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Homologue disjunction in mouse oocytes requires proteolysis of securin and cyclin B1

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Disjunction of pairs of homologous chromosomes during the first meiotic division (MI) requires anaphase-promoting complex (APC)-mediated activation of separase in budding yeast[1,2] and *C.elegans*[3,4,5] but not *X.laevis*[6,7], and it is not clear which model best fits the mammalian system. Here we show that homologue disjunction in mouse oocytes is dependent upon proteolysis of the separase inhibitor securin and the Cdk1 regulatory sub-unit cyclin B1. Proteolysis of both proteins was entirely dependent upon their conserved destruction box (D-box) motifs, through which they are targeted to the APC[8]. These data indicate that the mechanisms regulating homologue disjunction in mammalian oocytes are similar to those of budding yeast and *C.elegans.*

Completion of maternal MI is marked by formation of the first polar body (PB). Oocytes of MF1 mice produce PBs at 8-11 h after entry into MI at germinal vesicle breakdown (GVBD). We found that securin is expressed in mouse oocytes during progression through MI (GVBD + 4.5 h; Fig.1a). To determine whether it is degraded before exit from MI we performed timelapse measurements of GFP-tagged securin, which is a valid marker for endogenous securin in mammalian cells[9]. Timelapse fluorescence measurement of oocytes (n=26) injected with mRNA encoding either human securin::GFP (n=14) or mouse securin::GFP (n=12) showed that securin::GFP accumulated gradually for 7-9 h after GVBD, declined over the next 2-3 h, then increased again (Fig. 1b). PB formation occurred at the end of the decline phase. Preliminary experiments indicated that excess securin::GFP inhibited PB formation, which is consistent with reports on the effect of exogenous cyclin B1 in mouse oocytes[10]. We

therefore deliberately injected low levels of securin::GFP mRNA (~1 pg). Peak fluorescence intensities varied from 1-3 fold autofluorescence. These levels of exogenous securin did not inhibit PB formation, which was observed in 19/26 securin::GFP-injected oocytes compared with 14/18 uninjected control oocytes. The presence of DNA in the PB and in the oocyte (Fig. 1c), indicated that homologue disjunction was not inhibited.

To establish whether proteolysis of securin was required for homologue disjunction, we injected oocytes (n=17) with low levels of mRNA (~1 pg) encoding either a human (n=11) or mouse (n=6) D-box mutated securin variant (securin^{dm}::GFP). Timelapse fluorescence measurements showed that both human and mouse securin^{dm}::GFP were stable for the duration of the experiment (GVBD +14 hr; Fig. 1d). This contrasts with findings in mitosis showing that D-box mutated securin is degraded via its KEN box motif [9,11], and suggests that the APC activator Cdh1, which targets the KEN box[8], is either absent or inhibited in MI oocytes.

In contrast to wild type securin::GFP, PB formation was inhibited in oocytes expressing equivalent levels of securin^{dm}::GFP. The fluorescence intensities of securin^{dm}::GFP and securin::GFP at 7:40 h after GVBD, which corresponds to mean time of peak expression in securin::GFP-injected oocytes, were 1-2.9 times autofluorescence (mean 1.9 ± 0.5) and 1-3 times autofluorescence (mean 2.3 ± 0.18) respectively. However, PB formation was observed in only 2/17 securin^{dm}::GFP-injected oocytes compared with 9/13 uninjected controls (P=0.001). PBs formed by oocytes expressing securin^{dm}::GFP (n=2) were resorbed within 20 min of extrusion. DNA labeling showed that oocytes, including those that produced transient PBs, had a metaphase configuration (Fig.1e). Air-dried chromosomes spreads prepared at 16 h post GVBD showed that

anaphase onset was inhibited since the bivalents remained intact (n=7/7 oocytes; Fig. 1f). Both uninjected control oocytes and oocytes containing equivalent levels of securin::GFP had undergone homologue disjunction and extruded PBs at this time point. Tubulin immunolabelling of oocytes fixed at 14-16 h after GVBD revealed a bipolar spindle with chromatin aligned at the equator (n=6/6; Fig. 1g). Maintenance of the bipolar spindle was not due to inhibition of cyclin B1 proteolysis (See Supplementary Information, Fig. S1), but was likely due to synthesis of more cyclin B1 and its subsequent stabilization by the Mos/---/MAPK (mitogen-activated protein kinase) pathway. In support of this, MAPK activity is sustained during MI arrest in mouse oocytes (unpublished data).

