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

Mary Herbert^{1,5}, Mark Levasseur², Hayden Homer¹, Katie Yallop³, Alison Murdoch³ and Alex McDougall^{2,4,5}

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 *Caenorhabditis elegans*^{3,4,5}, but not *Xenopus laevis*^{6,7}. It is not clear which model best fits the mammalian system. Here we show that homologue disjunction in mouse oocytes is dependent on proteolysis of the separase inhibitor securin and the Cdk1 regulatory sub-unit cyclin B1. Proteolysis of both proteins was entirely dependent on their conserved destruction box (D-box) motifs, through which they are targeted to the APC⁸. 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. Oocytes of MF1 mice produce polar bodies at 8–11 h after entry into MI at germinal vesicle breakdown (GVBD). We found that securin was 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 green fluorescent protein- (GFP) tagged securin, which is a valid marker for endogenous securin in mammalian cells⁹. Timelapse fluorescence measurement of oocytes ($n = 26$) injected with mRNA encoding either human securin–GFP ($n = 14$) or mouse securin–GFP ($n = 12$) demonstrated that securin–GFP gradually accumulated for 7–9 h after GVBD, declined over the next 2–3 h, then increased again (Fig. 1b). Polar body formation occurred at the end of the decline phase. Preliminary experiments indicated that excess securin–GFP inhibited polar body formation, which is consistent with reports on the effect of exogenous cyclin B1 in mouse oocytes¹⁰. We therefore deliberately injected low levels of securin–GFP mRNA (approximately 1 pg). Peak fluorescence intensities varied from one-to-threefold autofluorescence. These levels of exogenous securin did not inhibit polar body formation, which was observed in 19 out of 26 securin–GFP-injected oocytes, compared with 14 out of 18 uninjected control oocytes. The presence of DNA in the polar body 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 (approximately 1 pg) encoding either a human ($n = 11$) or mouse ($n = 6$) D-box mutated securin variant (securin^{dm}–GFP). Timelapse fluorescence measurements demonstrated that both human and mouse securin^{dm}–GFP were stable for the duration of the experiment (GVBD + 14 h; Fig. 1d). This contrasts with mitosis, where D-box mutated securin is degraded through its KEN box motif^{9,11}, and suggests that the APC activator Cdh1, which targets the KEN box⁸, is either absent or inhibited in MI oocytes.

In contrast to wild-type securin–GFP, polar body formation was inhibited in oocytes that expressed equivalent levels of securin^{dm}–GFP. The fluorescence intensities of securin^{dm}–GFP and securin–GFP at 7 h 40 min 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, polar body formation was observed in only 2 out of 17 securin^{dm}–GFP-injected oocytes, compared with 9 out of 13 uninjected controls ($P = 0.001$). Polar bodies formed by oocytes expressing securin^{dm}–GFP ($n = 2$) were resorbed within 20 min of extrusion. DNA labelling demonstrated that oocytes, including those that produced transient polar bodies, had a metaphase configuration (Fig. 1e). Air-dried chromosome spreads prepared at 16 h post-GVBD demonstrated that anaphase onset was inhibited as the bivalents remained intact ($n = 7$ out of 7 oocytes; Fig. 1f). Both uninjected control oocytes and oocytes containing equivalent levels of securin–GFP had undergone homologue disjunction and extruded polar bodies at this time. Tubulin immunolabelling of oocytes fixed at 14–16 h after GVBD demonstrated a bipolar spindle with chromatin aligned at the equator ($n = 6$ out of 6; Fig. 1g). Maintenance of the bipolar spindle was not caused by inhibition of cyclin B1 proteolysis (see Supplementary Information, Fig. S1), but was probably caused by synthesis of more cyclin B1 and its subsequent stabilization by the Mos–MAPK (mitogen-activated protein kinase) pathway. In support of this, MAPK activity was sustained during MI arrest in mouse oocytes (H. H., M. H. and A. M., unpublished observations).

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 $\Delta 90$ –GFP, a D-box-truncated form of cyclin B1–GFP, which activates Cdk1 in MI ascidian oocytes¹³. In contrast with full-length cyclin B1 (see Supplementary Information, Fig. S2a), timelapse fluorescence measurements demonstrated that $\Delta 90$ –GFP was stable in MI oocytes (see Supplementary Information,

