Kluyveromyces lactis Zip1: A Novel Separation of Function Allele

by

Yashna Thappeta
Class of 2014
ACKNOWLEDGEMENTS

First and foremost, I would like to thank Dr. Amy MacQueen for her unwavering support and guidance over the last three years. Her passion for research has been inspiring and my time in her lab has shaped my college experience and my future aspirations.

I would also like to thank all of the members of the MacQueen lab, both past and present. First and foremost, I must thank Karen Voelkel-Meiman who has taught me to be a rigorous scientist at the bench and for readily answering all of my ‘off the record questions’. Cassandra O’Curran has also shaped a huge part of both my personal and professional lab life. Cassie and I first started working on this *K.lactis* Zip1 project together and she was a wonderful role model when I was just starting out in the MacQueen lab. Her contributions to our study have been phenomenal. I would also like to thank Michelle Cheng who has been a constant cheerleader in my life through the wonderful tension that comes with writing a thesis.

I would also like to thank my thesis readers, Dr. Scott Holmes and Dr. Michael Weir. Dr. Holmes has also served as my advisor and has been a calm source of wisdom whenever the stressful topic of my future arises. Dr. Weir was the instructor for my very first introduction to biology class during the fall of my freshman year. My experience in his classroom definitely shaped a large part of my interest in molecular biology and my desire to be involved with research.

I would like to thank Dhivya Perumal for her constant presence in my life for the last eleven years and especially in this last one month as I struggled through my thesis. Last but not least, I would like to thank my family for their understanding
when unending support and for always picking up my panicked phone calls. It never really seems like you guys are half a world away.
ABSTRACT

Homologous chromosomes must become associated during meiotic prophase in order to disjoin properly on the meiotic spindle. One protein responsible for aiding this process is Zip1, which assembles along the full length of an aligned homologous chromosome pair as a structural constituent of the synaptonemal complex (SC). To understand and characterize aspects of Zip1’s meiotic behavior and function, we have created a *Saccharomyces cerevisiae* strain, which expresses an ancestral version of the protein-*Kluyveromyces lactis* Zip1.

Our studies reveal that although *K. lactis* Zip1 is unable to elongate and form stable structures along the full length of paired chromosomes in *S. cerevisiae* cells as *S. cerevisiae* Zip1 does, it is able to promote some degree of meiotic function. We have studied Zip1’s non-SC meiotic functions and determined that *K. lactis* Zip1 is unable to robustly rescue centromere functions in *S. cerevisiae* cells. Surprisingly, *K. lactis* Zip1 was able to promote close to wildtype levels of interfering crossovers - a unique phenotype among non-SC forming mutants. Thus *K.l* Zip1 appears to be a separation of function allele, uncoupling Zip1 function in crossover formation from its capacity to build stable SC and to promote robust centromere interactions. Finally, we also found that deletion of a single copy of *PCH2*, an AAA+ ATPase, associated with a meiotic checkpoint, abolishes crossover interference in some intervals of strains expressing *K.lactis* Zip1.
TABLE OF CONTENTS

INTRODUCTION .................................................................................................................. 1
Meiosis and the math ........................................................................................................... 1
Meiosis specific nuclear dynamics during prophase I culminate in stable pair wise
interactions between homologous chromosomes .......................................................... 2
Homologous recombination is central to the homolog pairing process ................... 6
Crossover recombination creates a physical stabilization between homologs .......... 6
The synaptonemal complex links initial homolog pairing with pairing stabilization .... 7
Synapsis initiation at sites of crossover recombination and centromeres ............. 10
Synapsis preferentially initiation at centromeres ......................................................... 10
Role of the SC ................................................................................................................ 11
Zip1 plays multiple roles during meiotic prophase ...................................................... 12
Kluyveromyces lactis Zip1 ............................................................................................. 14

MATERIALS AND METHODS ...................................................................................... 17
Strain Construction ........................................................................................................ 17
Meiotic Chromosome Spreads ....................................................................................... 18
Genetic Analysis of crossovers ..................................................................................... 19

RESULTS ..................................................................................................................... 21
Klactis Zip1 provides some meiotic functions in S. cerevisiae cells ......................... 21
Klactis Zip1 does not form stable full length SC ....................................................... 24
Kl ZIP1 partially rescues the sporulation arrest triggered by Zip1-4LA .................... 27
Kl ZIP1 does not preferably associate with centromeres ........................................... 28
Kl ZIP1 fails to rescue centromere coupling function in S. cerevisiae .............. 30
haploids in early prophase ......................................................................................... 30
Kl ZIP1 partially rescue centromere coupling function in S. cerevisiae diploids in early
prophase ....................................................................................................................... 32
Centromere tethering is not rescued in meiotic haploids ......................................... 34
S. cerevisiae meiotic cells expressing K1 ZIP1 exhibit nearly wild-type crossover levels
and minimal loss of crossover interference in four spore viable .......................... 35
Crossover levels remain but interference is abolished when the dosage of PCH2 is
reduced in strains expressing K1 Zip1 ........................................................................ 39

DISCUSSION ................................................................................................................. 42
Klactis Zip1 and Chromosome Synapsis ..................................................................... 42
Klactis Zip1 and centromeres ....................................................................................... 44
Klactis Zip1 as a separation of function allele ........................................................... 45
Future Directions ......................................................................................................... 48

References .................................................................................................................... 50
LIST OF TABLES AND FIGURES

Figure 1. Meiotic prophase is divided into 5 stages ........................................3
Figure 2. The Synaptonemal Complex (SC) in budding yeast .......................9
Figure 3. Zip1 plays multiple roles during meiotic prophase I ......................13
Figure 4. *K. lactis* Zip1 staining in *K. lactis* cells ....................................15
Figure 5. Alignment of *S.cerevisiae* Zip1 and *K. lactis* Zip1 amino acid sequence .................................................................16
Figure 6. *K. lactis* Zip1 in *S.cerevisiae* cells promotes homolog disjunction 23
Figure 7. *K. lactis* Zip1 and Smt3, two central region proteins fail to assemble stable SC structure .........................................................25
Figure 8. Red1 localizes to chromosome axes in *S.cerevisiae* cells expressing *K. lactis* ZIP1 .................................................................26
Figure 9. *K. lactis* Zip1 partially rescues the sporulation arrest triggered by Zip1-4LA if expressed in early prophase but fails to do so in late meiotic prophase .................................................................27
Figure 10. *K. lactis* Zip1 does not preferentially associate with centromeres ......29
Figure 11. *K. lactis* Zip1 does not rescue centromere coupling in haploid cells ...31
Figure 12. *K. lactis* Zip1 partially rescues centromere coupling in diploid cells ....33
Figure 13. Centromere tethering in haploid cells ........................................35
Figure 14. *K. lactis* ZIP1 rescues crossover levels and interference in *S.cerevisiae* cells .................................................................37
Figure 15. Interference is abolished in *K. lactis* ZIP1 strain heterozygous for *PCH2* .40

Table 1. Table of primers used in this study ..............................................18
Table 2. Table of strains used in this study ...............................................20
Table 3. Measure of crossing over and crossover interference ......................38
Table 4. Measure of crossing over and crossover interference in a *PCH2* heterozygote .................................................................41
INTRODUCTION

Meiosis and the math

Sexual reproduction presents organisms with a novel problem with regard to chromosome ploidy. Offspring that result from sexual reproduction contain a set of genetic material from both their mom and dad. Thus, only one of two sets of chromosomes should be contributed to offspring. In order to create gametes with half the chromosome number, cells in a sexually reproducing organism must undergo a reductional cell cycle known as meiosis.

Like mitosis, meiosis begins with a DNA replication event, which results in the formation of two identical sister chromatids held together by cohesin proteins. However, meiosis consists of two rounds of cell division. During meiosis I, chromosomes become aligned with their homologous partners and are subsequently segregated apart from one another into two daughter cells. Each daughter then undergoes an additional cell division cycle that is analogous to mitosis - meiosis II - during which sister chromatids are separated to form four haploid gametes.

Although critical for proper ploidy reduction at the first meiotic division, the molecular mechanisms underlying how homologous chromosomes specifically align with one another during meiosis I are poorly understood.

Yeast is an ideal model organism for meiotic studies. Not only can diploid strains enter meiosis under certain stress conditions, but the haploid spores formed can also be studied. Furthermore, yeast is easily manipulated at a genetic level. Meiotic events in yeast are highly conserved with those of more complex eukaryotes (Kerr, Sarkar, & Arumugam, 2012). Thus an understanding of the principles
governing proper homolog segregation in yeast could provide insight for how the process can go awry in humans.

