

Mouse polo-like kinase 1 associates with the acentriolar spindle poles, meiotic chromosomes and spindle midzone during oocyte maturation

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Abstract. We have examined the dynamics of the localisation of the polo-like kinase 1 (Plk1) during maturation of the mouse oocyte. Levels of Plk1 protein increase following germinal vesicle breakdown, at which time the enzyme begins to accumulate at discrete positions on the condensing chromosomes and, subsequently, at the poles of the meiotic spindle, which moves towards the cortex of the egg. Interestingly, at metaphase in both meiotic divisions, Plk1 shows a punctate localisation along the broad spindle poles. Moreover, the punctate distribution of Plk1 on the meiotic chromosomes appears at early anaphase to correspond to the centromeric regions. The protein relocates to the spindle midzone during late anaphase and then associates with the midbody at telophase. We have confirmed the specific pattern of immuno-localisation seen in fixed preparations by observing the distribution of Plk1 tagged with green fluorescent protein in living oocytes. We discuss the localisation of the enzyme in light of the structure of the spindle poles, which are known to lack centrioles, and the highly asymmetric nature of the meiotic divisions.

Introduction

Maturation of the mammalian oocyte follows a temporally regulated programme of events in which spatial co-ordination of the positioning of the spindle and cleavage furrow lead to asymmetric cell division. The resumption of meiotic maturation is manifest by the breakdown of the germinal vesicle (GVBD) followed by chromosome condensation. This is accompanied by replacement of the interphase network of long microtubules by relatively short microtubules that radiate from the condensed chromatin. It leads to the formation of the first meiotic spindle, which, between 5 and 8 h after GVBD, migrates

to the cortex of the oocyte. p34^{cdc2} levels rise at the onset of GVBD, followed by an increase in MAP kinase activity a few hours later at the time of spindle formation (Verhac et al. 1994; Zernicka-Goetz et al. 1997a). The first meiotic division results in the extrusion of the first polar body, and is immediately followed by entry into the second division and arrest at metaphase II. Apart from a transient reduction in the level of p34^{cdc2} activity between the two meiotic divisions, the activity of both p34^{cdc2} and MAP kinases remains high. p34^{cdc2} activity is maintained at high levels during metaphase II arrest as a result of the equilibrium between cyclin B synthesis and degradation achieved by cytostatic factor (CSF). This requires the activity of *mos*, as the activator of the MAP kinase pathway (Colledge et al. 1994; Araki et al. 1996; Verhac et al. 1996). The relationship of these events to other aspects of meiotic regulation is poorly understood. Recently a new family of serine-threonine protein kinases, the polo-like kinases (plks), has emerged that appears to function at multiple steps in mitotic progression. The spatial and temporal separation of several specific phases of the meiotic cycle makes the maturing mouse oocyte an attractive model in which to study the role of murine Plk1 in meiotic progression.

The plks from yeasts, insects, amphibians, and mammals share a similar overall structure, exhibiting a high degree of sequence conservation both in the N-terminal catalytic kinase domain and in the non-catalytic C-terminal, suggesting that these molecules have a close evolutionary and, thereby, functional relationship (for reviews, see Glover et al. 1996; Lane and Nigg 1997). The plks appear to be required at several points during the progression through mitosis. A role in the activation of the major mitotic kinase p34^{cdc2} has been suggested by the finding that the *Xenopus* homologue, Plx1, can associate with and phosphorylate cdc25C. This enhances the cdc25C phosphatase activity required for p34^{cdc2} activation (Kumagai and Dunphy 1996; Abrieu et al. 1998; Qian et al. 1998). Consistently, Plx1 is activated slightly ahead of p34^{cdc2} both in mitosis and meiosis (Qian et al. 1998).

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In contrast, genetic studies with *Drosophila* and fission yeast have rather emphasised a role for plk activity in centrosome assembly and separation to form the bipolar spindle (Sunkel and Glover 1988; Ohkura et al. 1995). Moreover, microinjection of an antibody to human Plk1 blocks HeLa cells in a pseudomitotic state with monopolar spindles (Lane and Nigg 1996). Mutation of the gene for the budding yeast homologue Cdc5p, on the other hand, leads to arrest at late nuclear division. This appears to be due to a failure to activate the anaphase promoting complex (APC; Charles et al. 1998; Shirayama et al. 1998). This is consistent with finding that some APC targets are not proteolytically degraded in the absence of Plx1 function (Descombes and Nigg 1998), and that mouse Plk1 can directly phosphorylate APC components (Kotani et al. 1998). An additional consequence of disruption of the *plol* gene of fission yeast is a failure to establish the actin ring and septum prior to cytokinesis (Ohkura et al. 1995). A requirement for polo kinase to permit cytokinesis in *Drosophila* spermatogenesis has also been recently demonstrated (Carmena et al. 1998). The immunolocalisation of the plks in mitotically dividing cells is consistent with the enzyme having a role in the duplication or separation of the spindle poles, and cytokinesis. In budding yeast, fission yeast, insects, and mammals, plks have been found associated with the spindle poles (Adams et al. 1998; Shirayama et al. 1998; Mulvihill et al. in preparation; Golsteyn et al. 1995; Lee et al. 1995). Moreover at anaphase, the plks of animal cells relocate to the spindle midzone, a structure required for cytokinesis (Adams et al. 1995; Golsteyn et al. 1995).

