Investigating the Roles of NDJ1 and TID1 in Distributive Segregation Using Non-Exchange Chromosomes

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ABSTRACT

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Segregation Using Non-Exchange Chromosomes

Jonathan V. Henzel

Meiosis is a specialized cell division that leads to a reduction of ploidy in sexually reproducing organisms through segregation of homologous chromosomes at the first meiotic division. Improper segregation of chromosomes during meiosis results in anueploidy, which is usually fatal during embryonic development. The meiotic process is therefore tightly regulated. Typically, proper segregation of homologs at meiosis I requires pairing of homologous chromosomes, followed by crossover recombination between homologs. Crossovers enable proper chromosomal segregation during the first meiotic division in part by establishing tension in the meiotic spindle. However, in the absence of crossovers, some cells maintain the ability to direct homologous chromosomes to opposite spindle poles, through a poorly understood mechanism known as distributive segregation.

We are using the common brewers yeast *Saccharomyces cerevisiae* to determine possible roles of two genes in distributive segregation. The genes of interest, Ndj1 and Tid1, have been previously demonstrated to play a role in crossover interference, but their roles in distributive segregation are not well understood. Ndj1 has been shown to function in the tethering of telomeres to the nuclear envelope and may aid in the homology search chromosomes undergo. Tid1 has been characterized as a recombination accessory factor and may stimulate crossovers by directing recombinases to double strand break sites early in meiosis. To assay distributive segregation, we use yeast in which crossing over between one chromosome pair is prevented (due to sequence divergence). Using this system, we can assay the ability of yeast to carry out distributive segregation. Our results indicate that mutations in Ndj1 impair the ability of yeast to carry out distributive segregation, while mutations in Tid1 do not affect distributive segregation. These results, in turn, suggest that Ndj1 may play a role in distributive segregation. This experiment is part of a larger question to determine whether crossover assurance and crossover interference are independent mechanisms.

Keywords: meiosis, distributive segregation, nondisjunction, Ndj1, Tid1, yeast.

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Part I – A Review of Meiosis

1. Introduction

Common brewers yeast, *Saccharomyces cerevisiae*, is a model system which shares many cellular mechanisms with higher eukaryotes. *S. cerevisiae* are equally capable of a normal life cycle in both a haploid and diploid state, making them highly amenable to genetically based experiments. As most higher eukaryotes reproduce sexually, S. cerevisiae provides a unique platform for investigating the mechanisms of meiosis that result in haploid derivatives of diploid cells. In yeast, meiosis reduces a single diploid cell to produce four haploid spores, each of which is capable of life.

Yeast use meiosis, or sporulation, as a mechanism for long-term survival as a result of dietary deficiency or desiccation. While yeast, in general, provide an excellent model to study meiosis, there are phenotypic differences found in different strain isolates that present real differences in each strain's ability to carry out meiosis. Phenotypic differences among *S. cerevisiae* isolates range from delays in hitting meiotic checkpoints to chromosome missegregation to failure to form an ascus which contains the four spores produced by meiosis. If an ascus contains less than four spores which are capable of producing colonies, it can be inferred that meiosis did not carry through faithfully. Analysis of these deficient asci can then be used to speculate the likely causes of meiotic failure.

Here, we look at the ability of three different yeast isolates to segregate chromosomes that fail to form a crossover between chromosomes. The first chapter will provide an overview of the meiotic process and establish the importance of crossovers, which are essential for proper disjunction in meiosis I. The second chapter will discuss

the two proteins examined in this study, Ndj1p and Tid1p. The third chapter will provide our rationale for the experiment, including the results and a discussion involving the interpretation of those results.

2. Meiosis

Meiosis is a highly specialized form of cell division in sexually reproducing organisms that creates haploid gametes from diploid cells. The process of meiosis requires a dramatic reprogramming of nuclear organization in order to produce gametes containing only half of the genome. A new diploid individual is conceived from the fusion of two haploid gametes. In most sexually reproducing organisms, the new individual is formed through the inheritance of a full haploid set derived from each parent. Central to sexual reproduction in eukaryotes is the process of meiosis, a specialized form of cell division which generates unique haploid gametes from diploid parents. Sexual reproduction is a fundamental process through which most eukaryotic organisms generate new offspring. Unsurprisingly, this process is highly conserved among eukaryotes. As such, it is possible to use model organisms such as the brewers yeast Saccharomyces cerevisiae to gain insight into the complexities of an important process that is relatively poorly understood.

Meiosis begins with the duplication of the genomic content during S phase of the cell cycle producing pairs of identical discrete DNA molecules that remain physically connected at the centromere. These pairs are referred to as sister chromatids and each pair constitutes a single chromosome. Each chromosome has a homologous partner with which it interacts during meiosis. Homologs are architecturally identical chromosomes

that may or may not contain allelic differences with their respective counterparts. The nucleus of each gamete contains a haploid set of chromosomes. Upon fusion, the ploidy level is restored and a new, diploid organism is formed.

Meiosis is distinct from mitosis in that the process produces four unique daughter cells, rather than two identical cells seen in mitosis. Meiosis also differs from mitosis in that the nucleus of each new gamete produced during meiosis contains novel genotypes in the form of recombinant chromosomes. The creation of new, recombinant chromosomes results from crossing over between homologous chromosomes through the repair of programmed double strand breaks (DSBs) and is paramount to meiosis.

2.2 Stages of meiosis

Meiosis consists of two rounds of cell division: a reductional division, meiosis I (MI), in which homologous chromosomes disjoin from each other, and an equatorial division, meiosis II (MII), as sister chromatids segregate to opposite spindle poles. While meiosis II largely mimics the behavior of a mitotic cell, meiosis I is where the critical step of segregating homologous chromosomes occurs. MI is known as the reductional division because the ploidy level of the cell is reduced from diploid to haploid. Meiotic cells have developed a comprehensive program to encourage proper segregation so that each daughter cell has a complete haploid set of chromosomes. In order for the cell to maximize the likelihood of proper disjunction in MI each chromosome must establish a crossover with its homologous partner. In the absence of crossing over the incidence of missegregation of chromosomes (nondisjunction) during MI increases.

The two rounds of meiosis, like mitosis, are traditionally broken up into discrete phases based upon observational changes in appearance: prophase, metaphase, anaphase, and telophase with a final cleavage stage, cytokinesis, separating the newly formed cells from one another. Prophase I is most mechanistically distinct from its meiosis II and mitosis counterparts - in fact it can occupy almost 90% of the time of meiosis and its duration is longer than all other meiotic phases combined (Alberts et al.., 2002). It is during this stage in which crossovers are initiated and physical connections between homologous chromosomes are established. Prophase I proceeds into metaphase I as chromosomes congregate along the metaphase plate. Metaphase I gives way to anaphase I as homologous chromosomes segregate away from one another towards opposite poles established by the spindle pole bodies (SPB). The SPB is the microtubule organizing center in yeast and is functionally similar to the centrosome in other eukaryotes. Telophase I and cytokinesis occur concurrently as the nuclear envelope begins to reform, chromosomes assume a more relaxed state and the division of cytoplasm is completed as the cleavage furrow begins to pinch apart the daughter cells.

MII largely resembles the mitotic phases in that the new cells are formed after sister chromatids segregate away from one another. Prophase II establishes bipolar spindle attachment of the sister chromatids. Metaphase II involves the congregation of sisters along the metaphase plate before separation of the sisters to opposite poles at anaphase II. Similar to the first round, telophase II and cytokinesis occur concurrently, resulting in a total of four haploid daughter cells commonly referred to as gametes. In budding yeast, the final products of meiosis are four individual haploid spores that are

packaged into a protective sack known as an ascus. Each spore is capable of producing a colony. Collectively, the four spores are referred to as tetrads.

2.3 Meiosis requires crossovers

During meiosis I, critical events take place which contribute to successful homologous segregation. Most of the actions, such as pairing and crossing over between homologous chromosomes, have been demonstrated to be necessary for normal meiosis in numerous organisms (Baker et al.., 1976). These events also include chromatin restructuring, the search for and pairing of homologous chromosomes, initiation of recombination between the paired homologs and formation of the synaptonemal complex (SC), a ribbon-like tripartite proteinaceous structure that assembles between paired homologs and is thought to have a role in the formation of crossovers (Zickler and Kleckner, 1998). All of these events occur during prophase I. Reflecting the multitude of steps during prophase I, this stage has been further divided into temporal substages relating to the visual changes associated with the chromatin.

Leptotene is the first recognized substage of Pro I and is associated with the formation of condensed chromosomes that are noticeably separate (Zickler and Kleckner, 1998). During leptotene, the chromosomes are arranged in the characteristic Rabl orientation with centromeres clustered at the nuclear periphery with telomeres extended towards the nuclear center (Burgess, 2002). The transition into zygotene is discernible as the chromosomes undergo a coordinated movement resulting in a “bouquet” configuration with telomeres tightly clustered on the nuclear envelope near the spindle pole body (SPB) (Wanat et al.., 2008) (Figure 2). Zygotene begins with the assembly of

axial structural proteins along the length of the paired homologs and transitions into pachytene when the SC is fully formed and homologs are synapsed (Bascom-Slack et al.., 1997). Both zygotene and pachytene are of particular interest as telomere-mediated chromosome movement is persistent throughout (Wanat et al.., 2008). Diplotene is marked by desynapsis between homologs as the SC components disassemble (Lynn et al.., 2007) It is at this point at which chiasmata, discrete physical connections between two non-sister chromatids, can first be visualized (Bascom-Slack et al.., 1997).

Chiasmata was the term first applied to physical manifestation of crossovers when described by Frans Alfons Janssens in 1909 because of the cross-like appearance between recombinant chromatid arms. Diakinesis is a transition stage in which the cell prepares to line up the chromosomes in preparation for metaphase. In many animals, diplotene and diakinesis may be indistinguishable from one another (Alberts et al.., 2002). Crossovers are a hallmark of meiosis and play an important role in meiosis by establishing tension on the meiotic spindle (Nicklas, 1974). When homologous chromosomes are engaged in a crossover, they form an eight-armed structure known as a bivalent. Though the reason for crossing over can only be theorized, Bruce Nicklas (Nicklas, 1974) proposed a simple mechanical model for the CO requirement during meiosis. Through observations made during micromanipulation assays using grasshopper gonads, he saw that when the two kinetochores associated with a bivalent capture microtubules from opposite poles it is the chiasmata that keep the homologs from segregating prematurely. The resulting tension created from opposite pole forces directs the bivalent to the metaphase plate.

Crossovers that form between homologous chromosomes prevent their early separation resulting from kinetochore attachment to microtubules radiating from the SPB (Nicklas, 1974). The kinetochore is a protein complex which assembles at the centromere. It is this protein structure which facilitates microtubule attachment emanating from the spindle pole body. The kinetochore protein structure simultaneously binds to both microtubules and the DNA via specific histone protein variants. The attachment of microtubules to the kinetochore allows the SPB to direct chromosomes attached to the spindle via a microtubule-dependent poleward force (Theurkauf and Hawley, 1992).

2.4 Crossover Regulation

In studies concerning crossovers during meiosis, two distinct forms of crossover regulation have been identified; crossover assurance and crossover interference. Crossover assurance is an observed phenomenon in which the reciprocal DNA exchange between homologs is tightly regulated so that the cell ensures that each pair of chromosomes receives at least one crossover (Merriam and Frost, 1964). Crossover interference has been proposed from observations that have shown that crossovers are not randomly distributed along chromosomes; there is an uncharacterized mechanism that discourages crossovers from occurring within a certain proximity of an established exchange by reducing the frequency of nearby crossovers (Sturtevant, 1915). Although these two forms of crossover regulation, crossover assurance and interference, have been observed in a variety of sexually reproducing eukaryotes it is not known if these are

unrelated processes, or if they are mechanistically linked in order to promote proper chromosome disjunction.

Crossover assurance has been observed in S.cerevisiae. Despite the low overall number of crossovers, around twenty in S. cerevisiae, there is some mechanism that works to ensure each chromosome pair receives at least one crossover in yeast (Bascom-Slack et al.., 1997). If crossing over were a stochastic process, occasionally the low number of crossovers would result in chromosomes that fail to receive a single crossover. The term 'obligatory crossover' has been coined to explain the controlled formation of crossovers so that even the smallest chromosomes receive at least one crossover. This suggests that crossovers are critical to ensure proper disjunction of homologs in many organisms.

Crossover interference has been inferred to occur from observations made in both genetic and cytological studies (Zickler and Kleckner, 1998). It acts to alter the distribution of crossovers so that the probability of a crossover occurring close to another one is lower than would be expected if crossovers were resolved independently of each other. Although the exact mechanism of interference is not yet known, there are several proposed models to explain it. Although the models differ, there are three essential parameters the models account for. First, there is some signal generated by the crossover event or by some type of pre-crossover intermediate to alert the machinery that recombination is occurring at a particular locus. There must also be some type of signal amplification so that the signal can spread to other DSB repair sites. Finally, the signal must trigger effectors that prevent DSBs from being repaired as crossovers (Shinohara et al.., 2003).

2.5 Crossovers are essential for chromosome segregation during meiosis

Crossing over has been identified as an important step in ensuring normal segregation of homologs during meiosis. For example, yeast mutants that are defective in some part of the crossover pathway often show increased levels of nondisjunction at MI (Cromie et al., 2006). As homologous chromosomes pair and become juxtaposed, the formation of a series of programmed DSBs throughout the genome are catalyzed by Spo11p, a topoisomerase-like enzyme able to cut dsDNA (Keeney et al., 1997). In *S. cerevisiae*, it has been reported that approximately 175-260 DSBs are created throughout the entire genome, with a subset of these being repaired as crossovers (Peoples-Holst and Burgess, 2005). There is evidence that pairing precedes DSB formation - DSBs are observed less frequently in chromosomes that contain heterologies or lack a homologous partner altogether (Bascom-Slack et al., 1997). The meiotic chromatin architecture as well as meiosis-specific DNA repair pathways encourage the use of homologous chromosomes as templates to repair programmed DSBs. This is different from mitosis where sister chromatid mediated repair is preferred. While a subset of these DSBs result in a reciprocal exchange of DNA, the majority of DSBs will be repaired via the noncrossovers (NCO) pathway, which includes gene conversion events (Peoples-Holst and Burgess, 2005) (Figure 3). There is still some debate concerning how the cell determines which DSB repair sites undergo homologous exchange producing a crossover and which do not.

As MI progresses, only DSB repair sites which form a crossover will appear as chiasmata, an event that is seen in approximately 30-50% of all programmed DSBs in budding yeast (Whitby, 2005). In the subset of DSBs that result in a crossover, the

ssDNA is used to search for homologous sequences which results in single end strand invasion (SEI) forming transient stretches of heteroduplex DNA. As this interaction matures, the DNA molecules form a D-loop structure, a predicted intermediate in the Szostak model (Shinohara et al., 2003) (Figure 3). While the SEI event occurs regardless of whether a crossover will form or not, the formation of D-loop intermediates is associated with a majority of crossovers and is reliant on a class of proteins collectively known as the ZMM proteins. ZMM proteins include Zip1, Zip2, Zip3, Zip4, Mer3, and the MutS homologs Msh4 and Msh5. These proteins are highly conserved in sexually reproducing eukaryotes and complete synapsis is not achieved in budding yeast if any one of the ZMM proteins is absent (Lynn et al., 2007). *zmm* mutants also show a two- to three-fold reduction in the formation of crossovers compared to non-mutant, as well as increased levels of aneuploidy (Peoples-Holst and Burgess, 2005). Thus, *zmm* mutants provide additional anecdotal evidence that failure to properly regulate crossover formation is a factor in nondisjunction.

The programmed actions which occur prior to crossover formation between homologs are critical to ensure proper segregation. First, in order for an exchange to occur, homologs must recognize one another by proximally arranging themselves. This may occur prior to meiosis as is evidenced by cytological observations; chromosomes routinely adopt a position in which the centromeres are anchored near the SPB, an orientation named after Carl Rabl who first described the phenomenon in amphibian cells (Rabl, 1885). It is possible that the Rabl orientation promotes homologous pairing; by fixing the centromeres in location on the nuclear envelope, the physical location of allelic sequences on homologs will be equidistant from the nuclear envelope (Dresser, 2008).

