THE ROLES OF TID1, NDJ1, AND SPO16 IN DISTRIBUTIVE SEGREGATION DURING SACCHAROMYCES CEREVISIAE MEIOSIS

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TITLE: The Roles of Tid1, Ndj1, and Spo16 in Distributive Segregation During *Saccharomyces cerevisiae* Meiosis

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ABSTRACT

The Roles of Tid1, Ndj1, and Spo16 in Distributive Segregation During *Saccharomyces cerevisiae* Meiosis

Ethan Atticus Shaw

Meiosis is a specialized form of cell division in sexually reproducing eukaryotes. Crossovers are physical connections formed between homologous chromosomes during meiosis; these connections help ensure normal segregation of homologous chromosomes at meiosis I. However, the yeast *Saccharomyces cerevisiae* and other eukaryotes can still segregate homologs properly even in the absence of some crossovers. This is due to a backup mechanism known as distributive segregation, which correctly segregates non-crossover chromosomes at a higher rate than if segregation were completely random. To study distributive segregation, we have generated diploid yeast with one homeologous chromosome pair consisting of a *Saccharomyces cerevisiae* chromosome V and a *Saccharomyces carlsbergensis* chromosome V. This pair of chromosomes rarely recombine resulting in crossing over occurring in less than 3% of meiosis. Appropriate segregation of this chromosome pair during meiosis will depend on distributive segregation; we can then assess the possible roles of candidate proteins in distributive segregation through determination of the effect of mutation on segregation of this chromosome pair. Our work has focused on the roles of three proteins, Ndj1, Tid1, and Spo16. These three proteins affect meiosis in many ways, including the efficiency of crossover regulation and the overall timing of meiosis, but their roles during distributive segregation are not fully known.

A comparison of spore viability among WT, *ndj1*, and *tid1* strains reveals an elevated incidence of 2-spore-viable tetrads (suggestive of chromosome nondisjunction) in *ndj1*, but not *tid1*; these results suggest that the Ndj1 protein, but not the Tid1 protein, plays some role in distributive segregation. *spo16* strains seem to also show elevated levels of 2-spore-viable tetrads, but due to a lack of data no deductions can be made about the role of Spo16 in distributive segregation.

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1. Introduction

1.1 Meiosis

Meiosis is a specialized form of cellular division that produces gametes in sexually reproducing eukaryotes. It consists of two rounds of division, meiosis I and meiosis II, and results in one diploid cell eventually being divided into four haploid cells. During the first division homologous chromosomes are segregated causing a reduction in ploidy and during the second division sister chromatids are separated. The molecular mechanisms controlling chromosome segregation during meiosis are poorly understood, and the failure of successful segregation results in aneuploidy of the resulting cells. (Ohkura 2015) Aneuploidy occurs in a minimum of 5% of tested pregnancies making it the most common chromosomal irregularity in humans (Hassold and Hunt 2001). An aneuploid embryo frequently results in the loss of the pregnancy making nondisjunction the leading genetic cause of miscarriages (Hassold and Hunt 2001). Additionally, 1 in 300 liveborn infants are aneuploid causing abnormalities like Down syndrome or Klinefelter syndrome (Hassold and Hunt 2001).

Meiosis is divided into four phases: prophase, metaphase, anaphase, and telophase, which must be repeated in both meiosis I and meiosis II. Prophase I is the first and longest phase of meiosis where chromosomes must pair, synapse, and recombine, and it is divided into five stages: leptotene, zygotene, pachytene, diplotene, and diakinesis. Before entry into leptotene, a global clustering of centromeres occurs near the nuclear envelope. During leptotene the homologous chromosomes begin to condense and pair up with axial elements forming between sister chromatids. During the leptotene to zygotene transition there is a clustering of telomeres to the nuclear envelope creating a

"bouquet" formation that is thought to help promote homolog pairing. Additionally, during this time centromeres form non-homologous pairs. In zygotene the synaptonemal complex begins to form between the homologs and the non-homologous pairing of centromeres gives way to homolog alignment and synapsis. In pachytene synapsis is completed with the formation of the entire synaptonemal complex. After the completion of pachytene, homologous recombination takes place and the resulting chiasmata can be seen in the final diplotene and diakinesis stages. Lastly, prophase ends with the breakdown of the nuclear envelope (Page and Hawley 2003).

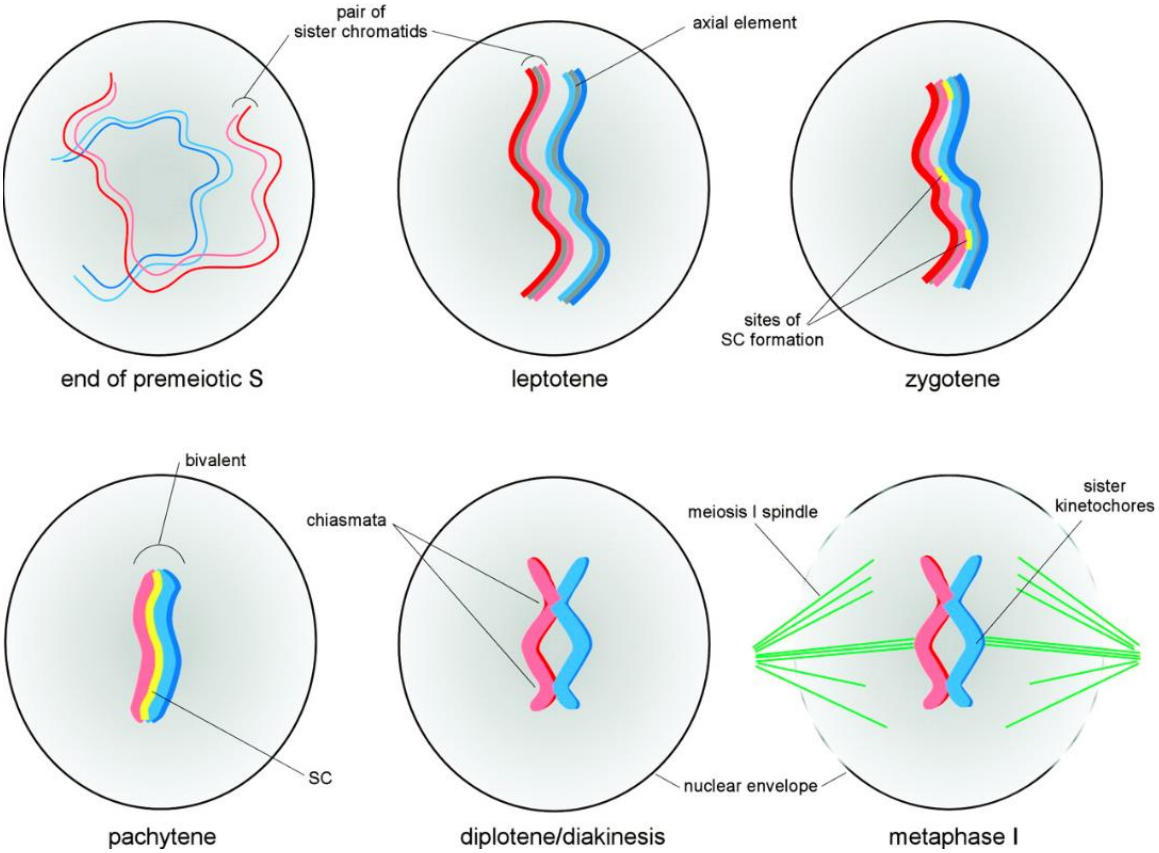

Figure 1- The stages of prophase I (Page and Hawley 2003).

After the completion of prophase I, metaphase I is marked with the chromosomes lining up along the metaphase plate where the sister kinetochores become attached to the spindle orienting the chromosomes to opposite sides of the cell. During anaphase I the

homologs are pulled to opposite poles, and telophase I ends meiosis I with the formation of two new nuclei. Each phase must be repeated in meiosis II for the formation of four haploid daughter cells. However, prophase II is much shorter than prophase I because there is no synaptonemal complex or crossover formation. The sister chromatids will be separated instead of homologous chromosomes during the second division.

1.2 Yeast

Saccharomyces cerevisiae is one of the most extensively studied eukaryotic model organisms within the fields of molecular and cellular biology. They double in number every 100 minutes under ideal conditions, but they will stop replicating when starved of nutrients or in the presence of a mating partner nearby (Herskowitz 1988). Yeast have 16 chromosomes that are duplicated during the cell cycle and segregated between the mother cell and the new bud during mitosis (Herskowitz 1988).

Yeast cells can be one of two mating types: a or α. The mating type is designated by the mating-type locus which causes yeast cells to synthesize different proteins involved in mating and sporulation. A cells secrete a-factor pheromones (Wilkinson and Pringle 1974), and α cells secrete slightly larger α-factor pheromones (Stotzler et al. 1976). If a haploid yeast cell comes into contact with the opposing mating type pheromone, the cell cycle will come to a halt in the G1 phase and synthesis of necessary proteins for mating will be initiated.

When an a and α cell are placed nearby they mate with nearly 100% efficiency (Herskowitz 1988). The mating results in a cellular and nuclear fusion creating a diploid cell from two haploids. These a/α diploid cells replicate by mitosis to form more diploid cells, and they cannot mate with either a or α cells. They do not produce the pheromones

or the pheromone receptors that a or α cells produce. Additionally, diploid yeast cells can undergo meiosis if starved of nutrients. However, it is not the diploidy itself that allows yeast to undergo meiosis. It has been shown that MATa/MATa and MATα/MATα diploids cannot sporulate. Only MATa/MATα yeast can go through meiosis forming four haploid spores inside of a sac, the ascus (Herskowitz 1988).

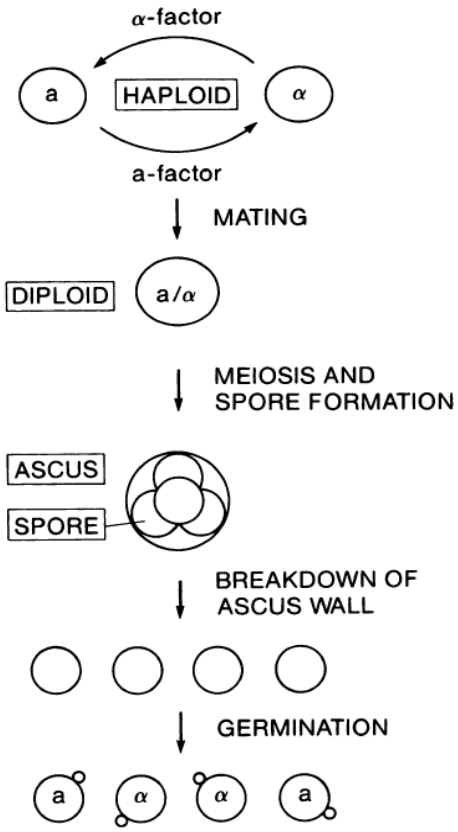

Figure 2 - The formation and breakdown of a yeast tetrad (Herskowitz 1988).

S. cerevisiae has been a model organism to study meiosis for many reasons including their ability to form these four spore tetrads. Tetrads give researchers the unique ability to obtain all four meiosis products from a single diploid cell allowing for in-depth analysis of recombination. A strain of diploid yeast can be plated on a nutrient deficient plate causing tetrad formation, and these tetrads can be dissected, which is when

four spores are separated from the ascus. The viability of the spores can be assessed in order to make inferences about the segregation of chromosomes during meiosis.

1.3 Double Strand Breaks / Crossovers

In *S. cerevisiae* meiotic recombination is initiated from the formation of DNA double strand breaks (DSBs) (Sun et al. 1989). There are many proteins that have been shown to have a direct role in the formation of DSBs in meiotic cells including Spo11, Mei4, Mer2, Rec102, Rec104, and Rec114 (Cao et al. 1990; Menees et al. 1992; Rockmill et al. 1995; Bullard et al. 1996). DSBs occur from a pair of nicks opposite of each other on a double stranded DNA molecule (Keeney 1997). It has been determined that Spo11 is the catalytic subunit of meiotic DNA cleavage activity, and Spo11 functions by utilizing a nucleophilic protein side chain that attacks the DNA backbone generating a phosphodiester linkage between Spo11 and the DNA (Keeney et al. 1997). Additionally, Spo11 has been shown to be a part of a widely conserved family of topoisomerase-like proteins (Keeney et al. 1997). A homolog to Spo11 has been found in fission yeast, (Lin and Smith 1994) flies, (McKim and Hayashhi-Hagihara 1998) nematodes, (Dernburg et al. 1998) mice, (Keeney et al. 1999; Romanienko and Camerini-Otero 1999) plants, (Hartung and Puchta 2000) and humans (Romanienko and Camerini-Otero 1999). This provides evidence that the mechanism of meiotic recombination initiation is evolutionarily conserved (Keeney et al. 1997).

