Supplemental Data

Resolution of Chiasmata in Oocytes

Requires Separase-Mediated Proteolysis

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Supplemental Results

Oocytes Lacking Separase Downregulate Cdk1-Cyclin B1 Activity

A key event that occurs at the onset of anaphase I in oocytes is a decline in the histone H1 kinase activity, which is presumably associated with Cdk1-cyclin B1 (Kubiak et al., 1992). Degradation of cyclin B1 is thought to cause this decline (Herbert et al., 2003). Re-synthesis of cyclin B1 after the first meiotic division restores activity to high levels by metaphase II. Both *Separase*$^{flox/flox}$ *Zp3-cre* (Sep Δ) and control *Separase*$^{flox/flox}$ (Sep f/f) oocytes gradually accumulate the H1 kinase activity from GVBD to 7 hr after GVBD corresponding to metaphase I with similar kinetics (Fig. S3A). Sep f/f oocytes, 90% of which undergo PBE, possess low levels of the activity immediately after PBE and re-accumulate it progressively towards metaphase II, when its activity remains high. On the contrary, only an average of 20% of Sep Δ oocytes extrude PBs. Oocytes that had extruded PBs were collected and their H1 kinase activity was measured. This revealed a decrease followed by a re-accumulation of the activity with similar kinetics to control oocytes (Fig. S3A). Therefore the degradation and re-accumulation of Cdk1 activity is not affected in the oocytes lacking separase that are still able to extrude PBs.

However, the majority of Sep Δ oocytes (80% on average) do not undergo PBE. Thus, it is important to ascertain the H1 kinase activity in those oocytes that fail to extrude PB. As oocytes normally start re-accumulating the activity soon after PBE, the low H1 kinase activity in post-metaphase I oocytes is only detected when oocytes are harvested at the time of PBE, because the deviation of timing of PBE (8 to 12 hr after GVBD) is greater then the duration of the low H1 kinase activity. To overcome this problem, we re-synchronized post-metaphase I oocyte culture by treating the oocytes with nocodazole (10μM) from 7 to 11 hr after GVBD and subsequently released them into drug-free medium. Seventy-nine percent of control Sep f/f oocytes (n=86) and 18% of Sep Δ oocytes (n=61) underwent PBE in a 30 min time window between 1.5 to 2 hr after the release from nocodazole containing medium. As shown in Fig. S3B, PB-extruded control oocytes at 2 hr after the release had low H1 kinase activity and then re-accumulated it with similar kinetics as in Fig. S3A, indicating that this transient nocodazole treatment allows a synchronous post-metaphase I oocyte culture and does not interfere with re-accumulation of the activity. When Sep Δ oocytes that had failed to extrude PBs were harvested at 2, 3.5 and 5 hr after the release and their H1 kinase activities were measured, they were found to decline and re-accumulate the activity with similar kinetics as in the control or in the mutant oocytes that had extruded PBs (Fig. S3A and B). These results indicate that oocytes lacking separase are able to decrease Cdk1 activity irrespective of whether they extruded PBs or not, suggesting that the absence of separase do not trigger metaphase I arrest.

Supplemental Experimental Procedures

Preparation of Chromosome Spreads

Chromosome spreads of prophase I mouse oocytes were prepared as follows. Fetal ovaries were dissected from E13.5 embryos, placed in a hypotonic buffer (30 mM Tris-Cl (pH 8.2), 50 mM sucrose, 17 mM sodium citrate) for 10 min and then transferred to 50 μl of 100 μM sucrose (pH 8.2). The ovaries were disaggregated using sharp forceps and pipetting to obtain a single cell suspension. The cell suspension was put on a slide glass that had been immersed in fixative (1% paraformaldehyde, 5 mM sodium borate (pH 9.2), 0.15% Triton X-100). The cells were dispersed on the slide by tilting and then slowly dried. Chromosome spreads from oocytes during meiotic maturation were prepared as follows. Zona pellucida was removed by incubating oocytes in M2 medium containing 10 mg/ml pronase (Calbiochem) at 37 °C for 5 min, followed by 3 washes in M2 medium. Zona pellucida-free oocytes (PBs had been detached and lost in most cases) were placed in a hypotonic solution (50% fetal calf serum diluted by water) at 37 °C for 10-30 min, placed in a drop of fixative (1% paraformaldehyde, 0.15% Triton X-100, 3 mM dithiothreitol, pH 9.2 adjusted by NaOH) on a slide glass and then slowly dried.