**Meiosis specific nuclear dynamics during prophase I culminate in stable pair wise interactions between homologous chromosomes.**

In order for proper meiosis I chromosome segregation to occur, homologous chromosomes must first establish stable pair-wise associations. Pairing must then be reinforced and maintained. These events occur during meiotic prophase I, which has been broken down into 5 stages based on cytological analyses of meiotic nuclei: *leptotene*, *zygotene*, *pachytene*, *diplotene*, and *diakinesis*. As chromatin begins to condense during the *leptotene* and *zygotene* stages of early meiotic prophase, the telomeres of chromosomes attach to the inner nuclear membrane and cluster together, resulting in a transient polarized chromosomal configuration referred to as the “bouquet” (Zickler & Kleckner, 1998). In some organisms, centromeres are also brought into close proximity to one another prior to homologous pairing. During *leptotene* and *zygotene*, recombination is initiated throughout the chromatin, and initial pairing events occur between homologous chromosomes. When the polarized configuration is lost and chromosomes disperse from the “bouquet” and enter the *pachytene* stage, they are aligned end to end with their homologous partners. Aligned homologous chromosomes at the mid-prophase stage of *pachytene* exhibit a proteinaceous structure, the synaptonemal complex (SC), assembled along their lengths (Page & Hawley, 2003; Zickler & Kleckner, 1999). SC is required to process recombination events into interhomolog crossovers; crossover recombination events in conjunction with sister cohesion serve to maintain the pairwise association of
homologs, after SC disassembly and throughout the subsequent chromosomal compaction processes that can occur during diplotene and diakinesis, and until their segregation on the meiosis I spindle. (Page & Hawley, 2003).

Figure 1: Meiotic prophase is divided into 5 stages: The red and blue sets of sister chromatids are homologs. By the end of premeiotic S phase, chromatin has begun condensing, and homologs are unpaired. By the end of leptotene, homologs have accurately paired. Synapsis initiates during zygotene and completed during pachytene. Chiasmata, the result of crossover events, maintain homolog associations during diplotene and diakinesis. Homologs segregate during the reductional division of metaphase I (Page & Hawley, 2003).
Dynamic chromosome movements facilitate the search homologous partners

The process by which homologs recognize one another is still fairly murky, however the predominant theory is that chromosomes actively search for their partner through transient associations that occur independent of homology (Maguire, 1974; Tsubouchi & Roeder, 2005). In yeast, these associations occur primarily at telomeres by bouquet formation and the instigation of ‘rapid prophase movements’, and at centromeres.

**Bouquet formation**

At the crux of late leptotene and early zygotene, telomeres attach to the inner nuclear membrane and transiently cluster, resulting in a chromosomal configuration called the “bouquet” (Zickler & Kleckner, 1998). However, in the absence of Ndj1, a telomere-associated protein, bouquet formation is lost and subsequent homolog pairing is merely delayed, not abolished in budding yeast. (Trelles-Sticken, Dresser, & Scherthan, 2000). The pairing delay phenotype of ndj1 mutants could indicate that while bouquet formation is not required for homolog pairing, it facilitates the search process by bringing chromosomes into close proximity.

**Rapid prophase movements**

Telomere associated proteins that play a role in bouquet formation, like Ndj1, are required for the rapid prophase movements (RPMs) that meiotic prophase chromosomes exhibit. These movements are driven via cytoplasmic motors outside the nuclear envelope, which link to telomeres through a group of proteins that span the nuclear envelope (Conrad, Lee, Wilkerson, & Dresser, 2007). Pairing rates are directly correlated with RPM activity but not bouquet formation in various meiotic
mutants, suggesting that RPMs facilitate at least some aspects of homologous pairing while the bouquet plays at most a minor role in *Saccharomyces cerevisiae* (Lee, Conrad, & Dresser, 2012).

**Centromere coupling in budding yeast**

Another early prophase chromosomal interaction mechanism that occurs in budding yeast may aid the homolog pairing process in a similar manner as bouquet and/or RPMs. This phenomenon is known as ‘centromere coupling’.

Coupling is a two-by-two association of centromeres that occurs prior to homolog pairing in early prophase meiotic nuclei. Early on, centromeres associate regardless of homology, but as homolog pairing develops, centromeres eventually associate with their homologous centromere partners. This transition form non-homologous coupling to homologous pairing is dependent on Spo11 (Tsubouchi & Roeder, 2005), a meiosis-specific gene product required for the introduction of the double strand breaks (DSBs) into DNA that initiate recombination (Keeney, 2001). (Homolog pairing appears to be mechanistically linked to recombination initiation in a number of organisms, including budding yeast).

Interestingly, early meiotic prophase centromere coupling is mediated by the SC protein Zip1, although other SC components are dispensable for coupling. Thus, as will be described in more detail below, the Zip1 protein mediates prophase chromosomal interactions in at least two mechanistically distinct manners, at centromeres and within the context of the SC.
Homologous recombination is central to the homolog pairing process

Homolog pairing is tightly linked to the process of homologous recombination. In many organisms, initial homolog pairing, perhaps even the homolog recognition process itself, is dependent on recombination initiation (DNA double strand break formation) (Tsubouchi & Roeder, 2005) (Roeder, 1997). Moreover, the resolution of interhomolog recombination events into crossovers are critical in most all organisms for maintaining paired associations between homologs until their segregation on the meiosis I spindle.

Crossover recombination creates a physical stabilization between homologs

Recombination is initiated by the introduction of double strand breaks (DSBs) in the DNA by the topoisomerase-like enzyme Spo11 (Neale & Keeney, 2006). Although Spo11 does not recognize a consensus site, DSBs tend to occur at “hot spots”, which typically occur in open chromatin such as promoter regions (Pan et al., 2011). DSBs are resected, exposing a single stranded 3’ end, which can then use a homologous DNA strand as a template to repair the break. There are three possible templates that can be used - sister or either of the nonsister chromatids. If the sister chromatid is used a template, no net alterations occur to the genetic information. If one of the two homologous nonsister chromatids belonging to the partner homolog is used, the recombination event can result in a crossover, involving the reciprocal exchange of genetic material between homologs. Alternatively, interhomolog recombinational repair can occur without reciprocal exchange, which results in a one-way transfer of information and is called a gene conversion or noncrossover. Only a
crossover results in the formation of chiasmata, which reinforces pairing (Bhalla & Dernburg, 2008).

Although there are three potential ways repair can occur, in yeast, little over half of DSBs seem to be resolved into interhomolog crossovers (Fung, Rockmill, Odell, & Roeder, 2004). However, every chromosome must undergo a crossover for proper segregation. When two or more crossover recombination events occur along a chromosome, they exhibit “interference”: if an event is present at one position, there is a reduced probability that another event will be found nearby. Interference can be assessed by computing the NPD ratio in a given linked genetic interval. NPDs are the result of a double crossover event, which are relatively rare between two linked genes. The NPD ratio measure observed NPD/expected NPD (E/O), where expected NPDs refers to the frequency of occurrence in the absence of interference (Poisson distribution). Analyses of crossover distribution in synapsis-defective meiotic mutants has raised the idea that crossover interference is mediated by the SC (Roeder, 1997).

The synaptonemal complex links initial homolog pairing with pairing stabilization

The processes of homolog pairing and inter-homolog recombination are linked by another meiosis-specific feature: the proteinacious structure known as the Synaptonemal Complex, or SC. SC normally assembles downstream of proper homolog recognition and the SC is required for a proper number and distribution of crossover recombination events (Storlazzi, Xu, Schwacha, & Kleckner, 1996; M Sym & G. Shirleen Roeder, 1994; Sym, Engebrecht, & Roeder, 1993).
The SC structure holds homologs close together along their full length during pachytene (Roeder, 1997). As chromatin condenses in early meiotic prophase prior to pachytene, meiosis specific proteins Red1 and Hop1 localize to chromosome cores, contributing to the formation of the axial elements of individual chromosomes (Smith & Roeder, 1997). In the context of the mature SC, axial elements are referred to as ‘lateral elements’ (Page & Hawley, 2004). In the absence of proper axial/lateral elements, the SC cannot form.

The lateral elements within the SC are connected by SC central region proteins, including Zip1 and SUMO (a small ubiquitin like modifier). Zip1 is a coiled coil protein that comprises the transverse filaments of the SC central region (Sym et al., 1993). Zip1’s α helical coiled coil is flanked by globular domains at the N and C terminus and it assembles parallel dimers that interact with one another at the N terminus and span the width of the SC (Dong & Roeder, 2000). This assembly is known to be facilitated by the Ecm11-Gmc2 complex, which also incorporate into the central region (Humphryes et al., 2013).
Figure 2. The Synaptonemal Complex (SC) in budding yeast: The SC is a proteinaceous structure that forms along the lengths of synapsed homologous chromosomes. It is composed of lateral elements (LE) like Red1 and Hop1 which associate with chromatin of the two sisters. They are bridged by Zip1 transverse filaments, which form the central region (CE). Figure from (Page & Hawley, 2003).
**Synapsis initiation at sites of crossover recombination and centromeres**

The proteins Zip2, Zip3, and Zip4 mark sites of synapsis initiation (Bhalla and Dernburg 2008). Furthermore, they all localize to crossover sites and also influence the distribution of crossovers. In a *zip2* null, synapsis does not occur and Zip1 foci appear between homologs, but they never elongate. Rather, a polycomplex of Zip1 appears in the nucleus. In the wild-type, Zip2 likely localizes to sites of synapsis initiation, as suggested by the high frequency of colocalization between Zip2 foci and nascent Zip1 foci (synapsis initiation events). It appears that these synapsis initiation sites are also sites of recombination, as Zip2 colocalizes with the Mre11 recombination protein in the wild-type. Zip4 exhibits a similar localization and null phenotype to that of Zip2. Given their requirement for normal synapsis initiation, Zip2/Zip3/Zip4 complexes are referred to as synapsis initiation complexes (SICs).