After Spo11-induced DSBs, the DNA undergoes 5’ to 3’ resection generating 3’ single-strand DNA overhangs that become bound by replication protein A (RPA) (Figure 3 Part 2) (Youds and Boulton 2011). Eventually RPA is displaced by either Rad51 or Dmc1 to form nucleoprotein filaments that aid in the DNA homology search within a

sister chromatid or homologous chromosome. When the sequence is identified single-end strand invasion occurs creating a displacement loop recombination intermediate (Figure 3 Part 3) (Hunter and Kleckner 2001). Next a double Holliday junction forms if the second end of the original DSB binds with the homologous chromosome (Figure 3 Part 5) (Youds and Boulton 2011). These Holliday junctions can either be resolved to generate a crossover or a non-crossover (Bishop and Zickler 2004). Additionally, the junctions can be processed through dissolution forming a non-crossover in a different manner. It is also possible that the strand invasion is short lived where after a small amount of DNA synthesis occurs and the invaded strand dissociates and binds its partner strand forming a non-crossover (Figure 3 Part 3b).

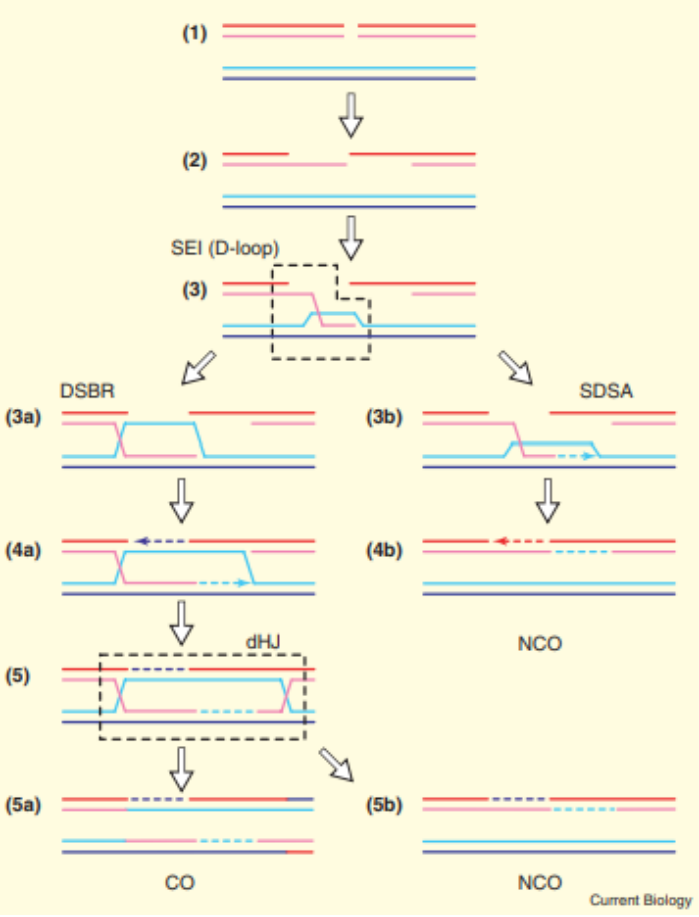

Figure 3 - The double-strand-break repair (DSBR) and synthesis-dependent strand annealing (SDSA) pathways. Recombination begins with single-end strand invasion

(SEI) forming a displacement loop (D-loop). The DSBR pathway results in the formation of a double holliday junction that can result in the formation of a crossover (CO) or noncrossover (NCO) product. The SDSA pathway always results in a NCO product (Heyer 2003).

1.4 Crossover Regulation

Crossovers lead to the formation of chiasmata between chromosomes that aid in the creation of tension between homolog pairs and ensure their proper segregation in meiosis I. Because of their importance in meiosis, the formation of crossovers is tightly regulated. Crossover assurance is one mechanism of regulation that ensures that every pair of homologs will achieve at least one crossover, the obligate crossover (Youds and Boulton 2011). Crossover interference is another mechanism that causes adjacent crossovers to be positioned farther apart than if the crossovers were randomly positioned (Youds and Boulton 2011). It has been thought that crossover interference and assurance could be outcomes from a single underlying mechanism (Shinohara et al. 2008).

However, assurance and interference seem to be regulated in slightly different ways between different species. For instance, in yeast it seems as though interference and assurance are mechanistically independent processes, (Shinohara et al. 2008) but in mice there is a lack of evidence for separate mechanisms generating interference and assurance (de Boer et al. 2007). *Caenorhabditis elegans*, a nematode, shows complete interference on its chromosomes where more than one crossover per homolog pair is a rare occurrence (Hillers and Villeneuve 2003; Lim et al. 2008). Studies on *C. elegans* have shown that crossover interference can be altered by either changing the balance between crossover and non-crossover repair of DSBs or by increasing the number of DSBs (Youds and Boulton 2011). This promotes the idea that there could be multiple levels of crossover interference in *C. elegans* (Youds and Boulton 2011).

1.5 Synaptonemal Complex

Before the formation of crossovers, the synaptonemal complex (SC) forms between homologs. The SC is an evolutionarily conserved structure that is formed during prophase I of meiosis. Formation of the SC results in the synapsis of homologous chromosomes- an important step to ensure the completion of meiotic recombination and the exchange of axial elements between the two connected chromatids (Page 2004). The SC is a tripartite proteinaceous structure composed of a central element and two lateral elements.

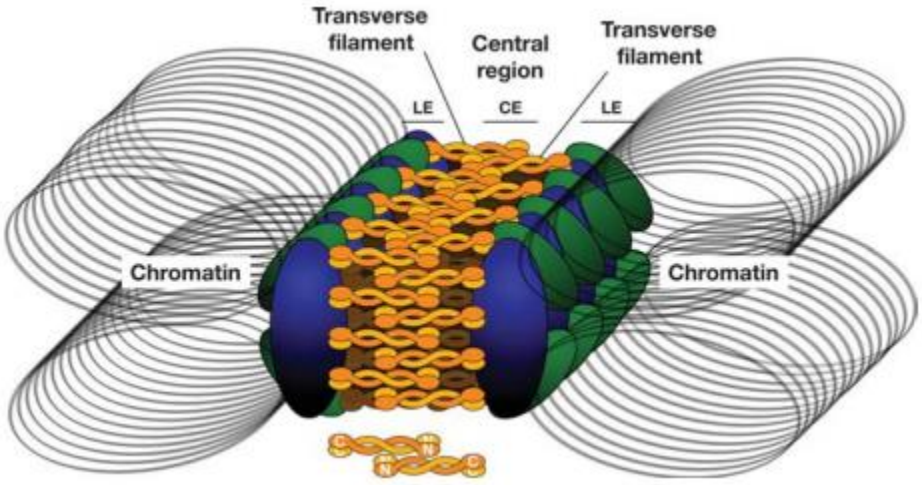

Figure 4 - A model of the synaptonemal complex. This tripartite structure is made up of two lateral elements (LE) and a central element (CE) (Page 2004).

The components of the SC have been identified in many species. In *S. cerevisiae*, many of the potential proteins involved were identified through cytological analysis of meiotic mutants (Heyting 1996). Red1 and Hop1 have been identified to form part of the axial cores, and Zip1 has been shown to make up a portion of the central element (Roeder 1995; Sym and Roeder 1995). The proteins that make up SC between species are similar, but in at least two species, *Schizosaccharomyces pombe* and *Aspergillus nidulans*, SC-

like structures have not been detected. In those organisms, meiosis still progresses in a similar manner except without the SC (Kohli and Baehler1994; Egel-Mitani et al.1982).

Additionally, in *S. cerevisiae*, plants, and mammals meiotic recombination initiates proper SC formation (Henderson and Keeney 2005). The full SC only forms in the presence of double strand breaks (Heyting 1996). Evidently, the double strand breaks allow for the nucleation sites that stabilize the axial cores (Sears et al. 1994). This contrasts with SC formation in *Drosophila melanogaster* and *Caenorhabditis elegans* where a normal SC can still form without the initiation of meiotic recombination (Mckim et al.1998; Dernburg et al 1998)

The formation of the SC happens in a stepwise fashion (Figure 5). First, the axial elements form on the homologous chromosomes (Henderson and Kenney 2005). In some organisms, including *S. cerevisiae*, axial elements begin to form at double strand break sites leading to the hypothesis that SC initiation occurs at recombination sites (Henderson and Keeney 2005). It has been shown that SC begins to form at sites that will eventually give rise to crossovers (Henderson and Keeney 2005). These sites begin to lengthen creating short stretches of SC eventually leading to the complete synapsis of homologs. Later in prophase I, the SC disassembles leaving the homologs connected by crossovers that direct proper segregation of homologous chromosomes in in meiosis I.

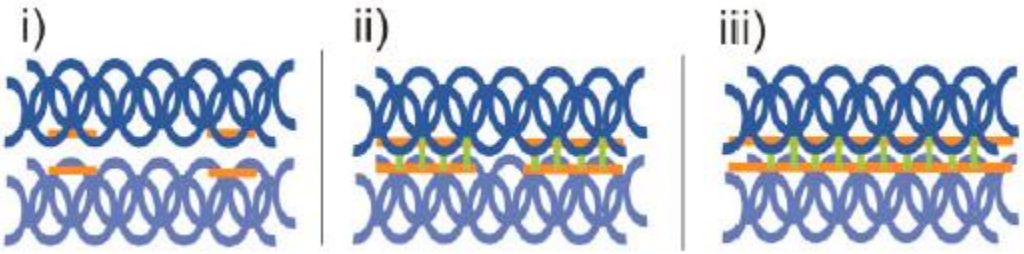

Figure 5 - The steps of synaptonemal complex formation. i) Axial elements begin to form ii) The central element attaches to the axial elements as the SC begins to lengthen iii) The SC fully extends between two homologous chromosomes (Henderson and Keeney 2005).

1.6 Distributive Segregation

Usually during prophase I synaptonemal complex formation is followed by crossing over between chromosomes, and in most species every pair of homologous chromosomes attain at least one crossover during meiosis (Jones et al, 2006). However, it has been observed in a few species of insects and in *S. cerevisiae* that proper segregation of homologs in meiosis I is still possible without crossovers. The mechanism to achieve this has been called distributive segregation (Hawley and Theurkauf 1993). The origins of this mechanism are unclear, but it has been proposed that substitutes for crossovers could have evolved, or backup mechanisms were already in place, in order to segregate homologs correctly (Wolf 1994). Additionally, Wolf proposed that it possibly functions through a modified synaptonemal complex, "stickiness" of heterochromatin, or adhesion of chromatids similar to somatic pairing (Wolf 1994). However, it is difficult to say with certainty how distributive segregation is occurring without comprehensive knowledge of chromosomes and spindle structure (Wolf 1994).

The ability for meiosis in *Drosphilia* to undergo a successful meiosis without crossovers has been studied for decades. It was observed over fifty years ago that homologous chromosomes in male *Drosphilia* do not form a SC (Meyer 1960) and rarely achieve crossovers (Cooper 1945). One possible explanation is that this occurred from a secondary loss of meiotic recombination through evolution (Wolf 1994). A mechanical basis for the segregation of these achiasmate chromosomes is unknown in male *Drosophila* (Thomas et al. 2005). However, it has been proposed that chromatid entanglement at heterochromatic regions may play a role in keeping homologs associated (Vazquez et al. 2002). Likewise, the mechanism for distributive segregation in *S.*

cerevisiae is largely unknown, but it has been hypothesized that centromere regions in yeast are able to undergo a sequence-independent pairing that allows for proper orientation of kinetochores (Kemp et al. 2004).

In female *Drosphilia* distributive segregation seems to occur in a different manner than in the males. Crossovers are generated between most homologs to direct segregation in females, but there also mechanisms in place to segregate achiasmate chromosomes (McKim et al. 2002). The X chromosomes in females do not achieve a crossover in 5% of meiosis, but surprisingly, meiosis I proper segregation still occurs 99% of the time overall (Carpenter 1991). It was determined that NOD, a protein in *Drosphilia* females, was one of the main components in the success of its distributive segregation capabilities (Carpenter 1973). When the nod gene is knocked out, the segregation abilities of the non-crossover chromosomes are severely diminished (Carpenter 1973). In *S. cerevisiae* there is no NOD ortholog, and the proteins involved in distributive segregation are still being investigated.

1.7 Proteins of Interest

Ndj1, Tid1, and Spo16 are three proteins that are involved in meiosis. Unpublished data from the Hillers lab has shown that *ndj1* and *tid1* mutants decrease overall spore viability. However, there was a distinctive increase in the instances of 2-spore-viable tetrads, which is indicative of meiosis I nondisjunction, in *ndj1* mutants but not in *tid1* mutants. These data along with other studies helped to elucidate that Ndj1 is an important factor in the segregation of homologous chromosomes in meiosis I while Tid1 seems to play a different role during meiosis. The Hillers lab became interested in how Ndj1 and Tid1 are involved in crossover regulation.

