Ipl1/Aurora B kinase coordinates synaptonemal complex disassembly with cell cycle progression and crossover formation in budding yeast meiosis

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http://sro.sussex.ac.uk
**A**

![Western Blot Image]

- **$\alpha$-3HA-Ipl1**
- **$\alpha$-Pgk1**

$\alpha$-Ipl1 and $\alpha$-Pgk1 Western blots from ipl1-mn strain. Mito 0-5 hours in SPM.

**B**

**Bar Graph**

- **Relative** to PGK1
- **Normalized to 0 hrs**

Hours in SPM: 0-5

**C**

- **$\alpha$-Histone H3**
- **$\alpha$-P-Ser10 Histone H3**

Wild type and ipl1-mn strains. Hrs in SPM: 0-8.

**D**

Wild type and ipl1-mn strains. Hrs in SPM: 0-8.
A. Tubulin dot + single DNA body

B. Short meta I spindle + 1 DNA body

C. Long meta I/early ana I + 1 DNA body

D. Anal + 2 DNA bodies

E. Short meta II spindles + 2 DNA bodies

F. Long meta II/early ana II + 2 DNA bodies

G. Anal + 4 DNA bodies

Legend:
- WT
- ipf1-mn

Proportion of cells vs. Hrs in SPM
**A**

- **pachytene**

- **diplotene**

- **metaphase I**

- **anaphase I**

**B**

**Mock-treated**

**Proportion**

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<th>7</th>
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**D**

**1-NA-PP1**

**Proportion**

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**C**

**Proportion**

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**E**

**Proportion**

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Figure S6

A. Wild type

B. *ipl1-mn*

C. 

\[ \lambda \text{ phosphatase} \]

\[ \text{inhibitors} \]

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<th>Time (Min. after NDT80 ON)</th>
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\[ + \] indicates the presence of a band; \[ - \] indicates the absence of a band.
A

**Xhol/EcoRI digest**

- **P2** 1.8 kb
- **NCO** 4.1 kb
- **CO1** 7.0 kb
- **DSB2** 1.6 kb
- **PROBE (180 bp)**

B

**Wild type**

C

**ipl1-mn**

D

**NCO**

E

**CO**

![Graphs showing DNA proportion over time](image)
A

metaphase I

WT

ipl1-mn

Nucleolar Cdc14-13Myc

19/20 16/16

B

anaphase I

WT

ipl1-mn

Nucleolar Cdc14-13Myc

0/9 0/25
Wild type

ipl1-mn
A

diplotene

metaphase I

anaphase I

B

H3-Ser10 → Ala

pachytene

diplotene

metaphase I
Supplementary Material.

Supplementary Figure legends.

**Supplementary Figure S1.** 3HA-Ipl1 protein levels and kinase activity during meiotic prophase. (A) Western blot analysis of 3HA-Ipl1 expressed from the CLB2 promoter. (B) 3HA-Ipl1 levels were quantified relative to Pgk1 (gray bars) and then normalized to the 0 hour time point (black bars). Ipl1 activity was measured by determining the phosphorylation of Ser10 Histone H3 compared to total cellular levels of histone H3 in wild type (C, Y1381) and ipl1-mn (D, Y1669).

**Supplementary Figure S2.** Nuclear divisions and spindle behaviour in ipl1-mn mutants suggest a metaphase-anaphase delay. Cells were assessed for nuclear as well as spindle morphology simultaneously to determine whether nuclear divisions had been decoupled from spindle morphology. Cells were divided into six categories, described as follows. Proportion of cells with a single DNA mass and a single focus of tubulin (A), representative of meiotic prophase cells, with a metaphase I spindle and a single DNA mass (B), representative of metaphase I, or a long metaphase I/early anaphase I spindle and a single DNA mass (C), representative of late metaphase I. In the wild type (Y940), < 5% of such DNA masses were ‘stretched’ at late metaphase I, whereas in ipl1-mn (Y1206), the majority displayed a ‘stretched’ phenotype. (D) Proportion of cells with two clearly separated DNA bodies and an anaphase I spindle, representative of cells having completed the metaphase I-anaphase I transition. (E) Cells with two, short metaphase I spindles, each
with a single body of DNA, representative of cells in metaphase II. (F) Proportion of cells with long metaphase II/early anaphase II spindles, each with a single body of DNA (late metaphase II). In the *ipl1-mn* mutant, the majority of these nuclei were stretched, as in (C). (G) Cells with two anaphase II spindles and four separate DNA bodies represented cells that had successfully completed both meiotic nuclear divisions. Arrows indicate spindles formed prior to meiotic entry. At least 200 cells were counted for each time point.