Meiotic roles for plks are, however, inferred from the mutant phenotypes of the genes in *Saccharomyces cerevisiae* (Schild and Byers 1980; Sharon and Simchen 1990) and in *Drosophila* (Sunkel and Glover 1988; Carmena et al. 1998; Herrmann et al. 1998). *S. cerevisiae* *cdc5* mutants are defective in both meiosis I and meiosis II: a complete spindle does not form during the first meiotic division, which can lack interconnecting spindle microtubules, and cells are unable to complete meiosis II. *Drosophila* *polo1* mutants show defective cytokinesis throughout spermatogenesis, but display non-disjunction primarily in the second meiotic division (Sunkel and Glover 1988; Carmena et al. 1998; Herrmann et al. 1998). Furthermore, molecular studies indicate biochemical roles for Plx1 during progression through meiosis in *Xenopus* (Abrieu et al. 1998; Descombes and Nigg 1998; Qian et al. 1998), although the subcellular distribution of Plx1 in *Xenopus* oocytes has never been examined. It is intriguing to know how plks are localised in the highly asymmetric cell divisions of vertebrate meiosis. We wished to determine whether mouse plk localises to the centrosomes in the early phases of the meiotic divisions and to the midbody region at the time of cytokinesis as it does in cultured mammalian cells. These questions are of interest not only in relation to the asymmetry of the cell, which is set up following migration of the spindle to the cortex of the oocyte during the first prophase, but also because the spindle poles are nucleated by acentriolar microtubule organising centres (MTOCs).

Materials and methods

Collection and culture of oocytes. Germinal vesicle (GV) stage oocytes were collected from ovaries of 4 to 6 week old MF1 mice in FHM medium (Speciality media, Lavallete, N.J.), containing 1 mg/ml of bovine serum albumin (BSA) (FHM+BSA). After removal of follicular cells, they were cultured in KSOM medium (Speciality media, Lavallete, N.J.), containing 1 mg/ml of BSA (KSOM+BSA), and supplemented with amino acids, under paraffin oil, at 37°C with 5% CO₂ in air. Only those oocytes that underwent GVBD during the first hour of culture were used. Samples of oocytes were then collected at various time after GVBD.

To obtain metaphase II oocytes, mice were superovulated by intraperitoneal injections of 5 i.u. pregnant mare's serum gonadotrophin (PSMF) and human chorionic gonadotrophin (hCG) 46 h apart. Eggs were retrieved from the ampullae at 15–16 h post-hCG into FHM+BSA. The cumulus cells were dispersed by brief exposure to 0.1 M hyaluronidase (Sigma).

Oocyte fixation and immunocytochemical staining. The zona pellucidae were removed with brief exposure to acid Tyrode's solution. Zona-free oocytes were placed in a specially designed chamber (Maro et al. 1984), coated with 0.1% poly-L-lysine (Sigma). They were fixed in 0.1% glutaraldehyde (Sigma) in PBS containing 1% Triton X-100 (Sigma). The samples were extracted with 2% Triton X-100 for 30 min at ambient temperature, incubated for three periods of 10 min in NaBH₄ (Sigma) in PBS and processed for immunofluorescence. Tubulin was visualised using rat monoclonal antibody to tyrosinated α -tubulin (YL1/2), and Texas Red-conjugated goat anti-rat antibody (Jackson ImmunoResearch Laboratories, West Grove, Pa., USA). Plk localisation was visualised using anti-HN185 antibody, and Alexa 488-conjugated goat anti-rabbit antibody (Molecular Probes, Leiden, The Netherlands). Chromatin was stained with 10 μ M TOTO-3 (Molecular Probes), in PBS containing 0.1% Tween-20 (Sigma). Samples were observed using a Bio-Rad MRC-1024 laser scanning confocal microscope.

Microinjection of MmGFP-plk1 mRNA. mRNA encoding for MmGFP-Plk1 was generated by in vitro transcription from the Sp6 promoter using a transcription kit (Ambion, Austin, Tex., USA), according to the manufacturer's instructions.

Oocytes were collected at the GV stage in FHM+BSA medium. Oocytes were observed on an inverted phase contrast microscope for microinjection. They were placed in a drop of FHM+BSA medium, in the centre of a depression slide and covered with paraffin oil. They were immobilised by applying a suction to a heat-polished holding pipette via a microinjector (Narashige) filled with heavy paraffin oil. Injection pipettes were made from capillaries with an internal filament (Clark Biomedical Supplies, UK) pulled on an automatic micropipette puller (Sutter, USA). Improved membrane penetration was achieved by using negative capacitance, and microinjections were performed using a constant flow system (Transjector, Eppendorf) as described by Zernicka-Goetz and Pines (1998). MmGFP-plk mRNA at a concentration of 1 mg/ml was microinjected into the cytoplasm. After microinjection, oocytes were cultured in KSOM+BSA medium, at 37°C with 5% CO₂ in air, and observed at various time after GVBD using a laser confocal microscope.

The configuration of the optical system on the confocal microscope was optimised for the recording of fluorescent images. The spindle and chromosomes of the oocytes, which can be discerned upon the recorded transmitted images presented in Figs. 6–8, were much clearer by direct observation. The positions of the spindle poles and chromosomes in relation to green fluorescent protein (GFP) fluorescence were confirmed by observation using DIC optics in conjunction with a CCD camera from Princeton Instruments (data not shown).

Generation and affinity purification of polyclonal antibodies: immunoblot analysis. DNA encoding a polypeptide corresponding to the internal 250 amino acids (residues 182–432) of *Xenopus* Plx1 was cloned into the expression vector pET23 A for expression in *Escher-*

ichia coli. The bacterially expressed polypeptide, HN185, was used to raise the rabbit antiserum anti-HN185. Anti-HN185 antibodies were purified as previously described (Harlow and Lane 1988) by affinity chromatography on a column of Sepharose CL-4B (Pharmacia) coupled to recombinant HN185 polypeptide purified from bacterial extracts. The purified antibodies were concentrated using the Ultrafree concentration system (Millipore).

