Fisher exact probability test).

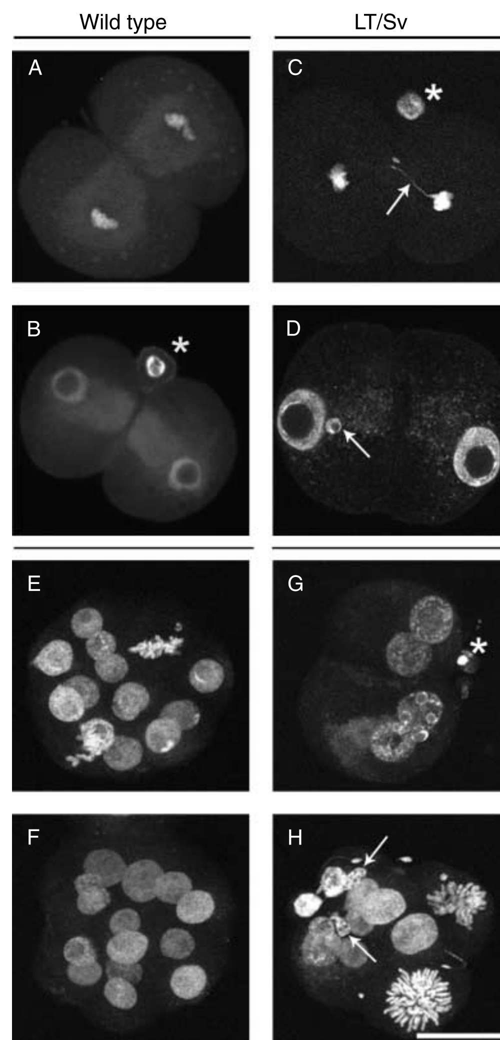

Figure 5 Chromatin organization in cleaving wild-type and LT/Sv embryos. (A) Wild-type embryo fixed at the telophase of the first mitotic division. (B) Wild-type embryo fixed at two-cell stage. (C) LT/Sv embryo fixed at the telophase of the first mitotic division, arrow indicates incorrectly separating chromosome. (D) LT/Sv embryo fixed at the two-cell stage, arrow indicates the micronucleus resulting from incorrectly separated chromosome or chromosomes. (E and F) Wild-type embryos at the morula stage composed of 14 blastomeres, fixed 80 h after hCG. (E) Two blastomeres undergo mitotic division. (G and H) Abnormally dividing LT/Sv embryos fixed 80 h after hCG. (G) Embryo arrested in two-cell stage with multinucleated blastomeres. (H) Embryo containing mitotically dividing blastomeres with abnormally high number of chromosomes and blastomeres containing micronuclei. Asterisks indicate polar body; arrows indicate abnormally positioned chromosomes or micronuclei. Bar 20 μm.

which varied from two blastomeres in the less advanced embryos, up to nine blastomeres in the most advanced ones (Fig. 5G and H). Moreover, this feature was accompanied by the presence of multinucleated blastomeres and blastomeres containing micronuclei (Fig. 5G and H). This observation clearly suggested deregulation of mitotic divisions of preimplantation LT/Sv embryos.

To study the potential impact of these abnormalities on the preimplantation embryo development 193 wild-type embryos and 63 LT/Sv embryos were cultured from one-cell stage (24 h after hCG) until the blastocyst stage (94 h after hCG). A group of 153 wild-type and of 25 LT/Sv embryos were cultured for additional 24 h i.e., until ~120 h after hCG. During the whole culture period LT/Sv embryos showed significant delay in their development (Fig. 6). Thus, not only the first and the second cleavage divisions were delayed as we described above, but also the subsequent ones, resulting in the retarded development of LT/Sv embryos. In consequence, while by 120 h after hCG the majority of wild-type embryos reached expanded blastocyst stage, a significant proportion of LT/Sv embryos were found at the morula stage and also a significant proportion underwent degeneration (Fig. 6). Such a hampered preimplantation development seems a logical consequence of the elevated frequency of abnormal chromosome separation in LT/Sv embryos.

To correlate the developmental problems observed during preimplantation period with the level of reproductive success of LT/Sv strain, we analyzed the size of litters of individual mating females of this strain and F1 controls. This analysis revealed that while all of the wild-type females ($n=68$) gave birth to live progeny at least two times, 31% (21/68) of LT/Sv females were infertile. Moreover, the average number of newborns in the first two litters was significantly reduced in LT/Sv females (Table 2).