**Supplementary Figure S3.** Stretched nuclei are observed in *zip1* and *ipl1-mn zip1*. Time course experiments of nuclear divisions and stretched nuclear phenotypes in wild-type (Y940), *ipl1-mn* (1206), *zip1* (Y1530), and *ipl1-mn zip1* (Y1658) mutants. At least 200 cells were counted for each time point.

**Supplementary Figure S4.** Meiotic nuclear divisions are delayed despite normal expression of Clb3-13Myc in *ipl1-mn* cells. (A and C) Proportion of ethanol-fixed cells containing a single nuclear body (1n), a stretched nuclear body (1n - stretched), or two distinct nuclear bodies (2n). (B and D) Proportion of cells with more than two nuclear bodies or containing two nuclear bodies of which at least one was stretched (2n – stretched). β-estradiol was added 6 hours after cells were transferred to SPM. Strains: wild type (Y1581), *ipl1-mn* (Y1582).

**Supplementary Figure S5.** Delayed SC disassembly in the *ipl1-as5* mutant (Y1583) treated with 1-NA-PP1. (A) Examples of surface-spread nuclei at
various stages. Zip1 is given in green and tubulin (tub) in magenta. (B) SC disassembly and PC occurrence (C) in mock-treated cells and 1-NA-PP1 treated cells (D and E). Bars: 2 µm. More than 100 nuclei were inspected for each time point.

Supplementary Figure S6. Zip1 is a phosphoprotein. Western blot analysis of Zip1 shows two bands in both wild-type (A, Y1602) and ipl1-mn (B, Y1538). Both bands are present prior to Ndt80 expression (0 minutes) and after Ndt80 expression. (C) Zip1 protein mock-treated (lane 1) treated with λ phosphatase (lane 2), or treated with both λ phosphatase and inhibitor.

Supplementary Figure S7. Schematic representation of the URA3-ARG4 ectopic recombination interval on Chromosome III (Allers and Lichten, 2001). XhoI digest of DNA yields the indicated sizes of parental molecules (P1 and P2), DSBs (DSB1 and DSB2) as well as crossovers (CO1 and CO2). Sequences flanking the insert at LEU2 and the insert at HIS4 are denoted by solid grey and dashed grey lines, respectively. The region recognized by the probe is shown in blue.

Supplementary Figure S8. Detection of crossover and noncrossover products at the URA3-ARG4 interval on Chromosome III (Allers and Lichten, 2001). (A) XhoI and EcoRI digest of DNA, probed with HIS4-specific sequences, yields the indicated sizes of parental (P2), double-strand break (DSB2), crossover (CO1) as well as noncrossover recombinants (NCO). The region recognized by the probe, which is specific to HIS4 sequences, is
shown in blue. M- marker. The sizes, in kilobases, are given adjacent to the
*ipl1-mn* blot. (B and C) Autoradiograms of typical wild-type (Y940) and *ipl1-mn*
(Y1206) meiotic time courses. (D and E) Quantification of NCO products (D)
and CO products (E).

**Supplementary Figure S9.** Cdc14-13Myc release from the nucleolus in
nuclei containing metaphase I or anaphase I spindles. Examples of nuclei at
metaphase I (A) and anaphase I (B). DNA is shown in blue, Cdc14-13Myc in
green, and tubulin (tub) in red. The proportion of nuclei with metaphase I (A)
spindle and nucleolar Cdc14-13Myc focus is shown to the right of the image
for wild type (Y1662) and *ipl1-mn* (Y1664). When nuclei were selected for
anaphase I spindles (B), virtually all showed absent Cdc14-13Myc staining of
the DNA, as expected. Arrows indicate Cdc14-13Myc staining in the merged
images. Bars: 2 µm.

**Supplementary Figure S10.** Surface-spread nuclei stained for Zip1 and Smt3
simultaneously. Individual channels obtained for the merged images shown in
Figure 6G and H. Bars: 2 µm. Strains: wild type (Y940), *ipl1-mn* (Y1206).