Histone H1 Kinase Assay

Histone H1 kinase activities were determined by *in vitro* reactions using 5 oocytes in a 10 μl reaction (25 mM HEPES (pH 7.4), 15 mM $MgCl_2$, 80 mM EGTA, 1 mM DTT, 0.1 mM ATP, 3 μCi of [γ-^{32}P]ATP (Amersham), 1 μg histone H1 (Roche)) incubated at 37°C for 20 min. Samples were boiled in sample buffer (1% SDS, 50 mM Tris-Cl (pH 6.8), 10% glycerol, 0.2% β-mercaptoethanol, 0.01% bromophenol blue), separated on a 15% SDS-polyacrylamide gel and radioactive signal was detected by PhosphorImager system (Molecular Dynamics).

Plasmid Construction

To generate a cDNA encoding separase with a site-directed mutation at its catalytic cysteine at amino acid 2028 replaced by serine (separase C2028S), QuikChange site-directed mutagenesis kit (Promega) was used with the following primers:
5'-GCCCTGCTGTTCGGCAGCAGCAGTGCAGCCC-3'
and 5'- GGGCTGCACTGCTGCTGCCGAACAGCAGGGC-3'.

For generation of a cDNA carrying a frameshift mutation at amino acid 212 (separase 1-212), wild-type separase cDNA was linearized by *Xba* I at its unique site in the ORF, treated with Klenow and then religated. Wild-type and mutant separase cDNAs were subcloned between 5'- and 3'-UTR sequences of the *Xenopus* β-globin gene on the pRN3 plasmid (Brunet et al., 1998) (gift of J. Moreau, Paris, France).

Supplemental References

Brunet, S., Polanski, Z., Verlhac, M. H., Kubiak, J. Z., and Maro, B. (1998). Bipolar meiotic spindle formation without chromatin. Curr Biol *8*, 1231-1234.

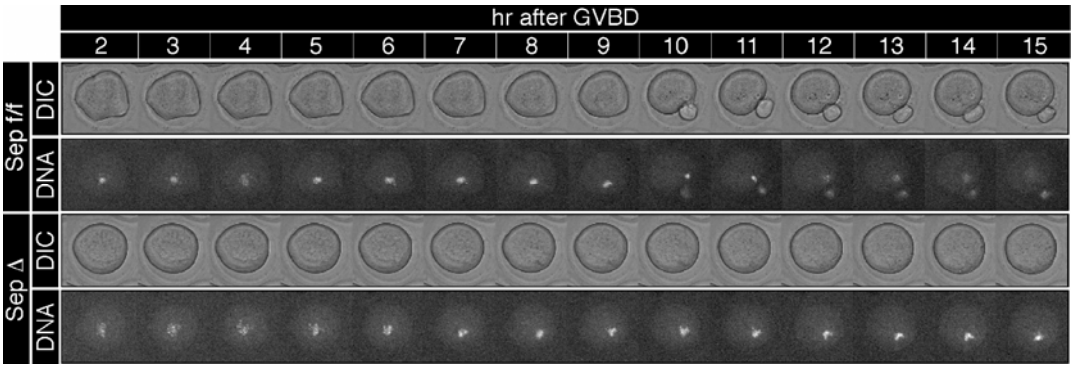

Figure S1. Oocytes Lacking Separase Fail to Extrude Polar Bodies or Segregate Chromosomes in Meiosis I

Chromosome movement of live *Separase*$^{flox/flox}$ *Zp3-cre* (Sep Δ) and *Separase*$^{flox/flox}$ (Sep f/f) oocytes observed by time-lapse microscopy. DIC images and fluorescence images of chromosomes (DNA) visualized by Hoechst 33348 in M2 medium are shown. The presented 1-hr interval images are selected from the original movie (Suppl. Movie 1).

