Stony Brook University

The roles of Mek1 and cohesins in suppressing meiotic intersister double-strand break repair

A Dissertation Presented

by

Tracy Liane Callender

to

The Graduate School

in Partial Fulfillment of the

Requirements

for the Degree of

Doctor of Philosophy

in

Molecular and Cellular Biology

(Cellular and Developmental Biology)

Stony Brook University

August 2010

Stony Brook University

The Graduate School

Tracy Liane Callender

We, the dissertation committee for the above candidate for the

Doctor of Philosophy degree, hereby recommend

acceptance of this dissertation.

Nancy M. Hollingsworth, Ph.D.
Dissertation Advisor
Professor, Department of Biochemistry and Cell Biology

Aaron Neiman, Ph.D.
Chairperson of Defense
Associate Professor, Department of Biochemistry and Cell Biology

Bruce Futcher, Ph.D.
Professor, Department of Molecular Genetics and Microbiology

J. Peter Gergen, Ph.D.
Professor, Department of Biochemistry and Cell Biology

Scott Keeney, Ph.D.
Member and Professor, Howard Hughes Medical Institute, Molecular Biology Program, Memorial Sloan-Kettering Cancer Center

This dissertation is accepted by the Graduate School

Lawrence Martin
Dean of the Graduate School

Abstract of the Dissertation

The roles of Mek1 and cohesins in suppressing meiotic intersister double-strand break repair

by

Tracy Liane Callender

Doctor of Philosophy

in

Molecular and Cellular Biology

(Cellular and Developmental Biology)

Stony Brook University

2010

Reciprocal exchange between homologs, in combination with sister chromatid cohesion, promotes proper segregation of homologs at Meiosis I. Missegregation of chromosomes during meiosis causes abnormal chromosome numbers in gametes. Errors in meiotic chromosome segregation can lead to infertility and, in humans, are responsible for genetic disorders like Trisomy 21.

Meiotic recombination is initiated by double-strand breaks (DSBs). Repair of these breaks is biased to occur by invasion of homologs, not sister chromatids. This bias is mediated both by a meiosis-specific recombinase, Dmc1 and by the suppression of Rad51/Rad54 mediated recombination between sister chromatids. In *dmc1Δ* mutants, DSBs fail to get repaired, triggering a recombination checkpoint resulting in a prophase arrest. Mek1, a meiosis-specific kinase activated by DSBs, is required to prevent DSB repair using sister chromatids.

To confirm that inactivation of Mek1 promotes intersister repair, two-dimensional gel analysis was used to look at intersister and interhomolog joint molecules (JMs). Inactivation of a conditional allele of Mek1 in a *dmc1*Δ background resulted exclusively in intersister JMs. To determine whether *MEK1* suppression of intersister repair requires the presence of homologs, DSB repair was analyzed in haploid strains containing *mek1*Δ and *dmc1*Δ. The finding that *dmc1Δ* haploids have unrepaired breaks that are repaired in the absence of *MEK1* demonstrates that the mechanism by which *MEK1* suppresses intersister repair is specific to sister chromatids. Interestingly, repair in wild-type haploids is either delayed or absent. Comparing DSB repair at different hotspots in disomic haploid and haploid strains, I found that DSBs are repaired only on the disomic chromosomes, indicating that repair on different chromosomes occurs independently.

REC8 encodes a meiosis-specific subunit of cohesin. DSB repair in *rec8Δ* diploids is impaired during meiosis. DSB repair was analyzed in diploid and haploid strains containing *rec8*Δ and *rec8*Δ *mek1*Δ. DSBs were efficiently repaired when *MEK1* is absent, indicating that *REC8* is specifically required for interhomolog repair.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

MI:	Meiosis I
MII:	Meiosis II
DSB:	Double-strand break
P:	Parental fragment
CO:	Crossover
NCO:	Non-Crossover
dHJ:	Double Holliday Junction
SDSA:	Synthesis Dependent Strand Annealing
SC:	Synaptonemal complex
AE:	Axial element
JM:	Joint Molecule
IH:	Interhomolog
IHJM:	Interhomolog joint molecule
ISJM:	Intersister joint molecule
as:	analog sensitive
ATP:	Adenosine 5'-triphosphate
1-NA-PP1:	4-amino-1-*tert*-butyl-3-(1'naphthyl) pyrazolo [3,4-*d*] pyrimidine
ssDNA:	single strand DNA
WT:	wild-type
1D:	One-dimensional
2D:	Two-dimensional
Spo:	Sporulation medium (2% potassium acetate)

List of Abbreviations continued

SPS:	pre-sporulation medium
SPM:	sporulation medium (1% potassium acetate)
YEPD:	rich medium
OD:	optical density
DAPI:	4’6-diamidino-2-phenylindole
UV:	ultraviolet
EDTA:	Ethylenediamine tetraacetic acid
ml:	milliliter
µl:	microliter
min:	minute
hr:	hour
µg:	microgram
mg:	milligram