Other studies had shown that *ndj1* mutants are defective in crossover interference, crossover assurance, and distributive segregation (Chua and Roeder 1997). Additionally, it has been shown that crossover interference was defective in *tid1* mutants (Shinohara et al. 2003). It has been hypothesized that the different aspects of crossover regulation are mechanistically linked, so next the Hillers lab checked if *tid1* mutants had a malfunctioning assurance mechanism as Chua and Roeder had shown in *ndj1* mutants. Unpublished data in a Hillers lab study ended up showing that *tid1* mutants did contain defective crossover assurance.

The Hillers lab wanted to follow up on investigating the effects these proteins had on crossover regulation and determine if both of these proteins had a role in distributive segregation. This would reveal if the same proteins involved in crossover regulation were also involved in distributive segregation. Unpublished data in the Hillers lab showed that Ndj1 but not Tid1 was involved in distributive segregation. However, these data were collected using yeast in the S288c background strain, and there were a large amount of 0-spore-viable tetrads in the wildtype S288c making the data from the *ndj1* and *tid1* mutant strains unreliable. This work repeats those experiments but in the SK1 background, a standard strain for meiotic studies, which is known to sporulate with higher efficiency.

Additionally, the Shinohara lab analyzed a protein, Spo16, that is directly involved in recombination and synaptonemal complex formation (Shinohara et al. 2008). However, unlike proteins with similar functions, crossover interference is not inhibited in the absence of the Spo16 (Shinohara et al. 2008). The Hillers lab became curious about whether or not this unique protein would affect the success of distributive segregation as well. In this study *ndj1*, *tid1*, and *spo16* mutants were all made in the SK1 background.

1.8 Ndj1

Ndj1, also known as Tam1, is a meiosis specific protein that is localized at the telomeres of chromosomes during prophase (Figure 6). It contains 352 amino acids and

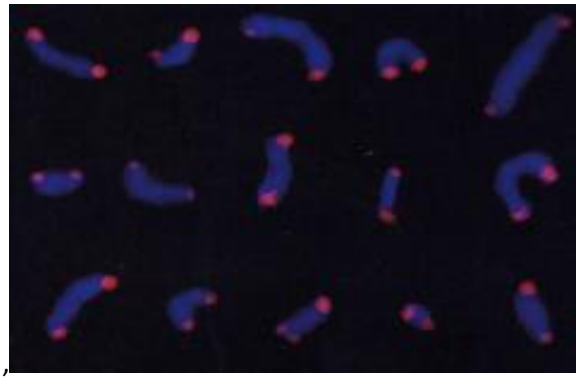

Figure 6 - Localization of Ndj1. A wildtype spread nucleus is shown as DNA is stained in blue and Ndj1 is stained in red (Chua and Roeder 1997).

seems to be unlike any other well-characterized protein (Conrad et al. 1997). During the leptotene-zygotene transition chromosomes normally form a bouquet where Ndj1 specifically tethers the telomeres to the nuclear envelope (Wu and Burgess 2006). It has been proposed that this bouquet may help homologous chromosomes pair by bringing them in closer proximity to each other (Fussell 1987; Dernburg et al. 1995). In *ndj1* mutants the telomeres fail to tether to the nuclear envelope disrupting this early organization of chromosomes (Wu and Burgess 2006).

ndj1 mutants show a delay in meiosis I completion. Many aspects of prophase I are slowed down including the formation of axial elements, the initiation of synapsis, and the turnover of DSBs (Chua and Roeder 1997; Conrad et al. 1997; Wu and Burgess 2006). The regulation of crossovers also seems to be affected by Ndj1. In the mutants the proportion of tetrads where crossovers were not achieved is increased showing that crossover assurance is being impaired (Chua and Roeder 1997). Additionally, crossover interference was shown to be weakened in *ndj1* mutants in the Roeder lab (Chua and

Roeder 1997) but crossover interference was completely functional in the mutants in the Dresser lab (Conrad et al. 1997). It was also shown that distributive segregation is faulty in *ndj1* mutants (Chua and Roeder 1997), so the data collected from *ndj1* mutants will be a positive control in this study.

Furthermore, Ndj1 has more recently been discovered to help maintain spindle pole body cohesion during meiosis in yeast (Li et al. 2015). Spindle pole bodies remain tethered for hours during prophase I unlike mitotic spindle pole bodies which separate within minutes (Li et al. 2015). It seems as though there are two separate assemblies of Ndj1 in yeast during meiosis - one that localizes to the telomeres and one that localizes to the SPBs. It is proposed that Ndj1 therefore regulates both telomere clustering and SPB cohesion (Li et al. 2015).

1.9 Tid1

Tid1, also known as Rdh54, is a homologue of Rad54 (Shinohara et al. 2003). Both proteins are part of the Swi2/Snf2 family of helicase related proteins that have DNA-unwinding activity (Eisen et al. 1995). They both function in DSB repair, but Rad54 predominantly functions in sister chromatid-based repair where Tid1 is not necessary (Ayelet et al. 1999). Tid1 has been shown to be present in vegetative cell growth, but it primarily plays a role in homologous chromosome-based repair functioning in a diploid-specific manner (Klein 1997; Dresser et al. 1997).

Tid1 plays a role in the colocalization of two proteins, Dmc1 and Rad51 (Shinohara et al. 2000). It interacts with both Dmc1 and Rad51 directly and has been shown to have a stronger interaction with Dmc1 than Rad51 (Dresser et al. 1997). Dmc1 and Rad51 are both homologs of RecA, a major bacterial strand exchange protein

(Shinohara and Ogawa 1999). They have somewhat redundant functions of forming joint molecules from DSBs at sites of recombination where they can each promote the formation of recombinants in the absence of the other (Bishop et al. 1992). However, Dmc1 is only present during meiosis, so it seems to be strictly involved in DSB repair using homologs (Bishop et al. 1992). Tid1 seems to organize the assembly of Dmc1 and Rad51 in addition to eliciting their timely removal from the chromosomes (Shinohara et al. 2000).

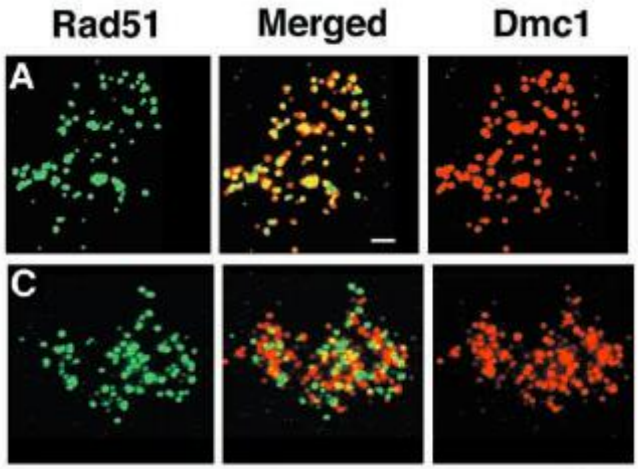

Figure 7 - The localization of Rad51 and Dmc1. The pictures were taken at 3 hours into meiosis with Rad51 in green and Dmc1 in red. A) WT C) *tid1* mutant (Shinohara et al. 2000).

tid1 mutants show a dramatic decrease in colocalization of Dmc1 and Rad51 (Shinohara 2000). In wildtype cells Rad51 promotes the assembly of Dmc1 to the DSB sites, but in *tid1* mutants Dmc1 is no longer directed to the Rad51 sites (Shinohara et al. 2000). Additionally, in *tid1* mutants crossover interference is impaired, (Shinohara et al. 2003) and crossover assurance is defective (Hillers lab unpublished).

1.10 Spo16

Spo16 is a member of a class of proteins known as ZMM proteins. ZMM proteins include the Zip, Msh, and Mer proteins which coordinate recombination and synaptonemal complex formation in *S. cerevisiae* (Shinohara et al. 2008). *zmm* mutants have been shown to contain a reduced number of crossovers and incomplete synaptonemal complex formation (Borner et al. 2004). These ZMM proteins localize along chromosomes in an interference distribution where they seem to specifically mark crossover sites (Fung et al. 2004).

Spo16 has been shown to facilitate Zip1 polymerization from recombination sites (Shinohara et al. 2008). *spo16* mutants display a 50-67% reduction in crossovers in comparison to wildtype (Shinohara et al. 2008). However, unlike the other *zmm* mutants, *spo16* mutants show a normal crossover interference distribution (Shinohara et al. 2008). It has been proposed that specific subcomplexes of ZMM proteins may function in either crossover differentiation or in crossover implementation (Shinohara et al. 2008).

In *spo16* mutants meiosis I was delayed 3 hours (Shinohara et al. 2008). It took longer for *spo16* mutants to repair DSBs, and Dmc1 took longer to disappear from the DSB sites in *spo16* mutants (Shinohara et al. 2008). Additionally, synaptonemal complex formation is defective in the mutants. In zygotene Zip1 foci form normally, but Zip1 is never fully polymerized leaving the homologs only partially synapsed (Shinohara et al. 2008). Shinohara proposed that unlike crossover interference, crossover assurance is only functional with all of the ZMM proteins and the entire synaptonemal complex (Shinohara et al. 2008).

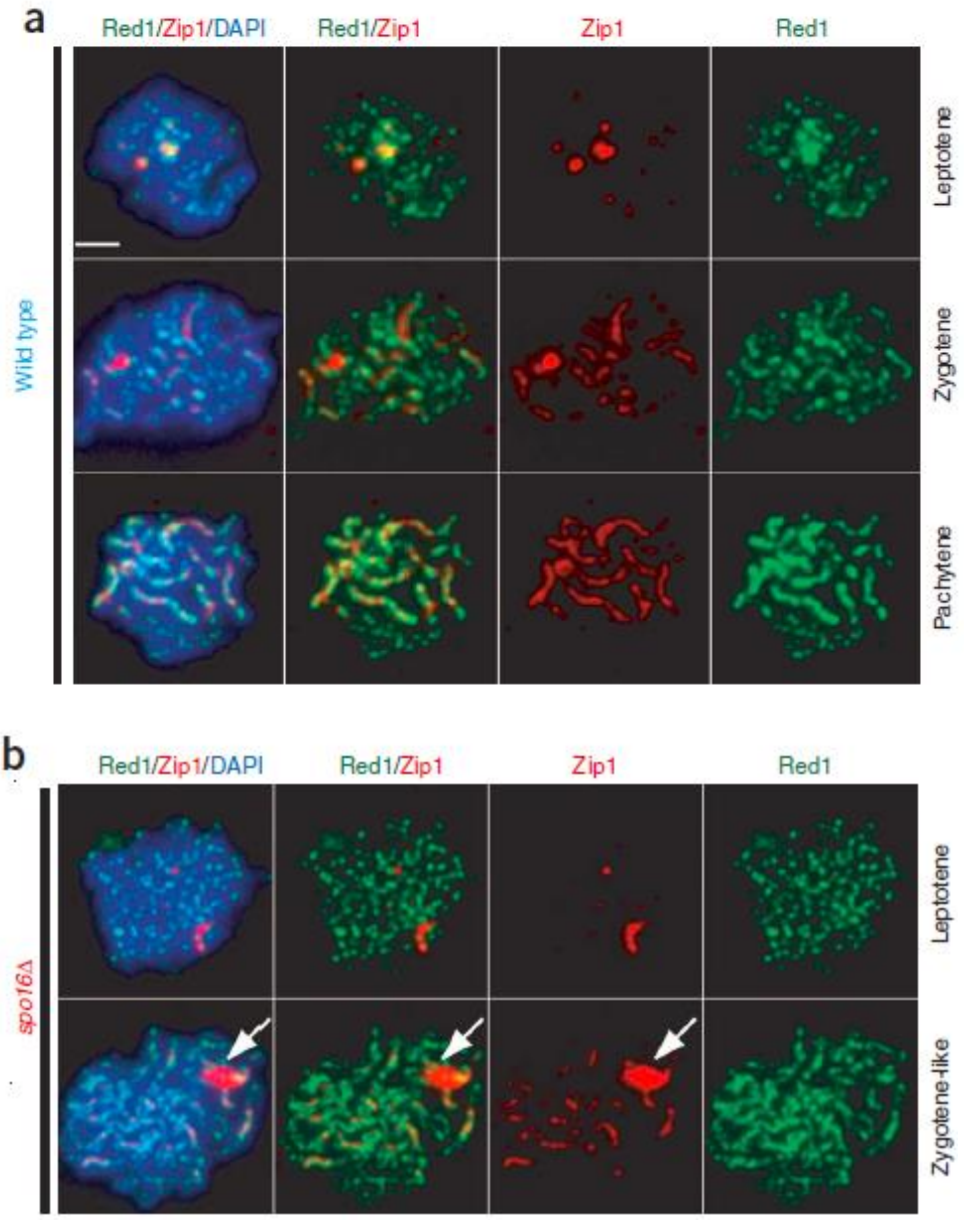

Figure 8 - Polymerization of Zip1. The difference is shown between a wildtype and a *spo16* mutant. DAPI shows DNA, Zip1 shows the central element of the synaptonemal complex, and Red1 shows the chromosome axes. A) In the wildtype, Zip1 elongates normally fully synapsing the homologs as meiosis progresses from leptotene to zygotene to pachytene. B) In the *spo16* mutant, Zip1 seems to form normally in leptotene but during a zygotene-like state Zip1 forms an aggregate and full synapsis is not achieved between homologs (Shinohara et al. 2008).