Discussion

We document here that the prolonged SAC activity is manifested by extended MAD2L1 presence on kinetochores and that it prolongs early mitotic M-phases, perturbs cleavage divisions, hampers preimplantation development, and correlates with the decreased reproductive success of LT/Sv mice. The SAC-related anomaly is certainly not the sole reason for the low reproductive performance of LT/Sv females. It coincides with the MI-arrest of oocytes, the development of ovarian teratomas (Stevens & Varnum 1974), spontaneous parthenogenetic activation of ovulated oocytes (Stevens

Downloaded from Bioscientifica.com at 10/31/2020 03:43:11AM
via free access

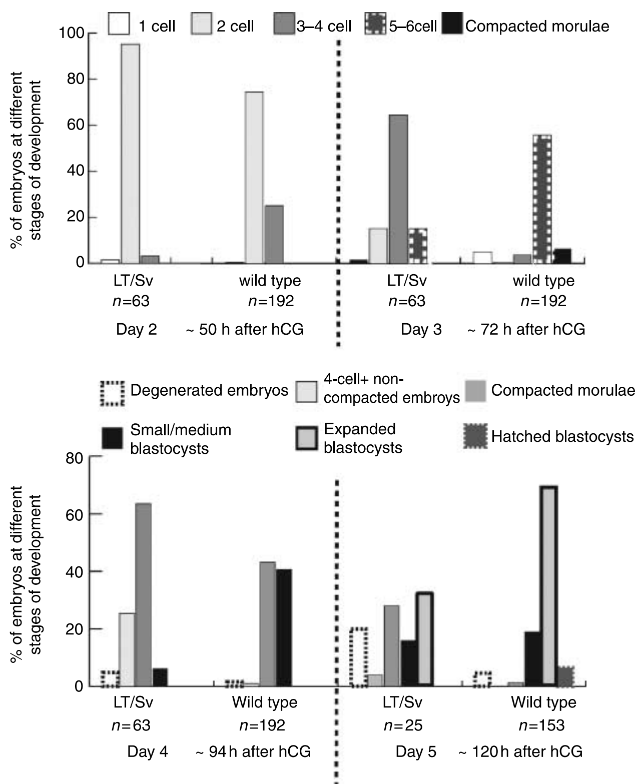

Figure 6 *In vitro* development of wild-type and LT/Sv embryos. Graphs show the proportion of wild-type and LT/Sv embryos in different stages of development during *in vitro* culture. Embryos were isolated at 24 h after hCG and scored at 50, 72, 94, and 120 h after hCG.

& Varnum 1974, Eppig 1978*a*), or formation of triploid embryos upon fertilization (Kaufman & Speirs 1987, Speirs & Kaufman 1988, Henery & Kaufman 1993*a*). As a result 30% of LT/Sv females do not reproduce at all and the remaining ones show a significant decrease in fertility (this study). It has to be noted, however, that triploidy does not affect the preimplantation development of wild-type mouse embryos that are able to reach the blastocyst stage and do not exhibit SAC-related phenotype of LT/Sv embryos described in our paper. (Niemierko 1975, Henery & Kaufman 1992, 1993*b*).

We show here that the length of the first and second mitosis in LT/Sv embryos is enormously prolonged. The prolonged presence of MAD2L1 on the kinetochores of LT/Sv embryos strongly suggests that the extension of mitotic division result from the inability to timely inactivate the SAC. In our previous study, we have shown that in the wild-type one-cell embryo (the same F1 hybrids as used in the present study) MAD2L1, whereas present on the kinetochores until 10 min after NEBD, is lost from the kinetochores of all analyzed embryos before 20 min after NEBD (Sikora-Polaczek *et al*. 2006). Since, on average, the one-cell embryos of F1 mice (wild-type) divide 105 min after NEBD, we estimate that, on average, 85 min elapses from the release of the kinetochore-located MAD2L1 to the cleavage (105−20 min; Hupalowska *et al*. 2008). On the other hand, in the LT/Sv mice MAD2L1 is present on kinetochores of all tested one-cell embryos 60 min after NEBD and in 25% of embryos even 90 min after NEBD. Thus, in one-cell LT/Sv embryos, the estimated time elapsing from the release of MAD2L1 from kinetochores to the cleavage is ∼93 min (183 min of average duration of the first mitosis −90 min) being very similar to this calculated for F1 control (93 vs 85 min). Thus, the prolongation of the first mitosis in LT/Sv embryos seems indeed to occur thorough the extension of this phase of mitosis when MAD2L1 localizes to kinetochores. This strongly suggests that the enormously long mitosis of LT/Sv embryos is exclusively SAC-dependent.

By evaluating the length of mitosis in the five randomly chosen inbred strains, we have found that the timing of the first mitosis can differ reasonably in embryos of different genotype. Previously, we estimated the timing of the first meiotic M-phase of oocytes (calculated as the time elapsing from the germinal-vesicle breakdown, which is an equivalent of NEBD in one-cell embryos, until cleavage) for four of these strains (Polanski 1997). As shown in Table 3, there is no correlation between the duration of the first meiotic M-phase and the duration of the first-embryonic mitosis in these four strains. However, in the LT/Sv mice the extremely prolonged first meiotic M-phase correlates with extremely extended first mitosis. This strongly suggests that in the LT/Sv oocytes and early embryos the defect is specific and common for meiosis and mitosis. Other characteristic LT/Sv anomalies, like prolonged presence of MAD2L1 on the kinetochores or a tendency to abnormal chromosome separation, are observed both at meiosis (Hupalowska *et al*. 2008) and mitosis (present study). This delivers an additional support for the hypothesis that the defect in the oocytes and early embryos of LT/Sv mice may have the same origin.

Table 2 Comparison of fertility of LT/Sv and wild-type mice.