For immunoblot analysis, samples were subjected to SDS-polyacrylamide gel electrophoresis and proteins were transferred to an Immobilon-P membrane. Membranes were preincubated in TBST buffer (20 mM TRIS-HCl, pH 8.2, 150 mM NaCl, 0.05% Tween 20) containing 10% (w/v) non-fat dried milk for 1 h to block non-specific binding of antibodies. They were then incubated with the anti-HN185 antibody in TBST containing 1% (w/v) dried milk for 1 h, washed, incubated with the peroxidase-conjugated secondary antibody for 1 h, and washed again in TBST. The procedure was carried out at ambient temperature. The secondary antibody was detected by enhanced chemiluminescence (Amersham).

Preparation of recombinant MmGFP-Plk1. For constructing a GFP-tagged Plk1 chimaera, the vector pMmGFP/RN3P (Zernicka-Goetz et al. 1996) was modified by the insertion in the Fok I restriction site (at the stop codon of the GFP gene) of the paired primers AATACGCTC-GAGTCGACGGGCCCAAGCTTAGGCCTGACTGACT and TAT TAGTCAGTCAGGCCTAAGCTTGGGCCCGTCGACTCGAGCG, creating a multicloning site (with XhoI, SalI, ApaI, HindIII, and StuI sites, followed by stop codons in the three reading frames). The mouse Plk1 cDNA (lacking DNA encoding the first 10 and last 2 amino acids) was then inserted into the XhoI–StuI sites. The ability of the construct to direct the synthesis of a protein of the correct size of the GFP-Plk1 chimaera in vitro was confirmed using the coupled transcription-translation TNT kit (Promega).

Results

We have used two approaches to follow the pattern of Plk1 distribution in relation to the changes that accompany the maturation of the mouse oocyte: immunostaining of fixed preparations, and direct observations of a GFP-tagged molecule in living oocytes. In the immunolocalisation studies, we used the anti-HN185 antibody, which specifically recognises a single M_r 71,000 protein in cultured mouse (3T3) cells (Fig. 1A, lane 1). Coupled in vitro transcription and translation of a cDNA encoding murine Plk1 produces a single co-migrating polypeptide that is also recognised by the anti-HN185 antibody (Fig. 1A, lane 3). When used to immunostain 3T3 cells, the antibody decorates centrosomes early in mitosis, and the central spindle late in mitosis in a similar pattern to that previously described in cultured mammalian cells (Golsteyn et al. 1995; Lee et al. 1995; our data, not shown). These observations, together with the correlation that we report below between the pattern of localisation observed by immunostaining or direct observation of GFP-tagged mouse Plk1, indicate that the M_r 71,000 molecular species recognised by the antibody is the mouse Plk1 protein. Plk1 is present in GV stage oocytes, but its level increases during the 5–8 h following GVBD (Fig. 1B).

The time at which the concentration of Plk1 increases correlates with the onset of formation and migration of the meiotic spindle to the cortex of the oocyte. We therefore attempted to determine whether there was any redistribution of Plk1 in the oocyte over this time period. We found that neither in GV stage oocytes (Fig. 2A) nor dur-

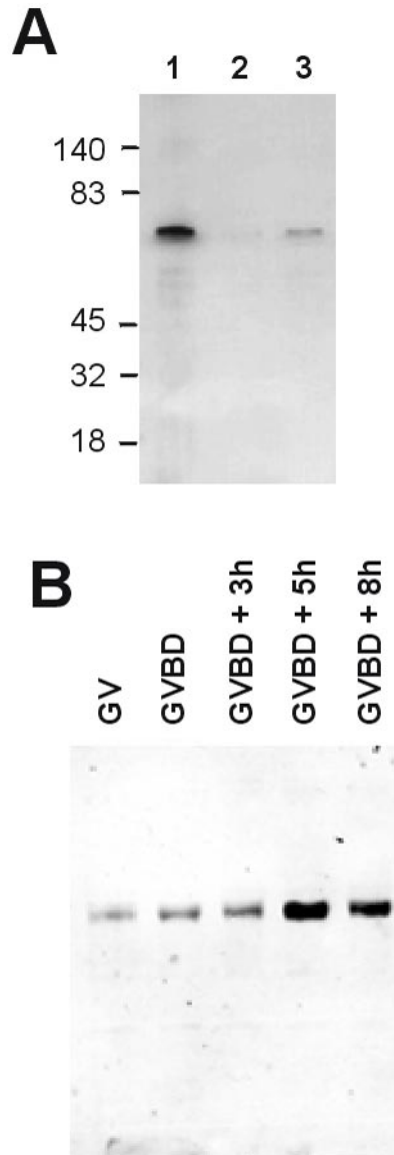


Fig. 1A, B. Characterization of the anti-HN185 antibody. **A** Immunoblot of mouse polo-like kinase 1 (Plk1). A 3T3 cell lysate was prepared for SDS-polyacrylamide gel electrophoresis (lane 1). An aliquot of the lysate was immunodepleted with excess HN185 antibody bound to protein A beads prior to SDS-polyacrylamide gel electrophoresis (lane 2). The product of the coupled in vitro transcription and translation of plasmid SPTK13 (containing the mouse Plk1 cDNA) was loaded onto lane 3. The separated proteins were transferred to Immobilon-P and probed with the anti-HN185 antibody. Molecular mass standards are indicated in kilodaltons. **B** Extracts of mouse oocytes, collected at different times after germinal vesicle breakdown (GVBD), were separated by SDS-polyacrylamide gel electrophoresis and, after transfer to Immobilon-P, probed with anti-HN185. Extracts of 40 oocytes were used in each lane

ing GVBD (Fig. 2B) is there a specific pattern of localisation of Plk1 in relation to microtubules or chromatin. Rather, the Plk1 seems to be distributed throughout the cytoplasm of the oocyte. However, a few hours after GVBD, when individual meiotic chromosomes become discernible, we begin to see the accumulation of Plk1 at distinct regions, but not along the entire length of the chromosomes (Fig. 3).