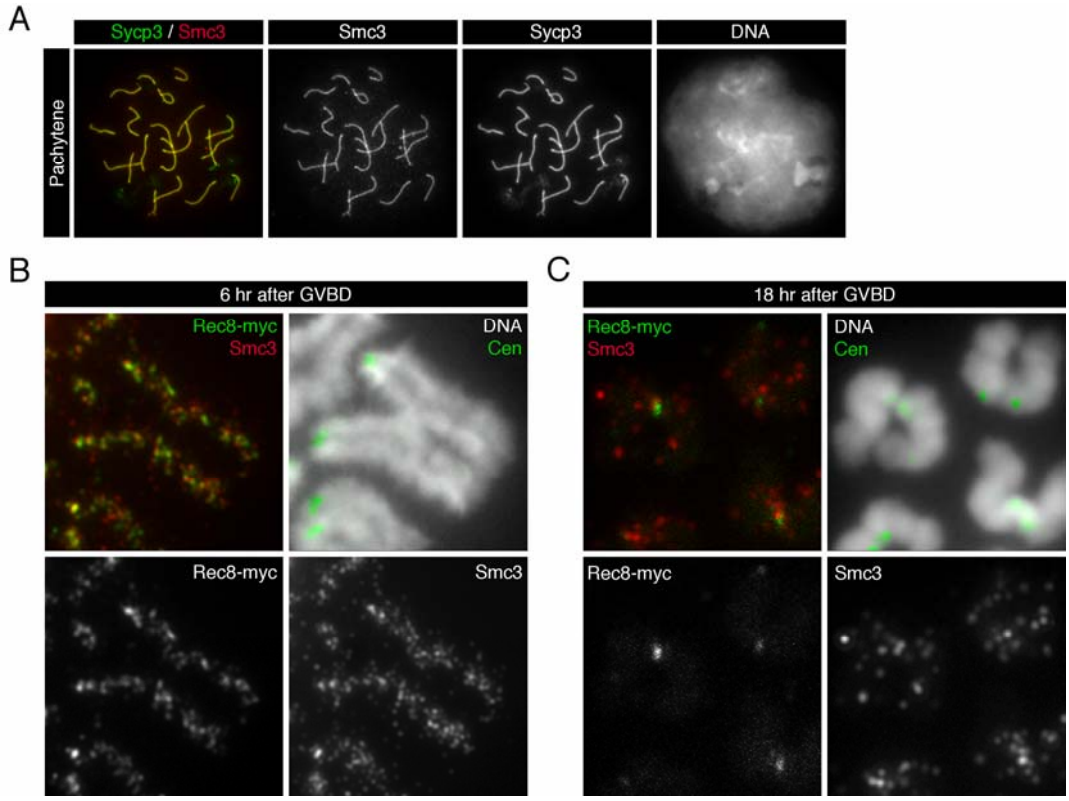

Figure S2. Anti-Smc3 Antibody Staining on Mouse Oocyte Chromosomes

(A) Anti-Smc3 antibody staining on pachytene nuclei of oocytes. Chromosome spreads from fetal ovaries of E13.5 embryos heterozygous for the *Rec8-myc* transgene were prepared and stained with anti-Smc3 (green) and anti-Sycp3 (red) antibodies. DNA was counterstained with DAPI.

(B and C) Anti-Smc3 antibody staining on the chromosome spreads of bivalents (B) and univalents (C) from oocytes heterozygous for *TG Rec8-myc* in culture for the indicated times after GVBD. Anti-Smc3 (red), anti-Myc (green) antibodies and CREST serum (green merged on DNA images) were used. DNA was counterstained with DAPI. The Smc3 signals are enriched at inter-chromatid axes and co-localize well with Rec8-myc signals on the bivalents. However, they defuse to chromatin loops sparsely. It is difficult to conclude that a certain fraction of Smc3 also localizes to the chromatin loops or not by the lack of background subtraction that is only possible in the Rec8-myc staining. In (C), some Smc3 signals co-localize with Rec8-myc signals in between two sister centromeres. Due to signals spread over the chromatin, it is difficult to ascertain that the Smc3 signals correspond to the centromeric population without co-staining of Rec8-myc.

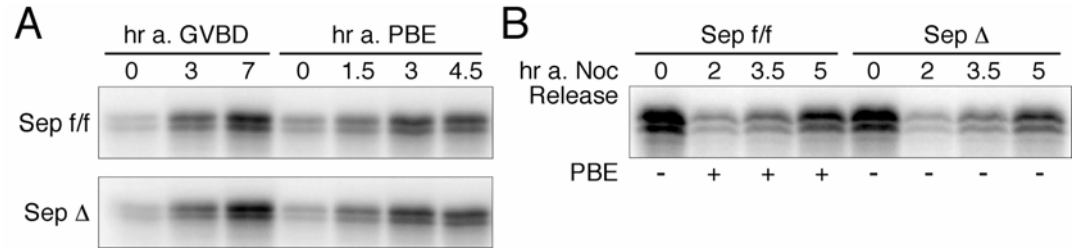

Figure S3. Oocytes Lacking Separase Downregulate Cdk1-Cyclin B1 Activity

Histone H1 kinase activities in oocytes at the indicated time in culture after GVBD, PBE or nocodazole release. In (B), oocytes were cultured for 7 hr after GVBD in M16 followed by a 4 hr treatment with 10 μM nocodazole, after which oocytes were cultured in nocodazole-free medium for another 5 hr. The oocytes that had undergone PBE (+) or not (-) are indicated (PBE). Autoradiographs of SDS-PAGE gels on which incubated samples were run are shown. Five oocytes were used for each reaction in these experiments.