Recent x-ray crystallography experiments have shown that Spo16 and Zip2 form a heterodimer (Arora and Corbett 2018). Zip2:Spo16 binds specific regions of DNA present in early meiotic recombination intermediates, and the heterodimer does not

contain an endonuclease site. It is hypothesized that Zip2:Spo16 binds and stabilizes meiotic intermediates, and another ZMM protein, Zip4, recruits additional crossover promoting factors (Arora and Corbett 2018).

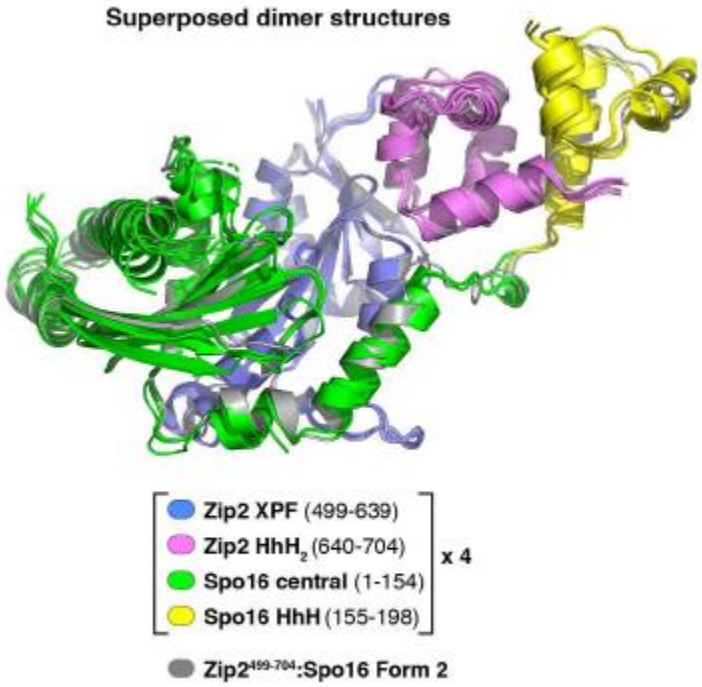

Figure 9 - The proposed structure of Zip2:Spo16 (Arora and Corbett 2018).

1.11 Experimental Overview

The roles that candidate proteins Ndj1, Tid1, and Spo16 have in distributive segregation were investigated. In order to directly test distributive segregation, strains of yeast were constructed that contained a pair of homeologous, or partially homologous, chromosomes. These homeologous chromosomes could potentially pair up with each other, but they do not share enough sequence homology for crossing over to occur. This forces the proper segregation of these homeologous chromosomes during meiosis I to rely on distributive segregation.

Separate homologous and homeologous strains of yeast were made with homozygous mutations for each of the candidate genes. Data was gathered after yeast cells went through meiosis and formed four spore tetrads. It was expected that if the chromosomes segregated correctly, there would be four-spore-viable tetrads. Each haploid cell at the end of meiosis would contain one of each of the 16 yeast chromosomes.

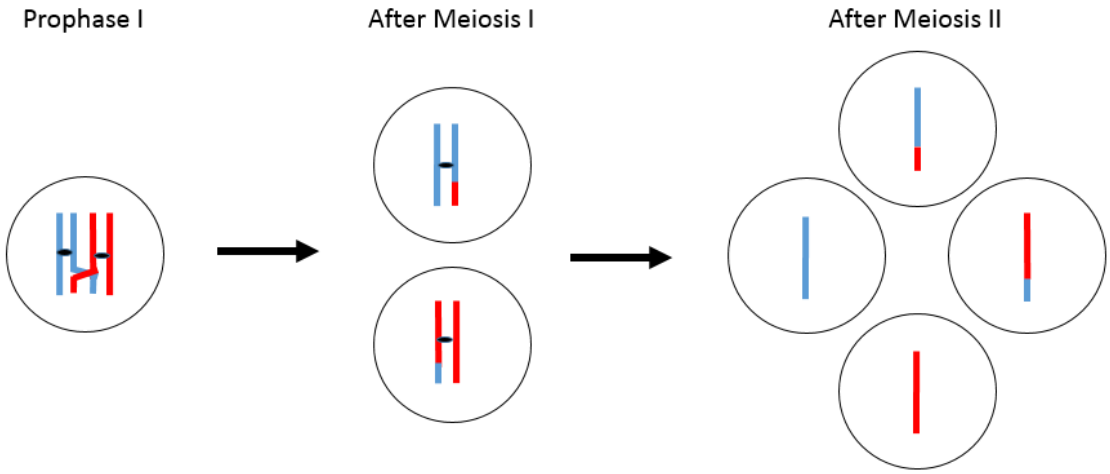

Figure 10 - The segregation of one pair of homologs (one red and one blue). The progression from meiosis I through meiosis II is shown. Chiasmata that are formed in prophase I ensure the proper segregation of chromosomes.

If meiosis I nondisjunction occurs between a pair of homologous chromosomes there would be two viable spores at the end of meiosis. The spores that were missing one of the chromosomes will not be viable. Additionally, if nondisjunction happens between more than one pair of homologs it is possible that there would be a 0-spore-viable tetrad.

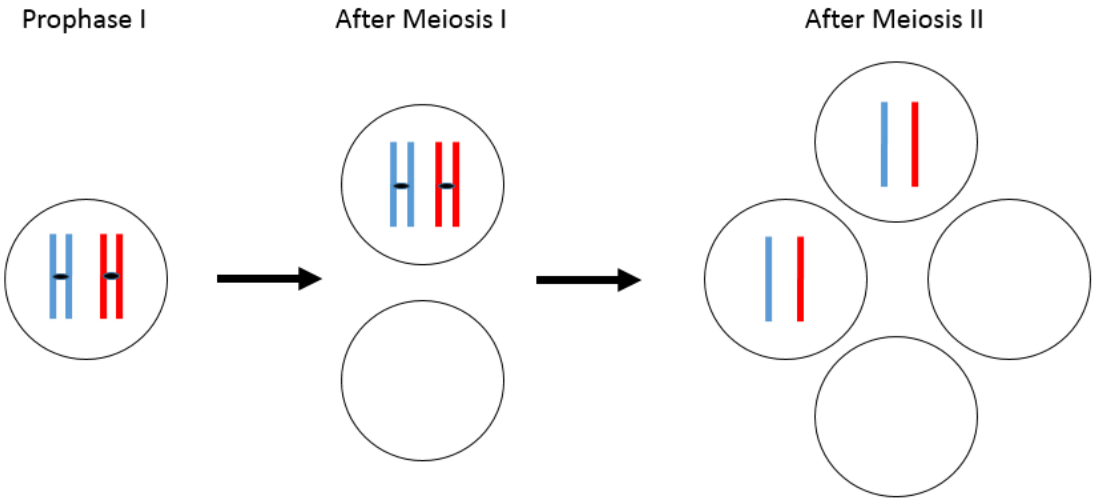

Figure 11 - Nondisjunction of a pair of homologs. When meiosis I nondisjunction occurs two cells at the end of meiosis II to be missing a chromosome. Without chiasmata formed in prophase I proper segregation of chromosomes in meiosis I is decreased.

By comparing the spore viabilities between the homologous and homeologous strains of each mutant, inferences can be made about the involvement each candidate protein plays within distributive segregation. If the homeologous mutant strains show increased levels of 2-spore and 0-spore-viable tetrads in comparison to their homologous counterparts and the wildtype homeologous strain then it can be concluded that distributive segregation is being impaired.

2. Methods

2.1 Rehydrating CRISPR plasmids

CRISPR plasmids bRA89, bRA90, pJH2970, pJH2971, and pJH2972 were received from the Haber lab via mail. The plasmids arrived on pieces of paper. Microcentrifuge tubes were obtained and labeled, and 15µl of sterile TE was added to each. The paper was cut into small pieces, and were added into 1.5ml tubes. The paper soaked up the TE immediately, so additional TE was added until there is a noticeable amount of liquid in the bottom of the tubes. The tubes were placed at 4°C for two days with the pieces of paper still inside. A NEB 5-alpha Competent *E. coli* high efficiency transformation protocol was then followed.

2.2 NEB 5-alpha Transformation

A tube of NEB 5-alpha competent *E. coli* cells was placed on ice for 10 minutes for each CRISPR plasmid. After the incubation 5µl of plasmid DNA was added into the cell mixture. The tube was then carefully flicked 4-5 times to mix cells and DNA, and the mixture was placed on ice for 30 minutes. The tubes were then heat shocked at 42°C for exactly 30 seconds. The tubes were placed on ice for 5 minutes, and 950µl of room temperature SOC was added. The cell tubes were placed at 37°C shaking at 250 rpms for 60 minutes. The selection plates used, LB + AMP, were placed at 37°C during this wait step to warm up. The cells were mixed thoroughly by flicking and inverting the tubes. 100µl of cells was spread onto LB + AMP plates and incubated at 37°C for at least 12 hours.

2.3 Plasmid Isolation

A Thermo Scientific GeneJET Plasmid Miniprep Kit was utilized to isolate the CRISPR plasmids.

2.4 Diagnostic Restriction Enzyme Digests

In order to ensure that the plasmids were correctly isolated, RE digests were utilized. Each RE digest was made by adding 1µl plasmid, 1µl 10x buffer, 7.5µl water, and 0.5µl enzyme into a PCR tube. The tubes were then placed into a thermocycler at 37°C for 2.5 hours. Table 1 shows each RE digest.

Table 1 – Restriction enzyme digests.

Plasmid	Restriction Enzyme	Expected Band Sizes (bp)
bRA89	HindIII	7,101, 2,459, 1,827
bRA90	EcoRV	7,493, 4,069, 291
pJH2970	Ndel	8,988, 2,033
pJH2971	NsiI	6,308, 4,594, 266
pJH2972	EcoRV	6,839, 4,032, 291

2.5 Freezing *E. coli* with CRISPR Plasmid Stocks

Overnights of 3 mls culture of *E. coli* that contained each CRISPR plasmid were grown at 37°C shaking at 200 rpms. The next day 750µl of *E. coli* and 250µl of 60% glycerol was added to a labeled 2ml Wheaton tube and stored at -80°C.

2.6 Making the Oligos for the Guide RNA

The protocol from "Cas9-Mediated Gene Editing in *Saccharomyces cerevisiae*" (Anand et al. 2017) was followed to make the oligos. In order for specific Cas9 targeting, 20nt sequences were selected to be inserted into the bRA89 plasmid to function as the gRNA sequence. The sequences were flanked by the Cas9 protospacer adjacent motif,

NGG. Each sequence was also tested with BLAST to make sure that it was unique within the SK1 *S. cerevisiae* genome. At the end of the forward oligo GTTTT was added to the 3' end, and at the end of the reverse complement sequence GATCA was added to the 3' end. These overhangs allow for the insertion of the oligo into the BpII cut CRISPR plasmid. The oligos utilized are shown in Table 2.

Table 2 - Oligos for CRISPR.

Name	Sequence
Tid1 Top oligo 2399-2418	5'-GACATGCTGGTGTGTGACGAGTTTT-3'
Tid1 Bottom oligo 2399-2418	5'-TCGTCACACACCAGCATGTCGGATCA-3'
Tid1 Top oligo 3870-3851	5'-TCTCTGAGACATATCTCGCCGTTTT-3'
Tid1 Bottom oligo 3870-3851	5'-GGCGAGATATGTCTCAGAGAGATCA-3'
Spo16 Top oligo	5'-GCGTTTGACACGGAACAGCGGTTTT-3'
Spo16 Bottom oligo	5'-CGCTGTTCCGTGCAAACGCGATCA-3'
Ndj1 Top oligo	5'-GTTTGCCCGCCGATGTTTAGGTTTT-5'
Ndj1 Bottom oligo	5'-CTAAACATCGGCGGGCAAACGATCA-3'

Each of the oligos were diluted in 1X TE to bring the concentration to 100µM. In order to duplex the oligos 5µl of one of the top oligos, 5µl of one of the bottom oligos, 2µl of ligase buffer, and 8µl of TE were added to a 1.5ml centrifuge tube. The solution was heated up to 100°C for 5 minutes and allowed to cool down to room temperature (Anand et al. 2017).