Homolog pairing may be further facilitated by specific telomere associations as telomeres cluster on the nuclear envelope as seen in the 'bouquet' configuration that first forms in leptotene (Zickler and Kleckner, 1998). Interestingly, this configuration has been observed in meiotic cells of various taxa, including plants, fungi and animals, perhaps underscoring the importance of telomere-directed interactions (Burgess, 2002). Indeed, cells that contain two linear homologs pair at a higher level than cells that contain one linear and one circular homolog in which the telomeric sequences were deleted (Chua and Roeder, 1997). To date, the only meiotic gene product that has been demonstrated to be required for bouquet formation is Ndj1p (Conrad et al., 1997).

2.6 Models of Double Strand Break Repair

According to the Szostak model, a classic model for recombination, DSBs are repaired via a single pathway. The model proposes that repair of DSBs either lead to crossovers in which homologs are reciprocally broken and rejoined, or NCOs such that most of the chromosome retains the original linkage (Szostak et al., 1983). Indeed, the two pathways can be observed genetically as either crossovers or gene conversion events in which one homolog repairs the DSB using the other homolog as a template without reciprocal exchange between the two homologous duplexes (Cromie et al., 2006). The DSB repair pathway is initiated after Spo11p activity by swift resectioning of the exposed 5' ends, revealing 3' overhangs around 600 nucleotides long (Sun et al., 1989). Following DNA resectioning, the Szostak model predicts one of the two processed ends will invade a paired homolog forming a displacement loop (D-loop) structure, temporarily forming a three-stranded DNA structure (Nimonkar et al., 2007). The other

processed end then anneals to the D-loop and the gaps are repaired via DNA synthesis using the invading strand as a template. These invading strands and subsequent DNA synthesis results in a stable four-strand intermediate held together by base pairing between homologs, forming a double Holliday junction structure (dHJ) in budding yeast. Cleavage of these junctions will either result in a crossover or NCO product, depending on the orientation of the cleavage event.

The Szostak model assumes the decision of whether to form a crossover or NCO is made late in the process, after repair has been initiated. Despite indirect cytological evidence supporting this model, recent observations show it to be relevant in the formation of crossovers but not in the NCO pathway (Cromie and Smith, 2007). Further disputing the Szostak model, evidence suggests the decision to form a crossover is made prior to strand invasion, likely during exit from zygotene (Zickler and Kleckner, 1998). The temporal and numerical formation of nodules at DSB sites as described by Carpenter (1994) can predict NCO destined DSB sites from ones that will be resolved as a crossover. As a result, a new model has been proposed, the synthesis-dependent strand annealing (SDSA) model, to describe the NCO pathway; shortly after the initial ssDNA strand invasion creating the D-loop, the invading ssDNA pulls out of the duplex after a brief spurt of synthesis and prior to dHJ formation (Nassif et al., 1994) (Figure 3). Further synthesis to close the remaining gaps take place using the sister chromatid template. The majority of DSBs will be repaired via the NCO pathway which are manifested as gene conversion events (Cromie and Smith, 2007).

2.6 Alternative segregation strategies

While crossing over has been shown to be important for proper segregation in the majority of organisms whose meiotic programs have been described, it is not an unconditional requirement. Proper segregation of non-exchange chromosomes at high frequencies has been observed in several species, including mantids, grasshoppers and potato bugs (Hughes-Schrader, 1969; Ault, 1986; Nokkola, 1986). The most notable research on chromosome behavior has been done in the fruit fly Drosophilamelanogaster in which it was first observed that every chromosome did not achieve a crossover (Cooper, 1948). Despite the apparent importance of the crossover in ensuring proper segregation, an alternative mechanism known as distributive segregation was proposed that functions to segregate non-exchange chromosomes (Grell, 1962). In meiotic Drosophilaoocytes the smallest chromosome (IV) never forms a crossover, yet achieves proper disjunction with remarkable efficiency (Hawley and Theurkauf, 1993). Cytological evidence of meiotic oocytes confirms that the homologous IV chromosomes are not physically joined at any point during MI (Puro and Nokkala, 1977). Also in Drosophilafemales, the X chromosomes fail to recombine in a full 5% of meioses, yet achieve proper segregation during MI 99% of the time (Carpenter, 1991). The other two autosomes also fail to recombine occasionally, as is the case with virtually all eukaryotes (Loidl et al., 1994).

As the system is so robust, it is no surprise that a gene locus, nod(no distributive disjunction), has been identified to function solely in Drosophilafemales during meiosis and is absolutely required for efficient segregation of non-exchange (E_0) chromosomes (Carpenter, 1973). NOD encodes for a kinesin-like protein which is believed to provide

an anti-poleward force to keep non-exchange chromosomes in the proximity of the chromosomal mass just after nuclear envelope breakdown and microtubule attachment (Theukauf and Hawley, 1992). Presumably, this anti-poleward force acts as a proxy for the lack of a crossover and allows the dividing cell to avoid premature segregation. nod mutants display odd meiotic behavior as non-exchange chromosomes are ejected out of the chromosomal mass that forms during prophase I in Drosophila In these mutants, non-exchange chromosomes remain as univalents and move to poles independently, often resulting in aneuploidy. In females, this mutation always affects the segregation pattern of chromosome IV as well as affecting the segregation pattern of the X chromosome in 5% of MI events (Carpenter, 1973). Interestingly, this experiment demonstrated that nod mutants do not experience any setbacks in chiasmate distribution but are unable to segregate E_0 chromosomes, suggesting the two distribution pathways are mechanistically independent.

Advances in cytological techniques such as fluorescent in situ hybridization (FISH) and electron microscopy (EM) have contributed to our understanding of the relationship between pairing and recombination (reviewed in Zickler and Kleckner, 1999). Burgess and Kleckner (1999) were able to demonstrate that collisions between allelic sequences on homologous chromosomes occurred twice that of non-allelic interactions. In yeast strains deficient in SC formation or where the ability to form DSBs is eliminated, homolog pairing is still detected by FISH (Loidl et al., 1994).

As previously stated, a crucial step in most organisms' meiotic program is the formation of crossovers, as chromosomes that fail to crossover have a significantly higher tendency to segregate randomly and are correlated with reduced gamete viability

(Bascom-Slack et al., 1997). Failure to properly segregate chromosomes (nondisjunction) will result in daughter cells with an incorrect chromosomal complement, a situation that is usually lethal and is referred to as aneuploidy (Zickler and Kleckner, 1998). Unsurprisingly, meiosis requires a tight coordination of proteins to ensure that homologous chromosome segregation in meiosis I is faithfully accomplished, yet it is unknown whether some of the same proteins that play a role in establishing crossovers are involved in distributive segregation. This study will look at the role of two meiosis-specific genes, NDJ1 and TID1, and whether they contribute to distributive, or achiasmate, segregation.

Ndj1p is a telomere associated protein that tethers the telomere to the nuclear envelope via a transmembrane protein, Mps3p (Dresser, 2008) and Rap1p, a protein which binds to repetitive telomere sequences (Kurtz and Shore, 1991). Tid1p is a DNA translocase which promotes interhomolog strand exchange by directing the recombinase Dmc1p to DSB sites (Nimonkar et al., 2007). Both proteins are discussed in greater detail in the next chapter.

Part II - Proteins of Interest

1. *NDJ1*

NDJ1 (nondisjunction protein), also referred to as TAM1 (telomere-associated meiotic protein), is a gene located on chromosome XV in S. cerevisiae. It is expressed specifically during meiosis and the protein localizes to discrete spots at the ends of condensed meiotic chromosomes during pachytene as observed in immunolocalization assays. Cytological assays using DAPI (a DNA dye that fluoresces under UV light) and immunofluorescent antibodies specific for Ndj1p show 96% of chromosome ends associate with Ndj1p foci in nuclear spreads, while no Ndj1p foci are detected at interstitial sites along the chromosomes (Chua and Roeder, 1997). Ndj1p is required for telomere reattachment to the nuclear envelope early in prophase I and is involved with telomere-mediated chromosomal movement that results in the appearance of the telomere bouquet during zygotene (Trelles-Sticken et al., 2000). This movement is facilitated by protein complexes on the nuclear envelope that mediate interactions between nuclear-associated actin cables of the cytoskeleton and the Ndj1p-bound telomere (Koszul et al., 2008). Failure to retether chromosomes to the nuclear envelope inhibits the first step of telomere reorganization and causes delays in multiple meiotic events that follow (Chua and Roeder, 1997).

ndj1 mutants show a delay in axial element formation, synapsis, and initiation of DSBs, although DSBs are eventually repaired at levels similar to what is seen in non-mutant strains (Conrad et al., 1997). In yeast, ndj1 mutants result in a decreased level of spore viability which may be a consequence of a decreased incidence or absence of crossovers, which denies the spindle the tension it requires to progress in meiosis (Wu

and Burgess, 2006). It has been demonstrated that interhomolog repair requires the two homologous DNA molecules to be within close proximity to one another (Petes and Pukkila, 1995). Interestingly, *ndj1* mutants show increased rates of ectopic recombination as well as disrupting the crossover interference mechanism, allowing crossovers to occur within close proximity to one another (Wu and Burgess, 2006). Ectopic recombination refers to the exchange of DNA between homologous sequences that are located on non-homologous chromosomes. This may produce aneuploid gametes as a result of deletions and insertions during ectopic recombination. Though the overall levels of crossing over are not reduced in *ndj1* mutants, the frequency of chromosomes that fail to achieve a crossover increases (Wu and Burgess, 2006). This suggests that telomere-mediated chromosomal movement, crossover assurance and interference are dependent on normal Ndj1p function. *ndj1* mutants also exhibit increased levels of nondisjunction of homologs at MI and precocious sister separation as seen by Conrad et al., as well as in this study.

There are two models proposed to explain the role of NDJ1 in crossover interference. One model proposes that crossover initiation is sensitive to stress built up between homologs and facilitated by synapsis. In this model, the loading of axial element proteins onto meiotic chromatin produces tension between paired chromosomes. Recombination is initiated once the stress between the synapsed chromosomes reaches a certain threshold. The crossover event relieves the stress locally and prevents another recombination attempt in the local area near the completed crossover. The role of Ndj1p in this model is to anchor the chromosomes to the nuclear envelope so that the chromosome is fixed, a necessary component for the establishment of tension. Therefore,

ndj1 mutants would lack tension between the homologs and fail to prevent crossovers from occurring at fixed distances from one another (Kleckner, 1996). While this model does suggest a possible interference mechanism, it does not include the occurrence of meiotic delays; most importantly the delay in synapsis between paired chromosomes.

Another model for the role of Ndj1p in the interference mechanism is that *ndj1* mutants are defective in homolog pairing, which then confers the delay seen in synapsis and ultimately SC formation. This view is based on temporal observations of chromosomal behavior. Chua and Roeder suggest that the retethering of telomeres aids in the homology search and failure to do so delays pairing. The delay in pairing causes a delay in SC-mediated synapsis, resulting in a lack of interference. Interference in this model is dependent on timely formation of the SC. This model assumes that an important role of the telomere bouquet is to reduce the homology search from three dimensions to two dimensions. Ndj1p has been demonstrated to play a role in chromosome movement during bouquet formation and so may have a role in assisting chromosome pairing (Trelles-Sticken et al., 2000).

2. *TID1*

After pairing, an important step in resolving DSBs is the active homology search of the resectioned ends using the homologous template. This is the purpose of the SEI after DSBs are processed. Recent experiments have shown Dmc1p, a RecA-like recombinase, to be required for DSB processing and complete synapsis. Dmc1p is a meiosis-specific recombinase and has been shown to promote strand invasion in order to complete the homology search for DSB repair. During meiosis, Dmc1p has been

demonstrated to colocalize with Rad51p at sites of Spo11p-induced DSBs and both proteins are required for proper crossover formation. It is believed that Rad51p mediates localization of Dmc1p to DSB sites (Holzen et al., 2006). In cytological assays performed by the Bishop lab, about 80% of Dmc1p foci are localized at, or immediately adjacent to Rad51p foci. It is unknown how the presence of Dmc1 affects SC formation; in the absence of Dmc1p, axial elements assemble properly but the central element does not (Bascom-Slack et al., 1997). In vivo, Dmc1p will preferentially bind to dsDNA, limiting the availability of the protein to be targeted to DSBs. Dissociation of Dmc1p from dsDNA to create a pool of unbound protein for translocation to DSB sites is dependent on another protein, Tid1p (Holzen et al., 2006).

TID1, also known as *RDH54*, is encoded by a gene located on chromosome II in *S. cerevisiae*. Tid1p is partially redundant to another DNA translocase, Rad54p. *TID1*, like *RAD54*, is a member of the Swi2/Snf2 family (Eisen et al., 1995) and has been shown to expose ssDNA by locally unwinding dsDNA using ATP hydrolysis (Shinohara et al., 2003). Tid1p is able to translocate along DNA at a rate of approximately 80 bp/s and has an observed processivity of about 10,000 bp. Preliminary studies suggest that Tid1p expends 1 molecule of ATP per bp transolcated. In addition to limiting Dmc1p association with dsDNA, this study proposed another role for Tid1p to promote DNA heteroduplex extension via translocation along dsDNA (Nimonkar et al., 2007).
Both Tid1p and Rad54p have been shown to promote dissociation of recombinases from dsDNA, however Rad54p seems to be primarily involved in mitosis while Tid1p is thought to be meiosis specific. Tid1p is detected in cells throughout the vegetative growth cycle, but *tid1* mutants only show an altered phenotype during meiosis. However,

the separation of function in different cell programs is not absolute as Rad54p and Tid1p are able to partially rescue each other when one of the proteins is missing or non-functional (Shinohara et al., 1997). It has been proposed that Tid1p and Dmc1p are functional predominantly in meiosis. This was based on experiments using CHEF electrophoresis and western blot assays to detect DSB repair in both mitosis and meiosis (Arbel et al., 1999).

In addition to the failure to release Dmc1p from dsDNA, tid1 mutants show delayed meiotic progression as well as a failure to fully resolve DSBs via the crossover pathway (Shinohara et al., 1997). It has been proposed that Tid1p plays an indirect role in the processing of DSBs into crossover events by releasing Dmc1p from dsDNA in order to make it available for its recombinase duty during meiosis (Holzen et al., 2006). Tid1p also plays a role in crossover interference as tid1 mutants decrease the proximity with which crossovers are completed in relation to each other. A similar result was obtained using dmc1mutant strains, suggesting a link between Tid1p and Dmc1p. The defect in the interference mechanism in tid1 mutants may be associated with defects in SC formation between homologs. In particular, tid1 mutants show an accumulation of the polycomplex (formation of the SC in the absence of paired homologs), as well as delays in Zip1p appearance. tid1 mutants are also severely delayed in dissolution of the SC (Shinohara et al., 2003).

Part III – Distributive Segregation

1. Nondisjunction in meiosis

Nondisjunction is a general term applied to a segregation failure following metaphase of either meiosis or mitosis. Nondisjunction results in aneuploid cells that do not contain the necessary genetic complement. If a single chromosome pair missegregates during MI, the outcome will result in one daughter cell receiving an extra copy of the missegregated chromosome while the other daughter cell will not contain any copies. Regardless of the outcome, both situations usually have severe consequences for the cells in terms of viability. Depending on the stage at which nondisjunction occurs (MI or MII), the outcome will show a characteristic spore viability pattern in yeast. If missegregation happens in MI, one sees an increase in two- and zero-spore viable tetrads at the expense of 4-spore viable tetrads. The outcome of missegregation in MII will result in an increase of three-viable tetrads.

In normal meioses, proper homolog disjunction during MI is achieved through the regulation of crossovers that form chiasmata: the repair of programmed DSBs, assurance that each homologous pair receives a crossover and interference to prevent two crossovers from occurring near each other. Both *ndj1* and *tid1* are known to be defective in the crossover interference mechanism but their roles in crossover assurance are unknown. Yeast strains that have lost the function of either protein also show defects in crossover interference yet retain a crossover frequency similar to non-mutant (Chua and Roeder, 1997; Shinohara et al., 2003). These results suggest that the total number of crossovers that occur in budding yeast is controlled by an independent mechanism and is not limited by crossover interference. Despite a sustained drive to understand these

complex processes, the relationship between crossover assurance and crossover interference is still unknown. In order to discern whether the two processes are linked, we have examined the ability for budding yeast to segregate chromosomes that do not form crossovers during MI.

