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# Supplemental Data

# Fpr3 and Zip3 Ensure that Initiation of Meiotic Recombination Precedes Chromosome Synapsis in Budding Yeast

**Amy J. MacQueen and G. Shirleen Roeder**

## Supplemental Experimental Procedures

### Strains

We replaced the *ZIP1* ORF using pEJW1, a *ZIP1-GFP* integrating plasmid marked with *URA3* [1]**,** and then selected for colonies resistant to 5-FOA to isolate a uracil-requiring strain carrying a single copy of *ZIP1-GFP.* Sporulation-competent haploid strains were constructed by using pB211 to integrate *MAT***a** at *THR1* in a *MAT*$\alpha$ haploid. *fpr3::KAN* and *dmc1::KAN* deletion alleles were constructed using dominant marker gene replacement [2]. The following gene disruptions were previously described: *CTF19* tagged with 13-*myc* [3], *zip3::URA3* [4], *zip2::LEU2* and *ZIP2-GFP:URA3* [5]**,** and *spo11::ADE2* [6]. Strains used to measure meiotic gene conversion carried heteroalleles at both *HIS4* (*his4-260/his4-Hpa*) and *ARG4* (*arg4-Nsp/arg4-Bgl*).

### Immunoprecipitation

Zip1 was immunoprecipitated from meiotic extracts as follows: 50 ul of Protein G Sepharose beads (GE Healthcare) were washed three times with Kellogg Extract buffer [7] (without LPC Protease Inhibitor mix), and incubated 1 hour with 4 ul of mouse anti-Zip1 polyclonal antibody [5]. For each immunoprecipitation, 0.25 g of mortar-ground, frozen yeast powder was solubilized in 0.5 ml of IP buffer [50 mM Tris-Cl pH 7.5, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 0.1% SDS, 0.5% sodium deoxycholate] containing Complete Protease Inhibitor mix (Roche). Solubilized extracts were sonicated on ice with three, 10-second pulses, using output control setting 2.5. Extracts were centrifuged at 14,000 x g (at 4°) for 10-15 minutes, and 0.5 ml of cleared extracts were mixed with the Protein G sepharose/Zip1 antibody preparations for 2 hours at 4°. Beads were then washed four times with Kellogg wash buffer [7] (containing Complete (Roche), in place of LPC, Protease Inhibitor mix).

### Genetic Screen

*MAT***a**/*MAT*$\alpha$ *ZIP1-GFP spo11 ndt80* haploid cells were transformed with a mini-Tn3 (*LEU2*) transposon library [8] and plated, at a density of 100-200 colonies per plate, on leucine-deficient medium (SC-Leu). Transformed colonies were replica-plated to sporulation medium for 50-60 hours. A line was drawn to divide each plate in half. Colonies belonging to one “half-plate” (approximately 50-100 colonies) were combined in water and screened (live) under a coverslip for Zip1-GFP distribution. For half-plate pools that contained a potentially interesting mutant,

individual colonies were then transferred to new SC-Leu plates and individually screened at 50-60 hours after introduction into sporulation medium. To continue analysis of interesting insertions, we selected for rare haploids that had lost the *MAT***a** information at *THR1*. We then used these *MAT*$\alpha$ haploids in genetic crosses to create diploid strains homozygous for the insertion. Insertion locations were identified with a standard plasmid rescue procedure, using the pRSQ2-*URA3* plasmid (GenBank U64694, http://ygac.med.yale.edu).

**Cytology and Imaging**

Centromere probes for FISH analysis were generated using amplified DNA from six ~1-kbp segments in the 5-kbp region flanking centromere of chromosome IV (*CENIV*). To minimize false positive signals, we hybridized two non-overlapping regions of *CENIV* with separate color probes. DNA from three *CENIV* segments was combined and used as template in a nick translation reaction using the BIONICK Labeling System (Invitrogen) with Cy3-dUTP (GE Healthcare), while DNA from the remaining three regions was combined and labeled in the same way using Cy5-dUTP (GE Healthcare). The following primary antibodies, diluted 1:100, were used: mouse anti-myc (clone 9e10, Covance), mouse anti-Nsp1 (Abcam), rabbit anti-Zip1 [9], mouse anti-Red1 [9], guinea pig anti-Smt3 [10], and rabbit anti-Fpr3. Antibodies against the amino terminus of Fpr3 and the carboxyl terminus of Fpr3 were gifts of J. Thorner [11]; each gave identical results in the experiment shown in Figure 2.