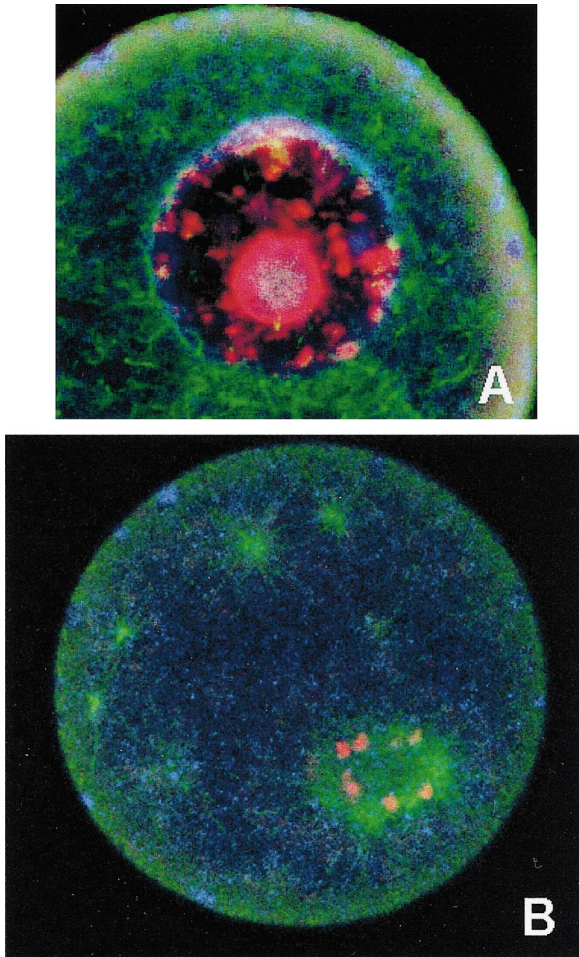


Fig. 2A, B. Immunolocalisation of Plk1 in GV (A) or GVBD (B) stage oocytes. Plk1 (blue) shows no specific pattern of localisation with respect to either chromatin (red) or microtubules (green)

When the spindle was fully formed and had become repositioned at the cortex of the oocyte, Plk1 was evident not only in a punctate pattern associated with chromosomes (Fig. 4A, arrows), but also at the spindle poles (arrowheads). A similar distribution of the enzyme was seen at metaphase of both the first and second meiotic divisions. There was no single concentrated focus of

Plk1 at the spindle poles in either metaphase or anaphase, but rather the enzyme appeared to be arranged in a string of foci along the broad poles. Astral microtubules are rarely seen associated with these meiotic spindles. However, when these were observed, they appear to emanate from larger MTOCs that also show an accumulation of Plk1 staining. Such a large focus with associated astral microtubules can be seen on the uppermost pole of the anaphase figure in Fig. 4B. The staining pattern of Plk1 early in anaphase I (Fig. 4B) suggests that its punctate distribution on the chromosomes is in the centromeric regions. The figure displayed in this panel is in early anaphase I; the chromosomes (red) appear pulled towards the spindle poles with Plk1 (blue) apparently present on the leading edge of each chromosome (arrows).

At late anaphase of meiosis I, Plk1 was found neither at the spindle poles nor associated with the chromosomes, but was associated with the midzone region of the spindle. At telophase, when the polar body was being extruded, Plk1 could be observed predominantly in the midbody (blue staining in Fig. 5).

To gain some insight into the pattern of distribution of Plk1 in living oocytes, we followed the distribution of the enzyme tagged with GFP. In our studies we used MmGFP, a form of GFP specifically developed for expression in the early mouse embryo, and which shows approximately 50-fold higher fluorescence than wild-type GFP in mammalian cells at 37°C (Zernicka-Goetz et al. 1996, 1997b). The chimaeric MmGFP-Plk1 gene was constructed in the vector pRN3P as it had been previously shown that synthetic mRNA transcribed from this plasmid appears to be efficiently expressed in blastomeres of the preimplantation mouse embryo (Zernicka-Goetz et al. 1997b). We injected synthetic mRNA encoding the MmGFP-Plk1 chimaera into oocytes at the GV stage, which were then allowed to mature in vitro before observation.

We found that by the time that the metaphase I spindle was positioned at the cortex of the oocyte, there was a low level of green fluorescence throughout the oocyte cytoplasm, and strong punctate fluorescence of MmGFP-Plk1 both at the spindle poles (Fig. 6, arrowheads), and on the chromosomes (arrow), structures that can be ob-

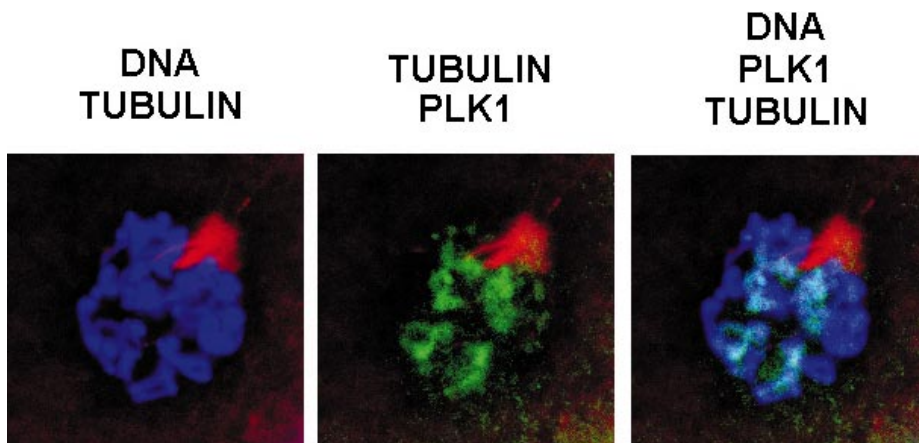


Fig. 3. Immunolocalisation of Plk1 on condensed chromosomes in a late GVBD stage oocyte. Plk1 (green) is associated with distinct foci on the newly condensed chromosomes (blue). Microtubules associated with the chromosomes are revealed by the red staining pattern