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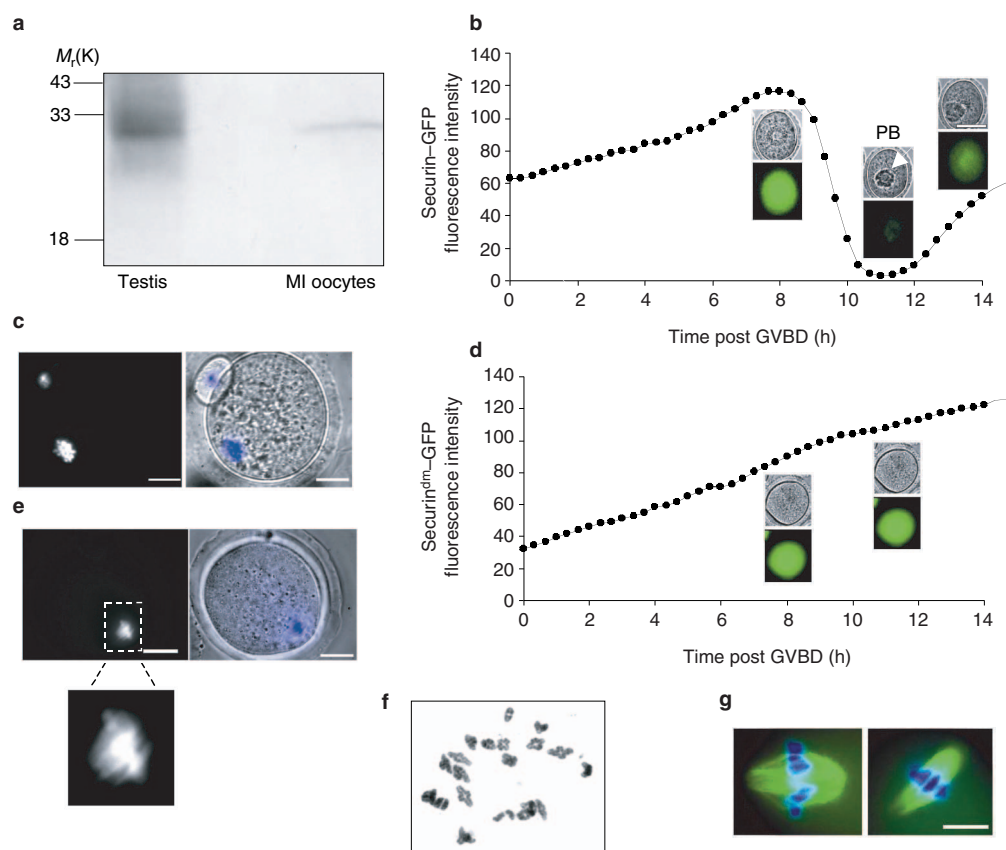


Figure 1. Proteolysis of securin is required for homologue disjunction. (a) Western blot showing presence of securin in MI oocytes (GVBD + 4.5 h; $n = 100$ oocytes). (b) Timelapse fluorescence measurement demonstrating securin-GFP expression in a representative oocyte ($n = 22$ out of 26). Insets show paired brightfield and fluorescence images. Polar body (PB) formation (arrow) occurred at the end of the decline phase ($n = 19$ out of 26). (c) Fluorescence and brightfield overlay images of a securin-GFP-injected oocyte stained with H333342 showing DNA in the polar body and in the oocyte.

(d) Timelapse fluorescence measurement of a representative oocyte ($n = 17$) demonstrating that securin^{dm}-GFP was stable. Insets show that polar body formation was inhibited ($n = 15$ out of 17). (e) DNA stained with H333342 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 out of 7 oocytes). (g) Immunolabelled spindles of securin^{dm}-GFP-injected oocytes (GVBD + 16 h). Microtubules are green and chromosomes are blue. Scale bars, 20 μ m.

Fig S2b) and polar body formation was inhibited. Polar bodies were observed in 0 out of 12 $\Delta 90$ -injected oocytes compared with 9 out of 12 oocytes expressing equivalent levels of full length cyclin B1-GFP. DNA staining ($n = 12$ oocytes) demonstrated 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 out of 7 oocytes; see Supplementary Information, Fig. S2d). Air-dried chromosome spreads of $\Delta 90$ -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 polar bodies at this time. Inhibition of homologue disjunction in the presence of $\Delta 90$ was independent of securin, as securin proteolysis was not inhibited in $\Delta 90$ -injected oocytes (see Supplementary Information, Fig. S2f).

As the effect of $\Delta 90$ is dose-dependent in mitotic systems^{9,12}, we determined whether very low levels $\Delta 90$ would inhibit homologue disjunction. Germinal vesicle stage oocytes ($n = 6$) were injected with $\Delta 90$ 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 oocytes¹⁴. Both polar body formation and homologue

disjunction were inhibited (6 out of 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 mitosis^{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, as such errors account for 80% of constitutive aneuploidies of our species¹⁵. □

Note: Supplementary Information is available on the Nature Cell Biology website.

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The authors declare that they have no competing financial interests.

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1. Buonomo, S. B. C. *et al. Cell* **103**, 387–398 (2000).
2. Cooper, K. F. *et al. Proc. Natl Acad. Sci.* **97**, 14548–14553 (2000).
3. Golden, A. *et al. J. Cell Biol.* **151**, 1469–1482 (2000).
4. Furuta, T. *et al. Mol. Biol. Cell* **11**, 1401–19 (2000).
5. Siomos, M. F. *et al. Curr. Biol.* **11**, 1825–1835 (2001).
6. Peter, M. *et al. Nature Cell Biol.* **3**, 83–87 (2001).
7. Taieb, F. E., Gross, S. D., Lewellyn, A. L. & Maller, J. L. *Curr. Biol.* **11**, 508–513 (2001).
8. Pfleger, C. M. & Kirschner M. W. *Genes & Devel.* **15**, 2396–2407 (2001).
9. Hagting, A. *et al. J. Cell Biol.* **157**, 1125–1137 (2002).
10. Ledan, E., Polanski, Z., Terret, M. E. & Maro, B. *Dev. Biol.* **232**, 400–413 (2001).
11. Zur, A. & Brandeis, M. *EMBO J.* **20**, 792–801 (2001).
12. Stemmman *et al. Cell* **107**, 715–726 (2000).
13. Levasseur, M. & McDougall, A. *Devel.* **127**, 631–641 (2000).
14. Kanatsu-Shinohara, M., Schultz, R. M. & Kopf, G. S. *Biol. Reprod.* **63**, 1610–1616 (2000).
15. Hassold, T. & Hunt, P. *Nature Rev. Genet.* **2**, 280–291 (2001).
16. Clouston, H. *et al. Hum. Genet.* **101**, 30–36 (1997).
17. Dominguez, A. *et al. Oncogene* **17**, 2187–2193 (1998).