In vertebrate mitosis, non-degradable cyclin B1 inhibits separation of sister chromatids in a dose-dependent manner [9,12]. To determine whether homologue disjunction was also inhibited in the presence of non-degradable cyclin B1 we injected mouse oocytes (n=12) with low levels of mRNA encoding Δ90::GFP, a D-box truncated variant of cyclin B1::GFP, which has previously been shown to activate Cdk1 in MI ascidian oocytes[13]. In contrast to full length cyclin B1 (See Supplementary Information, Fig. S2a), timelapse fluorescence measurements showed that Δ90::GFP was stable in MI oocytes (See Supplementary Information, Fig S2b) and PB formation was inhibited. PBs were observed in 0/12 Δ90-injected oocytes compared with 9/12 oocytes expressing equivalent levels of full length cyclin B1::GFP. DNA staining (n=12 oocytes) revealed that homologues maintained a metaphase or metaphase-like configuration (See Supplementary Information, Fig. S2c). Immunolabelling of tubulin in fixed oocytes showed an intact spindle with chromosomes at the spindle equator. However, misaligned chromosomes were occasionally present (2/7 oocytes; See Supplementary Information,

Fig. S2d). Air-dried chromosome spreads of Δ90-injected oocytes (n=7) showed intact bivalents at 15-16 h after GVBD (See Supplementary Information, Fig. S2e). Control uninjected oocytes and oocytes injected with full-length cyclin B1::GFP had produced PBs at this time point. Inhibition of homologue disjunction in the presence of Δ90 was independent of securin since securin proteolysis was not inhibited in Δ90-injected oocytes (See Supplementary Information, Fig. S2f).

Since the effect of Δ90 is dose dependent in mitotic systems [9,12], we were interested to determine whether very low levels Δ90 would inhibit homologue disjunction. GV stage oocytes (n=6) were injected with Δ90 protein to give an estimated oocyte concentration of 15-45 nM, which is 13-40 fold less than the estimated endogenous level of cyclin B1 in mouse oocytes[14]. Both PB formation and homologue disjunction were inhibited (6/6 oocytes).

Our findings indicate a dual system of regulation in which proteolysis of both securin and cyclin B1 is required for homologue disjunction in mouse oocytes. This is consistent with findings in vertebrate mitotis[9,12] and suggests regulation by separase. These data provide a framework for a mechanistic approach to the problem of segregation errors during maternal MI. This is an important issue in human reproductive health, since such errors account for 80% of constitutive aneuploidies of our species[15].

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Figure Legends

Figure 1. **Proteolysis of securin is required for homologue disjunction**. **a,** Western blot showing presence of securin in MI oocytes (GVBD+4.5 h; n=100 oocytes). **b,** Timelapse fluorescence measurement showing securin::GFP expression in a representative oocyte (n=22/26). Insets show paired brightfield and fluorescence images. PB formation (arrow) occurred at the end of the decline phase (n=19/26). **c,** Fluorescence and brightfield overlay images of a securin::GFP-injected oocyte stained with H33342 showing DNA in the PB and in the oocyte. **d,** Timelapse fluorescence measurement of a representative oocyte (n=17) showing that securin^{dm}::GFP was stable. Insets show that PB formation was inhibited (n=15/17). **e,** DNA stained with H33342 at GVBD+14 h shows that homologues maintained a metaphase configuration. **f,** Air-dried chromosome spread of an oocyte (GVBD+16 h) injected with securin^{dm}::GFP, showing intact bivalents (7/7 oocytes). **g,** Immunolabelled spindles of securin^{dm}::GFP-injected oocytes (GVBD+16 h). Microtubules are green and chromosomes are blue. Scale bars = 20 μm.