**Synapsis preferentially initiation at centromeres**

Synapsis initiation events also appear to occur at centromeres (Tsubouchi, MacQueen et al. 2008). Tsubouchi and colleagues demonstrated that over 80% of earliest Zip1 incorporation events occur near centromeres, suggesting that centromeres are preferred early sites for synapsis initiation. Moreover, Zip1 localization to centromeres occurs independently of Zip3, and centromere synapsis is less dependent on Zip3 than synapsis from recombination sites, indicating that synapsis initiation can occur at centromeres without the necessity of a canonical SIC (Tsubouchi, MacQueen et al. 2008).
Role of the SC

The SC is a widely conserved structural feature of eukaryotic meiotic chromosomes, and typically plays an essential role in meiosis, as evident by the observation that mutants defective in synapsis display reduced spore viability and sporulation efficiency (Agarwal & Roeder, 2000; Sym et al., 1993). In addition, the SC plays a role in facilitating crossovers. In mutants defective in synapsis, crossover recombination is diminished – in zip1 mutants show a three fold decrease while red1 mutants exhibit a ten fold decrease (Roeder, 1997). This could perhaps be attributed to the observation the maturation of DSBs into to crossovers seems to occur within the context of the SC (Borner, Kleckner, & Hunter, 2004). In addition, the SC is thought to facilitate the distribution of crossovers.

The critical role of crossovers is highlighted by the fact that each chromosome, regardless of its size, must have at least one crossover event. This distribution is ensured by crossover interference. In the absence of SC, for example in a zip1 background, interference is completely lost for the crossovers that remain, meaning that a subset of chromosomes may not experience a crossover event which could play a role in miss-segregation (M. Sym & G. S. Roeder, 1994).
**Zip1 plays multiple roles during meiotic prophase**

Zip1 is well documented as a major component of the central region of the budding yeast SC. As mentioned before, its role in the SC is tightly coupled with the ability to promote interfering crossovers. In a *red1* mutant, without axial elements, Zip1 fails to polymerize and SC is not formed and as expected, crossovers are lost. Interestingly, in a *zip1 red1* double mutant, there is an additive effect, where a greater number of crossovers are lost than in the *red1* single mutant alone (Storlazzi et al., 1996). This observation raised the idea that Zip1 may promote crossovers independent of its role in SC formation.

Zip1 also mediates centromere associations outside the context of the SC. As mentioned before, prior to double strand break formation, SC assembly and homolog pairing, Zip1 mediates centromere coupling. After SC disassembly, Zip1 is also implicated in playing a genetically distinct type of centromere interaction, centromere‘tethering’. Tethering is also the pair wise association of centromeres, but in late meiotic prophase I. Zip1 foci are found at the centromeres, potentially holding them together – playing a chiasmata like role. Thus centromere tethering has been hypothesized as a backup system to ensure segregation of chromosomes that do not experience a crossover (Newnham, Jordan, Rockmill, Roeder, & Hoffmann, 2010). For example, in haploid cells or diploid cells containing homeologs of a chromosome that are divergent in sequence, SC and chiasmata fail to form. However, in late meiotic prophase, centromeres pairs are found to be held together by Zip1 foci (Newnham et al., 2010).
At a molecular level, the multiple roles of Zip1 seem to be distinct. However, the extent to which chromosomes rely on each function for proper disjunction is unknown. In order to address this conundrum, we decided to study a *S.cerevisiae* Zip1 ortholog, exploring the possibility that one or more of these functions are products of evolution.(Beltrao & Serrano, 2005)

**Figure 3: Zip1 plays multiple roles during meiotic prophase:** The pair-wise association of chromosomes in early meiotic prophase is known as centromere coupling. Zip1 is found at centromeres. Once Spo11 introduces DSBs, chromosomes pair and SC assembles. In late meiotic prophase, after SC disassembles, paired chromosomes are held together by Zip1 foci in ‘centromere tethering’.
Kluveromyces lactis Zip1

*Kluveromyces lactis* diverged from *Saccharomyces cerevisiae* approximately 400 million year ago (Beltrao & Serrano, 2005) prior to a whole genome duplication event. Thus the *K.lactis* genome contains 6 chromosome pairs, while *S.cerevisiae* contains 16. *K.lactis* Zip1 is structurally very similar to *S.cerevisiae* Zip1, despite the fact that they are only 20% identical at the amino acid level. Both proteins consist of a coiled coil region flanked by two globular ends. *S.cerevisiae* Zip1 is 875 amino acids in length and is slightly larger than its ancestor, which is 755 amino acids long. Alignment of the amino acid sequences shows that a majority of the truncation is within the two globular ends, rather than the coiled coil (Figure 5).

Our interest in this particular ancestral version of Zip1 arose from Dr. Amy MacQueen’s preliminary observation that *K.lactis* Zip1 in *K.lactis* cells forms a single focus on the chromosomes rather than full length linear stretches that are made by *S.cerevisiae* Zip1 in *S.cerevisiae* cells. Thus *K.lactis* Zip1 perhaps does not participate in SC formation in *K.lactis* cells. In addition, it was particularly intriguing that there was a single Zip1 focus associated with a condensed chromosome (as seen in Figure 4) suggesting a centromeric role for Zip1 in this ancestral yeast strain.
Figure 4: *K.lactis Zip1* staining in *K.lactis* cells. Surface spread *K.lactis* nuclei have been stained with antibodies against *K.lactis* Zip1. Each nucleus has between 3 and 10 nuclei, more importantly there is a single *K.lactis* Zip1 focus per chromosome. This foci may potentially associate with centromeres.

In order to study *K.lactis* Zip1 and its potential to perform SC independent functions, Cassandra O’Curran (Wesleyan University 2013) replaced the *S.cerevisiae* Zip1 ORF in *S.cerevisiae* cells with the *K.lactis* Zip1 ORF. Thus our experimental *S.cerevisiae* strain expresses *K.lactis* Zip1 protein using endogenous *cis*-acting regulatory sequences.

Interestingly we found that *K.lactis ZIP1* was able to provide some meiotic function to *S.cerevisiae* cells. However, we were surprised to find that *K.lactis* Zip1 does not build SC. This observation raised an interesting question that we explore in this thesis: What non-SC functions does *K.lactis* Zip1 promote to facilitate proper disjunction? Could *K.lactis* Zip1 be relying on centromere dependant Zip1 function in the absences of SC?
Figure 5 Alignment of *S. cerevisiae* Zip1 and *K. lactis* Zip1 amino acid sequence: Sequence in white (top) is *S. cerevisiae* ZIP1 sequence while sequence in black (bottom) is *K. lactis* ZIP1 sequence. The blue region forms the globular domain while the green forms the coiled coil. It is interesting to note that a majority of the truncation exists in the globular domain.
MATERIALS AND METHODS

Strain Construction

All strains are in the BR1919 strain background and all genotypes are listed in Table 1. Yeast genetic manipulations were carried out via standard procedures.

A transformation was done to insert the \textit{K.lactis ZIP1} ORF into the \textit{S.cerevisiae} genome. The \textit{K.lactis ZIP1} ORF from the \textit{K.lactis} strain CBS2359 (provided by Dr. Angelika Amon, MIT) and the PCR product was transformed into a precise \textit{zip1::URA3} knockout #1069. Positive transformants (cells in which \textit{URA3} was replaced with \textit{K.lactis ZIP1}) were selected for on 5-FOA.

For coupling and tethering experiments, haploid strains expressing \textit{MAT\alpha} had \textit{MAT\alpha} integrated at the \textit{THR1} locus in a strain as described (Rockmill & Roeder, 1998).

Auxotrophic and drug markers to mark genetic intervals and measure crossover recombinations levels were inserted through transformations. \textit{NAT} at \textit{HMR} was amplified off of BAM211 using primers AJM1046 and AJM1047. \textit{THR1} at 193,424 and \textit{LEU2} at 152,000 on chrXI (http://www.yeastgenome.org/) were amplified using AJM1324, 1325 and AJM1321, 1322 respectively. \textit{TRP1} was transformed to mark \textit{SPO11} by amplifying it off of Lontine plasmid, pFA6A-TRP1, using primers AJM1234 and 1235.
<table>
<thead>
<tr>
<th>Primer Number</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1046</td>
<td>CATTTTAATAGAGCTGAGTACTTCTACATTTCTTTTGATATCGGTACGCTGCAGGTCGAC</td>
</tr>
<tr>
<td>1047</td>
<td>CGGTGTCTTAAAGGAAGAAGAATTTTACGACTACAGAAATGATGCAATGAGATCGAATTGGAGCTG</td>
</tr>
<tr>
<td>1234</td>
<td>GCTTTGGCTTTATAAAAAATGTTTTTTCAAGAATTTGAAAAACGTTACATTCGGATCCCCGGGGTTAAATTAA</td>
</tr>
<tr>
<td>1235</td>
<td>GTATTTTTTTTGGACTAGTATTTATCAAAAGATTCAATTGATATATTGATTTGAGCTAGCTGTTTTAAAC</td>
</tr>
<tr>
<td>1321</td>
<td>TATAAAGGTTTTTTAATAATTATTCGAGATGCTCTCGACGCTATCGGCCGAGCGGTCTAAGGCGCC</td>
</tr>
<tr>
<td>1322</td>
<td>ATTTGATGAAACAGCAATATAACCTACACCTAAAGTTGAATGAGATTTCGTTTTCTTTGAGTGCCCTCCTCCTTGTC</td>
</tr>
<tr>
<td>1324</td>
<td>TGGTGCGAAAAACCTTTATAAGTGAATCAGATTAAACTTCTTTGAAAGCTCTACCCCGATCGCTTTCGCG</td>
</tr>
<tr>
<td>1325</td>
<td>TATAACGGCGATGTACTTTACTTTAATTAAACTATGGCATTAATTCCGATAGAAAAAGACGGGTAGCCG</td>
</tr>
</tbody>
</table>

Table 1: Primer table

**Meiotic Chromosome Spreads**

Meiotic chromosome spreads, staining and imaging were carried out as described (Voelkel-Meiman, Moustafa, Lefrancois, Villeneuve, & MacQueen, 2012).