2.7 BpII Digest of BRA89 to add Insert

For this study the only CRISPR plasmid used was bRA89. The protocol from "Cas9-mediated gene editing in Saccharomyces cerevisiae" (Anand et al. 2017) was followed to digest BRA89. In order to cut BRA89, 66µl of TE buffer, 20µl of plasmid that had a yield

of 50-100ng/µl, 10µl Tango buffer, 2µl 50x SAM, and 2µl BpII enzyme were added into a 1.5ml microcentrifuge tube. The solution was then incubated overnight at 37°C. The cut in BRA89 was confirmed through gel electrophoresis with an uncut bRA89 control.

2.8 Gel Purification

The digested bRA89 was purified before ligation using gel purification. A QIAquick Gel Extraction Kit was utilized, and the cut plasmid was eluted with 35µl of EB buffer.

2.9 BRA89 Ligation and Transformation

The protocol was followed from "Cas9-mediated gene editing in Saccharomyces cerevisiae" (Anand et al. 2017) for ligating the gRNA inserts into bRA89. In order to ligate the duplexed oligo into BRA89 a 10µl solution was made with 3µl of ~5ng/µl gel purified vector, 5µl 25nM duplexed insert, 1µl 10x ligase buffer, and 1µl of NEB T4 DNA ligase. This solution was incubated at room temperature for an hour. All four duplexed oligos, two for TID1, one for NDJ1, and one for SPO16, were ligated into bRA89. Each ligated plasmid was transformed into *E. coli* following the NEB-5 alpha transformation protocol listed above. After the transformation, colony PCR was utilized to test for the presence of bRA89 with the correct insert.

2.10 *E. coli* colony PCR

About 8 colonies were chosen from potential transformants. The protocol for these 15µl PCR reactions called for the reagents shown in table 3.

Table 3 - Reagents for PCR.

Reagent	Volume	Final Concentration
5x GoTaq Buffer	3µl	1x
MgCl (25mM)	0.9µl	1.5mM
dNTPs (10mM)	0.18µl	0.12mM
Forward Primer (10µM)	1µl	0.67µM
Reverse Primer (10µM)	1µl	0.67µM
Taq Polymerase (5u/µl)	0.45µl	0.15 units
Template	Touch Colony	unknown
Water	8.7µl	unknown

Using a pipette, a chosen colony was touched and mixed into the PCR solution by pipetting up and down. Additionally, 1µl was taken from the PCR mixture and pipetted onto a labeled LB + AMP plate. This was done for every PCR reaction so that there would be a plate of colonies for all of the potential transformants. The forward primers utilized are shown in table 4, and the reverse primer that binds the ampicillin resistance gene was used for each reaction making an expected product size of 1359 bp. The reverse primer's sequence was 5'-GCCTCACTGATTAAGCATTGGTAAC-3'.

Table 4 – Forward primers for CRISPR plasmid check.

Forward Primer Name	Forward Primer Sequence
Tid1 Top oligo 2399-2418	5’-GACATGCTGGTGTGTGACGAGTTTT-3
Tid1 Top oligo 3870-3851	5’-TCTCTGAGACATATCTCGCCGTTTT-3’
Spo16 Top oligo	5’-GCGTTTGACACGGAACAGCGGTTTT-3’
Ndj1 Top oligo	5’-GTTTGCCCGCCGATGTTTAGGTTTT-3’

2.11 Sequencing BRA89 for Correct Insert

In addition to utilizing PCR to check the insert, bRA89 was also sequenced. GENEWIZ (New Jersey, USA) instructions were followed in order to make the pre-mixed sequencing solutions. bRA89 was diluted to ~100ng/µl in 10µl before being mixed with the primer for a total of 15µl with 25pmol of primer. The primers used to sequence the insert are shown in Table 5. Only two primers were needed because only the insert portion of bRA89 was sequenced.

Table 5 - Primers for insert check.

Primer Name	Primer Sequence
bRA Seq F	5’-TTTTGTAGTGCCCTCTTGGG-3’
bRA Seq R	5’-CACAGGAAACAGCTATGACC-3’

2.12 PCR for Gene Disruption

PCR products were made in order to be transformed into yeast to replace the target genes with CloNat resistance. For the template, a plasmid with a Clonat resistance gene, pAG25, was isolated from *E. coli* in the Hillers Bacteria box stored in the -80°C

freezer. Thermo Scientific's GeneJET Plasmid Miniprep Kit was used for plasmid purification. The plasmid was then diluted with sterile water until it was ~1ng/µl in order to be a more suitable template for PCR.

The primers used were constructed to be 90 nucleotides in length; 72 nucleotides were added onto the 5' side of the primer that were used for homology directed repair, and 18 nucleotides were added onto the 3' side of the primer that would bind to pAG25. The primers used are shown in table 6.

Table 6 - Primers for making PCR product for LiT transformations.

Primer Name	Primer Sequence
Ndj1-NAT Forward Primer	5'-ggtatatcaaaaatattgtcatgaactataccatatacaacttaggataaaaatacaggtagaaaaactataAGCTGAAGCTTCGTACGC-3'
Ndj1-NAT Reverse Primer	5'-cattaagcaagatttaaagtaaacagcaaagaaaagttttttttggttcagatgtaatatggatagcccgttAGGCCACTAGTGGATCTG-3'
Tid1-NAT Forward Primer	5'-aacgcgaagagctaaaaaaaaaaaagaaaacctactataaataaccgattagaatcgagttttgtattgaaAGCTGAAGCTTCGTACGC-3'
Tid1-NAT Reverse Primer	5'-taaacctattaaatacaatcagtgattaaataatagctattttatttagtatataagtgtccatatttggcgAGGCCACT AGTGGATCTG-3'
Spo16-NAT Forward Primer	5'-aaaatacgaaatagtgtaaaggttcaagagaataaacttctggaacaacatttctggaatggttcaatagaaAGCTGAAGCTTCGTACGC-3'
Spo16-NAT Reverse Primer	5'-aaacgagtacataaagaataagtaaataataattcaacaatacctgatttttttttttcttacatttttttaAGGCCACTAGTGGATCTG-3'

The protocol for these 50µl PCR reactions called for the reagents shown in table 7. The PCR reactions were placed in the thermocycler for 35 cycles with a 30 second

denaturation phase at 95°C, a 30 second annealing phase at 52.3°C, and a 90 second extension time at 72°C. The initial denaturation phase before the 35 cycles was set for 2 minutes at 95°C and the final extension after the cycles had ended was set for 5 minutes at 72°C. The expected PCR disruption product was 1439 nucleotides. Before the PCR products were transformed into yeast, they were first purified using Thermo Scientific's GeneJET PCR Purification Kit.

Table 7 - Reagents for PCR disruption product.

Reagent	Volume	Final Concentration
5x GoTaq Buffer	10µl	1x
dNTPs (10mM)	1µl	0.2mM
MgCl (25mM)	5µl	2.5mM
Forward Primer (10µM)	1µl	0.2µM
Reverse Primer (10µlM)	1µl	0.2µM
Template (~0.2ng/µl)	0.25µl	~1pg/µl
Taq Polymerase (5u/µl)	1µl	0.1 units
Water	30.75µl	x

2.13 LiT Transformation

A *Saccharomyces cerevisiae* Standard LiT Transformation protocol was followed in order to make mutant strains of yeast. First, yeast strains were grown overnight in 3 ml of YPAD broth at 30°C shaking at 200rpm. The next day 300µl of culture was added into 10 ml of YPAD broth and then incubated at 30°C shaking at 200rpm. The culture was grown until it reached an OD600 ~0.7, and then the cells were pelleted in 15ml conical tubes at 3000rpm for 3 minutes. The supernatant was poured off, and the cells were resuspended in 10 ml of sterile water. A centrifugation was then performed in the same

manner as before to pellet the cells. The supernatant was once again removed and the cells were resuspended in 10 ml of LiT (1ml LiAC, 1ml 10x TE, 8ml sterile water) with 10mM DTT. The cells were then incubated on a table-top rotator at room temperature for 45 minutes and then pelleted at the same speed as the previous centrifugations. The pellet was resuspended in 145µl of LiT per transformation.

The specific disruption PCR product and bRA89 with the corresponding insert were transformed into strains at the same time. Microcentrifuge tubes were prepared before the addition of the cells. The tubes contained 4µl plasmid and 8µl of PCR product, 5µl plasmid and 10µl of PCR product, or 6µl of plasmid and 12µl of PCR product. Dozens of transformations were performed with a large range of plasmid and PCR concentrations, but no specific concentrations were ever found to be optimal. Around 0.5µg of plasmid and 1.0µg of PCR product were typically added. Additionally, 5µl of sheared salmon sperm DNA was also added to each transformation tube. For the positive control, 0.1µg of plasmid pAG36 was added with 5µl of salmon sperm to a microcentrifuge tube. pAG36 was a plasmid that gives yeast Clonat resistance. It was isolated from *E. coli* in the Hillers Bacteria box stored in the -80°C freezer. For the negative control, only 5µl of salmon sperm was added to a microcentrifuge tube.

The DNA in each of the microcentrifuge tubes was then gently mixed with 145µl of the prepared cells. The solutions were left to incubate for 10 minutes at room temperature, and then 300µl of 50% PEG-LiT (1/10 volume LiT, 1/10 volume 10x TE, 8/10 volume 50% PEG) was added and gently mixed with a pipette. The tubes were then rotated at room temperature for 10 minutes before 15µl of DMSO was added. Immediately after the addition of DMSO, each tube was vortexed and placed at 42°C for

5 minutes. The heated tubes were then gently centrifuged at 4000rpm for 4 minutes. Following the pellet formation, instead of a typical 30-60 minute incubation, the yeast were added to 3 ml of YPAD broth and incubated overnight at 30°C shaking at 200rpm. The next morning 100µl of sterile water was added to YPAD plates followed by 100µl of 25mg/ml Clonat. The sterile water was added first in order to ensure that some Clonat will spread across the entire plate. The Clonat and water mixture was spread with sterile beads, and the plates were left for an hour at room temperature. After this wait step, the yeast from the overnight incubation was pelleted at 3000 rpms for 3 minutes. The supernatant was poured off, 150µl of yeast was pipetted onto the labeled Clonat plates, and the yeast was spread using the sterile beads that were still on the plate. The beads were then removed, and the plates were incubated for 72 hours at 30°C. Colonies that grew on the plates with Clonat were potential mutants.

2.14 Yeast Colony PCR to Check for Mutations

PCR was utilized to check the potential transformants for the replacement of the genes of interest with the Clonat resistance gene. In order to perform colony PCR on yeast, a pipette tip was touched to a yeast colony and transferred into a microcentrifuge tube with 25µl of 0.02M NaOH. Enough yeast was transferred to make the NaOH slightly turbid. The tube was placed into a heat block for a 15 minute incubation at 99°C. The 15µl PCR reactions were made up of the same reagents from table 3 except instead of an *E. coli* colony, 1µl of boiled yeast lysate was used as the template making only 7.7µl of water necessary. For every reaction the reverse primer was the same. It bound 58 bp into the Clonat resistance gene and had a sequence of 5'-CTGGCGCGCCTTAATTAACC-3'.

The forward primer varied depending upon which gene was being checked. The forward primers and expected PCR products are listed in Table 8.

Table 8 – Forward primers for mutation check.

Forward Primer Name	Forward Primer Sequence	Distance from Insert	Expected Product Size
Spo16 Test-F	5'-GCTCGAAAGAAGTGATGTG-3'	306 bp	364 bp
Ndj1-New-Test	5'-AGTTGGGCGGCTATTTTGAC-3'	98 bp	156 bp
Tid1newF1	5'-GGCATTTTTACTCGATCGCG-3'	223 bp	281 bp

2.15 Sequencing to check for mutations

The same PCR products made from yeast colony PCR were used for sequencing. Before being sequenced, the PCR products were purified using Thermo Scientific's GeneJET PCR Purification Kit. After purification the PCR products were diluted to 1ng/µl in 10µl. The same primers used from yeast colony PCR were also utilized for sequencing. Enough primer was added to make a total volume of 15µl with 25pmol of primer. The template and primer solutions were mailed to GENEWIZ for sequencing.

2.16 Tid1 Sequencing

A gDNA isolation was performed when sequencing was not confirming the replacement of Clonat with Tid1. The gDNA isolation protocol used was a standard yeast sorbitol gDNA prep. First, cells were grown overnight in 5ml YPAD broth at 30°C shaking at 200rpm. The next day 1.5ml of cells were added to a microcentrifuge tube, and the cells were pelleted at 3000rpm for 2 minutes. The supernatant was decanted and another 1.5ml of cells were added and centrifuged. The supernatant was once again decanted and the process was repeated with another 1.5ml of cells. The pellet was resuspended with 1ml of sterile water, and the cells were then centrifuged at 3000rpm for

2 minutes. The new pellet was resuspended in 500µl of 1M Sorbitol. Following the Sorbitol addition, 15µl of 0.5M DTT and 7.5µl of 10mg/ml Zymolyase were added. This solution was incubated at 37°C shaking at 200rpm for an hour.