Figure 1

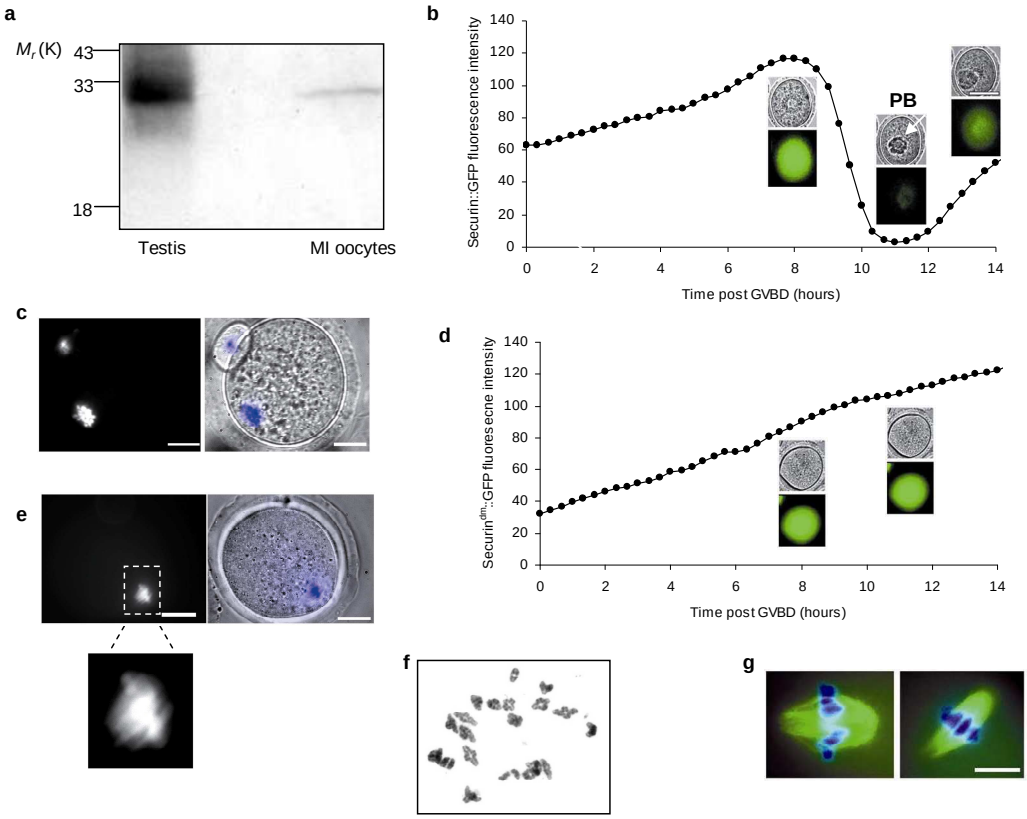
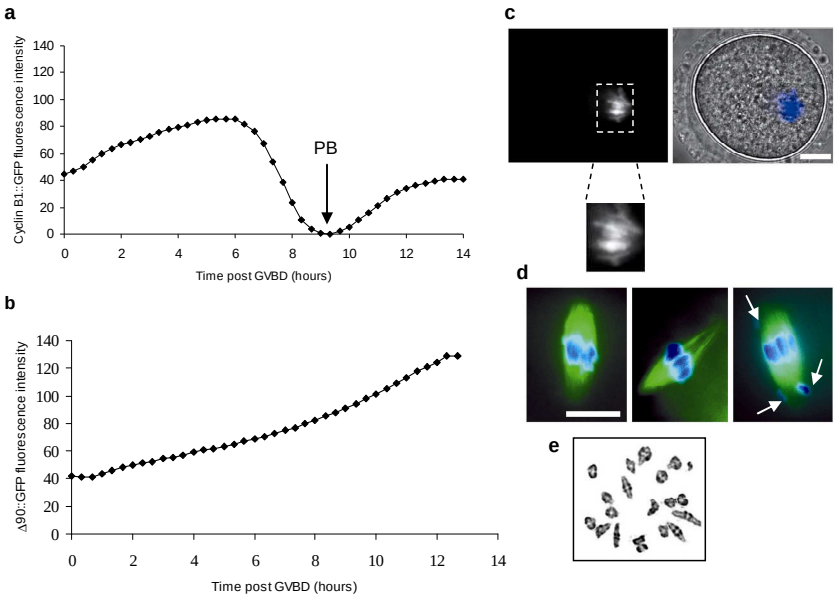

a
M_r (K)
43
33
18
Testis
MI oocytes
b
Securin:GFP fluorescence intensity
PB
Time post GVBD (hours)
c
d
Securin$^{\Delta m}$::GFP fluorescne intensity
Time post GVBD (hours)
e
f
g

Figure 2


a
Cyclin B1::GFP fluorescence intensity
PB
Time post GVBD (hours)
b
Δ90::GFP fluorescence intensity
Time post GVBD (hours)
c
d
e