Chromosomes that fail to form a crossover are known as non-exchange chromosomes (E_0) and show a higher frequency of nondisjunction compared to chromosomes that demonstrate crossing over. Chromosomes rely on sequence homology for efficient interhomolog crossover resolution with little tolerance for mismatches. Sequence divergence at recombination hotspots is known to decrease the incidence of crossovers at that location (Chambers et al., 1996). Syntenic chromosomes that lack sequence homology show dramatically decreased rates of recombination, while extreme sequence divergence virtually eliminates crossing over. Past studies have shown that crossovers are reduced 100-fold when paired chromosomes contain 9% divergence and a greater than 1000-fold reduction with 18% divergence (Chen and Jinks-Robertson, 1999).

2. Distributive segregation in yeast

Distributive segregation has been suggested to function in yeast, however the mechanism through which this achieved is not yet known. Although distributive segregation was previously demonstrated in insects, this mechanism was first proposed to occur in yeast by Dawson, Murray and Szostak (1986) using artificially constructed chromosomes containing limited regions of homology to one another. They found that these artificial chromosomes exhibited significantly lower levels of recombination compared to natural chromosomes, yet still achieved higher rates of proper disjunction

than would be expected if segregation was random. These results were not conclusive though as the higher rates of proper segregation may be an artifact of using artificial chromosomes.

The consensus is that although distributive segregation does appear to be functioning in yeast, it is about 100-fold less efficient in *S. cerevisiae* than in *Drosophila*. Whatever the mechanism, there is support that pairing occurs on some level prior to disjunction. Pairing during prophase I between nonhomologous chromosomes was observed cytologically using diploids monosomic for chromosomes I and III. In these double monosomic strains, Loidl et al. (1994) used silver staining and FISH to observe the two monosomic chromosomes form an unusual structure which was distinct from the SC during MI. They also report the two univalent chromosomes properly disjoin from one another approximately 90% of the time, not 50% as one would expect if segregation was random. Further evidence of nonhomologous exchanges in this study was provided by detection of ectopic recombination between homologous sequences on different chromosomes (Loidl et al., 1994).

In this study, we are examining the roles of Ndj1p and Tid1p in crossover control through the ability of cells to perform distributive segregation by eliminating crossovers between a pair of chromosomes using hybrid yeast strains. In hybrid diploids, a homeologous pair of chromosomes refers to the presence one native chromosome and one from a closely related species. Homeologs contain the same genes in the same order yet contain sequence polymorphisms at varying frequencies, especially in intergenic regions (Nilsson-Tilgren et al., 1986).

To address this question, we are comparing spore viability of the meiotic products in 3 different strain backgrounds, one native *S. cerevisiae* strain and two hybrid strains containing homeologs derived from evolutionarily divergent yeast strains. We will also measure crossover frequency in specific regions to determine whether the homeologs are truly achiasmate in the hybrid yeast strains. These homeologous chromosomes functionally replace the native chromosome, yet have a high enough sequence divergence from the *S. cerevisiae* counterpart so that recombination rate between homeologs is drastically reduced. By using homeologous strains to eliminate crossovers, we are testing the ability of these mutants to carry out distributive segregation.

In this study, diploids in the Y55 strain are heterozygous for chromosome III. The non-native chromosome III was derived from *S. paradoxus*, which has an estimated 80% homology to the *S. cerevisiae* III (Figure 5a). In a study done using a similar hybrid Y55 diploid strain, there was a 10- to 100-fold decrease in the meiotic recombination rate measured in four large genetic intervals on chromosome III compared to levels seen between homologs (Chambers et al., 1996). Diploids in the S288c strain are heterozygous for chromosome V. The non-native chromosome V was derived from *S. carlsbergensis*, which shares 71% sequence identity with its *S. cerevisiae* counterpart (Maxfield Boumil et al., 2003) (Figure 5b). The additional sequence divergence between the *S. carlsbergensis* and *S. cerevisiae* is enough to eliminate crossovers between the homeologs (Shubochkina et al., 2001). The other strain, SK1, contains a complete set of *S. cerevisiae* chromosomes. Crossover frequency will be measured on the homeologs using heterozygous markers to determine the level of crossing over between the divergent chromosomes.

We have examined the ability of *ndj1* and *tid1* mutants to segregate chromosomes during MI in three *S. cerevisiae* strain isolates. These mutants were compared to non-mutant versions to determine the effect of the mutation on spore viability. Two of the strains used contain a homeologous chromosome to increase the incidence of E_0 chromosomes. This allows us to determine if either protein has a role in distributive segregation, an alternative MI disjunction pathway. In all *ndj1* mutants analyzed, we demonstrate a spore viability pattern that is typical of MI nondisjunction. We failed to detect the same pattern in *tid1* mutants. In the context of the hybrid strains, this pattern is suggestive of a role for Ndj1p in distributive segregation.

Part IV – Materials and Methods

Yeast strains. Both *ndj1* and *tid1* mutants were generated in three different backgrounds (SK1, S288c and Y55) to create the test strains. Non-mutant strains in each background were used as control strains. The non-mutant SK1 strains were previously obtained by the Hillers lab through Nancy Kleckner's lab. KHY 112 was previously obtained by the Hillers lab through Doug Bishop. The S288c and Y55 non-mutant strain and ndj1::KanMX4 strains were gifts from Dr. Eva Hoffman. KHY 144 was a gift from Rhona Borts. The SK1 ndj1::KanMX4 was created by Jonathan Henzel as described under the yeast transformation heading. All tid1::cloNAT mutants except KHY 112 were created by Jonathan Henzel as described under the yeast transformation heading. KHY 148 and 149 were used to determine mating types of haploid spores following dissection. The mating type assay is described in further detail below. The genotypes of all strains used in this study are listed in Table 1.

Media. Yeast strains were maintained in Petri dishes containing the rich medium YPAD (1% Yeast Extract, 2% peptone (tryptone), 2% dextrose, 1.75% agar and 0.004% adenine sulfate). Selection of antibiotic resistance transformants occurred on YPAD plus 100 μL of the appropriate antibiotic (Geneticin for *ndj1* mutants and nourseothricin for *tid1* mutants). Synthetic dropout media (SD) was used to select for auxotrophic strains (0.64% Difco YNB, 2% dextrose, 1.75% agar plus appropriate amino acids). Nitrogen-deficient plates (SPO) were used to induce sporulation of diploid yeast strains (1% potassium acetate, 0.1% yeast extract, 0.05% dextrose, 0.025% complete amino acid mix). LB broth was used for bacterial growth for plasmid minipreps (1% tryptone, 0.5%

yeast extract, 0.5% NaCl). TE buffer was used to store extracted DNA (10 mM Tris pH 7.5, 1 mM EDTA).

Antibiotics. A cassette conferring antibiotic resistance was inserted into each gene of interest in this study. The gene to confer nourseothricin (cloNAT) resistance was inserted into the *TID1* gene. Nourseothricin is a polyketide antifungal compound produced naturally by the bacterium *Streptomyces nourse*i. The gene to confer Geneticin (G418) resistance was inserted into the *NDJ1* gene. Geneticin is an aminoglycoside antibiotic and is naturally produced by the gram-positive bacterium *Micromonospora rhodorangea*. Geneticin works to inhibit peptide synthesis in both prokaryotes and eukaryotes.

Transformations. Yeast gene transformations were performed via one-step gene replacement as described below as well as in Hillers Lab Procedures manual. As both *NDJ1* and *TID1* genes were disrupted with antibiotic resistance cassettes, a 12-24 hour outgrowth at 30°C in rich YPAD media was performed prior to plating onto YPAD+antibitotic for selection. All *ndj1* mutants were disrupted with the KanMX4 cassette, conferring Geneticin (G418) resistance. All *tid1* mutants were disrupted with the cloNAT cassette. cloNAT resistance cassette was cloned into *TID1* open reading frame using the pBluescriptII tid1::cloNAT plasmid built by Elaine Morlock. Transformed yeast cells were confirmed using both selective media with antibiotic and PCR. Once successful transformants were isolated and confirmed, PCR amplicons of the disrupted genes using primers flanking the desired DNA (described below) were used to transform remaining strains.

The ability to integrate foreign DNA was variable in different strains. SK1 strains transformed easily, a single transformation often produced many colonies of yeast cells that were confirmed as transformed cells. Y55 and S288c were less amenable to transformation, often requiring many attempts at transformation before yielding a lone colony confirmed to carry the disrupted gene of interest. Because there are many genotypic differences between the three strains, it is difficult to ascertain why there are differences in transformation efficiency. The strains used contain many laboratory-engineered deletions and mutation which may contribute to difficulties in the ability for the cells to integrate newly introduced DNA. Previous studies have shown single nucleotide polymorphisms (SNPs) to be responsible for gross differences in sporulation efficiency between two closely related strains SK1 and S288C (Ben-Ari et al., 2006). It is possible that additional SNPs may affect the strains receptivity to transformative DNA.

Glycerol Yeast Transformation

Grow yeast cells overnight at 30°C with shaking in 3mL of YPAD. Inoculate 25 mL YPAD broth with 750 μL of yeast cells from overnight culture and incubate at 30°C until the optical density at 600 nm is between 0.4 and 0.6. At this point, spin down cells in 50 mL conical tubes for 3 minutes at 3000 RPM and decant supernatant. Resuspend pellet in 25 mL ddH_20 and repeat centrifugation. Resuspend pellet in 10 mL Li-T solution (100 mM lithium acetate, 10 mM Tris pH 7.5) and repeat centrifugation again. Resuspend pellet in 1 mL 100 mM lithium acetate and transfer to microcentrifuge tube. Spin for 30 seconds at max speed and decant supernatant. Resuspend such that each transformation gets 50 μL 100 mM lithium acetate and aliquot 50 μL into fresh microcentrifuge tubes. Repeat centrifugation and decant supernatant. Make master mix so that each

transformation receives the following: 240 µL 50% PEG 3350, 36 µL 1 M lithium acetate and 50 µL boiled salmon sperm. Resuspend pellet with 325 µL of the master mix and add 30 µL of transforming DNA. Incubate at room temperature for 15 minutes occasionally mixing gently. Add 8 µL 60% glycerol and mix gently by inversion. Incubate an additional 30 minutes at room temperature. Heat shock at 42°C for 10 minutes and spin down for 1 minute at 3000 RPM. Resuspend pellet in 1 mL YPAD broth and transfer to test tube containing 2 mL YPAD broth. Incubate at 30°C with shaking overnight and plate onto appropriate selective media after 24 hours.

DNA isolation. Yeast genomic DNA (gDNA) was isolated using two different protocols; glass beads extraction and sorbitol extraction. The protocols were followed as found in Hillers Lab Procedures manual. Both methods gave equivalent yields and either method may be used indiscriminately.

Glass Beads/Phenol gDNA Extraction Protocol

Yeast cultures were grown in 5 mL YPAD broth overnight at 30°C with shaking and spin down in microcentrifuge tubes at max speed for 1 minute after sufficient growth was confirmed in the test tube. After decanting most of the supernatant, 0.3 grams of 425-600 micron acid-washed glass beads, 200 µL X-2-17 solution (0.2% 0.5 M EDTA pH 8.0, 1% 1 M Tris pH 8.0, 5% 5 M NaCl, 1% SDS, 2% Triton X-100)) and 200 µL phenol:chloroform. Perform phenol extraction 3 times, saving the aqueous layer each time. Precipitate DNA with 0.1 volumes of 3 M sodium acetate pH 5.2 and 2 volumes of 95% ethanol and incubate at -20°C for 10 minutes. Spin at max speed for 10 minutes and decant supernatant. Wash pellet with 70% ethanol by spinning for 1 minute at max speed

and decanting supernatant. Dry pellet in vacuum centrifuge and resuspend pellet in 50 µL TE buffer with 0.2 µL RNase (10mg/mL) and store in freezer.

Sorbitol gDNA Extraction Protocol

Yeast cultures were grown in 5 mL YPAD broth overnight at 30°C with shaking and spin down in microcentrifuge tubes at 3000 RPM for 2 minutes after sufficient growth was confirmed in the test tube. After decanting the supernatant, pellet was resuspended in 500 µL 1 M sorbitol. Add 15 µL DTT and 10 µL 100t zymolyase and incubate with shaking for 1 hour at 37°C. Add 200 µL TE and 70 µL 10% SDS and incubate at 65°C for 10 minutes. Add 350 µL 5 M potassium acetate, invert 6 times and ice for 30 minutes. Spin down for 6 minutes at max speed. Add no more than 650 µL of supernatant to prepared 2 mL microcentrifuge tubes containing 1 mL isopropanol and 200 µL 5 M ammonium acetate. Spin at 4000 RPM for 1 minute and discard supernatant completely. Dry pellet in vacuum centrifuge and resuspend pellet in 300 µL TE buffer with 1 µL RNase (10mg/mL) and store in freezer.

Plasmid isolation: Bacterial plasmid minipreps were performed using the alkaline lysis protocol as found in Hillers Lab Procedures manual.

Alkaline Lysis Plasmid Miniprep

Bacterial cells containing the pBluescript II tid1::cloNAT plasmid were grown overnight at 37°C with shaking in 5 mL of LB broth. After sufficient growth is confirmed, cells were pelleted in microcentrifuge tubes for 3 minutes at max speed and the supernatant was decanted. This was repeated and the resulting pellet was resuspended in 100 µL of cold GTE Solution I (50 mM glucose, 25 mM Tris-Cl pH 8.0, 10 mM EDTA), followed

by 200 μL of Solution II (0.2 M NaOH, 1% SDS) and mix by inverting tube 6 times. Add 150 μL ice-cold Solution III (5 M potassium acetate) and mix by inversion. Immediately place on ice for 10 minutes, followed by a 10 minute microcentrifuge spin at max speed. Decant supernatant into a new tube and perform phenol/chloroform extraction isolating the aqueous layer. Add 800 μL of ice-cold 95% ethanol, mix by inversion and leave on ice for 5 minutes. Pellet at max speed for 3 minutes, decant and add 500 μL ice-cold 70% ethanol and repeat centrifugation. Dry pellet in vacuum centrifuge and resuspend pellet in 50 μL TE buffer with 0.2 μL RNase (10mg/mL) and store in freezer.

Restriction Digest. Transforming DNA was excised out of the pBluescript II tid1::cloNAT plasmid using the restriction endonuclease NotI. NotI was purchased from New England Biolabs (NEB) and digests were followed using conditions described by NEB. Digests were confirmed using agarose gel electrophoresis.

PCR. Transformants were confirmed via PCR either through colony PCR or isolated gDNA. gDNA isolations were performed as described above and in Hillers Lab Procedures binder using either the glass beads protocol or sorbitol protocol. Both methods gave reliable gDNA yields that were suitable templates for PCR. Colony PCR consists of a 10 minute pre-incubation in 0.02M NaOH of a limited number of freshly-grown cells, followed by a normal PCR program. All PCR programs included a four minute hot start (94°C), followed by thirty seconds at 94°C, thirty seconds at the primer-dependent annealing temperature, and thirty seconds per 500 bp of expected PCR product

at 72°C (i.e. one minute and 30 seconds for an expected amplicon of 1500 bp). When applicable, master mixes were made so that each reaction received the appropriate volume as follows (all volumes in microliters):

ddH_2O – 7.7
5x GoTaq buffer – 3
GoTaq – 0.3
dNTPs – 0.18
25mM MgCl2 – 0.9
Fprimer – 1
R primer – 1

Primers. The sequences for each of the primers used are below. Ndj1TestF/TestR primers were used to extract DNA used for transformations as well as to confirm ndj1::KanMX4 transformants and required an annealing temperature of 56°C. Tid1F2/R3 primers were used to confirm tid1::CloNAT transformants and required an annealing temperature of 58°C.

Ndj1 primers
Ndj1TestF – AGAGTAATAGTTACAAATGG
Ndj1TestR – TCAAAGGAGAGCGGGATGAC

Tid1 primers
Tid1F2 – AGAGAAGATCCTACAGGAGG
Tid1R3 – TGAACCTGAATGAGTCGTGC

Mating. Individual patches of auxotrophic haploid cells were first moved onto YPAD using sterile wooden sticks. A patch of both haploid strains was mixed together in the center of the plate to select for prototrophic diploids. After an overnight incubation at 30°C YPAD plates were then replica printed onto synthetic defined media (SD - 0.64% Difco YNB, 2% dextrose, complete amino acid mix minus stated amino acid(s)) with appropriate amino acids added to create an auxotrophic environment limiting growth to diploid cells and grown overnight at 30°C. SD plates were then replica printed back onto

rich YPAD media to maximum growth of diploids for 1-2 days at 30°C. Diploids were then streaked onto SD double dropout media to select for single colonies. Single colonies were patched onto YPAD for growth prior to induction of sporulation. SK1 diploids were selected using SD-trp-leu. S288c diploids were selected using SD-trp-ura. Y55 diploids were selected using SD-ade-leu.