The cells were then taken out of the incubator and 200µl of TE was added along with 70µl of 10% SDS. The tube was then incubated at 65°C for 10 minutes and then 320µl of 5M KOAc was added. In order to mix the solution, the tube was inverted 6 times. The cells were then incubated on ice for 30 minutes and centrifuged for 6 minutes at max rpm. While the centrifuge was running, a 2ml microcentrifuge tube was prepared by adding 1ml of 100% Isopropanol and 200µl of 5M NH_4OAc. The centrifuged tube was obtained and 650µl of supernatant was added into the prepared 2ml microcentrifuge tube. In order to mix the solution, it was inverted 6 times. The tube was then centrifuged at 4000rpm for 1 minute, and the subsequent supernatant was decanted. The resulting pellet was allowed to air dry and then 100µl of TE and 2µl of 10mg/ml RNase were added resulting in isolated gDNA.

A PCR was performed utilizing the same reagents from table 3 except 1µl of gDNA (~0.4ng/µl) was used as the template making only 7.7µl of water necessary. GENEWIZ instructions were followed for sequencing the PCR product.

2.17 Yeast Strains Constructed

CRISPR was utilized on strains KHY 139, KHY 140, KHY 338, and KHY 369 in order to create new mutant strains. The two wildtype strains, KHY 139 and KHY 140, were constructed by Jonathan Henzel in the Hillers lab. KHY 338 was the haploid strain received from the Lichten lab that had a chromosome V from *Saccharomyces carlsbergensis* which allowed for the construction of homeologous diploid strains.

Additionally, some of the *tid1* mutant strains were not made in the lab, but they were received from the Bishop lab. All strains constructed and utilized were of the SK1 background and shown in table 9.

Table 9 - Yeast strains.

Catalog #	Background	MAT	Short Description	Genotype	Made By:
KHY 139	SK1	α	WT	ura3, trp1	Jonathan Henzel
KHY 140	SK1	a	WT	ura3, leu2	Jonathan Henzel
KHY 338	SK1	α	SK1 with carlsbergensis V	lys2, ho::LYS2, leu2, ura3 (carl chr V)	Lichten Lab
KHY 369	SK1	a	Swapped markers from KHY 139 and KHY 140	ura3, trp1	Ethan Shaw
KHY 370	SK1	α	KHY 139 *spo16* mutant	ura3, trp1, spo16::cloNAT	Ethan Shaw
KHY 371	SK1	a	KHY 140 *spo16* mutant	ura3, leu2, spo16::cloNAT	Ethan Shaw
KHY 372	SK1	α	KHY 338 *spo16* mutant	lys2, ho::LYS2, leu2, ura3, spo16: cloNAT (carl chr V)	Ethan Shaw
KHY 373	SK1	a	KHY 369 *spo16* mutant	ura3, trp1, spo16::cloNAT	Ethan Shaw
KHY 376	SK1	α	KHY 338 *tid1* mutant	lys2, ho::LYS2, leu2, ura3, tid1::cloNAT (carl chr V)	Ethan Shaw
KHY 382	SK1	a	DKB *tid1* mutant	ho::LYS2, lys2, leu2::hisG, ura3, trp1::hisG, his4B, tid1::leu2::TRP1	Bishop Lab

KHY 377	SK1	a	KHY 369 *tid1* mutant	ura3, trp1, tid1::cloNAT	Ethan Shaw
KHY 385	SK1	α	DKB *tid1* mutant	ho::LYS2, lys2, leu2:hisG, tid1::leu2::TRP1	Bishop Lab
KHY 378	SK1	α	KHY 139 *ndj1* mutant	ura3, trp1, ndj1::cloNAT	Ethan Shaw
KHY 108	SK1	a	ndj1::kanMX4	ura3, leu2, ndj1::kanMX4	Jonathan Henzel
KHY 380	SK1	α	KHY 338 *ndj1* mutant	lys2, ho::LYS2, leu2, ura3, ndj1::cloNAT (carl chr V)	Ethan Shaw
KHY 347	SK1	a	ndj1::kanMX4	trp1, ndj1::kanMX4	Polly Parks

2.18 Mating Yeast

In order to make diploid strains of yeast, the constructed haploid strains had to be mated. Table 10 shows which strains were mated to generate each diploid strain used in the study. In order to mate two strains of yeast, both haploids were first patched to a YPAD plate for an overnight incubation at 30°C. The next day one haploid was patched onto one side of a YPAD plate, and the potential mate was patched onto the other side of the same plate. Additionally, a small clump from each strain was also placed next to each other in the middle of the YPAD plate. The yeast in the middle of the plate were then mixed together and spread into a patch using a sterile stick. The YPAD plate was then placed into the 30°C incubator for overnight growth.

The next day the YPAD plate was replica printed to various amino acid dropout plates and incubated at 30°C overnight. Each strain of yeast is unable to synthesize specific amino acids as shown in table 9. Two strains of yeast that are mated together

must be able to complement each other. The diploids were selected for by growth on a double amino acid dropout plate that neither haploid parent could grow on.

However, the parents for the homeologous *tid1* mutant strain did not have genotypes that would complement each other in the diploid to allow for selection on a double amino acid dropout plate. For constructing this diploid, the mating was performed initially on a YPAD plate, but KHY 382 was mixed with 10x the amount of 376. The plate was incubated for ~24 hours before it was replica printed onto a SD –his plate. This plate was also allowed to incubate for ~24 hours until the diploid was selected for. In order to make sure that the yeast obtained was diploid, sporulation was also checked.

Table 10 - Yeast strains mated.

Strains Mated	Resulting Strain
KHY 139 + KHY 140	Wild type homologous control
KHY 338 + KHY 369	Wild type homeologous control
KHY 370 + KHY 371	Homologous *spo16* mutant
KHY 372 + KHY 373	Homeologous *spo16* mutant
KHY 377 + KHY 386	Homologous *tid1* mutant
KHY 376 + KHY 382	Homeologous *tid1* mutant
KHY 108 + KHY 378	Homologous *ndj1* mutant
KHY 347 + KHY 380	Homeologous *ndj1* mutant

2.19 Sporulating and Dissecting Yeast

The diploid yeast strains were freshly patched to a YPAD plate and grown overnight at 30°C. The yeast was then replica printed onto a nutrient deficient plate called a SPO plate. The SPO plate was left to incubate at 30°C for 3 days. After the incubation, 7µl of sterile water was pipetted into a microcentrifuge tube followed by 7µl of

zymolyase [10mg/ml]. A pipette tip was used to swipe across the yeast patch and mix it into the zymolyase solution. This was done multiple times until the solution was turbid. The yeast were incubated in the zymolyase for 10 minutes at room temperature. After the incubation, 1µl of the zymolyase solution was dropped onto the side of a YPAD plate. Using a pipette tip the drop was then spread in a line along the side of the plate. This plate could then be taken over to the Singer Instruments MSM Dissecting Microscope for tetrad dissection.

The plate was placed upside down with the lid removed on the stage of the microscope above the micromanipulator. The microscope light and the Singer touchpad attachment were turned on. "Singer set up" followed by "tetrad dissection" were chosen on the screen, and the microscope centered the plate. The plate moved around on the stage by using a joystick, and the 40x objective was used to located tetrads. When a tetrad was found the micromanipulator was raised up until it touched the tetrad. Once the tetrad was on the micromanipulator, it was lowered away from the plate. The plate was then moved so that a portion of the plate with no yeast was above the micromanipulator. The micromanipulator was raised back up to the plate, and the four spores could be separated from the ascus. The Singer touchpad guided the plate movement to produce an even amount of space between spores so there would be no overlap, and it was possible to distinguish between neighboring tetrads.

2.20 Data Collection

After the dissection, the spores were incubated for 3 days at 30°C. The spores were then replica printed to various amino acid dropout plates in order to check the genetic markers on each spore. This helped to ensure that tetrads were being dissected,

and that data was not collected on other cells that did not sporulate. It was expected from the matings achieved in this study that there would be 2 haploid cells that would not be able to grow on a specific amino acid dropout plate from every tetrad. If one parent couldn't synthesize leucine and the other parent could, then the tetrad produced had 2-viable-spores on a dropout plate deficient of leucine. If 4 colonies grew on an amino acid dropout plate then it was not a four spore tetrad that was separated during the dissection. The number of viable spores were counted up from each tetrad in order to compare spore viability patterns between strains.

2.21 Statistics

A 5x2 Chi-squared test was utilized to measure the difference in the distribution patterns of spore viability between each strain of yeast.

3. Results

3.1 Experimental Design

This study required constructing strains of yeast with CRISPR, mating these strains together, dissecting tetrads from each strain, and collecting spore viability data. Strains of yeast were constructed using CRISPR/Cas9 to replace the genes of interest with Clonat resistance. A CRISPR/Cas9 plasmid was received from the Haber lab, and it contained a BpII restriction site where a 20 nucleotide sequence could be inserted to make the gRNA. The plasmid also contained the gene to make the Cas9 protein. This plasmid was transformed into yeast in order to make a Cas9 complex with specific gRNA to the target genes. Additionally, a segment of DNA containing Clonat resistance was transformed into yeast at the same time in order for efficient targeted gene replacement (Figure 12).

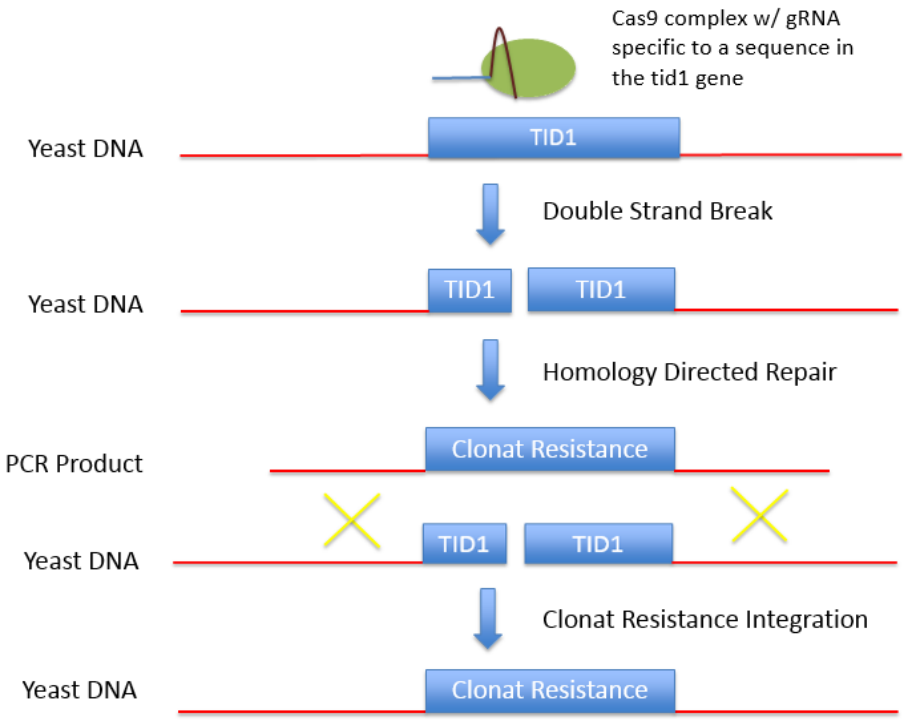

Figure 12 - The process of Clonat replacement of TID1.

The knockout strains that were constructed were haploid, so they had to be mated together in order to make diploid strains. Only diploid strains have the capability to divide meiotically. Each haploid contained a unique marker that could be checked by observing the growth or absence of growth on media lacking specific amino acids. For instance, most of the haploids had a mutation for either tryptophan synthesis or leucine synthesis. Therefore, the diploid constructed from a mating of one mutant that couldn't synthesize tryptophan and one mutant that couldn't synthesize leucine would be able to grow on a plate lacking both tryptophan and leucine while the two haploids could not (Figure 13).

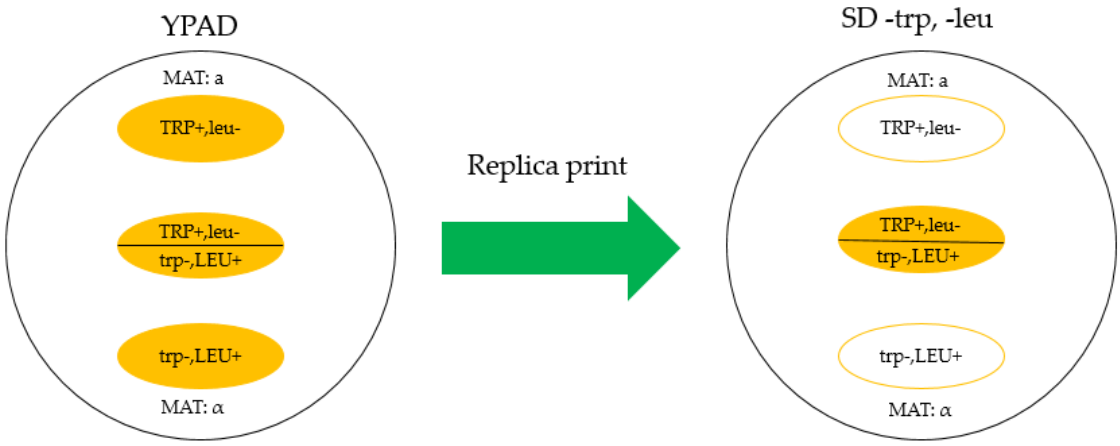

Figure 13 - An example mating between two strains. The top strain is mutant for tryptophan synthesis and the bottom strain is mutant for leucine synthesis. The yeast was transferred from a YPAD plate to an SD –trp –leu plate, which is an amino acid dropout plate missing both tryptophan and leucine.