Softworx deconvolution software, in conjunction with Deltavision RT imaging system (Applied Precision) adapted to an Olympus (IX70) microscope, was used for all image analysis. Zip1 linear segments were measured on chromosome spreads using the Softworx Measure Distance tool. Lengths of individual Zip1 stretches, exhibited by synapsing nuclei from mid-prophase cells, ranged from 0.35 to approximately 2.4 microns. For quantitation of the centromere-association of the most recently initiated Zip1 polymerization events, we selected those Zip1 stretches that were between 0.35 and 0.65 microns in length.

## Supplemental References


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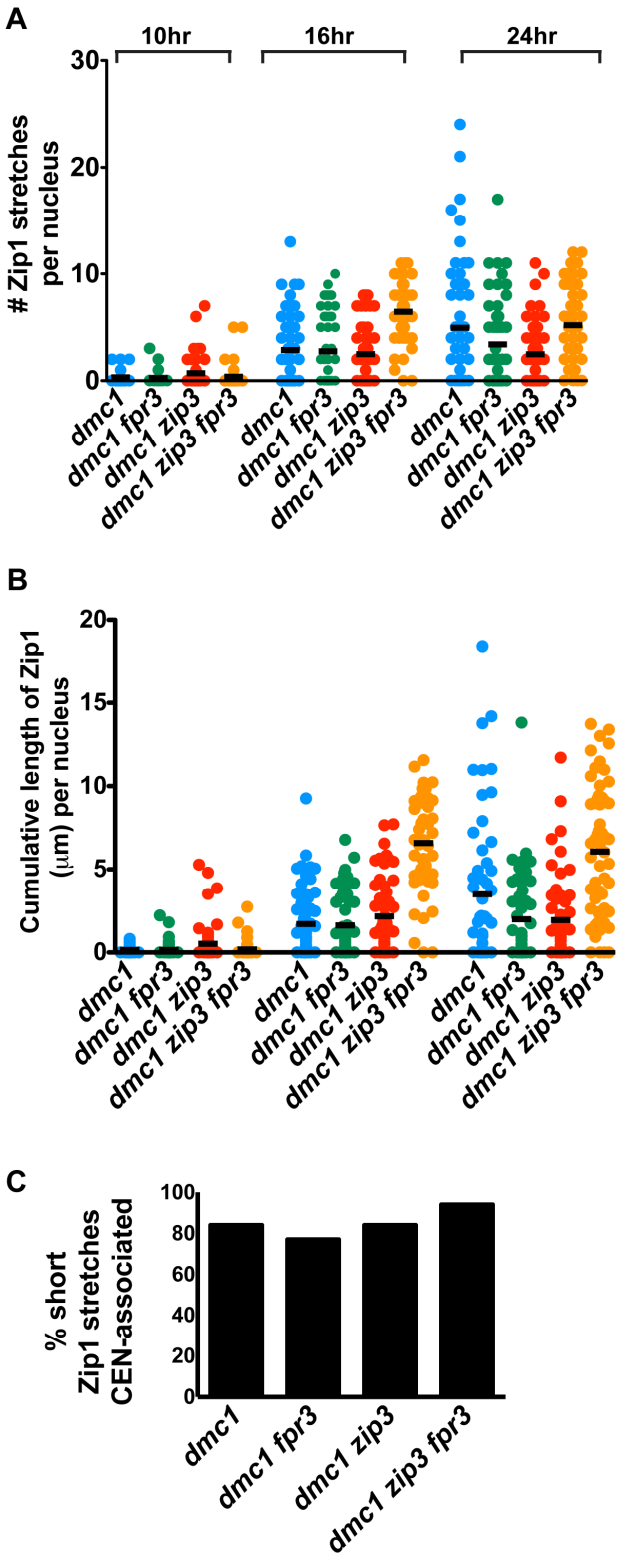
A
10hr
16hr
24hr
# Zip1 stretches per nucleus
30
20
10
0
dmc1
dmc1 fpr3
dmc1 zip3
dmc1 zip3 fpr3
B
Cumulative length of Zip1 (μm) per nucleus
20
15
10
5
0
C
% short Zip1 stretches CEN-associated
100
80
60
40
20
0

**Figure S1.**

**Figure S1. The *fpr3* Mutation Does Not Accelerate Synapsis in *dmc1* Meiotic Cells**

(A and B) Extent of Zip1 polymerization, at multiple time points, was measured for 50 or more nuclei from *dmc1* mutants and displayed on scatter plots. Nuclei were plotted as described in Figure 3G and H. A horizontal black bar depicts the mean for each category. At 16 hours, only *dmc1 zip3 fpr3* triple-mutant cells exhibit significantly elevated levels of Zip1 stretches (A) or cumulative length (B), compared to the other three strains (two-tailed Mann Whitney $p < 0.0001$).