Tetrad Dissection. Single colonies of selected diploids were grown on YPAD plates and incubated at 30ºC overnight. YPAD plates were then replica printed onto a sporulation (SPO) plate using sterile velvet and the SPO plate was incubated at 30ºC for 2-5 days, depending on the strain. When a maximum number of tetrads were seen using a standard light microscope, cells were placed in 10 μL of 5 mg/mL zymolyase for 10 minutes. After 10 minutes, 100 μL of ddH_20 was added to cells and a wooden stick was used to spread yeast cells with breached cell walls onto YPAD for dissection. Tetrads (yeast meiotic products) were then dissected using a Singer micromanipulator dissection microscope. The dissection plate was then incubated overnight at 30ºC or until sufficient growth was seen, usually 1-4 days.

Tetrad Analysis. After dissected spores produced sufficiently sized colonies, tetrads were scored for number of viable colony producing spores out of four possible based upon colony presence. Genetic analysis was done using dropout media to exploit auxotrophic mutations in the strains used. Dissection plates (master plates) were pressed onto a velvet topped cylinder just smaller than the diameter of the Petri dish. Fresh plates containing the appropriate growth media (dropout media or mating type test plates) were than pressed onto the velvet to adsorb cells left from the master plate. A fresh, sterile

velvet was used for each pair of plates (dropout media and rich media with antibiotic) that were replica printed.

Mating type assay. Determination of haploid mating type was done using strains previously described by Rine and Herskowitz, 1980. In order to determine the mating type of each spore resulting from a dissected tetrad, dissection plates were replica printed onto minimal media in the presence of a tester strain that carries a unique mutation in a metabolic pathway that is not found in any of the strains used for this experiment. The tester strains are otherwise prototrophic, so diploids will be able to grow on minimal media but haploid strains will not. One hour prior to replica printing, 200 μL of either tester strain Y70 (KHY 148) or Y227 (KHY 149) was spread using sterile beads onto separate SD plates and then incubated at 30ºC. Dissection plates were then replica printed onto each SD plate with tester strain lawn. Replica printed plates were incubated at 30ºC until sufficient growth was achieved to score tetrads based on the ability for the tester strain to grow.

Data Analysis

Map distance was calculated using the Perkins formula (1949):

$$cM = 100X = (100\ (6N + T))/(2(P + N + T))$$

where P indicates parental ditype (all four spores have parental marker arrangement), T indicates tetratype (two spores have parental marker arrangement, two spores do not) and N represents non-parental ditypes (all four spores have non-parental marker arrangement). Map distance is represented in centiMorgans (cM), a unit of measurement

for assessing genetic linkage of alleles on a single chromosome. One cM is equivalent to a single map unit and is measured as the chance that two markers on the same chromosome will be separated by a crossover between them.

Statistics

Fisher's exact two proportion test was used to determine whether there is a significant difference between each tetrad viability class in mutant strains vs. non-mutant. A chi-square test for independence was used to see whether the overall spore viability profile is affected by each mutation compared to the non-mutant version of the strains. Differences were considered significant using a maximum value of $P < 0.05$. All statistics were run using Minitab version 15.

Random Spore death

Random spore death was calculated for every strain variation used in this experiment. The calculation was done to confirm that spore death was not occurring in the dissected tetrads at random, there was a statistically significant pattern to the frequencies of spore viability. This calculation was used to demonstrate that the mutations constructed into isogenic strains were responsible for alterations in spore viability patterns and not some random force. The random spore death estimate was based off a random permutation calculation to generate expected values for each tetrad viability class. The formula used is:

$$P = (n!/a!*t!)*L^{a}D^{t}$$

where P is the expected probability calculated for each tetrad class, n is the number of potential spores in each tetrad, a is the number of live spores within the tetrad class being analyzed, t is the number of dead spores within the tetrad class being analyzed (i.e. if

calculating expected value for 3:1 tetrad class a = 3 and t = 1), L is the overall spore viability frequency observed for the strain and D is the overall spore death frequency observed for the strain.

Part V – Results

Yeast monosomic for a homeologous chromosome in a *S. cerevisiae* background were viable. The S228C MAT a hybrid strain used in this study had the native chromosome V replaced with chromosome V from S. carlsbergensis. The Y55 MAT α hybrid strain had the native chromosome III replaced with chromosome III from S. paradoxus. In both haploid strains, the homeologous chromosome derived from a different yeast species was able to substitute functionally for the native chromosome it replaced; colonies were viable and did not show any noticeable growth defects on either rich or defined media. Diploids created from these strains were also grown with no appreciable growth defects.

The spore viability of the hybrid yeast strains is significantly reduced. Both strains which carry a homeologous chromosome (hybrid S288C and hybrid Y55) have significantly reduced 4:0 (live:dead) overall spore viability ($P < 0.05$) compared to native SK1 or native Y55 strains after tetrad dissection (Table 2). This decrease in spore viability does not directly result from the presence of a homeologous chromosome as there is no deleterious effect seen in haploid strains carrying a homeolog. Rather, the reduced spore viability appears to stem from meiotic difficulties due to the presence of a homeolog. The overall pattern of spore viability in both of the non-mutant hybrid strains is significantly different from that of the non-mutant native strain ($P < 0.001$) (Figure 9, Table 2). The spore viability in all strains analyzed does not indicate random spore death ($P < 0.001$). An explanation of how random spore death was calculated is found in the Materials and Methods section.

Recombination rates are altered in the presence of homeologs. We measured crossover frequency between the homeologous chromosomes using heterozygous genetic markers to confirm that the homeologs were acting as non-exchange chromosomes. Recombination frequency within the interval was determined through tetrad analysis. Crossover frequency can be measured using a standardized genetic mapping equation developed by Perkins (1949). Only tetrads which produced 4-viable spores were used to collect crossover data (Table 3).

Crossover frequency in a non-mutant native Y55 strain was used to generate a benchmark with which to compare the non-mutant hybrid Y55 strain. The markers used in both native strains (SK1 and Y55) and the hybrid Y55 strain were located on chromosome III and covered the LEU2-MAT interval, spanning approximately 110 kB or 34.4% of the physical length of the chromosome. Past studies have demonstrated the LEU2-MAT interval to be approximately 34 cM or 22% of the chromosome III genetic map in a wild type SK1 background (Shinohara et al., 2003).

In this study, the same interval was determined to be 24 cM in the native Y55. The non-mutant hybrid Y55 strain, which carries a *S. paradoxus* chromosome III, experienced a suppressed level of recombination (6.16 cM) between the homeologs (Figures 13, 14 & 15). Compared to the native Y55, the frequency of recombination between the homeologs in the non-mutant Y55 hybrid is reduced 3.9-fold within the LEU2-MAT interval. In wild type strains, the interval measured covers over 20% of the genetic map and so the reduced crossover rate is not due to a failure to detect a crossover in meiosis. Therefore, we are making an assumption that crossovers within the LEU2-

MAT region occurred in the non-mutant hybrid Y55 strains approximately 4-fold less than in the native Y55 strain.

Low levels of recombination between the homeologs in the LEU2-MAT interval were seen in all of the hybrid Y55 strains; the genetic map in the hybrid Y55 *tid1* mutant (6.06 cM) was similar to the non-mutant version of the same strain, but the map distance increased to 9.5 cM in the *ndj1* mutant. This is a 1.5 fold increase in recombination frequency between *ndj1* mutants and non-mutants in the hybrid Y55 strain. At the time of this manuscript, we did not have measured rates for *ndj1* and *tid1* mutants in the native Y55 strain.

Crossover frequency was measured in the LEU2-MAT interval in the non-mutant and *ndj1* mutant native SK1 strains. In this study, the map distance in the non-mutant SK1 strain was found to be 26 cM. This was similar to that seen in the non-mutant native Y55 strain. The SK1 *ndj1* mutant experienced a 1.7-fold increase in map distance (45 cM) compared to the non-mutant SK1 strain. The increase in map distance between the *ndj1* mutant and non-mutant is similar in both the native SK1 and hybrid Y55 strains. No data was collected for the *tid1* mutant in the SK1 strain due to the lack of heterozygous markers.

Crossovers were also measured in the S288C homeologs encompassing a region known to be a hotspot for recombination (ILV1) between homologous chromosomes. The markers in the S288C strain were located on chromosome V and covered the URA3-TRP2 interval. This interval covers approximately 225 kB or 38.8% of the physical length of chromosome V (Maxfield Boumil et al., 2003). In the hybrid S288C strain, which carries a homeologous V derived from *S. carlsbergensis*, there was no

recombination detected in non-mutant or *ndj1* mutants and only a single crossover event was detected out of 56 4-viable spore tetrads (1.7%) in *tid1* mutant strains. The low number of crossovers is expected as the two homeologs are approximately 29% divergent. This is consistent with previously published data (Maxfield Boumil et al., 2003). Unfortunately, we were not able to acquire a native strain with markers similar to the S288C strain to compare.

The presence of a homeologous chromosome does not alter ability to properly complete meiosis. All non-mutant versions of the strains used in this study produced 4-spore viable tetrads at a higher frequency than their respective *ndj1* and *tid1* mutant counterparts. Tetrads that produce 4-viable spores are assumed to have undergone normal disjunction during MI as aneuploid cells are often inviable. Tetrads that result in a 2:2 profile are assumed to have experienced a missegregation event at MI. When both the frequency of 4:0 and 2:2 tetrads are compared within each strain analyzed, the data suggests that non-mutant strains do not have major difficulties in completing MI.

Other tetrad viability profiles are more difficult to characterize as they can arise from numerous errors throughout meiosis. For instance, 3:1 tetrads can arise from an MII nondisjunction event or precocious sister separation. Another possibility is the failure to properly repair DSBs in one chromosome prior to MI disjunction, resulting in a broken chromosome in which all or part of the broken chromosome is excluded from attachment to the spindle.

A single live spore may result from a nondisjunction event during MI followed by a second nondisjunction involving a different chromatid pair. It is also possible that a

failure to repair DSBs followed by nondisjunction events in either MI or MII may lead to 1:3 tetrads. Beyond the few possibilities mentioned, there are many situations that may produce the 1:3 tetrad class. It is for these reasons that we will not consider 1:3 tetrads when determining MI nondisjunction events.

Since tetrads with two viable spores are a convenient way to measure MI nondisjunction events and tetrads with three viable spores are thought to arise from something other than MI nondisjunction, we will use the ratio of 2:2 to 3:1 as an additional proxy for MI nondisjunction. Under the 2:3 live spore metric, values greater than 1 will be used to imply a MI disjunction defect. We have chosen to use a 2:3 ratio value of 1 as a threshold to gauge MI nondisjunction based on the data collected. This metric can be only applied when the incidence of 1:3 tetrads is relatively low and random spore death is not significantly high.

If overall spore death within a strain was high and spore death was random, one would expect an increase in tetrad classes with more dead spores, excluding the 0:4 tetrad class. That is to say that an increasing number of tetrads would be expected in tetrad classes with one live spore compared to two live spores, and 2:2 tetrads are expected to be greater than 3:1 tetrads. In this scenario where spore death is random and significantly greater than would be expected normally, we would also expect high 2:3 ratios except in this case it would also be coupled with increased levels of 1:3 tetrads. If MI nondisjunction was occurring in these tetrads, the event will be obscured because of some separate event(s) pushing 2:2 tetrads into the 1:3 tetrad class.

Using our model for calculating random spore death as described in the Materials and Methods section, a strain with low overall spore viability (~20%) would be expected

to have a 2:3 ratio of 6. While this ratio is very high, it is based off low expected values for these two spore viability classes. The expected frequency of tetrads with two live spores is 15.4% and only 2.6% of tetrads are expected to have three live spores. In contrast, 41% of tetrads are expected to have just one viable spore. We do not see a high 1:3 tetrad frequency in any case in which the 2:3 live spore ratio was greater than one. In addition, there was only one situation (hybrid Y55 *tid1* mutant) in which the rate of 1:3 tetrads was greater than the rate of 2:2 tetrads.

With an understanding of how different spore viability classes may originate, we have dissected tetrads produced by diploid yeast. Tetrad dissection allows us to mechanically separate individual spores within a single ascus and determine whether each spore is viable by growing them on rich media. Our results are described below. The SK1 strain, which contained all homologous chromosomes, was able to complete meiosis with few errors as 4:0 tetrads were produced at a high frequency (84.7%) compared to the incidence of 2:2 tetrads (2.9%) (Figure 6). This large contrast in frequency implies that MI disjunction occurred correctly in a large majority of tetrads analyzed. Most of the remaining tetrads analyzed produced the 3:1 tetrad class (8.8%). This is most likely to result from an error other than MI nondisjunction (Chambers et al., 1996). The 2:3 ratio in the SK1 strain was 0.33 (Figure 12).

The presence of an E_0 chromosome pair in the hybrid S288C strain had the largest effect on spore viability. The S288C strain produced 4 viable spores in only 59.4% of tetrads dissected (Figure 7). The frequency of 3:1 tetrads was 15.5%. Despite the lower frequency of 4:0 tetrads compared to the other strains in this study, there is no evidence to suggest that the inability of the hybrid S288C strain to produce 4:0 tetrads is due to an

MI nondisjunction event as only 5.7% of tetrads were 2:2. The 2:3 ratio in the hybrid S288C is 0.37 (Figure 12). The majority (74.8%) of tetrads produced in the non-mutant hybrid strain fell in both the 4:0 and 3:1 tetrad classes. In combination with the crossover data acquired in the URA3-TRP2 interval on the homeologous chromosome V, the data suggests that distributive segregation is functioning properly in disjoining E_0 chromosomes.

The low overall spore viability in the non-mutant hybrid S288C strain does not seem to be a relic of the presence of a homeolog. Compared to the occurrence of 4:0 tetrads, the non-mutant hybrid S288C still has a relatively low 2:2 tetrad frequency. This is in line with the frequency of 2:2 tetrads in all other strains analyzed in this study. Additionally, the 2:3 live spore ratio is similar to that seen in the non-mutant native strains used in this study. Taken together, these data are not suggestive of errors in MI segregation in the non-mutant hybrid S288C. In fact, the native S288C strain is notoriously bad at completing meiosis in general. The capability to properly carry out all phases of meiosis is reduced as measured by sporulation efficiency. Sporulation efficiency is one way to gauge meiotic aptitude and is simply the ability of the diploid yeast cell form an ascus containing four cells when induced into meiosis. Previous studies have shown sporulation efficiency in a S288C-derived strain at only 12%. In contrast, the same study measured the sporulation efficiency of an SK1-derived strain at 92% (Ben-Ari et al., 2006). The presence of a homeologous chromosome may compound and amplify these meiotic struggles.

The presence of a homeologous chromosome in the Y55 strain (hY55) results in a reduction in the frequency of 4:0 tetrads (71.0%) when compared to a native Y55 strain

(nY55) (84.9%) (Figure 8). Overall spore viability is 87.1% in hY55 and 91.2% in nY55, an insignificant difference. The 2:3 ratio in the hY55 strain is 0.46 and 0.29 in the nY55 strain, also insignificant (Figure 12). When taken together, the high 4:0 tetrad frequency paired with a relatively low 2:2 tetrad rate supports MI being completed successfully.

***ndj1* mutants are defective in MI disjunction**. All ndj1 mutant strains in this study produce 2:2 and 0:4 tetrads at a significantly higher rate than the non-mutant version of each respective strain. The increase in 2:2 and 0:4 tetrads are coupled with a statistically significant reduction in the number of tetrads that produce four viable spores in all strains tested. Remarkably, all three ndj1 mutant strains used in this study produced very similar frequencies of 2:2 tetrads. Additional evidence of a MI specific defect is that the number of 3:1 tetrads in ndj1 mutants is not significantly different from the non-mutant version in all strains analyzed. Overall, the spore viability pattern of ndj1 mutants suggests that Ndj1p has a role in promoting efficient MI disjunction.

Compared to the non-mutant version, the ndj1 mutant in the SK1 strain experienced a significant increase in tetrads in which only two spores were viable as well as 0:4 tetrads (Figure 6). This was concomitant with a significant decrease in 4:0 tetrads. The 2:3 live spore tetrad ratio was 1.69 (Figure 12), a 5.1-fold increase compared to the non-mutant. While the 0:4 tetrads may arise from a number of potential meiotic errors, the rise of 2:2 tetrads and high 2:3 ratio suggest a MI nondisjunction event. This is further supported by the observation that the other tetrad classes are similar to that seen in

the non-mutant. These results support a role for Ndj1p in promoting proper MI disjunction.