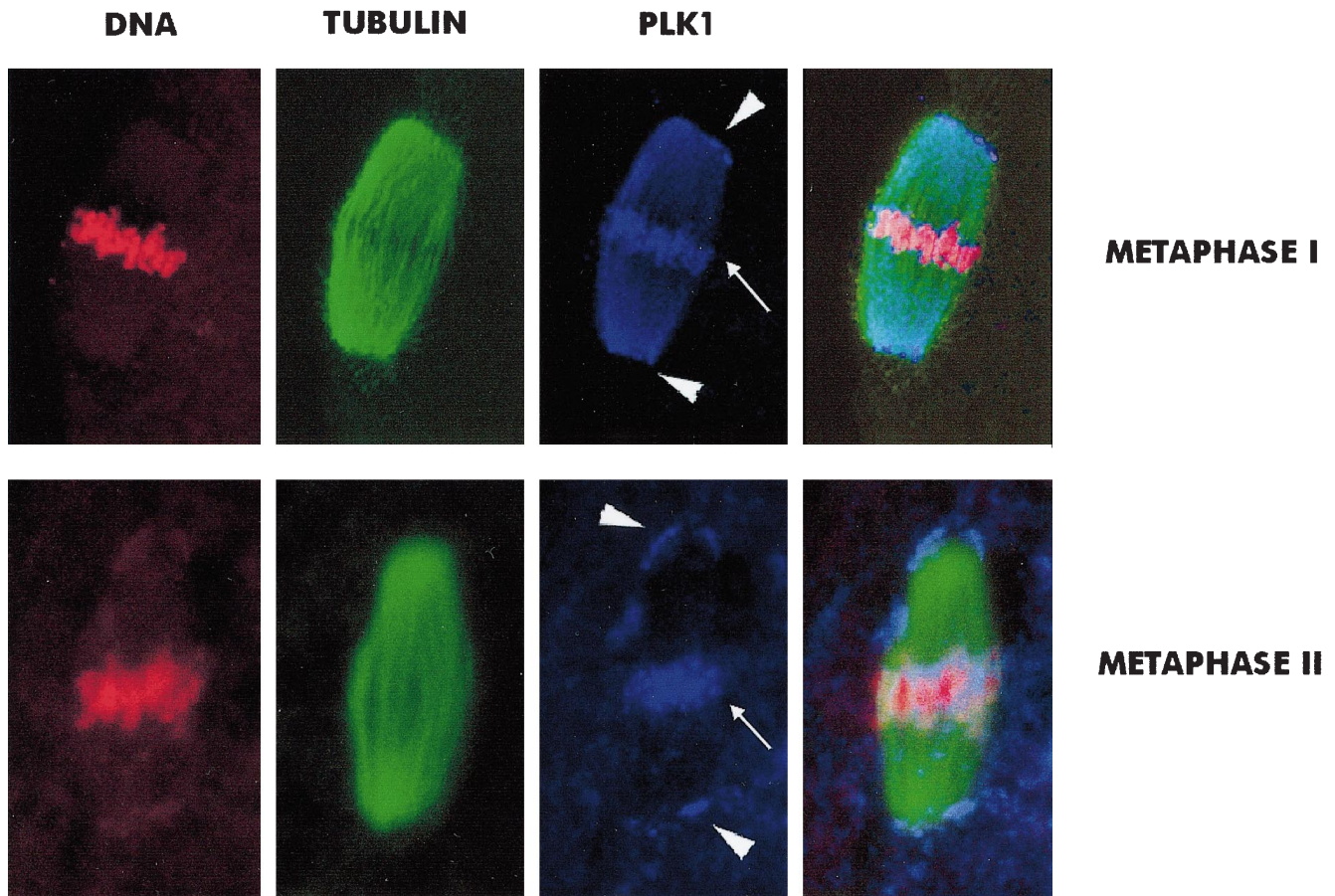
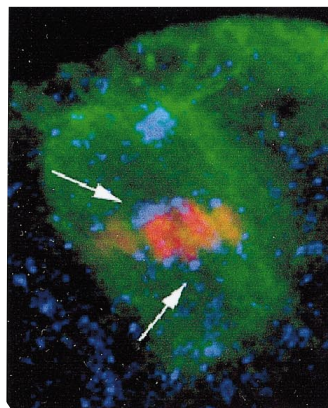
A**B**

Fig. 4A, B. Plk1 shows a punctate distribution at the polar microtubule-organising centres (*arrowheads*) and on the meiotic chromosomes (*arrows*) during metaphase and early anaphase. **A** Metaphase I and metaphase II oocytes triple stained to reveal Plk1 (*blue*), chromatin (*red*), and microtubules (*green*). **B** An oocyte in early anaphase (triple stained as in **A**), illustrating the punctuate distribution of Plk1 in the centromeric regions of the chromosomes

served by transmitted light (panels A and B). During cytokinesis of the first meiotic division (Fig. 7), strong MmGFP-Plk1 fluorescence was seen in the position of the midbody (arrows) at the junction of the oocyte with the extruded polar body. Finally, as oocytes arrest at metaphase II, Plk1 was once again seen in association with the spindle poles and chromosomes, showing punctate regions of green fluorescence (Fig. 8).