(C) Frequency of centromere-associated, short Zip1 stretches (0.35-0.65 microns in length) from nuclei at 10- and 16-hour time points. Similar to the *zip3* single mutant, centromere-associated synapsis initiation is increased in *dmc1* mutants, compared with wild-type (see Figure 5D for *zip3* and wild-type values). The number of short Zip1 stretches analyzed was 165, 151, 159 and 361 for *dmc1, dmc1 fpr3*, *dmc1 zip3*, and *dmc1 zip3 fpr3* cells, respectively. Significant differences were observed between the following pairs of strains (two sided $p < 0.0015$): *dmc1* vs. *dmc1 zip3 fpr3, dmc1 zip3* vs. *dmc1 zip3 fpr3, dmc1 fpr3* vs. *dmc1 zip3 fpr3*. The values exhibited by *dmc1*, *dmc1 fpr3* and *dmc1 zip3* are not significantly different.

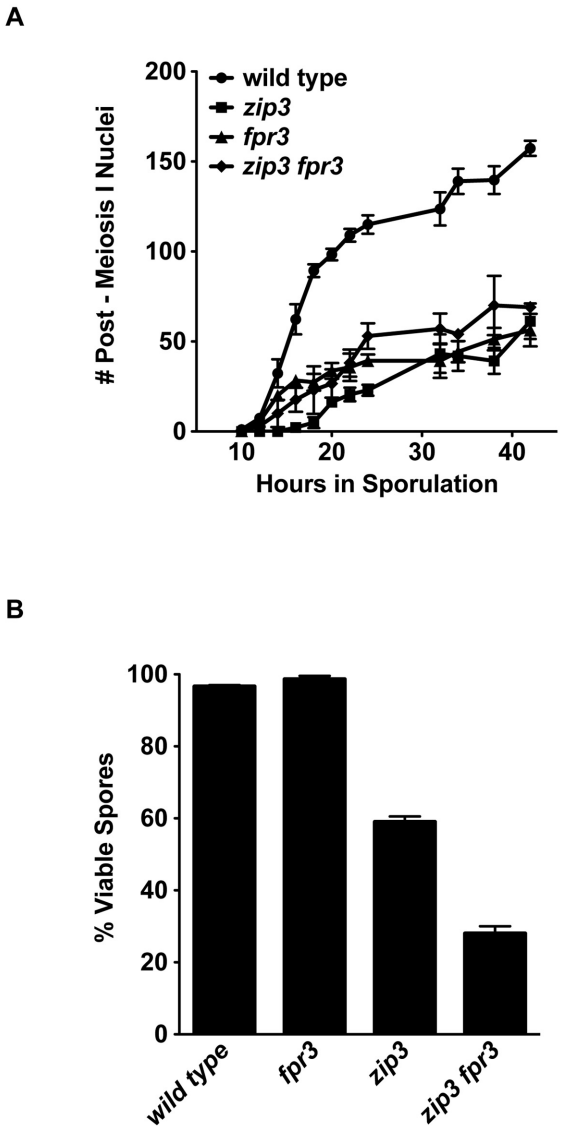


**Figure S2. Meiotic Time Course and Spore Viability**

(A) Meiotic nuclear divisions were examined at intervals throughout meiosis. At each time point, the number of bi- and tetra-nucleate cells, out of 200 total, was plotted.

(B) The histogram plots the % of viable spores for each genotype. For each genotype, between 60-90 tetrads (240-360 spores) were dissected for each of 3, independently isolated, diploids.