ACKNOWLEDGEMENTS

I would like to express a heartfelt thanks to my advisor Nancy Hollingsworth, who has been an exceptional mentor and friend during my graduate career. Throughout the years, she has given me incredible guidance scientifically, sometimes even more than is expected and I have gained so much knowledge from her. She has also been very supportive with my life and career choices and always has great advice to share. I would also like to acknowledge my committee members, Aaron Neiman, Peter Gergen, Scott Keeney and Bruce Futcher for their scientific guidance and suggestions over the past years. I would like to thank AGEP/TURNER for their support and mentorship. I would like to thank my previous and current lab members, Hengyao Niu, Emily Job, Caroline Park, Hsiao-Chi Lo, Allison Marullo, Yan Liu and honorary lab member, Marlies Rossmann for thoughtful suggestions, help with experiments and creating a wonderful lab environment. I want to specially thank Lihong Wan, for her patience and help with experiments and for all that she has taught me over the years. In addition I would like to thank the members of the Dean and Neiman labs for kindly sharing equipment and reagents and Neil Hunter for sharing strains and protocols. I would like to thank my family: my parents, Sheila and Roland, and sister, Tricia for their continued support in my educational endeavors; my aunt Esterlene who gave me the opportunity to come to the USA and pursue my dreams; Lisa, Karen and Deighton for always being there at times when I needed a shoulder to lean on. To Yamileh, Azad, Emily, Calvin, Banke and Ada thanks for being great friends and for great advice. Lastly, I would like to specially thank and share this accomplishment with Delon, my wonderful and supportive husband.

CHAPTER ONE

INTRODUCTION

I. Chromosome segregation in meiosis and its significance

Meiosis is the specialized cell division that produces the haploid gametes required for sexually reproducing organisms. In mammals these gametes are sperm and eggs and in yeast they are spores. Meiosis is characterized by one round of DNA replication followed by two consecutive rounds of chromosome segregation, termed meiosis I (MI) and meiosis II (MII) respectively. This causes a two-fold reduction in the chromosome number in the diploid parental cell to haploid progeny.

In vegetative cells, chromosomes are duplicated at S-phase and the sister chromatids are held together by conserved protein complexes called cohesins (GUACCI *et al.* 1997; MICHAELIS *et al.* 1997). For sister chromatids to segregate to opposite poles at mitosis, chromosomes are aligned at metaphase with sister centromeres directed to opposite spindle poles. This alignment is monitored by the spindle checkpoint which senses the tension created when the sister chromatids are pulled in opposite directions (LI and NICKLAS 1995). When all the chromosomes are aligned correctly, cohesin complexes are cleaved along the arms of the chromosomes and anaphase begins (CIOSK *et al.* 1998; UHLMANN *et al.* 1999; UHLMANN *et al.* 2000) (Figure 1-1A).

One of the hallmarks of meiosis is that in the first division, homologous pairs of sister chromatids segregate to opposite poles (Figure 1-1B-iii). In most organisms, proper segregation at MI requires that several meiosis-specific events occur. First, reciprocal recombination creates crossovers between homologous chromosomes. Secondly, meiosis-specific cohesin complexes are loaded onto chromosomes prior to replication (KLEIN *et al.* 1999). The combination of recombination and sister chromatid cohesion connects the homologs, such that tension can be created when the microtubules

from opposing spindle poles attach to the kinetochores of the connected homologs, allowing the correct alignment during Metaphase I (BASCOM-SLACK *et al.* 1997). Thirdly, sister centromeres must be mono-oriented to the same spindle pole. This is achieved by the localization of a protein complex known as monopolin to the kinetochores (TOTH *et al.* 2000). A failure in any of these processes results in chromosome missegregation at MI, causing homologous chromosomes to segregate randomly. This missegregation is responsible for infertility in humans and results in aneuploidy in gametes producing inviable progeny (ROEDER 1997). In the cases where aneuploidy can be tolerated, the resulting chromosome imbalances may cause disorders such as Trisomy 21 or Down syndrome, which is the leading cause of mental retardation in the United States (CHAMPION and HAWLEY 2002). It should be noted that there are some systems which do not require crossovers for segregation, such as male fruit flies and the female silk moth (SCOTT HAWLEY 2002).

II. Cohesins and segregation

Proper segregation in both mitosis and meiosis requires that chromosomes align properly during metaphase. For this to occur sister chromatids must be connected by protein complexes called cohesins (Figure 1-2) (GUACCI *et al.* 1997; MICHAELIS *et al.* 1997). The mitotic cohesin multi-subunit complex is comprised of four proteins: Scc1/Mcd1, Scc3 and two structural components Smc1 and Smc3 (Figure 1-2A) (GUACCI *et al.* 1997; LOSADA *et al.* 1998; MICHAELIS *et al.* 1997; TOTH *et al.* 1999). This complex forms a structure which has been proposed to form a ring around the DNA to prevent chromosome segregation until the onset of anaphase upon which the DNA is released