			No. of young in	
Mice	**No. of females**	**No. of fertile females** (%)*	1st litter[†]	2nd litter
Wild-type	68	68 (100)[a]	8.1±3.7[b]	7.0±2.5[c]
LT/Sv	68	47 (69)[a]	4.7±1.8[b]	4.2±2.0[c]

[a,b,c]Values marked with the same letter differ significantly (in all cases $P<0.0001$, [a]χ^2 test of independence, [b,c]*t*-test).
[*]Delivering at least one litter. [†]Infertile females excluded.

Downloaded from Bioscientifica.com at 10/31/2020 03:43:11AM
via free access

Table 3 The duration of the first meiotic M-phase and the first mitosis in different mouse inbred strains.

Strains order	**Duration** (min)	**Phenotype**
Length of first meiosis		
DBA	384	Fast
C3H	516	Intermediate
Balb/c	522	Intermediate
C57	528	Intermediate
KE	588	Slow
Length of first mitosis		
Balb/c	90	Fast
C3H	100	Intermediate
DBA	112	Slow
C57	116	Slow

The duration of meiotic M-phase is calculated as the time elapsing between germinal vesicle breakdown (GVBD, an equivalent of NEBD in embryos) and the first polar body extrusion (equivalent of mitotic cleavage). Strains are ordered from the shortest meiosis/mitosis to the longest one. KE strain (which due to extinction is no longer available) is shown to indicate that there exists the slow meiotic phenotype. Data for meiosis are taken from Polanski (1997).

SAC activity results from incorrect attachment between kinetochores and MTs. However, no apparent morphological differences in mitotic spindle architecture were found between maturing LT/Sv and control embryos. The only spindle malformation in LT/Sv mice was observed in MI-arrested LT/Sv oocytes as a characteristic increase in size of the meiotic spindle (Albertini & Eppig 1995, Ciemerych & Kubiak 1998). It cannot be excluded that the failure to timely inactivate SAC during embryonic mitoses could arise from discrete (and morphologically undetectable at the light microscopy level) anomalies in MT attachment to kinetochores during the spindle formation. Interestingly, in Ptk1 cells the depletion of NDC80 (HEC1) protein (involved in MT attachment to kinetochores) results in concomitant events: i) disorganization of the mitotic spindle often linked to the increase in its size, ii) a 40 min delay in anaphase, and iii) a decrease in MAD2L1 recruitment to the kinetochores (Martin-Lluesma *et al.* 2002, Guimaraes *et al.* 2008). NDC80 depletion in Ptk1 cells shows that SAC activity can be only temporarily prolonged with discrete morphological changes in the spindle, which resembles the phenotype observed in LT/Sv oocytes and embryos. Alternatively, the SAC defect in LT/Sv embryos may rely on a hampered SAC pathway itself, for example an incorrect and/or delayed transmission of signals in SAC regulatory system.

It is commonly acknowledged that the basic SAC mechanism acts similarly during both mitotic and meiotic division. However, mitotic and meiotic cellular environments differ significantly and modify SAC action. Most importantly, in oocytes, in contrast to embryos, the MOS/…/EMI2 (FBXO43) pathway inhibits APC/C in a similar way as does the SAC (Kubiak *et al.* 2008*c*). This results in a doubled system of APC/C inhibition upon MII arrest. To our knowledge, consequences of such apparently redundant inhibitory control of anaphase trigger were not studied in details so far. For this major cause, SAC deregulation may differently influence female meiosis and mitosis (Pesin & Orr-Weaver 2008).

It has been shown that MAD2L1, BUB3, BUB1B deficiency is fatal both for mouse embryogenesis and for the viability of somatic cells (Dobles *et al.* 2000, Kalitsis *et al.* 2000, Michel *et al.* 2001, Baker *et al.* 2004, Jeganathan & van Deursen 2006). As far as meiosis is concerned, ablation of BUB1B causes the failure of meiotic division (Baker *et al.* 2004, 2005). Loss of one *Mad2l1* allele results in significantly increased aneuploidy rate in MII arrested mouse oocytes, indicating that a single *Mad2l1* gene copy lowers the fidelity of the meiotic division (Niault *et al.* 2007). Both knockdown of MAD2L1 as well as interfering with BUB1 function result in premature MI/MII transition (Tsurumi *et al.* 2004, Homer *et al.* 2005). However, despite the involvement of the same key proteins in mitotic/meiotic SAC function some intriguing issues related to SAC remain. For example, deficiency of some SAC proteins was not shown to influence the course of meiosis and does not result in premature chromatid segregation (Jeganathan & van Deursen 2006, Pesin & Orr-Weaver 2008). Also the progression of meiosis in female XO mice is not affected by the presence of univalent chromosome X (LeMaire-Adkins *et al.* 1997), suggesting that the specific X-univalent structure permits the establishment of bi-oriented connections to the meiotic spindle. Furthermore, while mitotically-dividing cells arrest in prometaphase in the response to the presence of MT-disassembling drugs, the meiotically dividing mouse spermatocytes arrest at pachytene (Tepperberg *et al.* 1999). This suggests the presence of specific SAC effectors also during male meiosis. In meiotically dividing budding yeast in which chromosome exchange is affected, MAD3/BUB1B appears crucial for delaying prometaphase I rather than metaphase-anaphase transition (Cheslock *et al.* 2005). This, again, suggests a unique role of checkpoint proteins in meiotic progression. Aware of these differences the characteristics of mitotic SAC-related defects described in our present study resemble very closely the meiotic phenotype of LT/Sv oocytes (Hupalowska *et al.* 2008). Thus, it seems highly possible that the defects in SAC functioning observed in oocytes and early embryos might have the same molecular basis. We did not, however, address the question about the molecular mechanism underlying this anomaly and it remains to be elucidated.