Figures 7, 8: YT48 was spread after 24 hours of sporulation. For figure 7, nuclei were stained with the following primary antibodies: α-Zip1 in rabbit raised at YenZym Antibodies, LLC, against a C-terminal fragment of Zip1 as detailed in (Sym, Engebrecth et al. 1993) (1:100), affinity purified guinea pig anti-SUMO (1:200). Secondary antibodies were obtained from Jackson Immuno Research and used at a 1:200 dilution. For figure 8, only α-Red1 in rabbit, which was a kind gift of G.
Shirleen Roeder, Yale University, raised as detailed in (Smith and Roeder 1997) at 1:100 was used. For figure 10, $\alpha$-Zip1 in rabbit and $\alpha$-c-myc in mouse from invitrogen (1:200) were used as primary antibodies.

Haploid and diploids coupling experiments were conducted based on protocol developed by the Roeder lab (Tsubouchi & Roeder, 2005). Induction experiment using the $P_{\text{GAL}}$ was conducted as described by (Voelkel-Meiman et al., 2012).

**Genetic Analysis of crossovers**

Tetrads were dissected and only four spore viables were analyzed. Data was entered into Tetrad Analysis program, created by Dr. Eva Hoffman. For *K.l ZIP1* strains, data was first manually entered in duplicate and then only four spore viable tetrads were entered into the program (this was also done twice to ensure accuracy). *S.c ZIP1* data was directly entered. Map distances calculated using standard tetrad analysis, as per Perkins (Perkins, 1949), and were generated using the Stahl lab online tools: http://molbio.uoregon.edu/,fstahl/.
<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR1919-8b</td>
<td>$MAT_a$ leu2-3,112 his4-260,519 trp1-289 ura3-1 thr1-4 ade2-1</td>
</tr>
<tr>
<td>1919</td>
<td>$MAT_a$ $MAT_a$</td>
</tr>
<tr>
<td>CO9</td>
<td>$MAT_a$/$MAT_a$ $K.l$-ZIP1/$K.l$-ZIP1</td>
</tr>
<tr>
<td>CO10</td>
<td>$MAT_a$/$MAT_a$ zip1::URA3/zip1::URA3</td>
</tr>
<tr>
<td>CO49</td>
<td>$MAT_a$/$MAT_a$ zip1-4LA/zip1-4LA</td>
</tr>
<tr>
<td>CO51</td>
<td>$MAT_a$/$MAT_a$ zip1-4LA/zip1::URA3</td>
</tr>
<tr>
<td>YT9</td>
<td>$MAT_a$/$MAT_a$ zip1-4LA/ P$GAL^*$Sc ZIP1 $pkB80$-URA3/ura3 Ctf19-myc::KAN/ Ctf19+</td>
</tr>
<tr>
<td>YT11</td>
<td>$MAT_a$/$MAT_a$ zip1-4LA/ P$GAL^*$K.l-ZIP1 $pkB80$-URA3/ura3 Ctf19-myc::KAN/ Ctf19-myc::KAN</td>
</tr>
<tr>
<td>YT14</td>
<td>$MAT_a$/$MAT_a$ spo11::ADE2/spo11::ADE2 K.l-ZIP1/K.l-ZIP1 Ctf19-myc::KAN/ Ctf19-myc::KAN</td>
</tr>
<tr>
<td>YT15</td>
<td>$MAT_a$/$MAT_a$ spo11::ADE2/spo11::ADE2 Ctf19-myc::KAN/ Ctf19-myc::KAN</td>
</tr>
<tr>
<td>YT21</td>
<td>$MAT_a$/$MAT_a$ spo11::ADE2/spo11::ADE2 zip1::URA3/zip1::URA3 Ctf19-myc::KAN/ Ctf19-myc::KAN</td>
</tr>
<tr>
<td>YT23</td>
<td>$MAT_a$/$MAT_a$ spoill::ADE2 K.l-ZIP Ctf19-myc::KAN</td>
</tr>
<tr>
<td>YT24</td>
<td>$MAT_a$/$MAT_a$ spoill::ADE2 Ctf19-myc::KAN</td>
</tr>
<tr>
<td>YT25</td>
<td>$MAT_a$/$MAT_a$ spoill::ADE2 zip1::URA3 Ctf19-myc::KAN</td>
</tr>
<tr>
<td>YT35</td>
<td>$MAT_a$/$MAT_a$ zip1-4LA/ K.l-ZIP P</td>
</tr>
<tr>
<td>YT47</td>
<td>$MAT_a$/$MAT_a$ K.l-ZIP1/K.l-ZIP1 Ctf19-myc::KAN/ Ctf19-myc::KAN ndt80::LEU2/ndt80::LEU2</td>
</tr>
<tr>
<td>YT48</td>
<td>$MAT_a$/$MAT_a$ Ctf19-myc::KAN/ Ctf19-myc::KAN ndt80::LEU2/ndt80::LEU2</td>
</tr>
<tr>
<td>YT72</td>
<td>$MAT_a$/$MAT_a$ K.l-ZIP1/K.l-ZIP1 pch2::TRP1/PCH2 HIS4/ his4 HYG@CEN3/CEN3 THR1/thr1 ADE2@RAD18 NAT@HMR/HMR SPO11::KAN/SPO11 spo13::URA3/SPO13</td>
</tr>
<tr>
<td>YT76</td>
<td>$MAT_a$/$MAT_a$ pch2::TRP1/PCH2 HIS4/ his4 HYG@CEN3/CEN3 THR1/thr1 ADE2@RAD18 NAT@HMR/HMR SPO11::KAN/SPO11 spo13::URA3/SPO13</td>
</tr>
<tr>
<td>YT125</td>
<td>$MAT_a$/$MAT_a$ K.l-ZIP1/K.l-ZIP1 pch2::TRP1/PCH2 HIS4/ his4 HYG@CEN3/CEN3 THR1/thr1 ADE2@RAD18 NAT@HMR/HMR SPO11::TRP/SPO11 spo13::URA3/SPO13 THR1@193,424XI/thr1 LEU2@152kXI/leu2</td>
</tr>
<tr>
<td>YT131</td>
<td>$MAT_a$/$MAT_a$ pch2::TRP1/PCH2 HIS4/ his4 HYG@CEN3/CEN3 THR1/thr1 ADE2@RAD18 NAT@HMR/HMR SPO11::TRP/SPO11 spo13::URA3/SPO13 THR1@193,424XI/thr1 LEU2@152kXI/leu2</td>
</tr>
<tr>
<td>??*</td>
<td>$MAT_a$/$MAT_a$ K.l-ZIP Ctf19-myc::KAN</td>
</tr>
<tr>
<td>??*</td>
<td>$MAT_a$/$MAT_a$ Ctf19-myc::KAN</td>
</tr>
<tr>
<td>??*</td>
<td>$MAT_a$/$MAT_a$ zip1::URA3 Ctf19-myc::KAN</td>
</tr>
</tbody>
</table>

Table 1. Yeast strains table. All strains are isogenic with the BR1919-8b background (Rockmill and Roeder 1990).  
*strains used for haploid tethering experiment but have been lost.
RESULTS

*K. lactis* Zip1 provides some meiotic functions in *S. cerevisiae* cells

In order to analyze the extent to which *K. lactis ZIP1* can rescue *S. cerevisiae ZIP1* activity in *S. cerevisiae* cells, we created *S. cerevisiae* strains in which the ZIP1 open reading frame is replaced precisely with the open reading frame of *K. lactis ZIP1*. Such “*K.l ZIP1*”-expressing *S. cerevisiae* cells (hereafter referred to as *K.l Zip1*) were analyzed for various meiotic cell phenotypes in comparison with “*S. c. ZIP1*”-expressing, and zip1 null mutant *S. cerevisiae* cells.

Sporulation efficiency is a readout of a strain’s ability to progress through meiosis and form spores. Aberrant chromosome interactions during meiotic prophase can trigger a cell cycle checkpoint that can severely delay meiotic progression and the formation of spores. In order to measure the capacity for *K.l ZIP1*-expressing *S. cerevisiae* cells to sporulate, the frequency of spore products formed in over 4500 meiotic cells was assessed. As previously reported (Sym et al., 1993), zip1 mutants are defective in sporulation; only 5% of meioses resulted in spore formation in zip1 null mutants, as compared to 40% of meioses in a wild-type control. Interestingly, cells expressing *K.lactis ZIP1* displayed an intermediate sporulation efficiency of 18% (Figure 6A).