To construct the diploid mutant strains both haploid strains mated together were mutants for the particular gene of interest creating a homozygous mutation in the diploid. A homozygous mutation was necessary so that no protein of interest would be synthesized in the mutants. In order to construct the diploid homeologous strains, one of the haploid parents contained a chromosome V from *S. carlsbergensis*, constructed in the Lichten lab, while the other parent strain contained all *S. cerevisiae* chromosomes (Figure

14). The two homeologous chromosome Vs share about 70% sequence homology and form crossovers in less than 3% of meiosis forcing the segregation of these chromosomes to rely on distributive segregation (Kemp et al. 2004). Wildtype, *ndj1* mutant, *tid1* mutant, and *spo16* mutant homologous and homeologous strains were constructed.

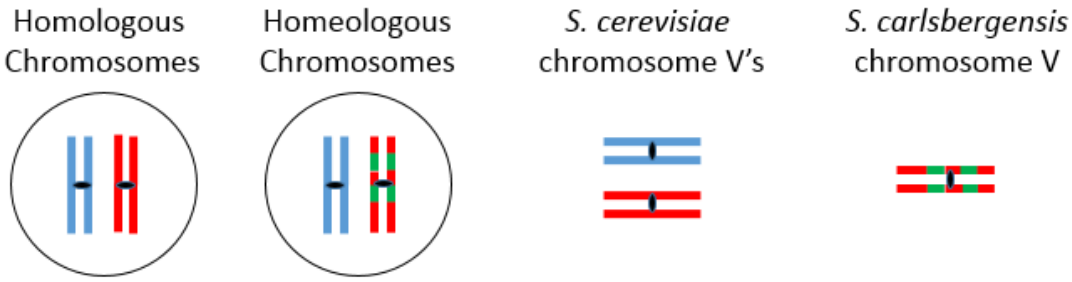

Figure 14 - A pair of homologous chromosomes V's and a pair of homeologous chromosomes V's. The homologous chromosome pair is shown in red and blue. The addition of the green color in the *S. carlsbergensis* chromosome V is indicating sequence divergence between the homeologous chromosome pair.

After strain construction was complete, data was collected from each strain in order to determine if the candidate proteins Ndj1, Tid1, and Spo16 had a role in distributive segregation. Individual spores were set out in a regular pattern from each tetrad, and spore viability was counted by looking at colony formation on each plate (Figure 15).

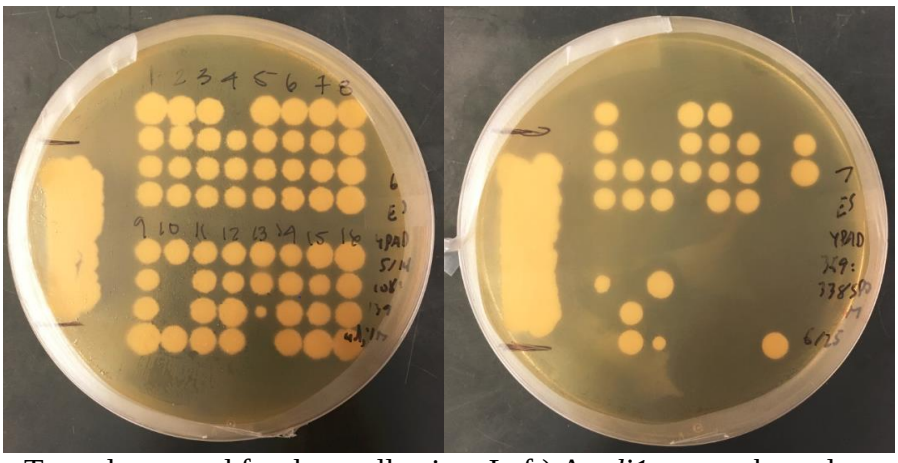

Figure 15 - Two plates used for data collection. Left) A *ndj1* mutant homologous strain of yeast. Right) A *spo16* mutant homeologous strain of yeast.

Before the data could be analyzed, markers were checked on each colony to make sure that they came from spores from a tetrad. The mating type and at least one nutritional marker were checked for each colony while looking for 2:2 segregation in 4-spore-viable tetrads. However, gene conversion events could cause 3 or 4 colonies to grow on an amino acid dropout plate or mating type test plate. After data analysis, strains could then be assessed for increased levels of nondisjunction in meiosis I. Since incidences of nondisjunction cause an increase of 2-spore-viable tetrads or 0-spore-viable tetrads these were the data that were primarily focused on.

3.2 Wildtype strains show high levels of spore viability

The wildtype homologous strain contained 88% 4-spore-viable tetrads, and the wildtype homeologous strain contained 63% 4-spore-viable tetrads. (Figure 16) Additionally, the wildtype homologous strain only had 7% 3-spore-viable tetrads and 3% 2-spore-viable tetrads. However, the wildtype homeologous strain had a 1.5 fold increase in 3-spore-viable tetrads and a 6 fold increase in 2-spore-viable tetrads. The increase in 2-spore-viable tetrads in the homeologous strain is indicative of meiosis I nondisjunction. There was a significant difference in the spore viability patterns between the homologous and homeologous strains (Figure 16).

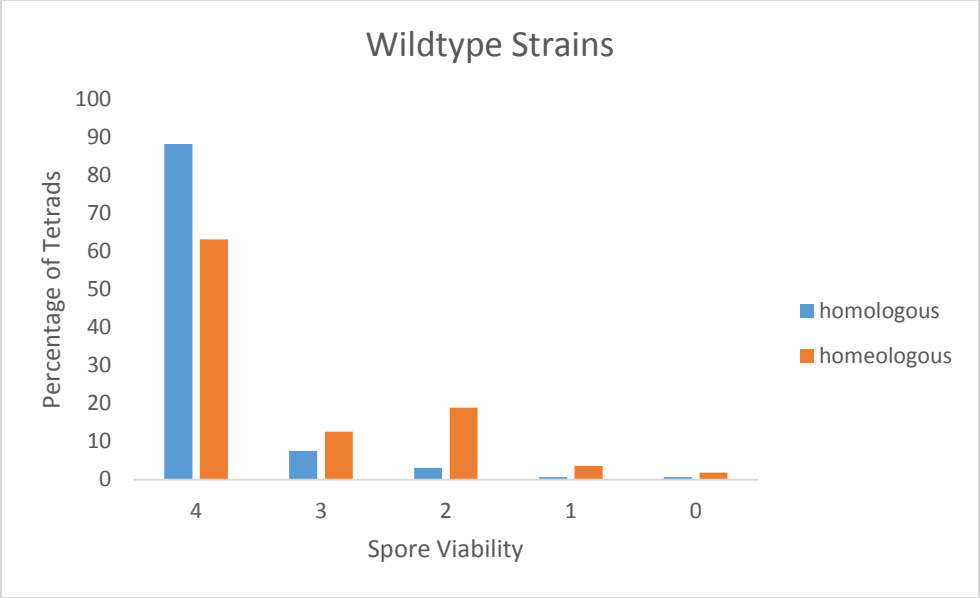

Figure 16 - The wildtype homologous strain and homeologous strain spore viability data (chi-squared; $\chi 2=28.08$, p-value<.001, df=4)

3.3 Homeologous *ndj1* mutant strain shows significantly more meiosis I nondisjunction than the homologous *ndj1* mutant strain

Both *ndj1* mutant strains had lower overall spore viability than their wildtype counterparts (Table 11). When compared with each other, the two *ndj1* mutant strains have spore viability patterns that are significantly different (Figure 17). There were 70% 4-spore-viable tetrads in the *ndj1* homolog strain and 23% 4-spore-viable tetrads in the *ndj1* homeolog strain. There was a large drop in 4-spore-viable tetrads in the *ndj1* mutant homeolog strain, and a corresponding increase in both 2-spore-viable tetrads and 0-spore-viable tetrads in the *ndj1* homeologous strain. There was a 3 fold increase in 2-spore-viable tetrads and a 4.5 fold increase in 0-spore-viable tetrads. This increase in 2- and 0-spore-viable tetrads suggests a much higher level of meiosis I nondisjunction occurring in the *ndj1* homeolog strain than in the *ndj1* homolog strain.

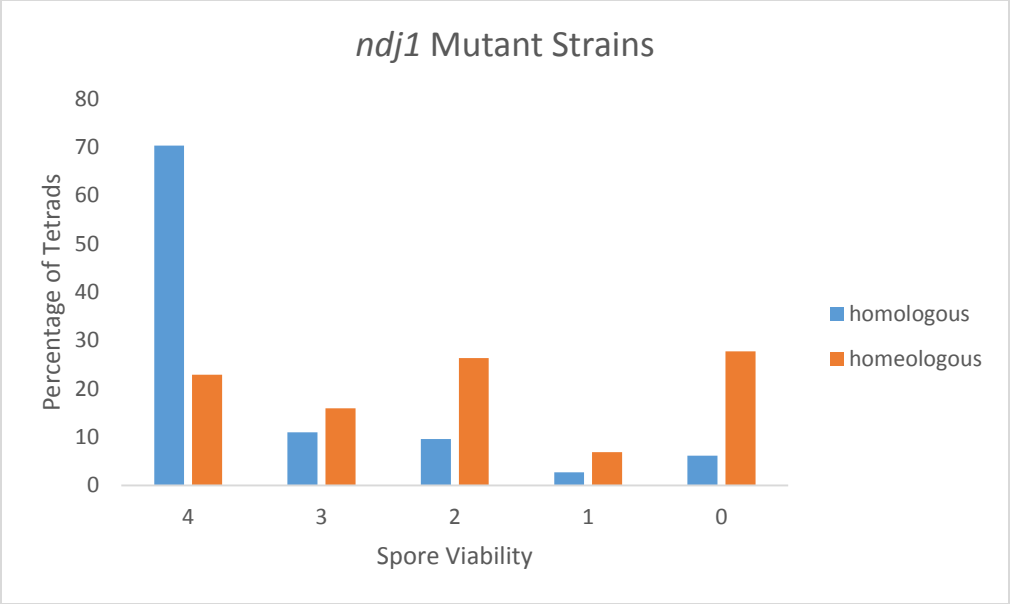

Figure 17 - The *ndj1* mutant homologous strain and homeologous strain spore viability data (chi-squared; $\chi 2=69.78$, p-value<.001, df=4)

3.4 Homologous and homeologous *tid1* mutant strains show a similar spore viability pattern

In comparison to their wildtype counterparts, both the homologous and homeologous *tid1* mutant strains had a decrease in 4-spore-viable tetrads and an increase in 3-, 2-, 1-, and 0-spore-viable tetrads (Table 11). Both of the *tid1* mutant strains had an increase from 3-spore-viable tetrads to 2-spore-viable tetrads. The homologous *tid1* mutant had 17% 3-spore-viable tetrads and 30% 2-spore-viable tetrads, and the homeologous *tid1* mutant contained 22.7% 3-spore-viable tetrads and 23.4% 2-spore-viable tetrads. Meiosis I nondisjunction seems to be occurring in both strains of *tid1* mutants with no additional increase of nondisjunction occurring in the homeolog. Although there seem to be some visible differences of spore viabilities between the two *tid1* mutant strains, there was no significant difference between the spore viability patterns (Figure 18).