Whereas the meiotic LT/Sv phenotype comprises permanent MI arrest of the majority of oocytes, we did not observe such stable arrest during early embryonic mitoses. The explanation for this comes from the fact that in MI arrested LT/Sv oocytes, at the time when SAC undergoes delayed inactivation, cytostatic factor (CSF) activity is already developed sustaining this arrest (Hirao & Eppig 1997, Ciemerych & Kubiak 1998). CSF is absent in early embryos, thus the delayed inactivation of

Downloaded from Bioscientifica.com at 10/31/2020 03:43:11AM
via free access

SAC results only in prolonged prometaphase, but not in permanent arrest.

The knowledge gained on the meiotic LT/Sv phenotype offers also an explanation to the variability in the length of mitoses in LT/Sv embryos. A fraction of LT/Sv oocytes (~30% in our recent report (Hupalowska *et al*. 2008)) progresses through the first meiotic division unperturbed. Similarly, the length of mitoses of some LT/Sv embryos does not differ from the length of respective mitoses of wild-type embryos. In the current study, 13 out of 53 of one-cell and 6 out of 37 of the two-cell LT/Sv embryos completed mitosis until 115 and 78 min respectively, i.e. at the time when the average wild-type embryos completed their mitoses (this study). The remaining LT/Sv embryos completed divisions gradually, at varying times after NEBD. This phenomenon reflects the progressive exit from the first meiotic division of LT/Sv oocytes (Ciemerych & Kubiak 1998), and a gradual release from meiotic MI arrest characteristic for oocytes with the $Mos^{-/-}$ mutation introduced to an LT/Sv background (Hirao & Eppig 1997).

In cells studied so far, SAC activity is transitory and is overcome if the perturbation persists over a critical period (Rieder & Palazzo 1992, Rieder *et al*. 1994). By analogy, such SAC recovery might be crucial for the completion of the mitotic divisions of LT/Sv embryos. The support for such hypothesis comes from the presence of anomalies in chromosome separation occurring during mitoses of LT/Sv embryos, suggesting that the delayed anaphase might occur without SAC satisfaction. Alternatively, SAC activity might finally cease as SAC-activating perturbation disappears. However, in such cases cleavage divisions should be unperturbed, yet we cannot exclude that more than one type of defect linked to SAC functioning acts in LT/Sv mice.

The high variability in the duration of embryonic mitoses could suggest that the relative success of reproduction of LT/Sv mice may rely upon those oocytes which pass only moderately perturbed M-phases and thus segregate chromosomes properly. However, the prolongation of embryonic mitoses occurring not only in embryos originating from MI but also from undisturbed MII oocytes clearly shows that normal completion of the first meiosis does not eliminate anomalies during the first mitosis. Therefore, the LT/Sv phenotype both in oocytes and embryos might comprise only meiotic elements of the cell-cycle control. Previous findings from our laboratories suggest that some meiotic pathways are functional also during the earliest cell-cycles of dividing embryos (Kubiak *et al*. 2008*c*). Thus, in LT/Sv mice, the defective meiotic elements of cell-cycle regulation could affect the SAC pathway not only during meiosis but also during the earliest embryonic divisions. Such defects, however, would disappear as soon as those meiotic components were replaced by mitotic ones, presumably as a result of embryonic genome expression. In LT/Sv embryos that successfully passed the critical meiotic and mixed meiotic/mitotic periods, further development could proceed unaffected. Alternatively, the defects may rely on maternal genes related to SAC function.

Materials and Methods

All animal studies presented were approved by Local Ethics Committee No. 1 in Warsaw, Poland and Regierungspraesidium in Freiburg, Germany, according to European Union Council Directive 86/609/EEC of 24 November 1986. All animals were raised on the premises.

Collection, parthenogenetic activation of ovulated oocytes and in vitro *culture of one- and two-cell embryos*

F1 (CBA/H×C57Bl/10) and LT/Sv females were superovulated by injection of 10 IU of eCG (‘Folligon’; Intervet, Boxmeer, The Netherlands) and 10 IU of hCG (‘Chorulon’; Intervet) 48–52 h apart. Ovulated oocytes were collected 17–18 h after hCG injection and cumulus cells were removed with hyaluronidase (Sigma–Aldrich, 300 units/ml PBS, Biomed, Lublin, Poland). LT/Sv oocytes were classified as being at MI or MII stage based on the absence or presence of the first polar body. For the parthenogenetic activation the oocytes were exposed to 8% ethanol (POCh, Gliwice, Poland) in M2 medium for 8 min at room temperature (Cuthbertson 1983), washed carefully and cultured in M2. Only those oocytes that extruded polar bodies and formed pronuclei were used for further analyses. Starting at 14 h after activation embryos were scored every 10 min to determine the timing of the NEBD and then collected for further analyses at different time after NEBD. Fertilized two-cell embryos were obtained from superovulated and mated females 49 h after hCG. Embryos were collected and cultured in M2 medium. Starting from 49 h after hCG embryos were observed every 10 min to determine the timing of NEBD and collected for further analyses at different times after NEBD.