We next measured spore viability, a readout for proper meiotic chromosome disjunction. 92% of wildtype spores were viable in our experiment (Figure 6B). Spores produced by zip1 mutants, on the other hand, showed 56% viability in this experiment. Interestingly, cells expressing *K.l* Zip1 exhibited 76% viability, indicating that *K.l ZIP1* partially rescues one or more Zip1 activities that promote proper homologous chromosome disjunction in *S. cerevisiae* cells (Figure 6B).
Zip1 has been shown to have dosage affects on both sporulation and viability (Klutstein et al., 2009). In order to assess whether *K. l. Zip1* is able to promote disjunction at a lower dosage, we quantified sporulation and viability in *K. l. ZIP1* hemizygotes (*S. cerevisiae* cells expressing a copy of *K. l. ZIP1* in *trans* to a *zip1* deletion allele). Sporulation efficiency was 4% and spore viability 64% when only a single copy of *K. lactis* Zip1 is expressed. Strains expressing a single copy of endogenous (*S. c.*) *ZIP1* exhibited a less severe defect with sporulation at 32% and viability at 89% (Figure 6C). While this intermediate viability phenotype of *K. l. ZIP1* hemizygotes is lower than the viability provided by a single copy of *S. c. ZIP1*, we note that it is higher than the viability exhibited by the *zip1* null
Figure 6: K. lactis Zip1 in S. cerevisiae cells promotes homolog disjunction  

A) Graph shows percentage of cells expressing different alleles of Zip1 that form spores. K. lactis Zip1 (CO9) partially rescues sporulation when compared to endogenous Zip1 (1919) and zip1 null (CO10).  

B) Bar graph shows percentage of viable spores resulting from meiosis in wild type (1919) or zip1 null (CO10) S. cerevisiae cells, or from S. cerevisiae cells expressing the K. lactis Zip1(CO9). At least 88 asci (>350 spores) per genotype were dissected.  

C) Summary of spore viability of S. cerevisiae with endogenous ZIP1 versus K. lactis ZIP1. Data collected by Cassandra O’Curran (Wesleyan University 2013)
**K. lactis** Zip1 does not form stable full length SC

In order to assess the association of *K.l* Zip1 with chromatin during meiotic prophase, we stained surface spread meiotic nuclei with an antibody against *S.c.* Zip1. An antibody raised against *K.l.* Zip1 (provided by A. Dernburg, UC Berkeley) was also evaluated and showed similar results. In pachytene arrested cells, chromosomes are condensed and fully paired. Endogenous Zip1 assembles along the full length of chromosome pairs, reflecting full length SC (Figure 7A). However, *K.l* Zip1 does not form similar full-length structures. Rather, the localization pattern of *K.l.* Zip1 falls into three categories: foci on chromatin with a polycomplex (an aggregate of Zip1 protein), Zip1 foci on chromatin without a polycomplex, and foci in conjunction with short linear stretches on chromatin (a “dotty-linear” phenotype) (Figure 7B, D and E respectively). Preliminary analysis indicates that polycomplex formation is prevalent (44 of 50 nuclei) in *S. cerevisiae* cells expressing *K.l.* ZIP1. Only 1 “dotty linear” *K.lactis* structure was observed in the 50 nuclei assessed. These observations agree with the quantitative analysis carried out by another MacQueen lab member, Karen Voelkel-Meiman. In a time course experiment investigating *K.l* Zip1 loading to chromosomes, Karen observed that in (<5%) of nuclei at any timepoint, short *K.l* Zip1 stretches are apparent on pachytene chromosomes, while the remaining nuclei exhibit foci of *K.l.* Zip1.

Furthermore, most nuclei from these strains failed to exhibit linear assemblies of other SC central region proteins, such as Smt3 (Figure 7) and Ecm11 (Karen Voelkel-Meiman; personal communication). It is interesting to note that Smt3 colocalizes with *K.l* ZIP1 in both the polycomplex and when forming discrete foci or dotty linear structures just as we seen in wild type cells.
However, our observations together indicate that K.l Zip1 is unable to form stable full length SC structures.

Figure 7: K.l Zip1 and Smt3, two central region proteins fail to assemble stable SC structure. (A) S.c Zip1 and Smt3 in S.cerevisiae cells (1919) colocalized forms full length SC. (B and C) K.l Zip1 in S.cerevisiae cells (YT47) and Smt3 form polycomplex and few foci localize to chromatin. (D) 10% of the time K.l Zip1 also forms foci without polycomplex and Smt3 forms dotty linear structures. (E) Even less frequently, K.l Zip1 and Smt3 form dotty linear structures.
Localization of the meiotic axis protein, Red1, in cells expressing *K.l.* Zip1 appeared similar to Red1’s localization on chromosome axes in zip1 null cells (Smith & Roeder, 1997). Unlike the dotty linear stretches of Red1 that form along the length of synapsed chromosomes in wild type, Red1 in zip1 nulls and in cells expressing *K.l.* ZIP1 forms along axes, thus we visualize two distinct Red1 stretches along a pair of synapsed chromosomes (Figure 8).

**Figure 8: Red1 localizes to chromosome axes in *S.cerevisiae* cells expressing *K.I.ZIP1*. Like in a zip null mutant, the expression of *K.I.ZIP1* causes Red1 to localize along chromosomal axis rather than a dotty linear structure as seen in cells expressing wildtype ZIP1(YT48).
**K.l Zip1 partially rescues the sporulation arrest triggered by Zip1-4LA**

*zip1-4LA* is a mutant *zip1* allele in which four leucine residues within the coiled coil region of Zip1 are substituted with alanine (Mitra & Roeder, 2007). A *zip1-4LA* homozygote fails to make spores, due to the *PCH2* associated meiotic checkpoint resulting in cell cycle arrest at pachytene after SC is assembled (Mitra & Roeder, 2007). It was also found that in a heterozygote, where only a single copy of *ZIP1* is expressed in *trans* with *zip1-4LA*, the sporulation arrest is rescued (Mitra & Roeder, 2007). Interestingly, a *K.l ZIP1(zip1-4LA)* hemizygote generated spores 15% of the time (Figure 9A). Cassandra O’Curran also collected a set of data measuring *K.l* Zip1 rescue and found a similar result (data not shown).

**Figure 9: K.l Zip1 partially rescues the sporulation arrest triggered by Zip1-4LA if expressed in early prophase but fails to do so in late meiotic prophase.**  
(A) A *zip1-4LA* homozygote (CO49) triggers a meiotic checkpoint, preventing formation of spores. The hemizygote with a single copy of *zip1-4LA* (CO51) exhibits of sporulation efficiency of 2.3%. Expression of *K.l ZIP1* (YT35) 15% of meioses result in spore formation. (B)

The *zip1-4LA* mutant has also been used to study the dynamic nature of the SC, wherein meiotic arrested cells containing *zip1-4LA* were induced to start expressing an inducible copy of *ZIP1*. Cells expressing only *zip1-4LA* form SC
and arrest at late prophase. However, late, post-SC formation ZIP1 expression allowed Zip1 protein to incorporate into the SC, and also to rescue sporulation (Voelkel-Meiman et al., 2012). We conducted a similar experiment and asked whether the induction of K.l ZIP1 can rescue sporulation at late (post-synapsis) timepoints. K.l Zip1 was placed under the control of the P_{GAL1} promoter which is only activated in the presence of B-estradiol (Benjamin, Zhang, Shokat, & Herskowitz, 2003). Therefore, without B-estradiol (uninduced), sporulation is similar to that of a zip1-4LA homozygotes (Figure 9B). After 22 hours of induction, P_{GAL1} [S.c Zip1]/ zip1-4LA showed 55% sporulation while P_{GAL}[K.l Zip1]/ zip1-4LA sporulation was negligible at 2%. Thus, while expression of K.l ZIP1 from early in meiosis can alleviate the sporulation arrest triggered by cells expressing zip1-4LA, expression of K.l ZIP1 late in meiosis is not sufficient to rescue this sporulation arrest.

**K.l Zip1 does not preferably associate with centromeres.**

Our interest in the potential centromeric role of K.l Zip1 initially stemmed from Dr. MacQueen’s observation that in K.lactis cells, a single K.lactis Zip1 focus appeared to localize to each condensed set of chromosomes. This observation raised the idea that perhaps K.l Zip1 performs only a centromeric function in K.l cells. This idea was in part fueled by the knowledge that, independent of SC assembly, Zip1 has also been shown localize to centromeres and play a role in pair-wise centromere associations during meiosis in *S. cerevisiae* (Tsubouchi & Roeder, 2005). In addition, centromeric regions have been shown to be preferred sites for synapsis initiation (Tsubouchi, Macqueen, & Roeder, 2008).
To see if *K.l* Zip1 in *S.cerevisiae* cells localizes to centromeres, I examined surface spread pachytene nuclei stained with an antibody to a centromere component, Ctf19, and Zip1. At pachytene, chromosomes are fully paired, so close to 16 centromere foci are seen per nucleus. Only nuclei that did not exhibit polycomplex were chosen in order to better visualize potential colocalization between *K.l* Zip1 foci and centromeres (Figure 10). Preliminary quantification of 13 nuclei show that less than 15% of centromeres are associated with *K.l* Zip1 foci. Pachytene *K.l* Zip1 foci do not appear to preferentially localize to centromeres.