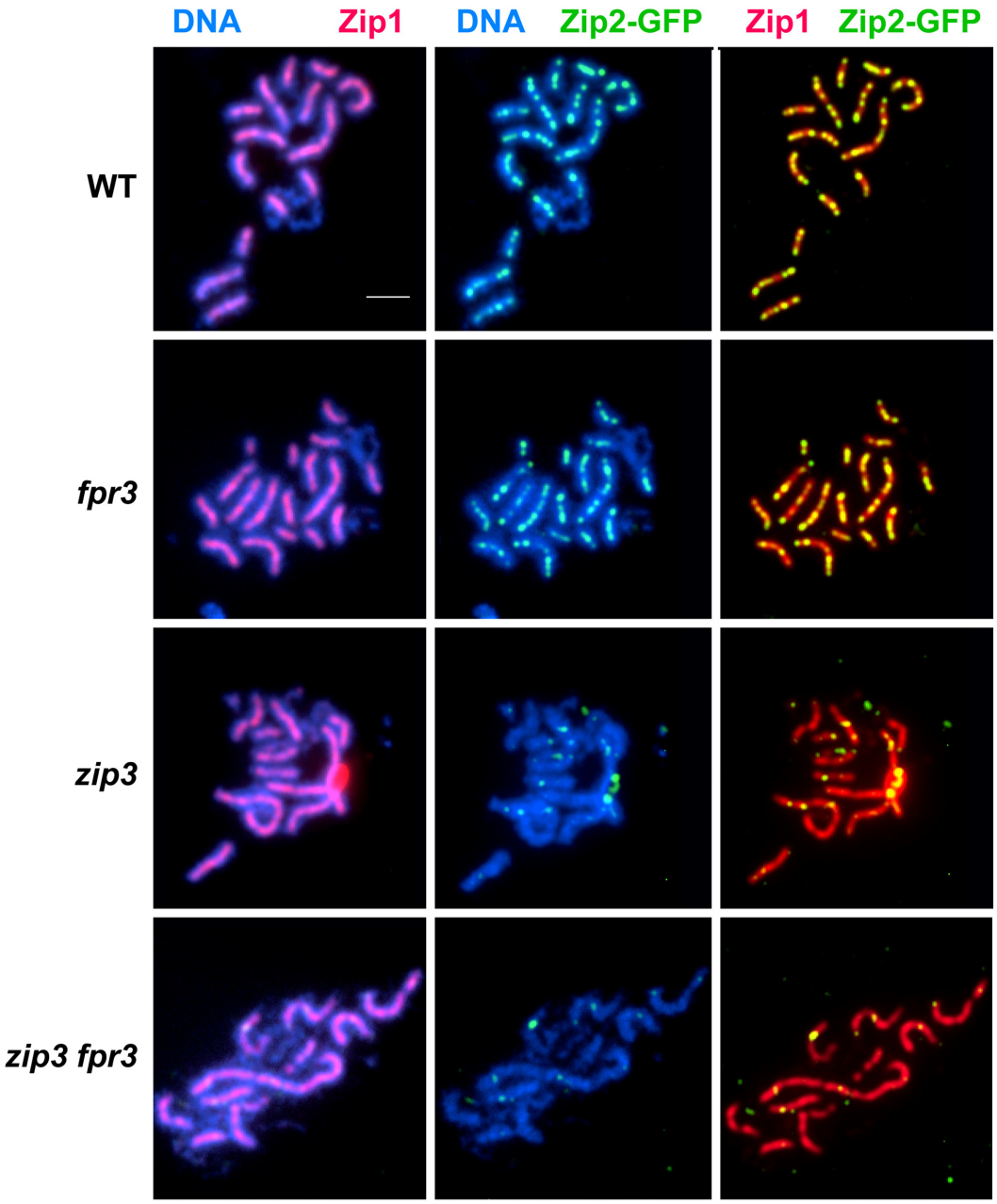


**Figure S3. Zip2-GFP Chromosomal Localization Is Diminished in *zip3* and *zip3 fpr3* Mutants**

Meiotic chromosome spreads from cells of four genotypes (labeled at left for the corresponding row) were stained with DAPI to visualize DNA (blue), anti-Zip1 (red) and anti-GFP to visualize Zip2-GFP (green). Each column shows a distinct set of 2 stains, as annotated at the top of the panels.. In *zip3*, the aggregate of Zip1 associated with large Zip2 foci reflects a polycomplex (PC). Bar, 2 microns.