Collection and in vitro *culture of fertilized one-cell embryos*

BALB/cPzhW, C3H/W, C57BL/Kw, DBA/2W (referred to throughout the text as BALB/c, C3H, C57 and DBA respectively), RI43, F1 (CBA/H×C57Bl/10), and LT/Sv females were superovulated as described above and mated with F1 males. Fertilized one-cell embryos were obtained from oviducts 24 h after hCG injection and then cultured in M2 medium. Embryos subjected to analysis of the length of first mitotic division were scored every 10 min after NEBD. Embryos subjected to long-term culture were scored every 24 h until 120 h after hCG.

Time-lapse recording of one- and two-cell embryos

One-cell and two-cell embryos obtained after mating of F1 and LT/Sv females with F1 males were recorded during the first and the second mitotic division. For the time-lapse recording, the culture dish (glass-bottom dish; WillCo Wells BV, Amsterdam,

Downloaded from Bioscientifica.com at 10/31/2020 03:43:11AM
via free access

The Netherlands) was placed in plastic chamber incubator XL (Zeiss; Carl Zeiss, Jena, Germany) mounted on a Zeiss Axiovert M200 inverted microscope (Zeiss, Carl Zeiss). The temperature in the incubator was maintained at 37 °C by a Tempcontrol 37-2 digital (Zeiss, Carl Zeiss). The microscope was connected to AxioCam Mrm camera (Zeiss, Carl Zeiss). Image acquisition was performed using differential interface contrast (DIC) microscopy (30–400 ms illumination) every 5 min under the control of AxioVision 3.4 software (Zeiss, Carl Zeiss). Figures were assembled using Adobe Photoshop 7.0.

Immunofluorescence

The embryos were fixed and processed as previously described (Bouniol *et al*. 1995). α-Tubulin was immunodetected with mouse MAB against α-tubulin (Sigma–Aldrich) followed by TRITC-labeled anti-mouse antibody (Jackson ImmunoResearch, West Grove, PA, USA). MAD2L1 was immunodetected by rabbit polyclonal antibody (gift from Dr K Wassmann; Wassmann *et al*. 2003), followed by FITC labeled anti-rabbit antibody (Jackson ImmunoResearch). Chromatin was visualized either with chromomycin A3 (Sigma–Aldrich) or propidium iodide (Vector Laboratories, Burlingame, CA, USA). The samples were mounted in Citifluor (Citifluor Ltd, London, UK) on glass slides and examined with laser scanning confocal microscope (Carl Zeiss). Figures were assembled using Adobe Photoshop 7.0.

Statistical analysis

Data were analyzed by χ^2 test of independence, Fisher's exact probability test or *t*-test. Differences at $P<0.05$ were considered significant.

Declaration of interest

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

Funding

This work was supported by grants from ARC (4900) and Ligue Contre le Cancer (Comité d'Ille-et-Vilaine et de la Vandée) to Jacek Z Kubiak.

Acknowledgements

The authors would like to thank Dr Katja Wassmann for anti MAD2L1 antibody and Anna Hupalowska for her help during the preparation of the figures.

References

Acquaviva C, Herzog F, Kraft C & Pines J 2004 The anaphase promoting complex/cyclosome is recruited to centromeres by the spindle assembly checkpoint. *Nature Cell Biology* **6** 892–898.

Albertini DF & Eppig JJ 1995 Unusual cytoskeletal and chromatin configurations in mouse oocytes that are atypical in meiotic progression. *Developmental Genetics* **16** 13–19.

Archacka K, Ajduk A, Pomorski P, Szczepanska K, Maleszewski M & Ciemerych MA 2008 Defective calcium release during *in vitro* fertilization of maturing oocytes of LT/Sv mice. *International Journal of Developmental Biology* **52** 903–912.

Artzt K, Calo C, Pinheiro EN, DiMeo-Talento A & Tyson FL 1987 Ovarian teratocarcinomas in LT/Sv mice carrying t-mutations. *Developmental Genetics* **8** 1–9.

Baker DJ, Jeganathan KB, Cameron JD, Thompson M, Juneja S, Kopecka A, Kumar R, Jenkins RB, de Groen PC, Roche P *et al.* 2004 BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. *Nature Genetics* **36** 744–749.

Baker DJ, Chen J & van Deursen JM 2005 The mitotic checkpoint in cancer and aging: what have mice taught us? *Current Opinion in Cell Biology* **17** 583–589.

Borsuk E & Tarkowski AK 1989 Transformation of sperm nuclei into male pronuclei in nucleate and anucleate fragments of parthenogenetic mouse eggs. *Gamete Research* **24** 471–481.

Bouniol C, Nguyen E & Debey P 1995 Endogenous transcription occurs at the 1-cell stage in the mouse embryo. *Experimental Cell Research* **218** 57–62.