**Supplementary Figure S11.** Red1 accumulation on spindles. (A)
Accumulation of Red1 at the poles of metaphase I spindles in wild type (~ 1/3,
Y940) and on anaphase I spindles in *ipl1-mn* (~ 1/3, Y1206). Bars: 2 µm.

**Supplementary Figure S12.** Hop1 dissociation from meiotic chromosomes in
wild type and *ipl1-mn*. Examples of Hop1 staining in nuclei at various stages
of meiosis I in wild type (Y940) and *ipl1-mn* (Y1206) (A and D). tub = tubulin. Bars: 2 µm. Quantification of Hop1 staining of meiotic chromosomes and aggregate formation in wild type (B and C) and *ipl1-mn* (E and F). > 50 nuclei were assessed for each stage.

**Supplementary Figure S13.** Rec8 is retained in anaphase I/telophase I nuclei of *ipl1-mn* that contain Zip1 staining. (A) Examples of wild-type (Y1485) nuclei stained for DNA (DAPI), Zip1 (red), tubulin (green), and Rec8-3HA (Rec8, blue). 30/30 spreads with clearly separated nuclei showed Rec8 staining at the spindle poles only. In contrast, 30/30 spreads that contained Zip1 staining in the *ipl1-mn* mutant (Y1551) also displayed significant non-polar Rec8 staining (B). Bars: 2 µm.

**Supplementary Figure S14.** Decoupling of cell cycle progression and SC disassembly in the *ipl1-mn*, but not the histone H3 Ser10→Ala mutant. (A) *ipl1-mn* (Y1175, S288c) shows delayed SC disassembly at diplotene, metaphase I and anaphase I. (B) SC disassembly occurs normally a mutant (and isogenic wild-type strain, Y1127) expressing histone H3 Ser10→Ala (Y1728, S288c). Zip1 is shown in green and tubulin (tub) in magenta. Bars: 2 µm.
## Supplementary Tables.