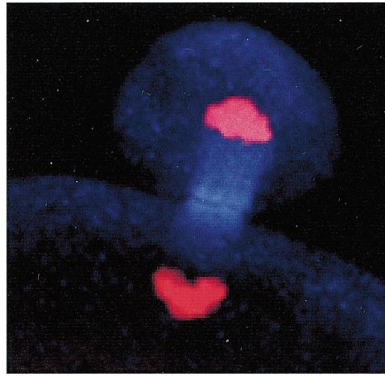
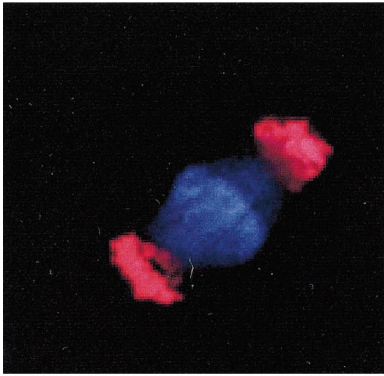
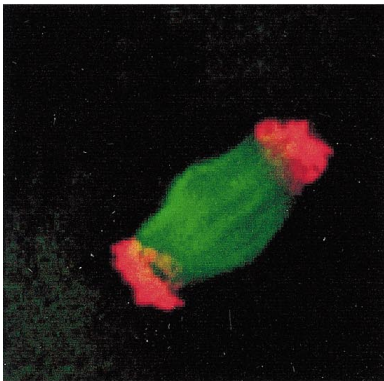
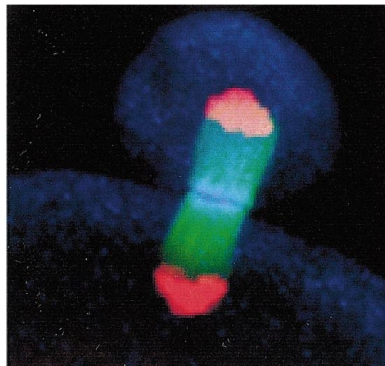
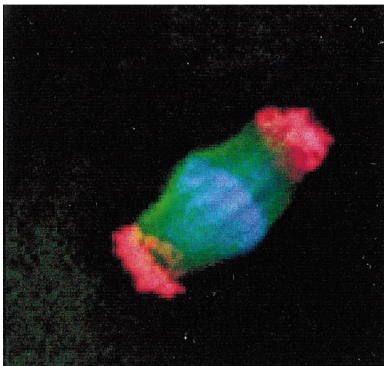
**LATE
ANAPHASE****TELOPHASE****DNA +
PLK1****DNA +
TUBULIN****DNA +
PLK1 +
TUBULIN**

Fig. 5. Plk1 accumulates in the spindle midzone in late anaphase-telophase. During late anaphase I, Plk1 (*blue*) accumulates in the spindle midzone, which becomes the midbody at telophase I. Chromatin is stained *red*, and the spindle microtubules, *green*

Discussion

Maturation of the mammalian oocyte occurs following a temporally regulated programme of events in which the spatial coordination of the positioning of the spindle and cleavage furrow are required to achieve the highly asymmetric divisions. Initiation of oocyte maturation is triggered by the rise in p34^{cdc2} levels that leads to GVBD. Formation of the first meiotic spindle is a prolonged process that correlates with activation of MAP kinase a few hours after p34^{cdc2} activation (Verhac et al. 1994; Zernicka-Goetz et al. 1997a). The first meiotic spindle forms in the centre of the oocyte and then, between 5 and 8 h after GVBD, it migrates to the oocyte surface. It is known from recent studies in *Xenopus* oocytes, that Plx1 can participate in the positive activation loop that

amplifies the onset of p34^{cdc2} kinase activity at the time of GVBD. However, although Plx1 can phosphorylate and thereby activate the p34^{cdc2}-activating cdc25C phosphatase, it is not clear whether it is the initiator of this process (Kumagai and Dunphy 1996; Abrieu et al. 1998; Qian et al. 1998). We have not addressed the activity of Plk1 in the mouse oocyte in the present study since, in contrast to *Xenopus*, it is extremely difficult to obtain sufficient numbers of synchronous oocytes for biochemical measurements. Our immuno blotting data indicate that levels of Plk1 protein increase well after the onset of GVBD. This of course does not argue against a role for Plk1 in activating Cdc25C and hence p34^{cdc2}, but it does suggest that a significantly higher concentration of enzyme is required later in meiosis. The transparency of the mouse oocyte gives considerable advantages over