Figure 10: *K.l* Zip1 does not preferentially associate with centromeres. *S.c* ZIP1 is seen to form short stretches prior to pachytene that associate with centromeres. *K.l* Zip1 associates with centromeres at less than 15% of the time.
**K.l Zip1 fails to rescue centromere coupling function in *S. cerevisiae* haploids in early prophase**

One of the Zip1 mediated centromere functions is ‘centromere coupling’, which describes the pair-wise associations of centromeres that occur during early meiotic prophase prior to double strand break formation. Zip1 mediated centromere coupling is not dependent on homology (Tsubouchi & Roeder, 2005). We assessed centromere coupling by counting centromere foci, once again marked by Ctf19, in a *spo11* haploid strain, which is genetically engineered to enter meiosis. The haploid strain contains 16 chromosomes; ~8 Ctf19 foci are visualized if coupling is intact. 3 sets of 50 meiotic nuclei were examined (selected based on Red1 staining), for strains expressing *S.c. Zip1, zip1* null or *K.l Zip1*.

In a *spo11* haploid, an average of 8 foci are counted, indicating that chromosome have paired independent of homology. In a *zip1 spo11* haploid, closer to 16 foci were observed (Figure 11), indicating a loss of centromere coupling. *K.l Zip1 spo11* haploids also demonstrated an average of 16 foci, indicating that *K.l Zip1* is unable to rescue centromere coupling in haploid cells.
Figure 11: K. l Zip1 does not rescue centromere coupling in haploid cells: spo11 mutants carrying either S. c. ZIP(YT24), K. l. ZIP1(YT23) or a zip1 null (YT25) allele were analyzed. Bar graphs are depicted next to images of nuclei visualized by DAPI and centromeres marked with Ctf19-myc (A) Intact centromere coupling results in predominantly 8 centromere foci per nucleus while spo11 zip1 mutants display approximately 16 centromere foci (B). (C) K. l Zip1 is significantly different from S. c Zip1 and resembles the zip1 spo11 double mutant phenotype.
**K.l Zip1 partially rescue centromere coupling function in S. cerevisiae**

diploids in early prophase

Because meiotic haploid cells are potentially an artificial cellular context, coupling was also assessed in diploid *spo11* strains. Coupling was assessed in diploid cells in a similar manner to the haploid experiment. 4 sets of 50 meiotic nuclei were scored for each strain. Diploid *S.cerevisiae* cells have 32 chromosomes. Thus, close to 32 centromere foci are exhibited when coupling is abolished, like in *zip1 spo11* (Figure 12B). In *spo11*, *S.cerevisiae* Zip1 promotes coupling and closer to 16 foci are seen (Figure 12A). Cells expressing *K.l Zip1 spo11*, however, appear to exhibit an intermediate phenotype, with nuclei exhibiting fully coupled, partially coupled or uncoupled centromeres. However, it is interesting to note that when one of the four data sets is removed, *K.l Zip1* appears to rescue coupling function in diploids (Figure 12D).
Figure 12: *K.l* Zip1 partially rescues centromere coupling in diploid cells: (A) (YT15) Intact centromere coupling results in predominantly 16 centromere foci per nucleus. (B) spo11 zip1 mutants (YT21) display approximately 32 centromere foci. (C) (YT14) Graph includes all four data sets *K.l* Zip1 exhibits an intermediate phenotype – as reflected in the image. (D) Removal of one anomalous data set reveals that coupling in diploid cells may be rescued by *K.l* Zip1 – thus we see closer to 18 foci.
Centromere tethering is not rescued in meiotic haploids

Centromere tethering describes the pairwise association of centromeres that occur after SC disassembly in budding yeast meiotic cells. Centromere tethering relies on Zip1, but occurs independently of SC formation (Falk, Chan, Hoffmann, & Hochwagen, 2010; Newnham et al., 2010). Therefore, despite the fact that *K.l* Zip1 is unable to form stable SC structure, it could still play a role in tethering centromeres during late meiotic prophase. In order to study centromere tethering independent of SC formation, previous studies have used a haploid meiotic system similar to the one used to assess centromere coupling. A previous undergraduate in our lab, Cassandra O’Curran, spread meiotic nuclei, which were marked with Ctf19-myc, to visualize centromeres. Unlike coupling, centromere tethering is measured in a *SPO11*+ background. As expected, Cassie found that wildtype nuclei exhibited tethering with an average of 8 Ctf19-MYC foci in the 22 nuclei scored. An average of 15 Ctf19-MYC foci were observed in *zip1* (n=21), indicating a loss of tethering. Cells expressing *K.l* ZIP1 exhibited an average of ~15 Ctf19-MYC foci (n=31) although a higher frequency of nuclei exhibiting lower numbers of Ctf19-MYC foci were observed in comparison to the *zip1* null. Thus, *K.l* Zip1 minimally rescues the centromere tethering function of Zip1 in *S.cerevisiae* cells.
S. cerevisiae meiotic cells expressing K.l ZIP1 exhibit nearly wild-type crossover levels and minimal loss of crossover interference in four spore viable

SC formation and meiotic crossover recombination are closely coupled. In mutants that disrupt SC formation, including the zip1 mutant, interhomolog crossover levels fall to approximately half of their wildtype levels but interference, the non-random distribution of crossovers, is completely abolished (M Sym & G. Shirleen Roeder, 1994; Tsubouchi, Zhao, & Roeder, 2006). In order to ask whether K.l Zip1 can rescue the function of S.c. Zip1 in promoting crossovers in S. cerevisiae cells, I measured the level and distribution of interhomolog crossovers in six intervals distributed over three chromosomes. Four intervals were on chromosome III: HIS4-CEN3, CEN3-MAT, MAT-RAD18 and

Figure 13: Centromere tethering in haploid cells: Bar graphs quantify number of nuclei exhibiting a given number of Ctf19 foci of S.cerevisiae cells with three distinct genotypes at the ZIP1 locus. (A) Endogenous Zip1=8.3 foci; tethered (B) zip1 exhibits an average of 15.3 foci; untethered. (C) K.l Zip1 minimally rescues tethering.
RAD18-HMR. The two additional intervals SPO11-SPO13 and THR1@193,424-
LEU2@152,000 were on chromosomes VIII and XI respectively. Crossovers
were measured genetically by tetrad analysis. 276 four spore viable tetrads from
cells homozygous for K.l ZIP1, and 140 four spore viable tetrads from strains
homozygous for S.c. ZIP1, and carrying markers that define 6 crossover intervals,
were analyzed for the number of recombinant spores.

Only one of the intervals measured, MAT-RAD18, displayed a significant
difference between crossover levels (10 cM) in cells expressing S.c. ZIP1 versus
K.l. ZIP1 (Figure 14A). In the other intervals tested, cells expressing K.l. ZIP1
exhibited similar levels of crossing over as cells expressing S.c. ZIP1.

The tetrad data from the six genetic intervals also allowed us to assay for
crossover interference. Interference describes a non-random distribution of
crossovers such that the frequency of two crossovers occurring close together is
lower than expected for a random distribution, and is characteristic of meiotic
recombination. Crossover interference can be detected as a deficit in the number
of double crossover events (NPDs) within a genetically marked interval, and is
measured from the NPD ratio = observed NPDs/expected NPDs (Snow, 1979).
The frequency of NPDs expected in the absence of interference was calculated for
our tetrad data using the standard calculation given in (Papazian, 1952). A ratio of
less than 1 indicates that crossover interference is operating, dispersing the
occurrence of exchanged genetic material. A ratio closer to 1 (increase in NPDs
observed) reflects a loss in interference.
**Figure 14: K.l. ZIP1 rescues crossover levels and interference in S.cerevisiae cells:** Comparing crossover levels and interference in wild type S.c Zip1 (YT131) and K.l Zip1 (YT125) expressed in S.cerevisiae cells. Crossover levels are quantified by map distance, measured in centimorgans (cM). Quantities reflected in each interval of both bar graphs are detailed in Table 1. (A) Crossover levels are similar to wild type in all but one interval (MAT-RAD18). (B) K.l Zip1 intervals show a small relative loss of interference.

Crossovers measured in tetrads from cells expressing K.l Zip1 exhibit a very slight loss of interference as compared to the crossovers measured in cells expressing S.cerevisiae Zip1. In CEN3-MAT interference exhibits no change. In the interval THR-LEU on chromosome XI, interference cannot be quantified because the two loci are tightly linked and the probability of a double crossover event (expected NPD) is negligible (and no NPDs were observed). In sum, these data show that K.l ZIP1 is able to promote interfering crossovers in S. cerevisiae cells, a surprising result based on the fact that interfering crossovers have been previously linked by genetic analyses, to the formation of mature SC.
**Table 3: Crossing over and crossover interference.** Strains YT131 (wildtype-S. c ZIP1) and YT123 (K.l ZIP1) were used to examine the HIS4-CEN3, CEN3-MAT, MAT-RAD18 and RAD18-HMR, SPO11-SPO13 and THR1@193,424chrXI-LEU2@152,000chrXI (ChrXI THR-LEU). In wildtype, spore viability was 93.4% and 82.3% contained four viable spores and n=140. In K.l ZIP1 spore viability was 68.92% and 39.2% contained four viable spores and n=276.