Cheslock PS, Kemp BJ, Boumil RM & Dawson DS 2005 The roles of MAD1, MAD2 and MAD3 in meiotic progression and the segregation of nonexchange chromosomes. *Nature Genetics* **37** 756–760.

Chesnel F, Bazile F, Pascal A & Kubiak JZ 2006 Cyclin B dissociation from CDK1 precedes its degradation upon MPF inactivation in mitotic extracts of *Xenopus laevis* embryos. *Cell Cycle* **5** 1687–1698.

Chesnel F, Bazile F, Pascal A & Kubiak JZ 2007 Cyclin B2/cyclin-dependent kinase1 dissociation precedes CDK1 Thr-161 dephosphorylation upon M-phase promoting factor inactivation in *Xenopus laevis* cell-free extract. *International Journal of Developmental Biology* **51** 297–305.

Ciemerych MA 1995 Chromatin condensation activity and cortical activity during the first three cell cycles of a mouse embryo. *Molecular Reproduction and Development* **41** 416–424.

Ciemerych MA & Kubiak JZ 1998 Cytostatic activity develops during meiosis I in oocytes of LT/Sv mice. *Developmental Biology* **200** 198–211.

Ciemerych MA, Tarkowski AK & Kubiak JZ 1998 Autonomous activation of histone H1 kinase, cortical activity and microtubule organization in one- and two-cell mouse embryos. *Biology of the Cell* **90** 557–564.

Ciemerych MA, Maro B & Kubiak JZ 1999 Control of duration of the first two mitoses in a mouse embryo. *Zygote* **7** 293–300.

Cuthbertson KS 1983 Parthenogenetic activation of mouse oocytes *in vitro* with ethanol and benzyl alcohol. *Journal of Experimental Zoology* **226** 311–314.

Dobles M, Liberal V, Scott ML, Benezra R & Sorger PK 2000 Chromosome missegregation and apoptosis in mice lacking the mitotic checkpoint protein Mad2. *Cell* **101** 635–645.

Draetta G, Luca F, Westendorf J, Brizuela L, Ruderman J & Beach D 1989 Cdc2 protein kinase is complexed with both cyclin A and B: evidence for proteolytic inactivation of MPF. *Cell* **56** 829–838.

Eppig JJ 1978*a* Developmental potential of LT/Sv parthenotes derived from oocytes matured *in vivo* and *in vitro*. *Developmental Biology* **65** 244–249.

Eppig JJ 1978*b* Granulosa cell deficient follicles: occurrence, structure, and relationship to ovarian teratocarcinogenesis in strain LT/Sv mice. *Differentiation* **12** 111–120.

Eppig JJ, Wigglesworth K, Varnum DS & Nadeau JH 1996 Genetic regulation of traits essential for spontaneous ovarian teratocarcinogenesis in strain LT/Sv mice: aberrant meiotic cell cycle, oocyte activation, and parthenogenetic development. *Cancer Research* **56** 5047–5054.

Fang G, Yu H & Kirschner MW 1998 The checkpoint protein MAD2 and the mitotic regulator CDC20 form a ternary complex with the anaphase-promoting complex to control anaphase initiation. *Genes and Development* **12** 1871–1883.

Golas A, Dzieza A, Kuzniarz K & Styrna J 2008 Gene mapping of sperm quality parameters in recombinant inbred strains of mice. *International Journal of Developmental Biology* **52** 287–293.

Greda P, Karasiewicz J & Modlinski JA 2006 Mouse zygotes as recipients in embryo cloning. *Reproduction* **132** 741–748.

Guimaraes GJ, Dong Y, McEwen BF & DeLuca JG 2008 Kinetochore–microtubule attachment relies on the disordered N-terminal tail domain of Hec1. *Current Biology* **18** 1778–1784.

Downloaded from Bioscientifica.com at 10/31/2020 03:43:11AM
via free access

Hampl A & Eppig JJ 1995 Analysis of the mechanism(s) of metaphase I arrest in maturing mouse oocytes. *Development* **121** 925–933.

Henery CC & Kaufman MH 1992 Cleavage rate of diandric triploid mouse embryos during the preimplantation period. *Molecular Reproduction and Development* **32** 251–258.

Henery CC & Kaufman MH 1993*a* Cellular and nuclear volume of primitive red blood cells in digynic and diandric triploid and control diploid mouse embryos. *European Journal of Morphology* **31** 237–249.

Henery CC & Kaufman MH 1993*b* The cleavage rate of digynic triploid mouse embryos during the preimplantation period. *Molecular Reproduction and Development* **34** 272–279.

Hirao Y & Eppig JJ 1997 Analysis of the mechanism(s) of metaphase I arrest in strain LT mouse oocytes: participation of MOS. *Development* **124** 5107–5113.

Hoffmann S, Tsurumi C, Kubiak JZ & Polanski Z 2006 Germinal vesicle material drives meiotic cell cycle of mouse oocyte through the 3′UTR-dependent control of cyclin B1 synthesis. *Developmental Biology* **292** 46–54.

Holloway SL, Glotzer M, King RW & Murray AW 1993 Anaphase is initiated by proteolysis rather than by the inactivation of maturation-promoting factor. *Cell* **73** 1393–1402.