The *ndj1* mutant in the hybrid S288C strain shows a similar pattern in tetrad viability to the SK1 *ndj1* mutant except the effect is considerably more pronounced (Figure 7). The hybrid S288C strain is virtually unable to produce tetrads in which each spore is euploid. Four spore viable tetrads made up 4.6% of tetrads analyzed. There was a 4.8-fold increase in the frequency of 2:2 tetrads (22.2%) compared to the non-mutant strain. The majority of tetrads (55.6%) analyzed in this background resulted in four inviable spores. The 2:3 live spore ratio was 3.0 (Figure 12), an 8.1-fold increase compared to the non-mutant. This is strong evidence that a MI nondisjunction event produced 2:2 tetrads. In addition, all other tetrad class frequencies were similar to that seen in the non-mutant version. Using the recombination frequency as an indication that crossing over is eliminated in the homeologous chromosomes, the data strongly suggests that Ndj1p is required for proper segregation in distributive disjunction. This is based on the assumption that the number of crossovers remained constant in the native chromosomes. Thus, the increased frequency of 2:2 tetrads is likely due to problems with distributive segregation during MI.

The hybrid Y55 *ndj1* mutant also shows a significant increase in the rate of 2:2 and 0:4 tetrads compared to the non-mutant hybrid Y55 strain, as well as 1:3 tetrads (Figure 8). These increases are accompanied by a significant decrease in 4:0 tetrads. Although there was no significant difference in the 3:1 class of tetrads between the *ndj1* mutant and non-mutant, the frequency of 3:1 tetrads was higher than the frequency of 1:3 tetrads, preserving the validity of the 2:3 ratio as a measure of MI nondisjunction. The

2:3 ratio in the ndj1 mutant was 1.38 (Figure 12), a 3-fold increase compared to the non-mutant. The high 2:3 ratio suggests MI nondisjunction in hybrid Y55 ndj1 mutants. It is possible that other meiotic events are obscuring our ability to detect the true rate of nondisjunction. We may be underestimating the level of MI nondisjunction as 1:3 tetrads may be a result of MI nondisjunction coupled with an error during MII disjunction.

***tid1* mutants are not defective in MI disjunction**. The spore viability pattern of tid1 mutants do not suggest that Tid1p plays a role in the distributive disjunction pathway. While there is a significant reduction in the number of 4-viable spore tetrads in tid1 mutants in all three strains assayed, the reduction was not accompanied solely by the expected increase in the frequency of 2:2 tetrads if there was an error in MI disjunction. Instead, the decrease in spore viability was spread out over different tetrad classes in each strain analyzed. Despite the apparent lack of a pattern between all tid1 mutant strains used in this study the data does not suggest that spore death is random (all strains - $P < 0.001$).

In the native SK1 tid1 mutant, there were significant increases in the rate of 3:1 (28.3%) 2:2 (19.3%) and 1:3 (10.4%) tetrads but not 0:4 tetrads (0.7%) (Figure 6). The 2:3 ratio was 0.68 in the SK1 background (Figure 12). The pattern of spore viability in the SK1 strain, which includes high rates of 3:1 and 1:3 tetrads, does not correlate with expected frequencies from MI nondisjunction errors. Additionally, the low 0:4 tetrad frequency coupled with the low 2:3 live spore ratio also fails to support a MI disjunction error.

The hybrid S288C tid1 mutant only had a significant increase in the frequency of 3:1 tetrads (28.0%) and had a 0.22 2:3 ratio (Figure 12). The low incidence of 2:2 tetrads is not indicative of a MI missegregation event (Figure 7). In fact, the data suggest that tid1 mutants are able to segregate E_0 chromosomes with a comparable efficiency to that seen in the non-mutant homeologous S288C strain. Using the S288C strain as the true measure of distributive segregation, the data do not support that Tid1p is required for achiasmate disjunction.

The hybrid Y55 tid11 mutant also had statistically similar levels of 2:2 tetrads to the non-mutant hybrid Y55 and had a low 2:3 ratio (0.52) (Figure 12). The hybrid Y55 tid1 mutant only had significant increases in the 1:3 (13.8%) and 0:4 (23.6%) tetrad classes (Figure 8). These results were unexpected as neither a MI nor MII nondisjunction pattern emerges in this mutant. There is no single expected event that would result in either a 1:3 or 0:4 class of spore viability. A high incidence of tetrads in these spore viability classes may occur through a combination of different meiotic errors, including MI nondisjunction. Although it is possible that our ability to determine MI nondisjunction is masked by additional meiotic defects in the hybrid Y55 background, we do see evidence to suggest MI disjunction is working. The majority of tetrads dissected were either 4:0 or 3:1, both of which are only possible when MI is functioning properly.

***tid1* mutants produce 3-viable spore tetrads at a significantly higher rate than non-mutant in SK1 and S288c strains**. Tetrad dissection of tid1 mutants showed 3:1 spore viability patterns at a significantly higher rate in the native SK1 strain ($P < 0.001$) and hybrid S288C strain ($P < 0.05$). There was a higher frequency of 3-viable spore tetrads in

the hybrid Y55 strain, but the difference was not significant ($P < 0.114$). Tetrads with 3 viable spores cannot occur from a MI nondisjunction event. The most likely scenarios which give rise to 3-viable spore tetrads are precocious sister chromatid separation or a MII nondisjunction event.

Part VI – Discussion

Two aspects of crossover regulation are crossover assurance and interference. In a typical meiosis, crossover assurance works to ensure that each chromosome pair receives a crossover. Crossover interference is derived from an observation that crossovers are mechanistically prevented from forming within a certain proximity of an established crossover event. In other words, crossovers do not seem to be distributed randomly throughout the genome. It is not known if crossover assurance and interference are different aspects of the same mechanism or if they act independently. Most known mutants that affect interference are thought to also have an effect on assurance.

In this study, we examined the effects of two mutations in proteins known to cause interference defects, Ndj1p and Tid1p, on meiotic segregation of both homologous and homeologous chromosomes. While both *ndj1* and *tid1* mutants have an altered crossover distribution resulting from defects in the crossover interference mechanism, the overall number of crossovers is not reduced relative to wild type strains. Though they have both been demonstrated to cause interference defects, *ndj1* and *tid1* mutants seem to have different effects on spore viability; *ndj1* mutants have been shown to have elevated levels of 2:2 and 0:4 tetrads (Chua and Roeder, 1997), *tid1* mutants do not show the same effect (Shinohara et al., 2003). At face value, the difference in spore viability suggests that *ndj1* mutants are less able to segregate homologs during MI than are *tid1* mutants. However, these experiments were done using different strain backgrounds, complicating direct comparisons between them. We are the first to directly compare the effects of *ndj1* and *tid1* mutants using isogenic strains.

A common interpretation of defects in MI disjunction is that chromosomes which segregate improperly have failed to form a crossover. Failure to form crossovers will result in a higher frequency of E_0 chromosomes. According to this model, E_0 chromosomes arise from a defect in the crossover assurance system. In our study we saw evidence that MI disjunction was working in *tid1* mutants but not in *ndj1* mutants. Applying this model to our results suggests that the increased occurrence of MI nondisjunction in *ndj1* mutants (relative to *tid1* mutants) is due to an increased occurrence of E_0 chromosomes in *ndj1* mutants (relative to *tid1* mutants). In other words, *ndj1* mutants are defective in crossover assurance while *tid1* mutants are not defective in crossover assurance.

However, this model assumes that organisms are incapable of segregating chromosomes in the absence of a crossover. This is not the case. Alternative distribution mechanisms to segregate chromosomes without forming a crossover do exist. This allows for the possibility that the difference in the ability to complete proper MI disjunction may not be due to a defect in crossover assurance, but in the ability to segregate achiasmate chromosomes.

Our lab is working to distinguish whether the difference in the ability to segregate chromosomes during MI in these mutants is due to crossover assurance defects or in the ability to segregate non-exchange chromosomes. The main goal of this investigation was to look at the roles of these proteins in distributive segregation. In a related study, the Hillers lab is investigating whether chromosomes receive the obligate crossover (assurance) in *ndj1* and *tid1* mutants by mapping crossovers along the entire length of a single chromosome.

Before looking at distributive segregation using hybrid strains, we first constructed isogenic *ndj1* and *tid1* mutants in a native strain of *S. cerevisiae* (SK1) to confirm that there is a significant difference in spore viability. Wild type SK1 yeast have been demonstrated to be efficient in the completion of meiosis and are competent in the formation of crossovers. In our study, we found that *ndj1* and *tid1* mutants had different effects on spore viability; *ndj1* mutants have elevated levels of 2:2 and 0:4 tetrads, while *tid1* mutants do not. In addition, the ratio of 2:2 tetrads to 3:1 tetrads was high for *ndj1* mutants (1.69) and below 1 for *tid1* mutants (0.69). The pattern of spore viability in *ndj1* mutants is suggestive of MI nondisjunction. The spore viability pattern in *tid1* mutants is not suggestive of MI nondisjunction. Based on the data from the SK1 strain, it is reasonable to conclude that *ndj1* mutants are defective at MI disjunction. *tid1* mutants do not seem to be defective at MI disjunction.

Nondisjunction in the native SK1 strain can be interpreted in two ways. One interpretation is that *ndj1* mutants result in an increased incidence of E_0 chromosomes, suggesting a defect in the crossover assurance mechanism. This was first proposed by Chua and Roeder (1997). Another interpretation is that *ndj1* mutants also have a diminished ability to segregate chiasmate chromosomes. In order to determine whether crossover assurance and interference are independent mechanisms of crossover regulation, we must look at the incidence of E_0 in *tid1* mutants. If *tid1* mutants retain the ability to secure a crossover for each chromosome pair, we should not see an increase in the incidence of E_0 chromosomes. This will be done in a follow-up experiment by mapping crossovers along the entire length of a single chromosome.

To examine the roles of *NDJ1* and *TID1* in distributive segregation, we generated isogenic *ndj1* and *tid1* mutants in two different strain backgrounds (S288C and Y55), each containing a single homeologous chromosome in an otherwise *S. cerevisiae* background. The homeologous chromosome present in each strain is not expected to form crossovers with its *S. cerevisiae* partner due to sequence divergence. Based on genetic data collected using heterozygous markers on the homeologous chromosomes in the S288C strain, we were able to confirm that recombination events between the homeologs were very infrequent; only one crossover occurred within the interval measured out of 134 4:0 tetrads.

We saw the same spore viability patterns in the mutant hybrid strains as we saw in the mutant native SK1 strain; high levels of 2:2 and 0:4 tetrads in *ndj1* mutants but not in *tid1* mutants. This suggests that *ndj1* and *tid1* mutants differ in their abilities to segregate E_0 chromosomes. Because 0:4 tetrads may arise from a combination of meiotic errors, we will focus on the 2:2 tetrad pattern to focus on MI disjunction. If the elevated levels of 2:2 tetrads are indeed due to MI nondisjunction, *ndj1* mutants must fail to segregate E_0 chromosomes at a higher frequency than *tid1* mutants.

However, since we saw the same spore viability patterns in both homologous and homeologous strains; we propose the possibility that the elevated 2:2 spore viability pattern seen in the hybrid *ndj1* mutants may be due to an inability to segregate chromosomes that fail to form crossovers. In these hybrid strains, the effect of a *ndj1* mutation is compounded when there is a defect in crossover assurance, not independent of it. In other words, in situations in which a pair of chromosomes is liberated from receiving an obligate crossover, we still see MI nondisjunction in *ndj1* mutants.

The hybrid S288C strain best provides us with a model to assay distributive segregation. The hybrid S288C strain used in this study was derived from strains first described by Nilsson-Tillgren et al. (1986). They measured recombination frequency along the entire length of chromosome V and failed to detect crossovers between the homeologs. This was supported by several subsequent studies using the same set of homeologs in the S288C background (Shubochkina et al., 2001; Maxfield Boumil et al., 2003). Based on a failure to detect recombination in the URA3-TRP2 interval on chromosome V in this study, the homeologs in the hybrid S288C strain were assumed to be non-exchange chromosomes. In this study, the pattern of spore viability in hybrid S288C *ndj1* mutants is similar to that seen in native SK1 *ndj1* mutants. If similar spore viability patterns are seen in both the native SK1 and hybrid S288C strains, there is the possibility that *ndj1* mutants have difficulty segregating achiasmate chromosomes. This is in addition to a defect in the crossover assurance mechanism in *ndj1* mutants. As a result, we suggest that one cannot use spore viability patterns as a proxy for crossover assurance.

From this study we were able to conclude that *ndj1* and *tid1* null mutants have different effects on spore viability in isogenic strains. This supports previous experiments but was the first to confirm these results within isogenic strains. We have also demonstrated that *ndj1* and *tid1* mutants are able to segregate non-exchange chromosomes with different efficiency; *ndj1* mutants are defective in distributive segregation while *tid1* mutants do not show the same defect. This is based on the spore viability patterns obtained from three different strain backgrounds. In each strain background, we found that the pattern of spore viability was consistent for *ndj1* mutants; a significantly reduced rate of 4:0 tetrads concomitant with elevated levels of 2:2 and 0:4

tetrads. We failed to detect a similar pattern in any of the *tid1* mutants generated for this study. Instead, the spore viability pattern for *tid1* mutants differed in each strain background. Interestingly, in the hybrid S288C strain (true E_0 chromosomes) we saw a decrease in 4:0 tetrads coupled with an increase in 3:1 only. This spore viability pattern cannot arise through MI nondisjunction, suggesting *tid1* mutants are competent at MI disjunction. The frequency for the rest of the tetrad classes were statistically similar to that seen in the non-mutant strain.

In addition to this study, we will further investigate crossover assurance through a more direct assay. Using strains derived from the ones used in this study, our lab is constructing heterozygous markers along the length of certain chromosomes to build genetic maps in *ndj1* and *tid1* mutants. This will allow us to more directly measure whether crossovers are forming between the homeologs by looking along the entire length of the homeologs.

By using homeologs to increase the incidence of E_0 chromosomes, we have shown that *ndj1* mutants are defective in their ability to carry out distributive segregation. In conjunction with the results from this investigation, the crossover data collected along the length of the homeologs will help us to answer whether these mutants are also defective in crossover assurance. Together, these studies will help elucidate whether two observed phenomena, crossover assurance and crossover interference, are distinct mechanisms working to regulate crossovers during meiosis.

Tables and Figures

Table 1: Haploid yeast strains used in this study.