### Supplementary Table S1: Strains used in this study.

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<td>MATα his4::URA3-arg4-EcPal(1691) LEU2 MATα HIS4 leu2::URA3-ARG4 ura3Δ arg4Δ lys2 ho::LYS2 ura3Δ arg4Δ lys2 ho::LYS2</td>
<td>(Allers and Lichten, 2001)</td>
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<tr>
<td>Y1175 (S288c)</td>
<td>MATα leu2-3,112 his3Δ lys2::BglII MATα leu2-3,112 his3Δ lys2::BglII arg4 ilv1-Kpn, PAC2::[pD174::LEU2 lacO array] arg4 ilv1-Kpn, PAC2::[pD174::LEU2 lacO array] rad3 trp2 KANMX6-P&lt;sub&gt;CLB2&lt;/sub&gt;-3HA-IPL1 rad3 trp2 KANMX6-P&lt;sub&gt;CLB2&lt;/sub&gt;-3HA-IPL1</td>
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<td>Y1206</td>
<td>MATα his4::URA3-arg4-EcPal(1691) LEU2 MATα HIS4 leu2::URA3-ARG4 ura3Δ arg4Δ lys2 ho::LYS2 ura3Δ arg4Δ lys2 ho::LYS2 KANMX6-P&lt;sub&gt;CLB2&lt;/sub&gt;-3HA-IPL1 KANMX6-P&lt;sub&gt;CLB2&lt;/sub&gt;-3HA-IPL1</td>
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<td>MATα leu2::hisG his3::hisG trp1::hisG ura3 lys2 MATα leu2::hisG his3::hisG trp1::hisG ura3 lys2 ho::LYS2 ho::LYS2</td>
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<td>MATα leu2::hisG his3::hisG trp1::hisG ura3 lys2 MATα leu2::hisG his3::hisG trp1::hisG ura3 lys2 ho::LYS2 PDS1-18MYC::TRP1 ho::LYS2 PDS1-18MYC::TRP1 REC8-3HA::URA3 REC8-3HA::URA3</td>
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<td>(Clyne et al., 2003)</td>
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<td>ho::LYS2  PDS1-18MYC::TRP1 ho::LYS2  PDS1-18MYC::TRP1 RE8C-3HA::URA3  KANMX6-PSCC1-3HA-CDC5 RE8C-3HA::URA3  KANMX6-PSCLB2-3HA-CDC5</td>
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<td>MATα leu2::hisG his3::hisG trp1::hisG ura3·lys2 MATα leu2::hisG his3::hisG trp1::hisG ura3·lys2 ho::LYS2 zip1Δ ho::LYS2 zip1Δ</td>
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| Y1658 | \( \text{MAT}\alpha \text{ leu2::hisG his3::hisG trp1::hisG lys2} \)  \\
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|     | This work |
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|     | \( P_{\text{GAL1\/NDT80}}::\text{TRP1} \text{ CLB1-13MYC::TRP1} \)  \\
|     | \( P_{\text{GAL1\/NDT80}}::\text{TRP1} \text{ CLB1-13MYC::TRP1} \)  \\
|     | This work |
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|     | \( \text{ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3} \)  \\
|     | \( P_{\text{GAL1\/NDT80}}::\text{TRP1} \text{ CDC14-13MYC::TRP1} \)  \\
|     | \( P_{\text{GAL1\/NDT80}}::\text{TRP1} \text{ CDC14-13MYC::TRP1} \)  \\
|     | This work |
| Y1663 | \( \text{MAT}\alpha \text{ leu2::hisG his3::hisG trp1::hisG lys2} \)  \\
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|     | \( \text{ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3} \)  \\
|     | \( \text{ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3} \)  \\
|     | \( P_{\text{GAL1\/NDT80}}::\text{TRP1} \text{ CLB1-13MYC::TRP1} \)  \\
|     | \( P_{\text{GAL1\/NDT80}}::\text{TRP1} \text{ CLB1-13MYC::TRP1} \)  \\
|     | This work |
| Y1664 | \( \text{MAT}\alpha \text{ leu2::hisG his3::hisG trp1::hisG lys2} \)  \\
|     | \( \text{MAT}\alpha \text{ leu2::hisG his3::hisG trp1::hisG lys2} \)  \\
|     | \( \text{KANMX6-P}_{\text{CLB2-3HA-IPL1}} \)  \\
|     | \( \text{KANMX6-P}_{\text{CLB2-3HA-IPL1}} \)  \\
<p>|     | This work |</p>
<table>
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| Y1669  | MATα leu2::hisG his3::hisG trp1::hisG lys2  
ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3  
ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3  
P_{GAL1}-NDT80::TRP1  
P_{GAL1}-NDT80::TRP1  
KANMX6-P_{CLB2}-3HA-IPL1 | This work |
| Y1727 (S288c) | MATα leu2::hisG his3::hisG trp1::hisG ura3 lys2  
MATα leu2::hisG his3::hisG trp1::hisG ura3 lys2  
ho::LYS2  
ho::LYS2 | (Liu et al., 2005) |
| Y1728 (S288c) | MATα leu2  
ht1-hhf1Δ::KAN  
ht1-hhf1Δ::KAN  
hta1-htb1Δ::HPH  
hta1-htb2Δ::NAT  
hta1-htb1Δ::HPH  
hta2-htb2Δ::NAT | (Liu et al., 2005) |
| Y2030  | MATα leu2::hisG his3::hisG trp1::hisG lys2  
MATα leu2::hisG his3::hisG trp1::hisG lys2  
ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3  
ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3  
P_{GAL1}-NDT80::TRP1  
P_{GAL1}-NDT80::TRP1  
KANMX6-P_{CLB2}-3HA-IPL1  
KANMX6-P_{CLB2}-3HA-IPL1 | This work |
| Y2262  | MATα leu2::hisG his3::hisG trp1::hisG lys2  
MATα leu2::hisG his3::hisG trp1::hisG lys2 | This work |
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<td>ndt80::HPHMX6 P_{GAL1}-CDC5::TRP1</td>
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<tr>
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<td>MATα leu2::hisG his3::hisG trp1::hisG lys2</td>
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<td>ho::LYS2 ura3::pGPD1-GAL4(848).ER::URA3</td>
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<td>ndt80::HPHMX6 P_{GAL1}-CDC5::TRP1</td>
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<td>KANMX6-P_{CLB2}-3HA-IPL1</td>
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This work

1 Strains were SK1 or, when indicated, S288c.

**Supplementary References.**