<table>
<thead>
<tr>
<th>Interval</th>
<th>Strain</th>
<th>PD²</th>
<th>TT¹</th>
<th>NPD¹</th>
<th>NPD exp ¹</th>
<th>cM³</th>
<th>Interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIS4-CEN3</td>
<td>S. c ZIP1</td>
<td>75</td>
<td>60</td>
<td>2</td>
<td>5</td>
<td>26.3</td>
<td>0.4056</td>
</tr>
<tr>
<td></td>
<td>K. l ZIP1</td>
<td>157</td>
<td>103</td>
<td>4</td>
<td>7</td>
<td>24.1</td>
<td>0.5645</td>
</tr>
<tr>
<td>CEN3-MAT</td>
<td>S. c ZIP1</td>
<td>83</td>
<td>54</td>
<td>2</td>
<td>4</td>
<td>23.7</td>
<td>0.5418</td>
</tr>
<tr>
<td></td>
<td>K. l ZIP1</td>
<td>139</td>
<td>117</td>
<td>5</td>
<td>10</td>
<td>28.2</td>
<td>0.5007</td>
</tr>
<tr>
<td>MAT-RAD18</td>
<td>S. c ZIP1</td>
<td>53</td>
<td>78</td>
<td>5</td>
<td>11</td>
<td>39.7</td>
<td>0.4677</td>
</tr>
<tr>
<td></td>
<td>K. l ZIP1</td>
<td>137</td>
<td>101</td>
<td>5</td>
<td>8</td>
<td>27</td>
<td>0.6541</td>
</tr>
<tr>
<td>RAD18-HMR</td>
<td>S. c ZIP1</td>
<td>85</td>
<td>51</td>
<td>1</td>
<td>3</td>
<td>20.8</td>
<td>0.3052</td>
</tr>
<tr>
<td></td>
<td>K. l ZIP1</td>
<td>145</td>
<td>102</td>
<td>3</td>
<td>8</td>
<td>24</td>
<td>0.3998</td>
</tr>
<tr>
<td>SPO11-SPO13</td>
<td>S. c ZIP1</td>
<td>62</td>
<td>73</td>
<td>2</td>
<td>9</td>
<td>31</td>
<td>0.2348</td>
</tr>
<tr>
<td></td>
<td>K. l ZIP1</td>
<td>124</td>
<td>126</td>
<td>5</td>
<td>13</td>
<td>30.6</td>
<td>0.3931</td>
</tr>
<tr>
<td>ChrXI THR-LEU</td>
<td>S. c ZIP1</td>
<td>111</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>10.7</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>K. l ZIP1</td>
<td>217</td>
<td>41</td>
<td>0</td>
<td>0</td>
<td>7.9</td>
<td>ND</td>
</tr>
</tbody>
</table>

² Observed parental ditype tetrads
¹ Observed tetratype tetrads
³ Observed nonparental ditype tetrads
⁴ NPD tetrads expected (Papazian, 1952); rounded to the nearest whole number
⁵ Map distance in centimorgans
⁶ Interference based on NPD ratio = observed NPD/expected NPD
Crossover levels remain but interference is abolished when the dosage of

**PCH2 is reduced in strains expressing K.l Zip1**

Defects in SC assembly often trigger a *PCH2*-dependant cell cycle checkpoint. This checkpoint leads to cell cycle arrest that prevents progression beyond meiotic prophase I (MacQueen & Hochwagen, 2011). Mutation of *PCH2* in an SC defective mutant like *zip1* causes arrested cells to bypass the checkpoint, resulting in higher sporulation efficiency. (San-Segundo & Roeder, 1999).

In our initial crossover analysis our strains were missing one copy of *PCH2*. Interestingly, in these experiments, cells expressing *K.l. ZIP1* showed similar levels of crossing over as cells expressing *S. c. ZIP1*, but interference was abolished in three of the five intervals assessed. From 542 four spore viable tetrads of *K.l ZIP1 pch2/PCH2* and 498 of *ZIP1 pch2/PCH2* was quantified (Figure 15, Table 2). Data was collected over five genetic intervals; four on chromosome III: *HIS4-CEN3, CEN3-MAT, MAT-RAD18, RAD18-HMR* and one on chromosome VIII, *SPO11-SPO13*.

Crossover levels were close to similar in all intervals except *MAT-RAD18*, in which the *S.c ZIP1* cells underwent a substantially greater number of crossovers. However, in *K.l ZIP1* expressing cells, the NPD ratio was close to 1 in three of the five intervals.
Figure 15: Interference is abolished in \textit{K.l ZIP1} strain heterozygous for PCH2. Comparing crossover levels and interference in \textit{S.c} Zip1 \textit{pch2}/PCH2+(YT76) and \textit{K.l} Zip1 pch2/PCH2+(YT72) expressed in \textit{S.cerevisiae} cells. Crossover levels are quantified by map distance, measured in centimorgans (cM). Quantities reflected in each interval of both bar graphs are detailed in Table 1. (A) Crossover levels are conserved in all but one interval (MAT-RAD18). (B) \textit{K.l ZIP1} express loss of interference in 4 intervals \textit{HIS4-CEN3}, \textit{MAT-RAD18}, \textit{RAD18-HMR} and \textit{SPO11-SPO11}
Table 3: Crossing over and crossover interference in a PCH2 heterozygote. Strains YT76 (wildtype-S.c ZIP1 pch2/PCH2) and YT72 (K.l ZIP1 pch2/PCH2) were used to examine the HIS4-CEN3, CEN3-MAT, MAT-RAD18 and RAD18-HMR and SPO11-SPO13. In wildtype, spore viability was 90% and 75% contained four viable spores and n=498. In K.l ZIP1 spore viability was 70% and 40.2% contained four viable spores and n=542. 

<table>
<thead>
<tr>
<th>Interval</th>
<th>Strain</th>
<th>PD</th>
<th>TT</th>
<th>NPD</th>
<th>NPD exp</th>
<th>cM</th>
<th>Interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIS4-CEN3</td>
<td>S.c ZIP1</td>
<td>264</td>
<td>222</td>
<td>4</td>
<td>19</td>
<td>25.1</td>
<td>0.208</td>
</tr>
<tr>
<td></td>
<td>K.l ZIP1</td>
<td>302</td>
<td>192</td>
<td>8</td>
<td>13</td>
<td>23.9</td>
<td>0.6236</td>
</tr>
<tr>
<td>CEN3-MAT</td>
<td>S.c ZIP1</td>
<td>275</td>
<td>204</td>
<td>10</td>
<td>16</td>
<td>27</td>
<td>0.644</td>
</tr>
<tr>
<td></td>
<td>K.l ZIP1</td>
<td>287</td>
<td>213</td>
<td>6</td>
<td>16</td>
<td>24.6</td>
<td>0.365</td>
</tr>
<tr>
<td>MAT-RAD18</td>
<td>S.c ZIP1</td>
<td>193</td>
<td>286</td>
<td>11</td>
<td>41</td>
<td>35.9</td>
<td>0.2688</td>
</tr>
<tr>
<td></td>
<td>K.l ZIP1</td>
<td>317</td>
<td>163</td>
<td>10</td>
<td>9</td>
<td>22.8</td>
<td>1.117</td>
</tr>
<tr>
<td>RAD18-HMR</td>
<td>S.c ZIP1</td>
<td>284</td>
<td>203</td>
<td>6</td>
<td>15</td>
<td>24.2</td>
<td>0.3962</td>
</tr>
<tr>
<td></td>
<td>K.l ZIP1</td>
<td>308</td>
<td>178</td>
<td>14</td>
<td>11</td>
<td>26.2</td>
<td>1.3044</td>
</tr>
<tr>
<td>SPO11-SPO13</td>
<td>S.c ZIP1</td>
<td>216</td>
<td>268</td>
<td>7</td>
<td>33</td>
<td>31.6</td>
<td>0.213</td>
</tr>
<tr>
<td></td>
<td>K.l ZIP1</td>
<td>260</td>
<td>220</td>
<td>26</td>
<td>18</td>
<td>37.2</td>
<td>1.455</td>
</tr>
</tbody>
</table>

a Observed parental ditype tetrads  
b Observed tetratype tetrads  
c Observed nonparental ditype tetrads  
d NPD tetrads expected(Papazian, 1952); rounded to the nearest whole number  
e Map distance in centimorgans  
f Interference based on NPD ratio = observed NPD/expected NPD
DISCUSSION

The Zip1 protein is important for promoting proper disjunction of chromosomes during meiosis in *S.cerevisiae* cells. It has been characterized to play several roles, each of which may play a part in facilitating proper chromosome segregation. These roles include SC formation, promotion of crossover recombination in the context of the SC and centromere associations. However, the degree to which chromosomes rely on each of these functions for homolog segregation is unknown. Here we have analyzed an ancestral version, *K.lactis* Zip1, for its capacity to carry out the functions of Zip1 in *S.cerevisiae* cells.

*K.lactis* Zip1 and Chromosome Synapsis

We found that while *K.lactis* Zip1 does promote meiotic chromosome segregation, it does not assemble stable SC structures on chromosomes in *S. cerevisiae* cells. Rather, it predominantly forms polycomplex structures, along with foci and short stretches on chromosomes. A polycomplex is an aggregate of SC material in the nucleus (Sym & Roeder, 1995). In this strain background (BR) they are relatively rare, and are usually observed when Zip1 has been over-expressed (Sym & Roeder, 1995) or in mutants defective in SC assembly (Tung & Roeder, 1998).