Strain	Background	Genotype	Source[1]
KHY 140	SK1	MAT a, ho::LYS2, ura3, leu2	NKY 291[2]
KHY 108	SK1	Isogenic to KHY 140 with ndj1::kanMX4	
KHY 123	SK1	Isogenic to KHY 140 with tid1::cloNAT	
KHY 139	SK1	MAT α, ho::LYS2, ura3, trp1	NKY 290[3]
KHY 107	SK1	Isogenic to KHY 139 with ndj1::kanMX4	
KHY 134	SK1	Isogenic to KHY 139 with tid1::cloNAT	
KHY 112	SK1	ho::LYS2, lys2, leu2::hisG, ura3, hom3-10, trp2, tid1::LEU2	DKB 1612[4]
KHY 115	S288C	MAT a, his3Δ, leu2-3, 112 lys2Δ-Bgl2, *S. carlsbergensis* chr. VI v1-Kpn, PAC2::[pD174::LEU2, lacO array]), RAD3, TRP2	TB 105[5]
KHY 105	S288C	Isogenic to KHY 115 with ndj1::kanMX4	
KHY 129	S288C	Isogenic to KHY 115 with tid1::cloNAT	
KHY 116	S288C	MAT α, his3Δ, leu2-3, 112 ade1::ARG4, trp2, cyh2-1, arg4-HpaI, cup1::ura3::THR3; rad3, *S. cerevisiae* chr. VI v1-92, sec3::[pBK13::LEU2 lacO array], ura3::HIS3::[PAFS512:URA3, PrCYC-GFP-lacI], rad3, trp2	TB 109[6]
KHY 127	S288C	Isogenic to KHY 116 with ndj1::kanMX4	
KHY 100	S288C	Isogenic to KHY 116 with tid1::cloNAT	
KHY 113	Y55	MAT a, HIS4-HhaI, leu2-R1, met13-2, lys2-c, ura3-1	Y128[7]
KHY 102	Y55	Isogenic to KHY 113 with ndj1::kanMX4	
KHY 135	Y55	Isogenic to KHY 113 with tid1::cloNAT	
KHY 114	Y55	MAT α, (Y55 with *S. paradoxus* chromosome III) ade1-1, ura3-Nco cyh2R, met13-2, kar1-13	Y597[8]
KHY 138	Y55	Isogenic to KHY 116 with ndj1::kanMX4	Y710[9]
KHY 136	Y55	Isogenic to KHY 115 with tid1::cloNAT	
KHY 144	Y55	MAT α, his4-ATC, trp5-1, CyhR, lys2-c, ade1-1, ΔHO, ura3-1	Y2863[10]
KHY 148	N/A	MAT α, thr 3-10	
KHY 149	N/A	MAT a, lys1-1	

[1]: Parental source of strains used.
[2]: Parental strain derived from NKY 291 supplied by Nancy Kleckner
[3]: Parental strain derived from NKY 290 supplied by Nancy Kleckner
[4]: Parental strain derived from DKB 1612 supplied by Doug Bishop
[5]: Parental strain derived from TB 105 supplied by Dean Dawson via Eva Hoffman
[6]: Parental strain derived from TB 109 supplied by Dean Dawson via Eva Hoffman
[7]: Parental strain derived from Y128 supplied by Eva Hoffman
[8]: Parental strain derived from Y597 supplied by Eva Hoffman
[9]: Parental strain derived from Y710 supplied by Eva Hoffman
[10]: Parental strain derived from Y2863 supplied by Rhona Borts

Table 2: Tetrad analysis of diploid strains

Strain	Test	Number of asci analyzed	Spore Viability (live:dead) 4:0	3:1	2:2	1:3	0:4	Overall Spore Viability (%)[1]	2:3 Ratio[2]
SK1	WT	137	116	12	4	4	1	93.4	0.33
		(% of total)	84.7	8.8	2.9	2.9	0.7		
SK1	ndj1Δ	119	56	13	22	10	18	66.6	1.69
		(% of total)	47.1	10.9	18.5	8.4	15.1		
SK1	tid1Δ	145	60	41	28	15	1	74.8	0.68
		(% of total)	41.4	28.3	19.3	10.3	0.7		
S288C	WT	123	73	19	7	4	20	74.6	0.37
		(% of total)	59.4	15.5	5.7	3.3	16.3		
S288C	ndj1Δ	128	6	9	29	13	71	23.8	3.0
		(% of total)	4.7	7.0	22.7	10.2	55.5		
S288C	tid1Δ	132	57	37	8	3	27	67.8	0.22
		(% of total)	43.2	28.0	6.1	2.3	20.5		
hY55[3]	WT	219	154	35	16	3	6	87.1	0.46
		(% of total)	72.0	16.4	7.5	1.4	2.8		
hY55[3]	ndj1Δ	214	75	32	44	26	42	58.2	1.38
		(% of total)	34.3	14.6	20.1	11.9	19.2		
hY55[3]	tid1Δ	122	33	29	15	18	27	54.1	0.22
		(% of total)	27.1	23.8	12.3	14.8	22.1		
nY55[4]	WT	99	84	7	2	0	6	91.2	0.29
		(% of total)	84.8	7.1	2.0	0	6.1		

[1]: Overall spore viability is calculated by dividing the total number of live spores by four times the total number tetrads dissected.
[2]: 2:3 ratio is calculated by dividing the number of 2:2 tetrads by the number of 3:1 tetrads.
[3]: Hybrid Y55 containing homeologous III derived from S. paradoxus
[4]: Native Y55 containing all homologous chromosomes

Table 3: Genetic analysis of diploid strains

Strain	Test	Interval[1]	Number of tetrads[2] PD	TT	NPD	Total	Map Distance[3] (cM)	Fold Increase[4]
SK1	WT	LEU2-MAT	66	47	2	115	25.65	–
SK1	ndj1	LEU2-MAT	26	26	4	56	44.64	1.74
S288C	WT	URA3-TRP2	73	0	0	73	0	–
S288C	ndj1	URA3-TRP2	5	0	0	5	0	N/A
S288C	tid1	URA3-TRP2	55	1	0	56	0.89	N/A
hY55[5]	WT	LEU2-MAT	133	12	1	146	6.16	–
hY55[5]	ndj1	LEU2-MAT	60	14	0	74	9.46	1.53
hY55[5]	tid1	LEU2-MAT	29	4	0	20	6.06	0.98
nY55[6]	WT	LEU2-MAT	54	28	2	84	23.81	–

[1]: Genetic interval tested for recombination frequency. See Figures 3&4 for schematic description.
[2]: Number of tetrads with given segregation pattern as determined through dissection; PD – parental ditype; TT – tetratype; NPD – non-parental ditype (See Materials and Methods for descriptions).
[3]: Map Distance was calculated using the Perkins formula (1949) and is described in detail in the Materials and Methods section.
[4]: Ratio of map distance calculated in mutants to map distance in non-mutant isogenic strains.
[5]: Hybrid Y55 containing homeologous III derived from S. paradoxus
[6]: Native Y55 containing all homologous chromosomes.

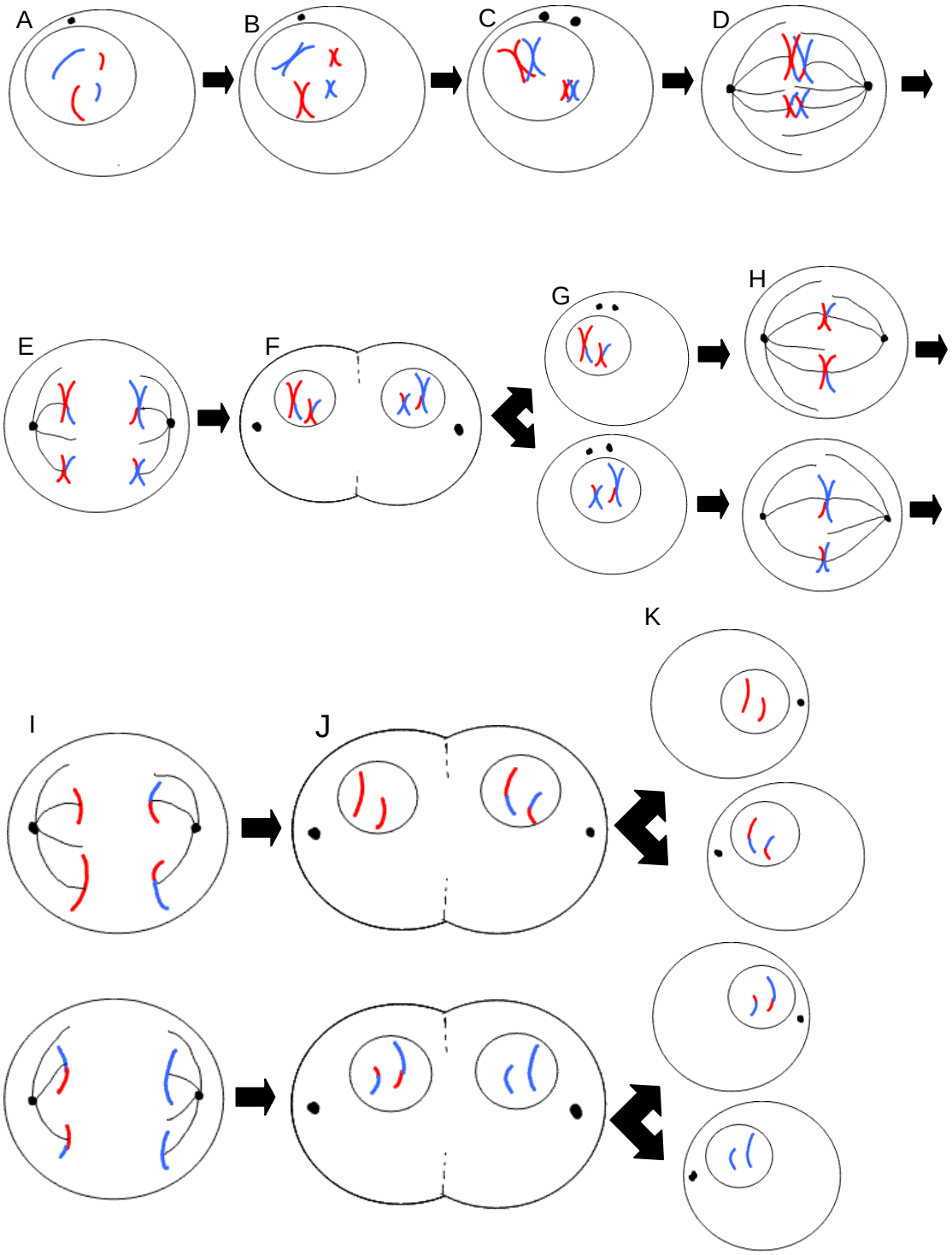

Figure 1 – General stages of meiosis of a generic 2n diploid cell. A - Interphase cell prior to chromosome duplication. B – S phase cell after duplication of DNA. C – Prophase I cell where chromosome pairing and recombination occur. It is through recombination between homologous chromosomes that tension is established during this phase. The spindle pole body (black dots) is also duplicated. D – Metaphase I cell with paired homologs lined up on the metaphase plate down the middle of the cell. Microtubules emanating from the spindle pole bodies attach to centromere proteins to set

the cell up for the reductional division. E – Anaphase I cell with homologous chromosomes segregating away from one another. F – Telophase I/Cytokinesis in which the cell begins to divide itself to become two haploid cells. The nucleus begins to reform and cell membrane is deposited to fully divide the two cells. G – Prophase II cell is similar to a mitotic prophase cell. The spindle pole body is duplicated and the chromosomes adopt a condensed structure. H – Metaphase II cell in which chromosomes line up on the metaphase plate. Microtubules from the spindle pole bodies attach to centromere of chromosomes in order to separate sister chromatids. I – Anaphase II cell where sister chromatids are pulled towards opposite poles. J – Telophase II/Cytokinesis where the cells begin to separate to create four new daughter cells. K – Upon the completion of meiosis a single diploid cell has generated four unique haploid cells. In yeast, these cells are packaged into an ascus; a protective sack to help spores survive stressful conditions.

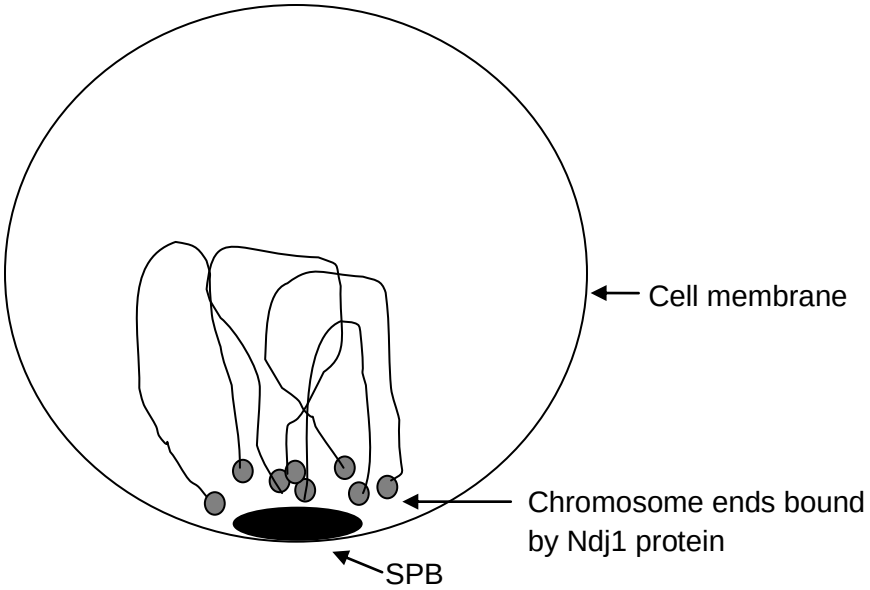

Figure 2 – Telomere Bouquet formation during zygotene. Figure adapted from Burgess, 2002.

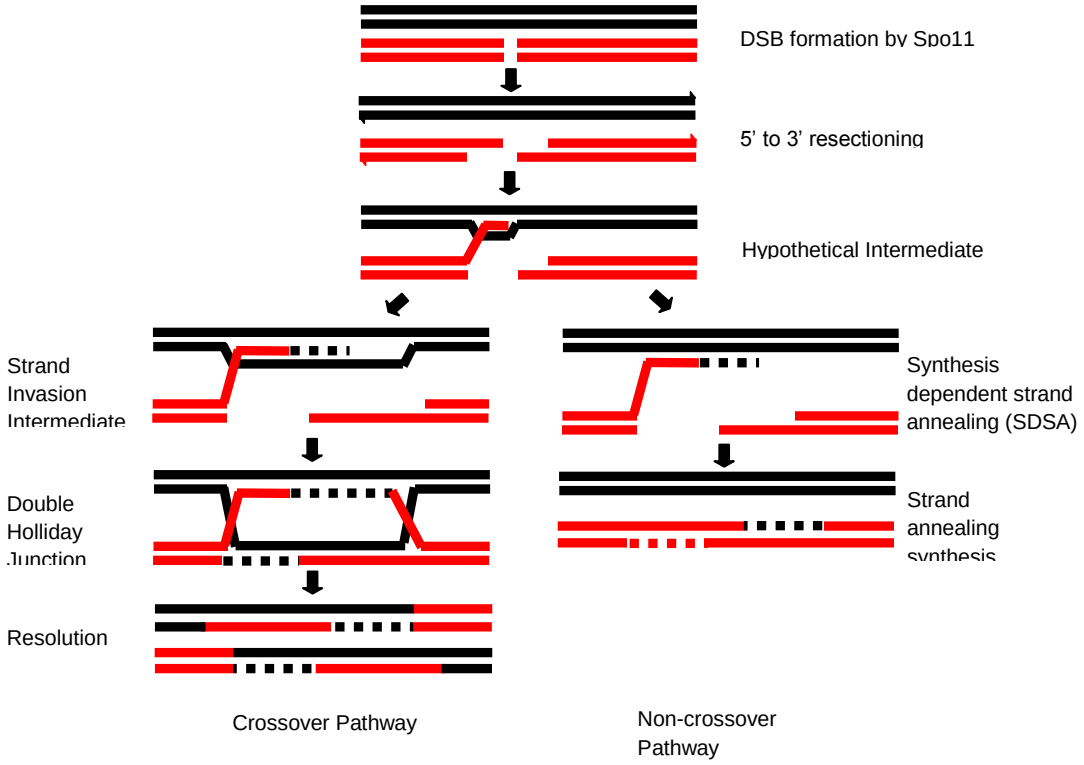

Figure 3 – Double strand break repair leading to crossovers or non-crossovers. Figure adapted from original description by Szostak et al. (1983) and revised by Nassif et al. (1994).