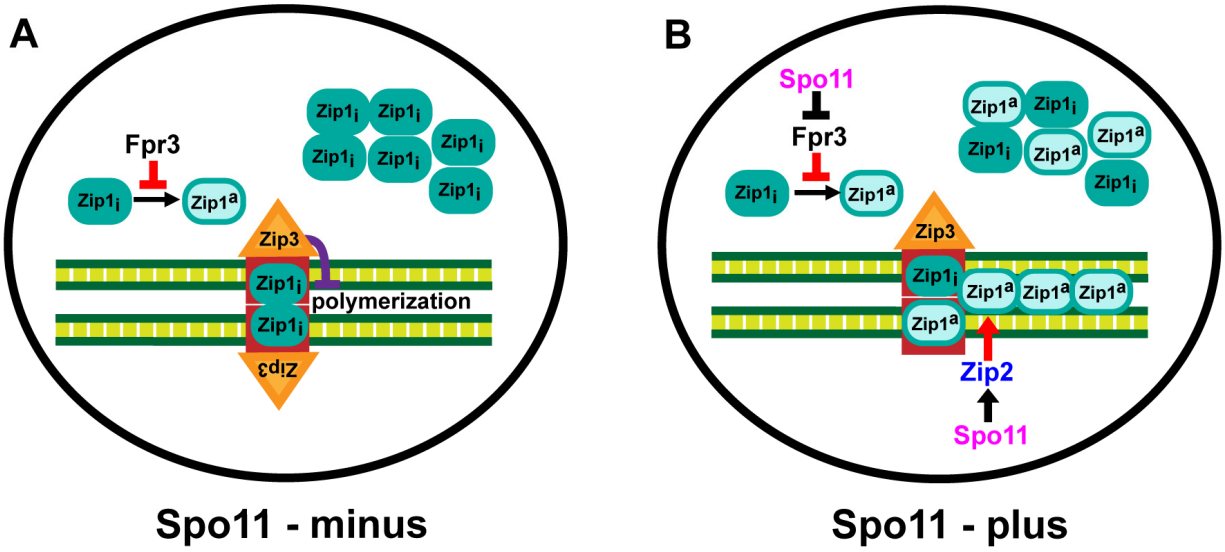


**Figure S4. Cartoon Model of Synapsis Regulation at Centromeres**

(A) Fpr3 activity regulates the distribution of Zip1 in the nucleoplasm of *spo11* mutant cells. We therefore propose that, prior to Spo11 activity, Fpr3 acts in the nucleoplasm to regulate Zip1, perhaps promoting an inactive form ($Zip1_i$) that is refractory to linear assembly on chromatin. Fpr3 could act either by repressing the transformation of Zip1 from an inactive ($Zip1_i$) to an active ($Zip1^a$) form, or by promoting the transformation of $Zip1^a$ to $Zip1_i$. On the other hand, Zip3 prevents Zip1 polymerization locally, at centromeric chromosomal regions, prior to Spo11 activity. Zip3 might discourage Zip1 polymerization through an effect on Zip1 protein itself or through regulating nearby chromosomal proteins.

(B) Signals downstream of recombination initiation, in conjunction with Zip2, are normally required for synapsis initiation. We propose that Spo11 signaling triggers Zip2 to localize to centromeres and reverse the inhibitory effects of Fpr3 and Zip3 on Zip1 polymerization. In addition, Spo11 signaling might inhibit Fpr3 activity to gradually increase the level of active Zip1 in the nucleoplasm.

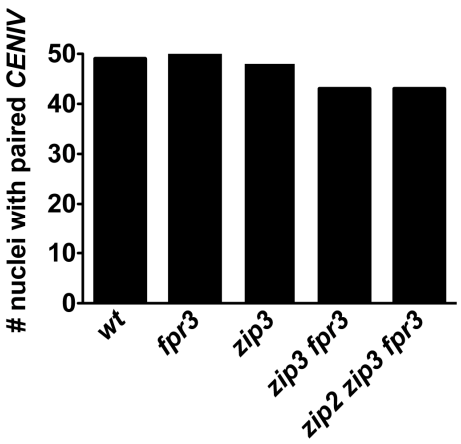


**Figure S5. Homologous Synapsis of Centromere IV**

Zip1 localization was monitored in conjunction with the position of a centromere IV (*CENIV*) FISH probe. Nuclei were scored only if a Zip1 stretch encompassed at least one FISH signal. For all genotypes, Zip1 linear stretches almost always associated with paired *CENIV* FISH signals. For each genotype, 50 nuclei were scored. The slight reduction in homologous synapsis observed in *zip3 fpr3* cells is not significant (two-sided p = 0.059).