Homer HA, McDougall A, Levasseur M, Yallop K, Murdoch AP & Herbert M 2005 Mad2 prevents aneuploidy and premature proteolysis of cyclin B and securin during meiosis I in mouse oocytes. *Genes and Development* **19** 202–207.

Hupalowska A, Kalaszczynska I, Hoffmann S, Tsurumi C, Kubiak JZ, Polanski Z & Ciemerych MA 2008 Metaphase I arrest in LT/Sv mouse oocytes involves the spindle assembly checkpoint. *Biology of Reproduction* **79** 1102–1110.

Jeganathan KB & van Deursen JM 2006 Differential mitotic checkpoint protein requirements in somatic and germ cells. *Biochemical Society Transactions* **34** 583–586.

Kalab P, Kubiak JZ, Verlhac MH, Colledge WH & Maro B 1996 Activation of p90rsk during meiotic maturation and first mitosis in mouse oocytes and eggs: MAP kinase-independent and -dependent activation. *Development* **122** 1957–1964.

Kalitsis P, Earle E, Fowler KJ & Choo KH 2000 Bub3 gene disruption in mice reveals essential mitotic spindle checkpoint function during early embryogenesis. *Genes and Development* **14** 2277–2282.

Kallio M, Eriksson JE & Gorbsky GJ 2000 Differences in spindle association of the mitotic checkpoint protein Mad2 in mammalian spermatogenesis and oogenesis. *Developmental Biology* **225** 112–123.

Kaufman MH & Howlett SK 1986 The ovulation and activation of primary and secondary oocytes in LT/Sv strain mice. *Gamete Research* **14** 255–264.

Kaufman MH & Speirs S 1987 The postimplantation development of spontaneous digynic triploid embryos in LT/Sv strain mice. *Development* **101** 383–391.

Kubiak JZ & Ciemerych MA 2001 Cell cycle regulation in early mouse embryos. In *The Cell Cycle and Development. Novartis Foundation Sysmposium 237*, pp 79–89. (discussion 89–99). Eds G Bock, G Cardew & JA Goode. Chichester: John Wiley & Sons Ltd.

Kubiak JZ, Bazile F, Pascal A, Richard-Parpaillon L, Polanski Z, Ciemerych MA & Chesnel F 2008*a* Temporal regulation of embryonic M-phases. *Folia Histochemica et Cytobiologica* **46** 5–9.

Kubiak JZ, Chesnel F, Richard-Parpaillon L, Bazile F, Pascal A, Polanski Z, Sikora-Polaczek M, Maciejewska Z & Ciemerych MA 2008*b* Temporal regulation of the first mitosis in *Xenopus* and mouse embryos. *Molecular and Cellular Endocrinology* **282** 63–69.

Kubiak JZ, Ciemerych MA, Hupalowska A, Sikora-Polaczek M & Polanski Z 2008*c* On the transition from the meiotic to mitotic cell cycle during early mouse development. *International Journal of Developmental Biology* **52** 201–217.

Lacefield S & Murray AW 2007 The spindle checkpoint rescues the meiotic segregation of chromosomes whose crossovers are far from the centromere. *Nature Genetics* **39** 1273–1277.

Lee GH, Bugni JM, Obata M, Nishimori H, Ogawa K & Drinkwater NR 1997 Genetic dissection of susceptibility to murine ovarian teratomas that originate from parthenogenetic oocytes. *Cancer Research* **57** 590–593.

LeMaire-Adkins R & Hunt PA 2000 Nonrandom segregation of the mouse univalent X chromosome: evidence of spindle-mediated meiotic drive. *Genetics* **156** 775–783.

LeMaire-Adkins R, Radke K & Hunt PA 1997 Lack of checkpoint control at the metaphase/anaphase transition: a mechanism of meiotic nondisjunction in mammalian females. *Journal of Cell Biology* **139** 1611–1619.

Li Y & Benezra R 1996 Identification of a human mitotic checkpoint gene: hsMAD2. *Science* **274** 246–248.

Ma J, Svoboda P, Schultz RM & Stein P 2001 Regulation of zygotic gene activation in the preimplantation mouse embryo: global activation and repression of gene expression. *Biology of Reproduction* **64** 1713–1721.

Maleszewski M & Yanagimachi H 1995 Spontaneous and sperm-induced activation of oocytes in LT/Sv strain mice. *Development, Growth and Differentiation* **37** 679–685.

Marangos P & Carroll J 2008 Securin regulates entry into M-phase by modulating the stability of cyclin B. *Nature Cell Biology* **10** 445–451.

Martin-Lluesma S, Stucke VM & Nigg EA 2002 Role of Hec1 in spindle checkpoint signaling and kinetochore recruitment of Mad1/Mad2. *Science* **297** 2267–2270.

Michel LS, Liberal V, Chatterjee A, Kirchwegger R, Pasche B, Gerald W, Dobles M, Sorger PK, Murty VV & Benezra R 2001 MAD2 haplo-insufficiency causes premature anaphase and chromosome instability in mammalian cells. *Nature* **409** 355–359.

Mohammed AA, Karasiewicz J & Modlinski JA 2008 Developmental potential of selectively enucleated immature mouse oocytes upon nuclear transfer. *Molecular Reproduction and Development* **75** 1269–1280.