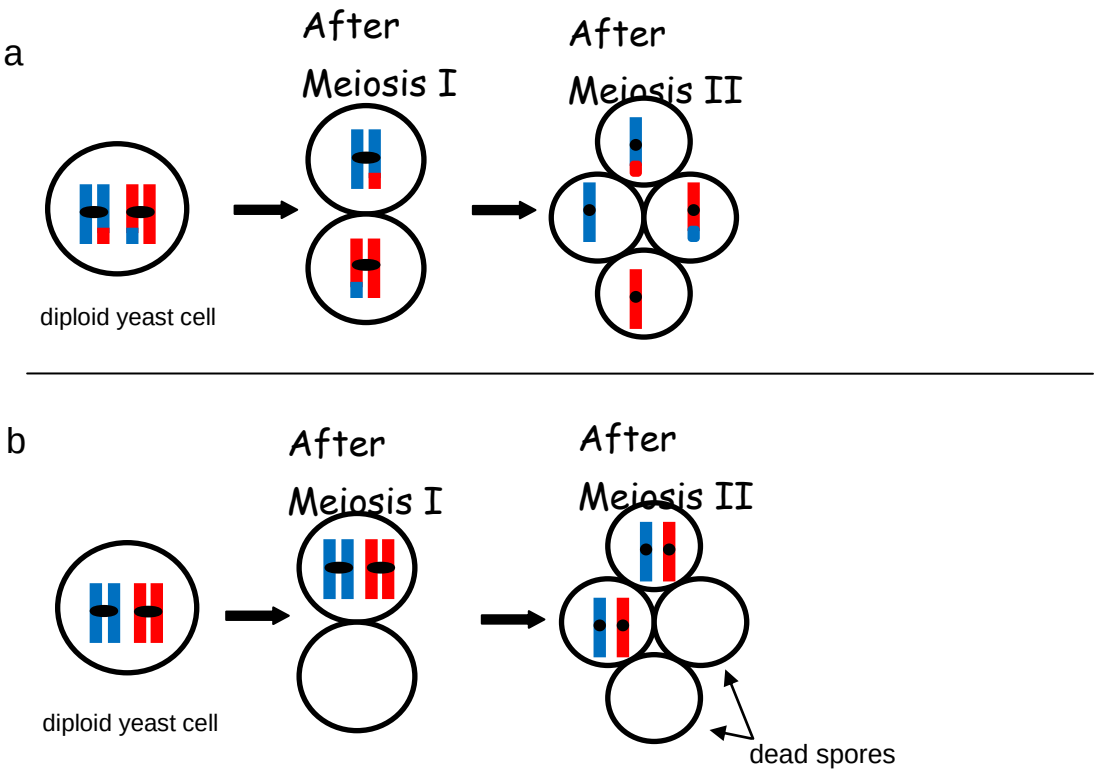

Figure 4 – Chromosome segregation patterns during Meiosis I. Diploid yeast cells are induced into meiosis and produce four haploid cells that are packaged into an ascus and known as a tetrad. In panel a, a crossover between homologous chromosomes precedes normal MI disjunction and produces four viable spores. In panel b, a failure to form a crossover between one pair of chromosomes results in MI nondisjunction with homologs migrating to the same pole. This results in a tetrad with only two viable spores.

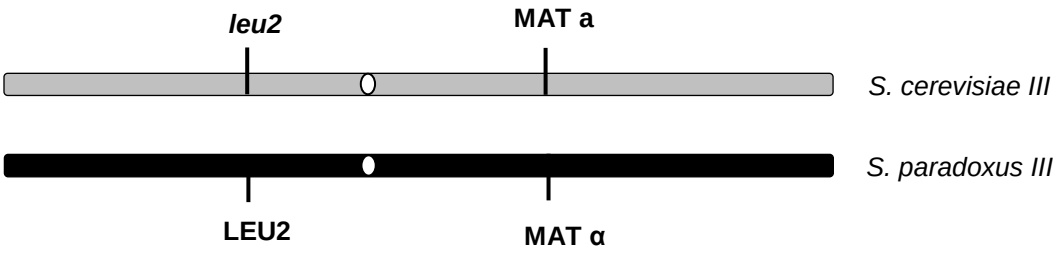

Figure 5a – Homeologous chromosomes used in the hybrid Y55 strain.

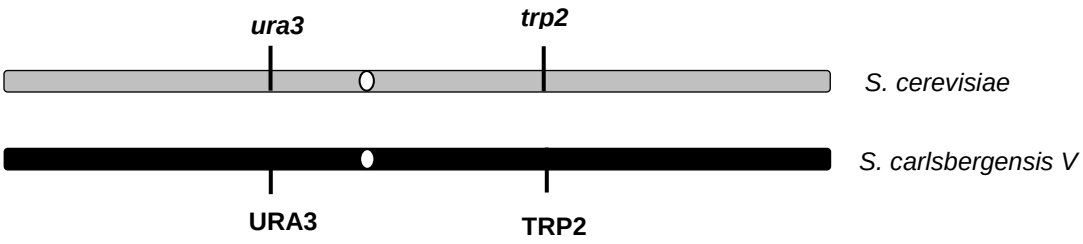

Figure 5b – Homeologous chromosomes used in the hybrid S288C strain.

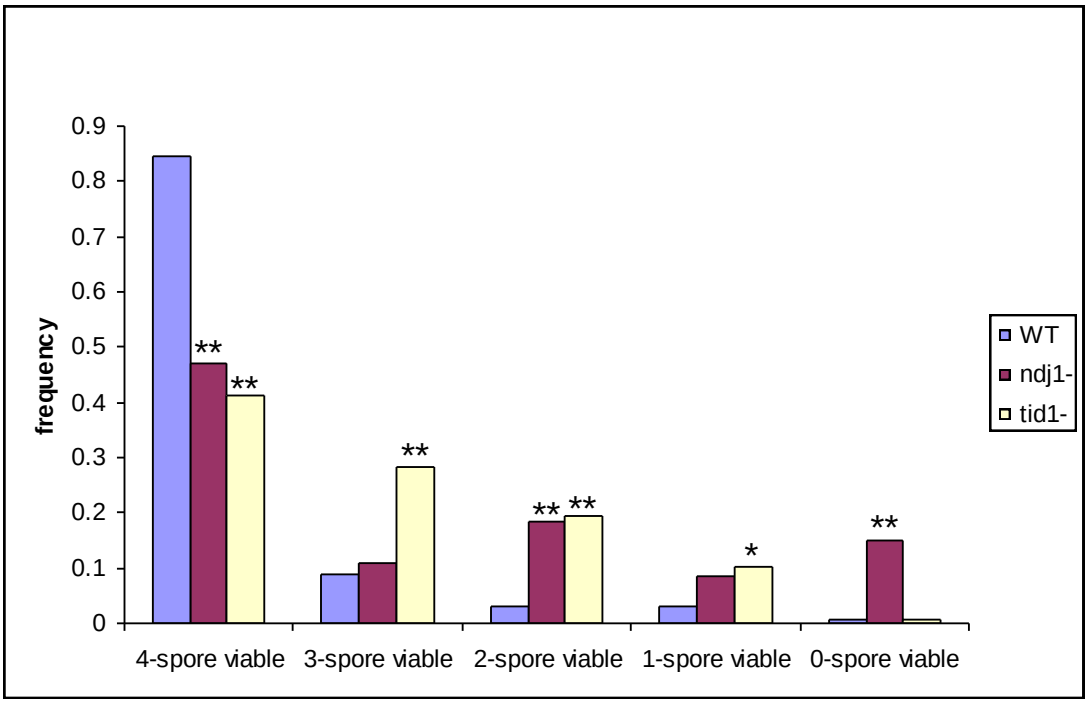

Figure 6 – Spore viability of isogenic native SK1 strains. Native SK1 strains contain all homologous chromosomes. Isogenic ndj1 and tid1 mutants were constructed and spore viability was measured through tetrad analysis. Asterisks represent significant differences compared to the non-mutant strain (* = $P < 0.05$; ** = $P < 0.001$).

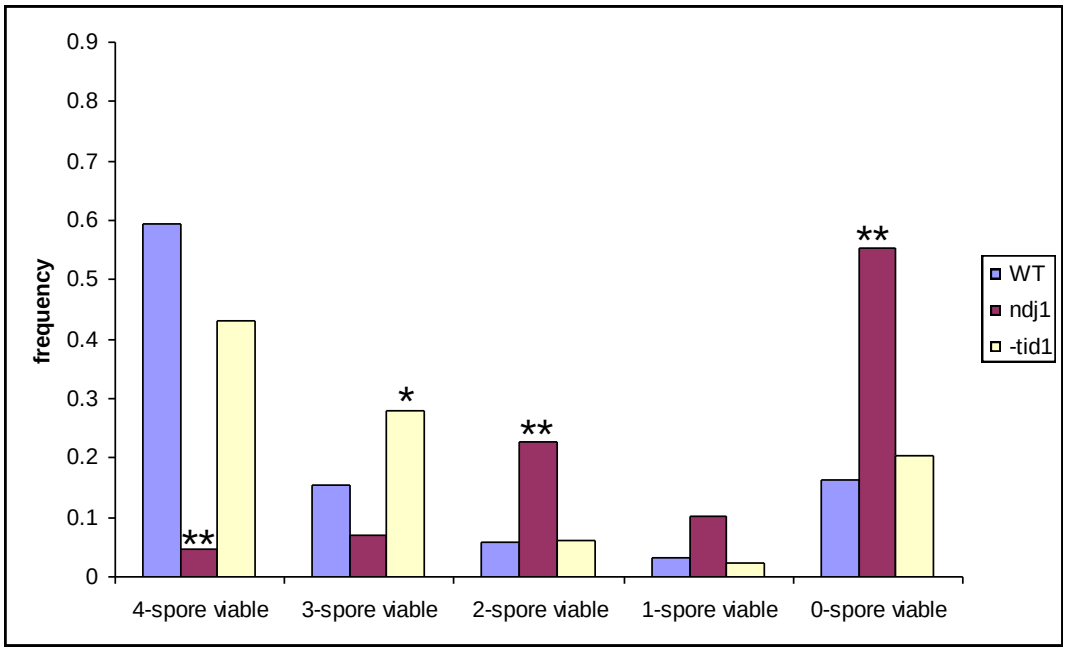

Figure 7 – Spore viability of the hybrid S288C strains. Hybrid S288C strains contain a homeologous chromosome V derived from *S. carlsbergensis* in an otherwise *S. cerevisiae* background. Isogenic *ndj1* and *tid1* mutants were constructed and spore viability was measured through tetrad analysis. Asterisks represent significant differences compared to the non-mutant strain (* = $P < 0.05$; ** = $P < 0.001$).

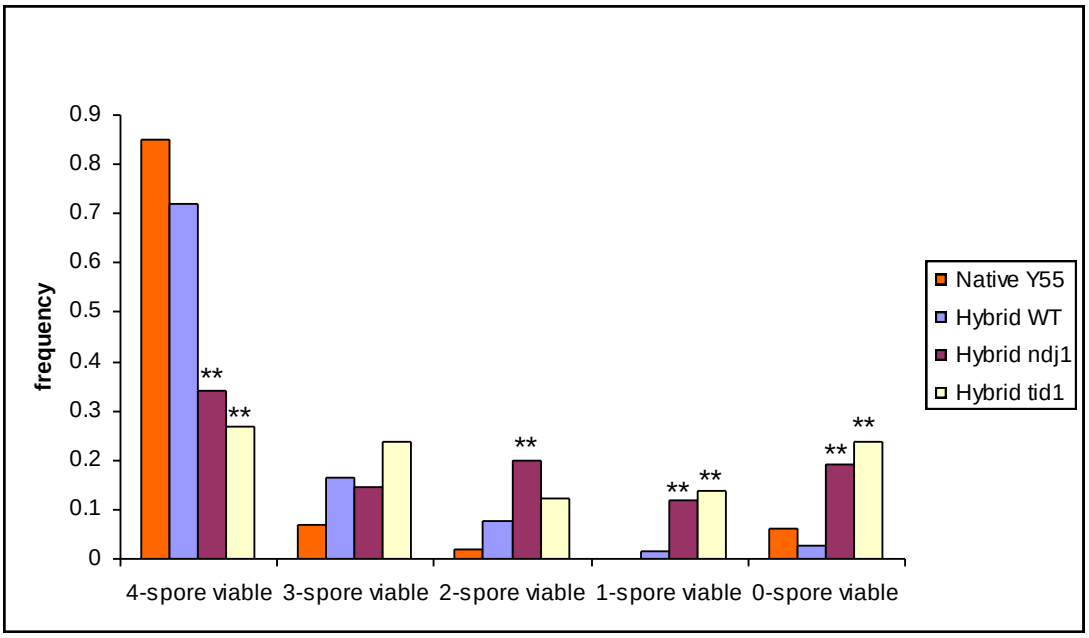

Figure 8 – Spore viability of the Y55 strains. Native Y55 strains contain all homologous chromosomes. Hybrid Y55 strains contain a homeologous chromosome III derived from *S. paradoxus* in an otherwise *S. cerevisiae* background. Isogenic *ndj1* and *tid1* mutants were constructed and spore viability was measured through tetrad analysis. Asterisks represent significant differences between mutant hybrid strains and the non-mutant hybrid (** = $P < 0.001$).

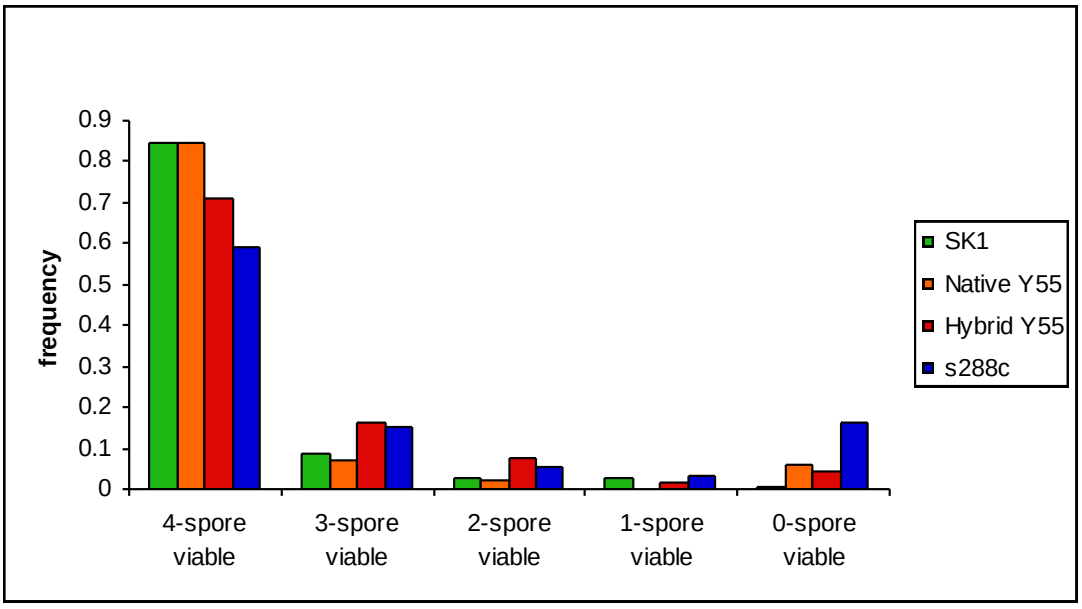

Figure 9 – Comparison of spore viability between non-mutant versions of all strains used in this study. Native Y55 strains contain all homologous chromosomes. Native SK1 strains contain all homologous chromosomes. Hybrid Y55 strains contain a homeologous chromosome III derived from *S. paradoxus* in an otherwise *S. cerevisiae* background. Hybrid S288C strains contain a homeologous chromosome V derived from *S. carlsbergensis* in an otherwise *S. cerevisiae* background.

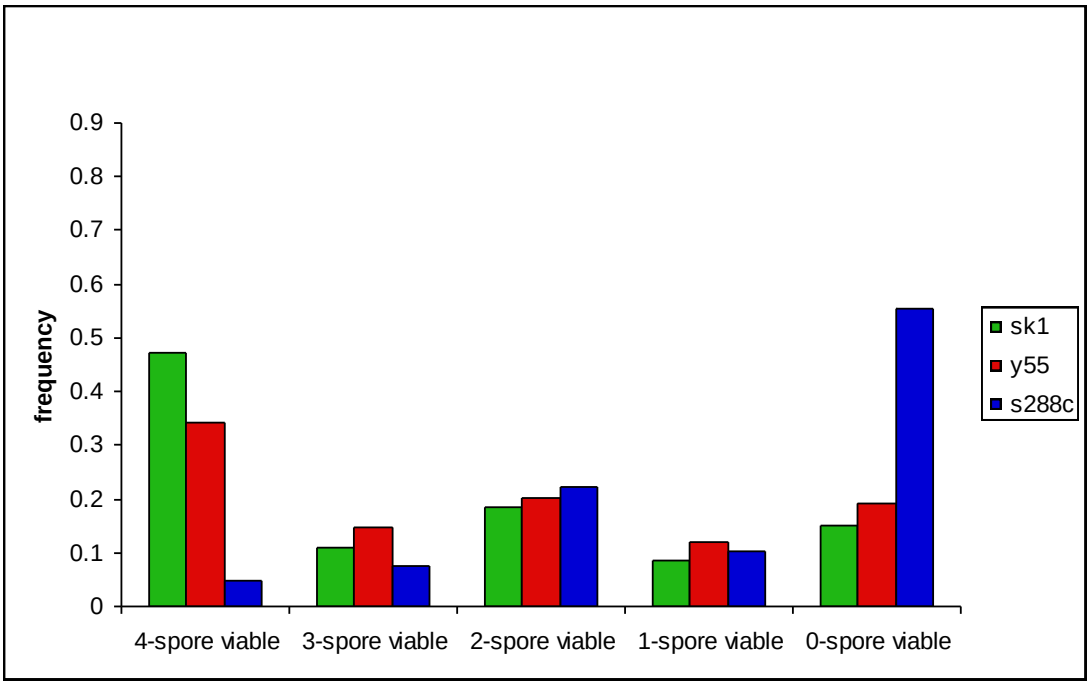

Figure 10 – Comparison of spore viability between *ndj1* mutant versions of all strains used in this study. Native Y55 strains contain all homologous chromosomes. Native SK1 strains contain all homologous chromosomes. Hybrid Y55 strains contain a homeologous chromosome III derived from *S. paradoxus* in an otherwise *S. cerevisiae* background. Hybrid S288C strains contain a homeologous chromosome V derived from *S. carlsbergensis* in an otherwise *S. cerevisiae* background.