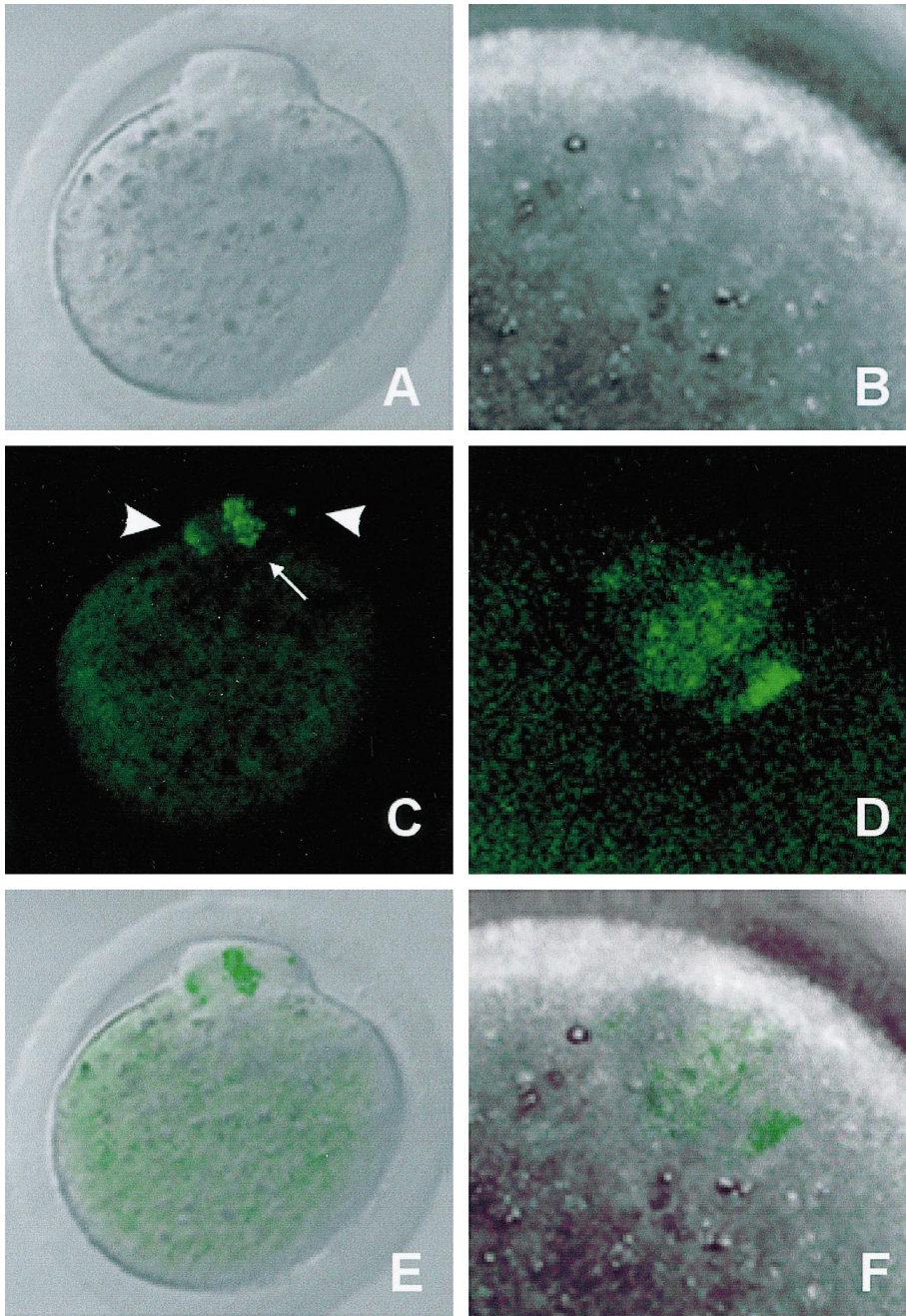


Fig. 6A–F. Green fluorescent protein-labelled Plk1 (MmGFP-Plk1) localises to the broad spindle poles (*arrowheads*) and punctate regions of the chromosomes (*arrow*) in the first meiotic metaphase. The transmitted light (**A, B**), projected confocal images of green fluorescence (**C, D**), and merged images (**E, F**) are shown from two oocytes that had been injected with MmGFP-Plk1 mRNA

the *Xenopus* oocyte for the study of the localisation of cell cycle regulatory proteins. It has enabled us to observe that newly synthesised Plk1 first associates with the meiotic chromosomes after GVBD, and then is transported to the cortex of the egg as a passenger upon the meiotic apparatus.

The pattern of localisation of Plk1 in these meiotic divisions is very similar to that observed during mitosis in cultured mammalian cells, but shows some significant features that reflect the specialised structure of the meiotic apparatus. Of these the most striking is the absence of centrioles at the spindle poles. As a consequence the spindle microtubules appear to be nucleated from a broad band rather than from a well focused centrosome as seen

in the tapered spindles of mitosis. Our studies show that Plk1 is distributed throughout the broad poles of the mouse meiotic spindle in tiny foci that, from the distribution of microtubules, appear to correspond to a string of small MTOCs. The enzyme is seen on the spindle pole bodies of the yeasts, and in animal cell centrosomes that either contain or lack centrioles (Golsteyn et al. 1995; Lee et al. 1995; Adams et al. 1998; Shirayama et al. 1998; Mulvihill et al. in preparation; our present data). Thus, irrespective of the highly diverged architecture of spindle poles in different organisms and cell types, the need to maintain the localisation of plks on this region of the spindle has been conserved. It is notable, however, that Plk1 does not appear to be present on all MTOCs. The cy-

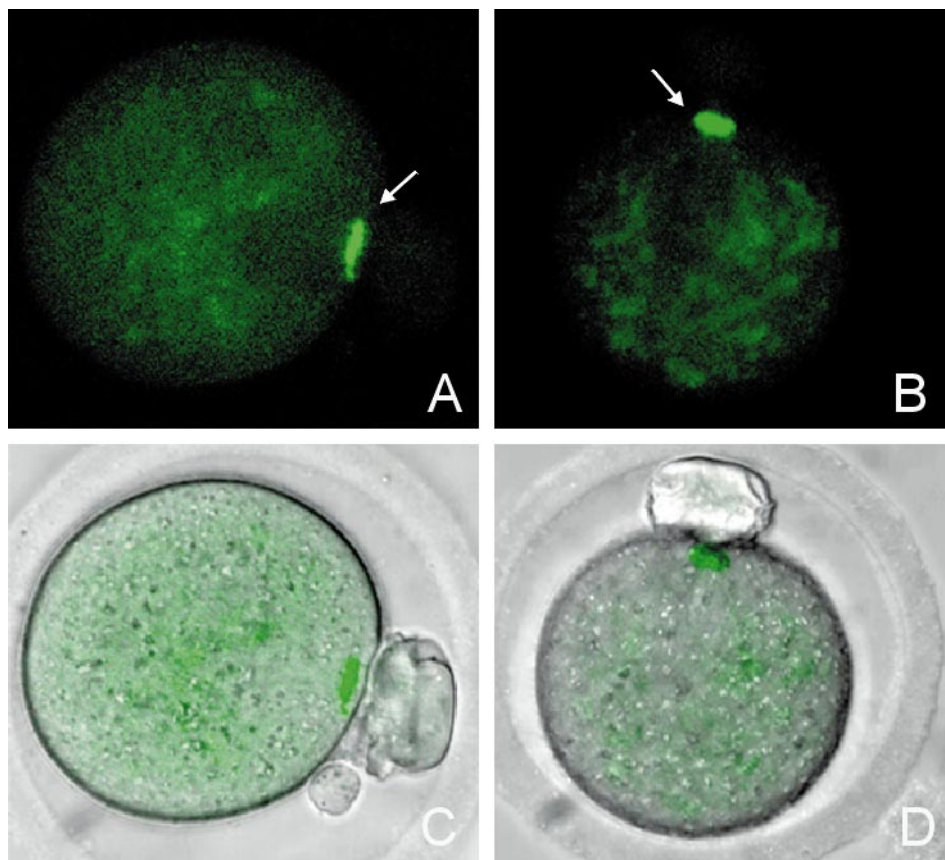
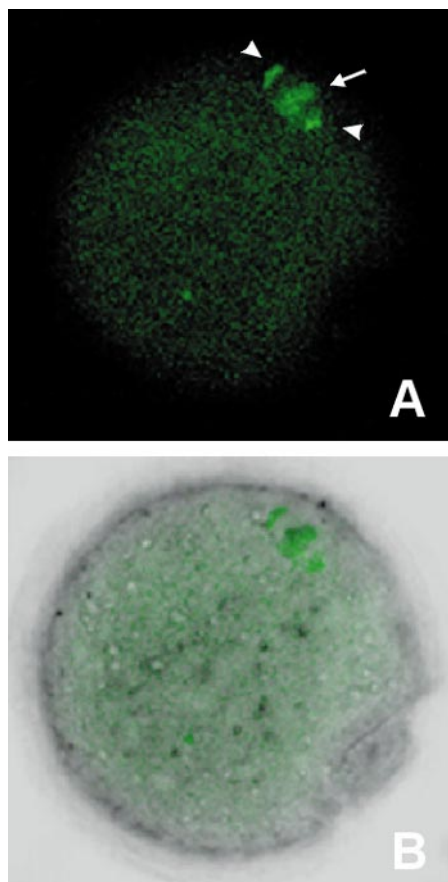