Musacchio A & Salmon ED 2007 The spindle-assembly checkpoint in space and time. *Nature Reviews. Molecular Cell Biology* **8** 379–393.

Niault T, Hached K, Sotillo R, Sorger PK, Maro B, Benezra R & Wassmann K 2007 Changing mad2 levels affects chromosome segregation and spindle assembly checkpoint control in female mouse meiosis I. *PLoS ONE* **2** e1165.

Niemierko A 1975 Induction of triploidy in the mouse by cytochalasin B. *Journal of Embryology and Experimental Morphology* **34** 279–289.

Nilsson J, Yekezare M, Minshull J & Pines J 2008 The APC/C maintains the spindle assembly checkpoint by targeting Cdc20 for destruction. *Nature Cell Biology* **10** 1411–1420.

Oh B, Hwang S, McLaughlin J, Solter D & Knowles BB 2000 Timely translation during the mouse oocyte-to-embryo transition. *Development* **127** 3795–3803.

O'Neill GT & Kaufman MH 1987 Ovulation and fertilization of primary and secondary oocytes in LT/Sv strain mice. *Gamete Research* **18** 27–36.

Pesin JA & Orr-Weaver TL 2008 Regulation of APC/C activators in mitosis and meiosis. *Annual Review of Cell and Developmental Biology* **24** 475–499.

Polanski Z 1997 Strain difference in the timing of meiosis resumption in mouse oocytes: involvement of a cytoplasmic factor(s) acting presumably upstream of the dephosphorylation of p34cdc2 kinase. *Zygote* **5** 105–109.

Polanski Z, Hoffmann S & Tsurumi C 2005 Oocyte nucleus controls progression through meiotic maturation. *Developmental Biology* **281** 184–195.

Ram PT & Schultz RM 1993 Reporter gene expression in G2 of the 1-cell mouse embryo. *Developmental Biology* **156** 552–556.

Rieder CL & Palazzo RE 1992 Colcemid and the mitotic cycle. *Journal of Cell Science* **102** 387–392.

Rieder CL, Schultz A, Cole R & Sluder G 1994 Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitors sister kinetochore attachment to the spindle. *Journal of Cell Biology* **127** 1301–1310.

Sikora-Polaczek M, Hupalowska A, Polanski Z, Kubiak JZ & Ciemerych MA 2006 The first mitosis of the mouse embryo is prolonged by transitional metaphase arrest. *Biology of Reproduction* **74** 734–743.

Speirs S & Kaufman MH 1988 Effect of exogenous hormones on the ovulation of primary and secondary oocytes in LT/Sv strain mice. *Gamete Research* **21** 179–184.

Stevens LC & Varnum DS 1974 The development of teratomas from parthenogenetically activated ovarian mouse eggs. *Developmental Biology* **37** 369–380.

Downloaded from Bioscientifica.com at 10/31/2020 03:43:11AM
via free access

Taylor SS & McKeon F 1997 Kinetochore localization of murine Bub1 is required for normal mitotic timing and checkpoint response to spindle damage. *Cell* **89** 727–735.

Tepperberg JH, Moses MJ & Nath J 1999 Colchicine effects on meiosis in the male mouse. II. Inhibition of synapsis and induction of nondisjunction. *Mutation Research* **429** 93–105.

Tsurumi C, Hoffmann S, Geley S, Graeser R & Polanski Z 2004 The spindle assembly checkpoint is not essential for CSF arrest of mouse oocytes. *Journal of Cell Biology* **167** 1037–1050.

Uhlmann F 2003 Chromosome cohesion and separation: from men and molecules. *Current Biology* **13** R104–R114.

Waksmundzka M, Krysiak E, Karasiewicz J, Czolowska R & Tarkowski AK 1984 Autonomous cortical activity in mouse eggs controlled by a cytoplasmic clock. *Journal of Embryology and Experimental Morphology* **79** 77–96.

Wang QT, Piotrowska K, Ciemerych MA, Milenkovic L, Scott MP, Davis RW & Zernicka-Goetz M 2004 A genome-wide study of gene activity reveals developmental signaling pathways in the preimplantation mouse embryo. *Developmental Cell* **6** 133–144.

Wassmann K, Liberal V & Benezra R 2003 Mad2 phosphorylation regulates its association with Mad1 and the APC/C. *EMBO Journal* **22** 797–806.

Winston NJ & Maro B 1995 Calmodulin-dependent protein kinase II is activated transiently in ethanol-stimulated mouse oocytes. *Developmental Biology* **170** 350–352.

Yamamoto A, Kitamura K, Hihara D, Hirose Y, Katsuyama S & Hiraoka Y 2008 Spindle checkpoint activation at meiosis I advances anaphase II onset via meiosis-specific APC/C regulation. *Journal of Cell Biology* **182** 277–288.

Yu HG, Muszynski MG & Kelly Dawe R 1999 The maize homologue of the cell cycle checkpoint protein MAD2 reveals kinetochore substructure and contrasting mitotic and meiotic localization patterns. *Journal of Cell Biology* **145** 425–435.

Received 16 January 2009
First decision 9 February 2009
Accepted 11 March 2009

Downloaded from Bioscientifica.com at 10/31/2020 03:43:11AM
via free access