Another important observation is that Smt3 co-localizes with *K.l* Zip1 and the mere fact that *K.l* Zip1 is recognized by antibodies raised against *S.c* Zip1, indicates that *K.l* Zip1 interacts with at least some of the same meiotic machinery that *S.c* Zip1 associates with. However, in order to eliminate the possibility that punctate *K.lactis* Zip1 incorporation in the SC is merely due to insufficient antibody compatibility, we
have created strains carrying an internal epitope tag (V5) inserted into the *K.lactis ZIP1* ORF. Insertion of the V5 tag in the coiled coil domain triggered a meiotic checkpoint that prevents sporulation (data not shown). This first location for the tag was chosen because insertion of GFP at the equivalent locus in the *ZIP1* ORF resulted in minimal loss of function (White, Cowan, Cande, & Kaback, 2004). We have chosen a second location to insert the tag, which will hopefully yield a *K.l* Zip1-V5 protein that promotes chromosome segregation to a similar degree as untagged *K.l* Zip1.

The V5 tagged *K.l* Zip1 protein will be a useful tool that we can use to cytologically assess whether *K.l* Zip1 interacts with *S.cerevisae* Zip1 in the SC. For instance, one of our studies concluded that expression of *K.l. ZIP1* from early in meiosis can alleviate the sporulation arrest triggered by cells expressing *zip1*-4LA, while expression of *K.l. ZIP1* late in meiotic prophase is not sufficient to rescue this sporulation arrest. It is known that Zip1 protein incorporates into SC on a continual basis, even after SC is fully formed (Voelkel-Meiman et al., 2012). We would like to assess whether *K.l* Zip1 incorporates into SC built of S.c. Zip1 (or Zip1-4LA) in either the early or late expression context.

In addition, *K.l* Zip1-V5 localization patterns on the SC could also reveal information about *K.l* Zip1 and the *PCH2*-dependent checkpoint. If *K.l* Zip1-V5 forms linear stretches that colocalize uniformly with Zip1-4LA SCs, this sheds light on *K.l* Zip’s ability to form stretches and potentially dimerize with Zip1-4LA, creating a hybrid SC component. However, if *K.l* Zip1-V5 localizes as foci, this
raises the possibility that the \textit{PCH2}-dependent checkpoint is regulated at particular sites along chromosomes.

\textit{K.lactis Zip1 and centromeres}

Given the observation \textit{K.l} Zip1 is unable to form stable SC, we hypothesized that the \textit{K.l} Zip1 protein is able to promote chromosome disjunction by its function at centromeres. We considered this theory in part because Zip1 mediated centromere associations in late meiotic prophase may have the capacity to hold centromeres together in the absence of SC and/or crossover formation (Newnham et al., 2010). Moreover, preliminary observation of a single \textit{K.lactis} Zip1 focus per chromosome in \textit{K.lactis} meiotic cells raised the possibility that Zip1 in \textit{K.lactis} may only perform centromeric functions.

One centromeric function of Zip1 is to promote centromere coupling in early meiotic prophase, prior to SC assembly. Centromere coupling was not rescued by \textit{K.lactis} Zip1 in meiotic \textit{S.cerevisiae} haploid cells, however it did show an incomplete capacity to promote pair-wise centromere associations in diploid cells. While it is not clear why \textit{K.l} Zip1 functioned better in diploids versus haploid cells, our observations suggest that one cannot assume that chromosome interactions in haploid and diploid cells are equivalent. One theory that could explain why centromere coupling occurs in diploids but not in haploids, is that the diploid cells contain two copies of \textit{K.l ZIP1} – suggesting that centromere coupling is regulated by a dosage effects of the \textit{K.l} Zip1 protein.

The other Zip1 mediated centromere function for which we evaluated \textit{K.lactis} Zip1 is centromere tethering. Because centromere tethering is proposed to be a
mechanism for non-exchange chromosomes to associate and ensure their proper segregation, it was tantalizing to imagine that robust centromere tethering is the basis for the additional viable spores in cells expressing *K.lactis ZIP1*. However, our assay so far for tethering suggests little rescue of this activity by *K.l.* Zip1. However this data is preliminary and could be strengthened by a larger data set. In addition, given our conflicting conclusions in the haploid and diploid experiments in our haploid coupling studies, we will assess tethering in diploid cells.

Given our failure to observe a robust capacity for *K.l.* Zip1 to promote centromere associations in *S.cerevisiae* and our inability to detect *K.l.* Zip1 at centromeres on *S. cerevisiae* meiotic chromosomes, our original speculation that *K.lactis ZIP1* is an allele that uncouples the centromere function from Zip1’s functions in SC formation and crossover recombination is now less compelling. Instead, we were surprised to find that despite the fact that *K.l* Zip1 does not form SC, it promotes crossovers at wildtype levels. Furthermore, the crossovers we measured in strains expressing *K.l.* Zip1 exhibited a minimal loss of interference. Although, it ought to be noted that this is a preliminary result as we have only assessed a limited data set, this finding has interesting implications.

**K.lactis ZIP1 as a separation of function allele**

Most known mutants defective in SC formation, including *zip1* mutants (Tung & Roeder, 1998), and mutants missing SIC proteins like Zip4 or Zip2 (Chua & Roeder, 1998; Tsubouchi et al., 2006), exhibit diminished interference and crossovers, in conjunction. The *ndj1* null was found to have a unique phenotype, given that it rescues crossovers to a wildtype level but shows a decrease in crossover
interference (Chua & Roeder, 1997). In this case the diminished interference phenotype was attributed to a delay in full length SC formation. The phenotype exhibited by *S. cerevisiae* cells expressing *K.l* Zip1, interfering crossovers in the absence of SC, is unique. This finding first, suggests a model in which Zip1 promotes crossover maturation independent of its role as a structural component of the SC. Second, this unique phenotype indicates that interference and SC formation are not inextricably linked.

The idea that Zip1’s role in building SC might be independent of Zip1’s role in crossover recombination was previously suggested by an earlier genetic study Storlazzi and Kleckner in 1994. These authors observed a reduction in crossovers in a *zip1 red1* double mutant, as compared to a *red1* single mutant, despite the fact that neither the double nor the single mutant formed SC. *K.l.* Zip1’s capacity to rescue interfering crossover formation in the absence of SC bolsters the idea that Zip1’s role in crossover formation is independent of its capacity to assemble SC *per se*, and furthermore provides us with a platform for future studies that will investigate the molecular features of the Zip1 protein that specifically interface with the crossover pathway.

Our crossover data thus far suggests that the loss of a single dose of *PCH2* results in loss of interference in cells expressing *K.l ZIP1*. Previous data has shown that in a *pch2* null, interference is abolished (Joshi, Barot, Jamison, & Borner, 2009) at least in certain chromosomal regions. In our experiments, deletion of a single copy of *PCH2* showed no affect on interference in otherwise wildtype cells, but in cells expressing *K.l. ZIP1*, deletion of a single copy of *PCH2* resulted in a loss of
interference in three out of five intervals, and diminished interference in the remaining two intervals assessed. The observation that in cells expressing *K.l. ZIP1* interference is greatly diminished through loss of just a single dose of *PCH2*, suggests that the processes that give rise to interfering crossovers in *S.cerevisiae* cells expressing *K.lactis ZIP1* involve both Zip1 and Pch2, perhaps via redundant functions.

In conclusion, *K.l* Zip1 appears to be a separation of function allele, uncoupling Zip1 function in crossover formation from its capacity to build stable SC and to promote robust centromere interactions. *K.l* Zip1 is 40% similar and 25% identical at the amino acid level to *S.c. Zip1*; it will be interesting to learn which areas of the Zip1 protein are responsible for *K.l. Zip1*’s capacity to interface with *S.cerevisiae* crossover machinery.
Future Directions

We are interested in using *K.l* Zip1 as a starting point to unravel the machinery involved in crossover resolution independent of SC formation. As mentioned, the SIC proteins Zip2, Zip3, and Zip4, and the mismatch repair and recombination proteins Msh4, Msh5, and Mer3, are required for synapsis, mark presumed crossover sites, and are required for the generation of interfering crossovers (Agarwal & Roeder, 2000; Chua & Roeder, 1998; Fung et al., 2004; Lynn, Soucek, & Borner, 2007). SICs have also been proposed to be mediators between synapsis and recombination (Agarwal and Roeder 2000). We can discern the extent to which each SIC protein plays a role in recombination versus SC formation using the *K.l. ZIP1* allele, which supports crossovers but not SC assembly.

Given that *K.l ZIP1* separates crossover recombination function from other known functions of *S.c* Zip1, we can also compare the *S.c* and *K.l* Zip1 protein sequences to identify a specific domain of *K.l* Zip1 that might play a role in crossovers only. Our results also raise questions about how Pch2 interfaces with *K.l* Zip1 in order to regulate crossover interference.

In order to gain further insight into *K.l* Zip1, we also plan to study the protein in *K.lactis* cells. We have several factors to assess within this context. First and most importantly, we would like to create a system through which we can visualize centromeres in *K.lactis* cells in order to investigate whether our original assumption that the single *K.lactis* Zip1 focus per chromosome in *K.lactis* cells indeed localizes to centromeres. In this context, we also hope to assess centromere tethering and
coupling in *K. lactis* cells. This would be done by knocking out both copies of *K.l ZIP1* and comparing the phenotype to that of wildtype *K. lactis* cells.
References