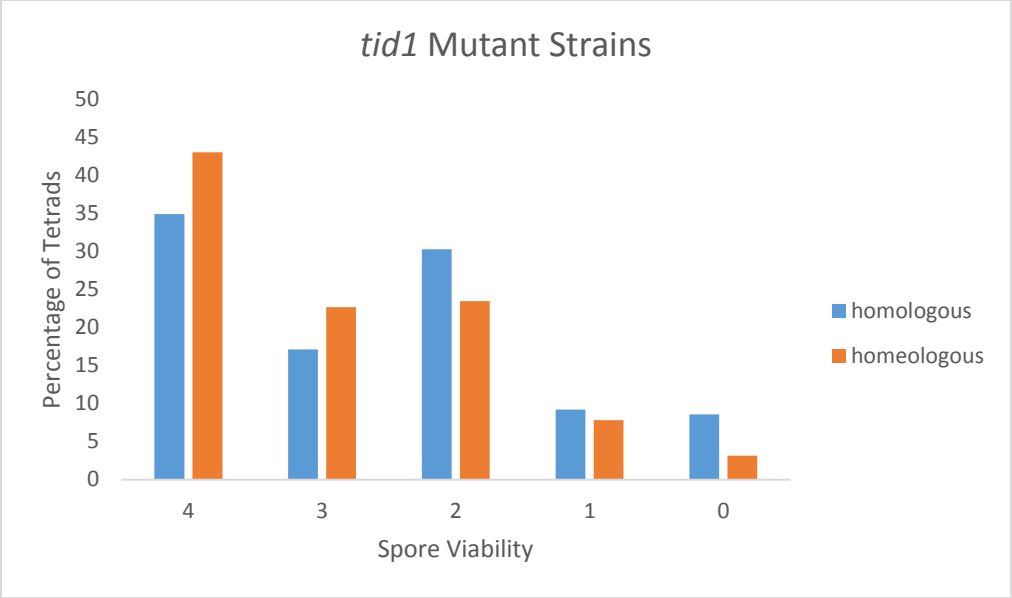

Figure 18 - The *tid1* mutant homologous strain and homeologous strain spore viability data (chi-squared; $\chi 2=6.99$, p-value=.136, df=4)

3.5 Both *spo16* mutant strains show meiosis I nondisjunction

When comparing all of the homologous strains the *spo16* mutant homologous strain has the lowest overall spore viability at 51% (Table 11). Additionally, the *spo16* mutant homeologous strain has the lowest spore viability in comparison to every other homeologous strain investigated at 39% (Table 11). The homologous *spo16* mutant had 37% 0-spore-viable tetrads, which is at least a 3 fold increase in 0-spore-viable tetrads in comparison with any other homologous strain (Table 11). This indicates that yeast missing Spo16 have the most difficulty properly segregating chromosomes during meiosis I. There is a significant difference when comparing the spore viability patterns of the two *spo16* mutants (Figure 19). The two strains have almost identical 0-spore-viable tetrads, but the *spo16* mutant homeolog has a 2.5 fold increase of 2-spore-viable tetrads. This indicates that the *spo16* mutant homeolog has even more meiosis I nondisjunction occurring than the homolog.

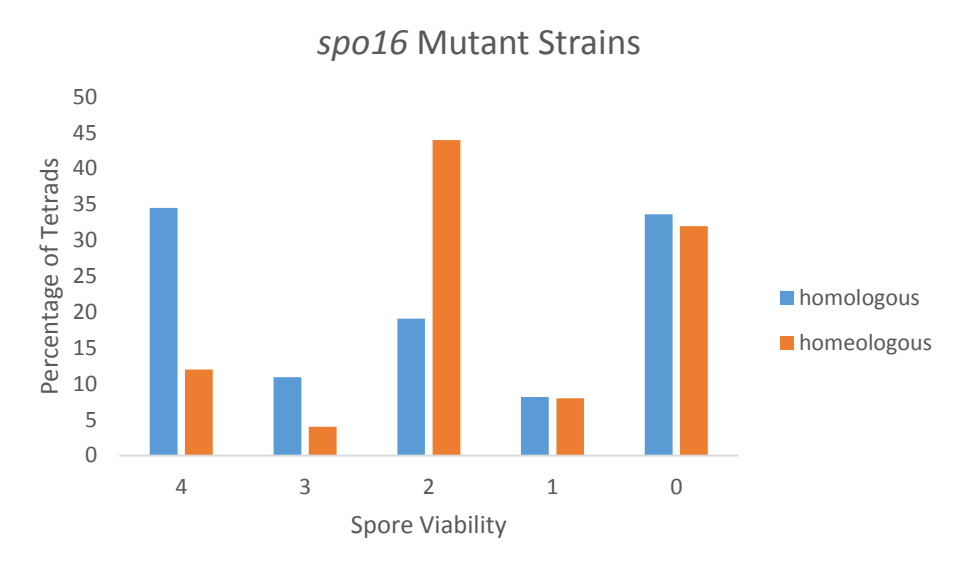

Figure 17 - The *spo16* mutant homologous strain and homeologous strain spore viability data (chi-squared; $\chi 2$=10.08, p-value=0.039, df=4)

Table 11 - Spore viability data.

Strain	n	4 s.v. # (%)	3 s.v. # (%)	2 s.v. # (%)	1 s.v. # (%)	0 s.v. # (%)	Overall s.v. %
WT homologous	160	141 (88.1)	12 (7.5)	5 (3.1)	1 (0.6)	1 (0.6)	95.5
WT homeologous	111	70 (63.1)	14 (12.6)	21 (18.9)	4 (3.6)	2 (1.8)	82.9
tid1 homologous	152	53 (34.9)	26 (17.1)	46 (30.3)	14 (9.2)	13 (8.6)	65.1
tid1 homeologous	128	55 (43.0)	29 (22.7)	30 (23.4)	10 (7.8)	4 (3.1)	73.6
ndj1 homologous	145	102 (70.3)	16 (11.0)	14 (9.7)	4 (2.8)	9 (6.2)	84.1
ndj1 homeologous	144	33 (22.9)	23 (16.0)	38 (26.4)	10 (6.9)	40 (27.8)	49.8
spo16 homologous	117	38 (34.5)	12 (10.9)	21 (19.1)	9 (8.2)	37 (33.6)	51.1
spo16 homeologous	25	3 (12.0)	1 (0.04)	11 (44.0)	2 (0.08)	8 (32.0)	39.0

4. Discussion

The wildtype homologous strain of yeast seems to undergo meiosis efficiently with about 90% 4-spore-viable tetrads and only a small amount of spore death. The homeologous wildtype strain shows chromosome segregation problems in meiosis I with a slight increase of 2-spore-viable tetrads in comparison to the homologous wildtype strain. This was expected because proper segregation of chromosome V is reliant on distributive segregation in the homeologous strain, and distributive segregation is less efficient than crossing over in ensuring proper segregation of chromosomes in meiosis I. If chromosome V was segregating randomly in meiosis I with no other factors being involved, we expect 50% 4-spore-viable tetrads and 50% 2-spore-viable tetrads. However, this is not the case with 63% 4-spore-viable tetrads and 19% 2-spore-viable tetrads observed in the wildtype homeologous strain. These data show that distributive segregation is functioning as there is nonrandom segregation of chromosomes in meiosis I occurring about 68.4% of the time in the homeologous strain.

The spore viability of *tid1* mutant strains has been previously studied, but varying results have failed to unambiguously define the role of Tid1 in segregation. One study using yeast in the SK1 background showed that spore viability was only slightly reduced in *tid1* mutants with an 82% overall viability (Shinohara et al. 1997). In that study the percentage of tetrads dropped with the number of viable spores, so the highest percentage of tetrads were 4-spore-viable and the lowest percentage were 0-spore-viable (Shinohara et al. 1997). Another study showed a lower overall spore viability with an equal distribution between 4-, 3-, 2-, 1-, and 0-spore-viable tetrads using the W303 yeast background (Klein 1997). Our data, which was collected using yeast in the SK1

background, seem to lie between these two previous studies. The *tid1* mutant homologous strain shows a decreasing percentage of tetrads from 4- to 0-spore-viable with a spike in 2-spore-viable tetrads.

The previous studies combined with our data show that when Tid1 is missing spore viability decreases. Our data also indicate that Tid1 has a potential role in the segregation of chromosomes during meiosis I, which is not apparent in the other studies. Additionally, when comparing the homeologous *tid1* mutant data with the homologous *tid1* mutant data there is not a significant difference in the pattern of spore viability. Since the data suggests that there is no more nondisjunction occurring in the homeolog mutant than the homolog mutant it can be concluded that Tid1 does not play a role in distributive segregation.

Ndj1 has also been examined in other studies. It has been shown that in *ndj1* mutants there is reduced spore viability (Chua and Roeder 1997). Specifically, this is due to a significant increase in meiosis I nondisjunction and precocious separation of sister chromatids (Chua and Roeder 1997). Our data show only a slight increase in 3 spore and 2-spore-viable tetrads in the *ndj1* mutant which only marginally supports the Chua and Roeder findings. Additionally, Chua and Roeder checked if Ndj1 has a role in distributive segregation by using two sets of yeast artificial chromosomes- a homologous pair and a nonhomologous pair. They found a significant increase in nondisjunction in both pairs (in the homologous pair only nonrecombinant YACs were used) (Chua and Roeder 1997). Our study looked at a homeologous pair of chromosomes which also showed a significant increase in nondisjunction. In the *ndj1* homeologous strain there was a large increase in both 2-spore and 0-spore-viable tetrads in comparison with the homologous mutant strain

making these spore viability patterns significantly different. These data in conjunction with the Chua and Roeder study confirm that Ndj1 has a role in distributive segregation.

Spo16 has been shown to have a role in meiosis. It was observed in the Shinohara lab that *spo16* mutants have a temperature dependent defect in sporulation leading to increased sporulation at 23°C compared to 30°C, and overall spore viability was lower than at 33.1% (Shinohara et al. 2008). Tetrads with 4, 2 and 0 viable spores were most common in the mutant from the Shinohara data (Shinohara et al. 2008). Our data were collected at 30°C in order to control for temperature-dependent effects on yeast meiosis between our different strains. At 30°C sporulation still occurred but at a lower rate than at 23°C. Our data from the *spo16* mutant homologous strain were consistent with Shinohara's data with mostly 4-, 2-, and 0-spore-viable tetrads indicating that homolog nondisjunction may be the main source of spore death in *spo16* mutants.

Collecting data on the *spo16* homeolgous strain was extremely difficult. The sporulation percentage was so low that it was difficult to collect and dissect a sufficient number of tetrads for a statistically significant study. Throughout the study the homeologous strains seemed to have a lower sporulation rate than the homologous strains presumably due to the presence of the *S. carlsbergensis* chromosome V. Additionally, the *spo16* homologous mutant already had a low sporulation rate. Without Spo16 there are less crossovers forming and the synaptonemal complex between homologs is incomplete compounding problems during meiosis. It is also possible that the unusually low sporulation percentage in the *spo16* mutant homeologous strain was due to a mutation in one of the haploid parent genomes.

There was a significant difference between the spore viability patterns of the homeologous *spo16* mutant and the homologous *spo16* mutant. The homeologous *spo16* mutant had almost no 4-spore-viable tetrads, a 2.5 fold increase in 2-spore-viable tetrads, and no significant change in 0-spore-viable tetrads. One possible explanation for the lack of an increase in 0-spore-viable tetrads in the *spo16* homeolog strain is that the 0-spore-viable tetrads in the *spo16* homolog strain are occurring from a meiotic problem other than nondisjunction in meiosis I. Then when the homeologous pair of chromosomes is added, nondisjunction is occurring more frequently causing an increase in 2-spore-viable tetrads which could result from a defective distributive segregation mechanism. However, because only 25 tetrads were dissected in the homeolog this is not conclusive evidence of Spo16 having role in distributive segregation. More tetrads need to be dissected in order to have a more convincing sample size. *spo16* mutants are temperature sensitive, so it might be necessary in the future to incubate the *spo16* homeolog mutants at 23°C in order to collect more tetrads.

The three candidate proteins Tid1, Ndj1, and Spo16 were all tested to see if they had a role in distributive segregation. Our data show no evidence that Tid1 affects the success of distributive segregation while Ndj1 is necessary for a functioning distributive segregation mechanism. We lack sufficient evidence to determine if Spo16 directly affects distributive segregation because of the small sample size. These results align with the proposed mechanism for distributive segregation by Dawson et al. (2004). It was suggested that yeast centromeres cluster in the beginning of meiosis and, once homologs begin to synapse, their centromeres are pulled away from the initial cluster (Kemp et al. 2004). If there is one pair of chromosomes that cannot synapse, such as the chromosome

V homeologs in this study, they are the last centromeres left in the cluster and become default partners (Kemp et al. 2004). The sequence independent centromere pairing helps to orient the kinetochores so that they are likely to encounter microtubules from opposite poles (Kemp et al. 2004).

Ndj1 could have a direct effect on this process because it has been proposed that clustering of telomeres by Ndj1 during the beginning of meiosis helps in the initial chromosome pairing which could in turn affect centromere clustering. The bouquet formation and centromere pairing occur at the same time during meiosis. Furthermore, without Spo16 homologous chromosome pairs cannot fully synapse. This can cause faulty segregation of homologous chromosomes as seen in the spore viability data. It is plausible that if the homologous chromosomes do not fully synapse then the transition between centromere pairing between non-homologous chromosomes to centromere pairing between homologs would be hindered causing a faulty distributive segregation. However, Tid1's primary function of Dmc1 and Rad51 recruitment to DSBs would not likely affect centromere pairing and therefore have no effect on distributive segregation. Our study assisted in elucidating the roles of Ndj1, Spo16, and Tid1 in distributive segregation and helped support the proposed hypothesis that centromere pairing initiates the proper segregation of achiasmate chromosomes.

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