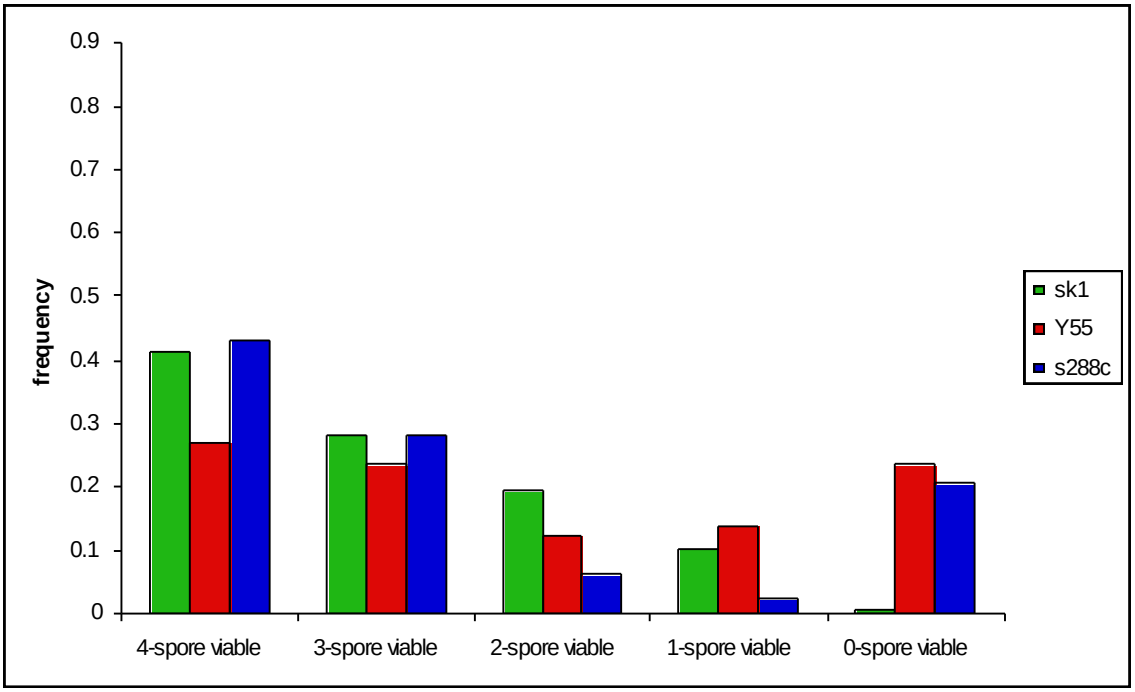

Figure 11 – Comparison of spore viability between *tid1* mutant versions of all strains used in this study. Native Y55 strains contain all homologous chromosomes. Native SK1 strains contain all homologous chromosomes. Hybrid Y55 strains contain a homeologous chromosome III derived from *S. paradoxus* in an otherwise *S. cerevisiae* background. Hybrid S288C strains contain a homeologous chromosome V derived from *S. carlsbergensis* in an otherwise *S. cerevisiae* background.

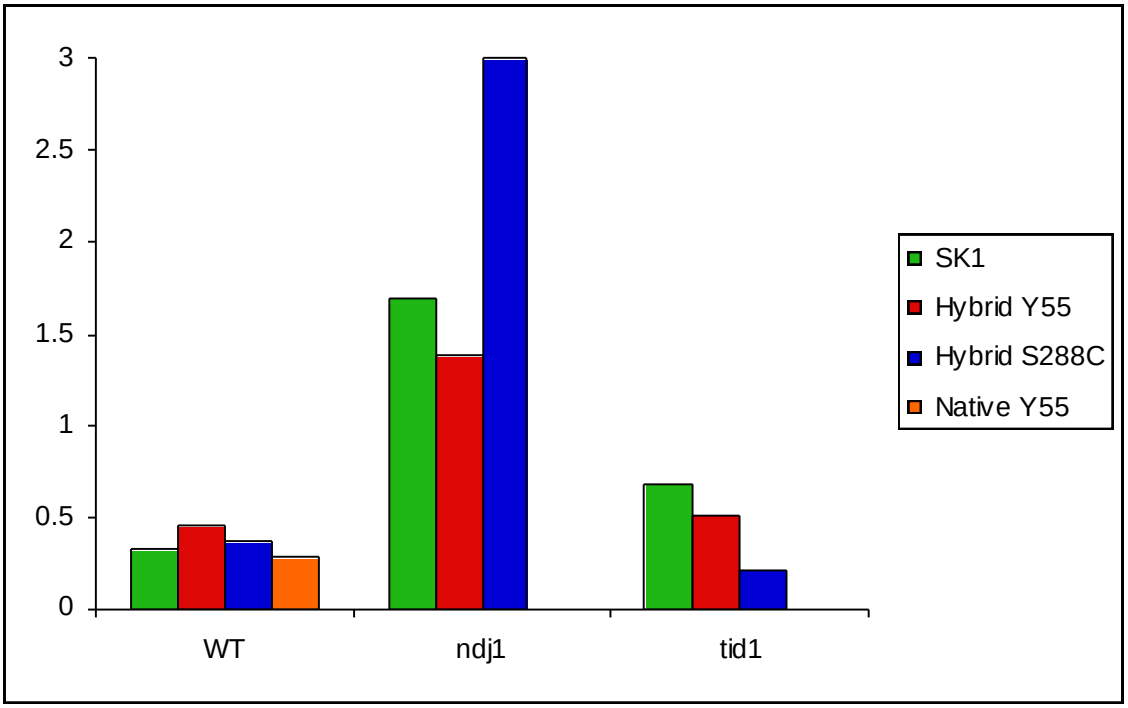

Figure 12 – Comparison of 2:3 live spore ratios in all strains used in this study. The number of 2:2 tetrads was divided by the number of 3:1 tetrads produced within each strain. Native Y55 strains contain all homologous chromosomes. Native SK1 strains contain all homologous chromosomes. Hybrid Y55 strains contain a homeologous chromosome III derived from *S. paradoxus* in an otherwise *S. cerevisiae* background. Hybrid S288C strains contain a homeologous chromosome V derived from *S. carlsbergensis* in an otherwise *S. cerevisiae* background.

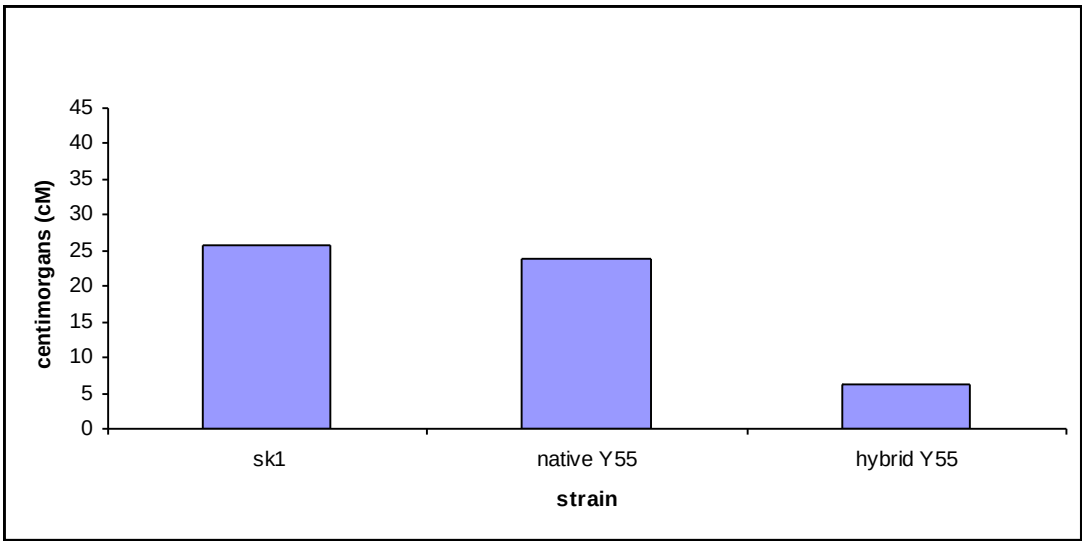

Figure 13 – Comparison of map distance (cM) within the native SK1 and Y55 strains and the hybrid Y55 strain in the LEU2-MAT interval on chromosome *III*. Native Y55 strains contain all homologous chromosomes. Native SK1 strains contain all homologous chromosomes. Hybrid Y55 strains contain a homeologous chromosome III derived from *S. paradoxus* in an otherwise *S. cerevisiae* background.

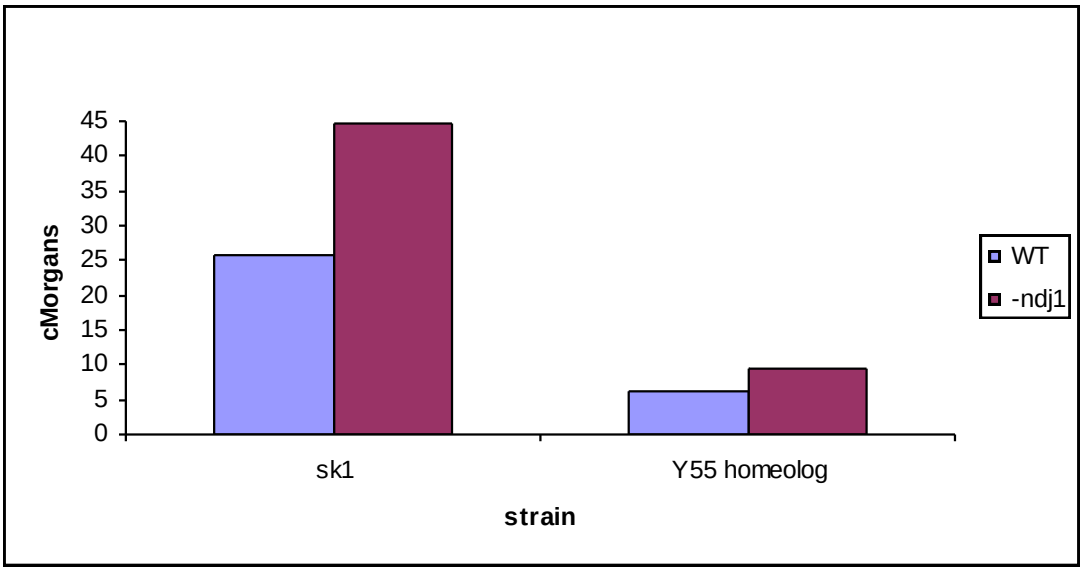

Figure 14 – Comparison of map distance (cM) within the native SK1 and hybrid Y55 strains in the LEU2-MAT interval on chromosome *III*. Native SK1 strains contain all homologous chromosomes. Hybrid Y55 strains contain a homeologous chromosome III derived from S. paradoxus in an otherwise S. cerevisiae background.

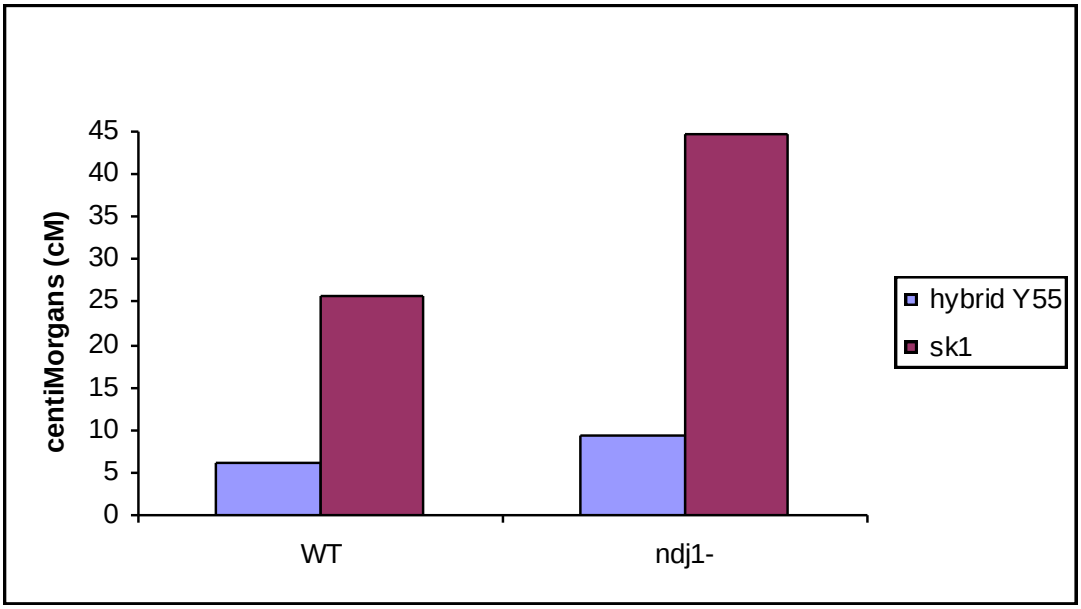

Figure 15 – Comparison of map distance (cM) separated by mutation in the LEU2-MAT interval on chromosome *III*. Native SK1 strains contain all homologous chromosomes. Hybrid Y55 strains contain a homeologous chromosome III derived from *S. paradoxus* in an otherwise *S. cerevisiae* background. Genetic markers were not analyzed in the *tid1* SK1 strain

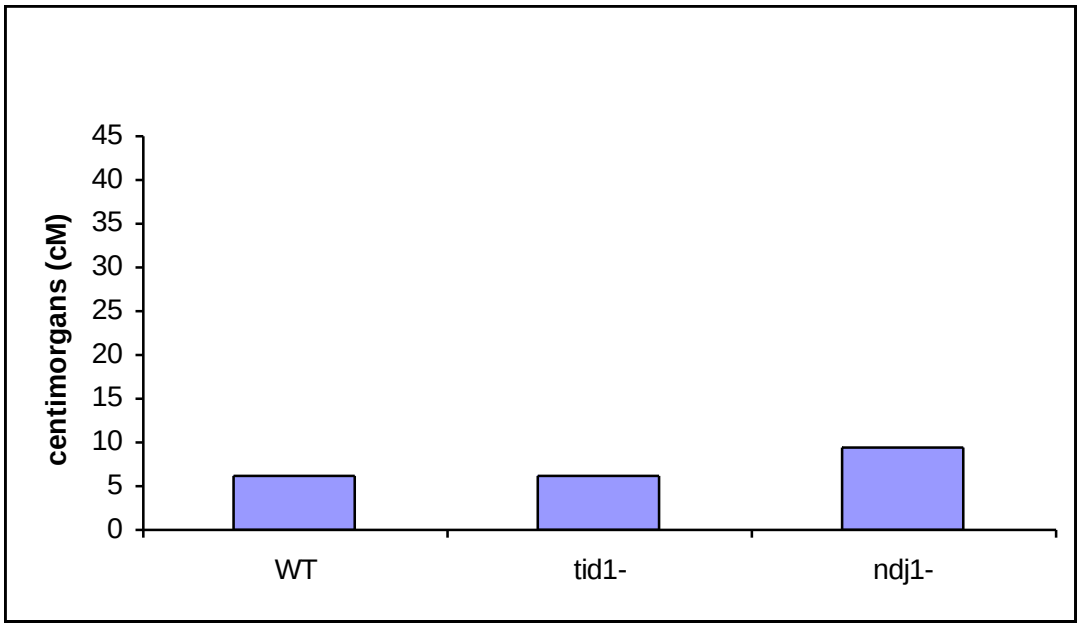

Figure 16 – Comparison of map distance (cM) within the hybrid Y55 strain only in the LEU2-MAT interval on chromosome *III*. Hybrid Y55 strains contain a homeologous chromosome III derived from S. paradoxus in an otherwise S. cerevisiae background.

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