Fig. 7A–D. MmGFP-Plk1 is localised at the position of the midbody (*arrows*) at the time of polar body extrusion. Projected confocal images of green fluorescence are shown independently (**A**, **B**), and after merging with the corresponding transmitted light image (**C**, **D**) for two oocytes that had been injected with MmGFP-Plk1 mRNA



toplasm of the mouse GVBD oocyte contains a number of transient asters of organised microtubules quite distinct from the nascent meiotic spindle, examples of which can be seen in Fig. 2B. None of these shows any significant accumulation of Plk1, suggesting that the association of the enzyme with MTOCs is a specific feature of the poles of the spindle.

The redistribution of Plk1 prior to cytokinesis at the first meiotic division is similar to that seen to occur in other metazoan cells during mitosis. At late anaphase, it associates with the midzone region of the spindle, which resolves into the midbody at telophase-cytokinesis (Golsteyn et al. 1994, 1995; Lee et al. 1995). A number of observations indicate that the reorganisation of this region of the spindle during late mitosis is a necessary prerequisite for cytokinesis. Wheatly and Wang (1996) have shown that in cultured cells induced to have multi-polar spindles, the formation of the cleavage furrow absolutely depends upon the presence of central spindle microtubules. Furthermore, creation of a barrier between the central spindle and the cortex of cultured cells can prevent cleavage (Cao and Wang 1996). Indeed, in *Drosophila*,

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Fig. 8A, B. MmGFP-Plk1 localises to the broad spindle poles (*arrowheads*) and punctate regions of the chromosomes (*arrow*) in the second meiotic metaphase. A projected confocal image of green fluorescence is shown independently (**A**), and after merging with the corresponding transmitted light image (**B**) for an oocyte that had been injected with MmGFP-Plk1 mRNA

mutations that disrupt the structure of the midzone of the late spindle, including polo, lead to cytokinesis defects (for example, Williams et al. 1995; Adams et al. 1998; Giansanti et al. 1998; Carmena et al. submitted). Thus the plks appear to be localised in metazoan cells at sites appropriate for a function in cytokinesis.

It is interesting that Plk1 shows a similar pattern of localisation in metaphase I and metaphase II in spite of the fundamental differences in cellular physiology at these two divisions, and in the manner of chromosome segregation. After a number of hours, metaphase I is resolved by the completion of the division allowing the oocyte to become arrested in metaphase II with high levels of p34^{cdc2} until it is activated parthenogenetically or by fertilisation. Maintenance of the metaphase II arrest appears to require the establishment of an equilibrium between cyclin B synthesis and degradation, and is achieved by CSF. This requires the activity of mos, as the activator of the MAP kinase pathway. It will be of future interest to examine Plk1 activity in relation to these events.

The association of plks with chromosomes has received very little comment in published studies of the subcellular localisation of the protein during mitosis. The punctate nature and position of Plk1 we now describe on the meiotic chromosomes suggests that it is associated with the centromeric regions. This is entirely consistent with a contemporaneous study by Logarinho and Sunkel (1998), which shows that polo kinase contributes to the generation of MPM2-reactive epitopes on centromeres in *Drosophila* cells. The presence of the enzyme on this region of the chromosome during both meiotic divisions suggests that it is required for a general aspect of kinetochore function, rather than a process that distinguishes the segregation of homologues at the first meiotic division from the separation of sister chromatids at meiosis II. Although this could relate to the proposed involvement of plks inactivating the APC (see Introduction), we have at present no way of addressing this in the mouse oocyte.

The temporal regulation of meiosis and subsequent mitoses of the mouse egg together with its exquisite cytology make this an attractive system in which to study the role of Plk1 in the cell biology of these divisions. The approach we describe offers particular potential as a means of dissecting the functional domains that regulate the localisation and activity of Plk1 during these processes. Moreover the introduction of MmGFP-labelled proteins into the maturing mouse oocyte offers a new and potentially powerful way to observe the dynamic changes in the localisation of other regulatory molecules during meiosis.

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