(GRUBER *et al.* 2003; UHLMANN *et al.* 1999). One model proposed for how cohesins load onto chromosomes is through the opening of the Smc1 and Smc3 hinge (GRUBER *et al.* 2006). It is thought that cohesins load onto chromosomes just prior to replication, but it is during replication that the connections needed to create cohesion are established, this is known as S-phase cohesion (UHLMANN and NASMYTH 1998). When all the chromosomes are correctly attached to the spindle, the Mcd1 subunits of the cohesin complex are cleaved to trigger anaphase (Figure 1-2B) (UHLMANN *et al.* 1999; UHLMANN *et al.* 2000). This cleavage is done by a cysteine protease called separase, whose activation is mediated by a multi-subunit ubiquitin ligase called the anaphase promoting complex (APC) or cyclosome. Inactive separase exists in a complex with an inhibitory protein, securin (CIOSK *et al.* 1998). Once bi-orientation has occurred, the APC complex is activated by Cdc20, which causes degradation of securin via the proteasome, relieving the inhibition of separase to cleave the α-kleisin subunit Mcd1 (the α-kleisin subunit is the key target of separase in yeast cells and it is replaced during meiosis), allowing release of sister chromatids (COHEN-FIX *et al.* 1996; UHLMANN *et al.* 2000).

Since there are two rounds of chromosome segregation in meiosis, cohesin cleavage must be regulated so that cohesion can be lost in a stepwise manner. This two step removal is possible because of the replacement of Mcd1, with a meiosis-specific subunit, Rec8 (KLEIN *et al.* 1999). The other proteins in the complex remain the same (Figure 1-2A). At anaphase I, separase cleaves the Rec8 localized along the arms of the chromosomes, allowing homologs to separate reductionally (Figure 1-1B-iv) (BUONOMO *et al.* 2000). At this time, the centromeric Rec8 cohesin complexes are protected from cleavage by two proteins, Spo13 and Shugoshin (Sgo1) (KATIS *et al.* 2004; KITAJIMA *et*

al. 2004; LEE *et al.* 2004). This protection of centromeric Rec8 cohesins during the first division is important to prevent sister chromatids from separating precociously in MI (KLEIN *et al.* 1999). In metaphase II, the sister kinetochores on each homolog are bi-oriented and the centromeric cohesins are then cleaved to allow the sister chromatids to segregate equationally upon the onset of anaphase II (Figure 1-1B-v).

Recently, it was discovered that in addition to holding sister chromatids together, cohesins also play a role in DNA repair in vegetative cells. When a double-strand break (DSB) is made during the G2 phase of the cell cycle, cohesins are recruited to the break and this 'DSB cohesion' is enough to hold the sister chromatids together even if S-phase cohesion is inactive (STROM *et al.* 2004; UNAL *et al.* 2004). This G2 cohesion has been shown to be mediated by the recruitment of Mcd1 cohesin complexes to the DSBs.

III. Recombination

A. Mitotic Recombination

One of the differences between DNA repair in mitotic and meiotic cells is the template choice through which DSBs are repaired. During vegetative growth, DSBs can be created from errors that occur during replication or from exogenous DNA damage caused by ultraviolet light, X-rays or chemicals. For the cell to maintain its genomic integrity, there is a bias towards using sister chromatids to repair DSBs, perhaps because their identical sequences make repair very conservative (BZYMEK *et al.* 2010; KADYK and HARTWELL 1992). This repair is mediated by the RecA-like recombinase Rad51 (PAQUES and HABER 1999). During mitotic recombination, the 5' ends of a DSB are resected to produce 3' single stranded ends. These ends are covered by the single-strand

binding protein, RPA, which removes secondary structure. Rad51 replaces RPA, with the help of the mediator proteins, Rad52 and Rad55/57 (GASIOR *et al.* 1998). Rad51 is then able to catalyze the invasion of sister chromatids, in combination with the accessory protein, Rad54, which is required primarily for sister chromatid recombination (Figure 1-3) (ARBEL *et al.* 1999; PAQUES and HABER 1999; PETUKHOVA *et al.* 1998). Following strand invasion, Rad54 has also been shown to remove Rad51 from the DNA duplex to allow for DNA synthesis and ligation (SOLINGER *et al.* 2002; SUGAWARA *et al.* 2003).

B. Meiotic Recombination

In contrast to mitotically dividing cells, during meiosis DSBs are deliberately created by an evolutionary conserved, meiosis-specific, topoisomerase-like enzyme, Spo11 (KEENEY *et al.* 1997). Spo11 introduces DSBs non-randomly throughout the genome at regions on the DNA known as recombination 'hotspots'. Spo11 cleavage has been shown to occur primarily in nucleosome free areas, but is not sequence dependent (BLITZBLAU *et al.* 2007; BUHLER *et al.* 2007; DE MASSY and NICOLAS 1993; WU and LICHTEN 1995). These programmed breaks initiate reciprocal exchange of DNA between homologous chromosomes. Resection is initiated upon the removal of the covalently linked Spo11, attached to a short oligonucleotide, by an endonucleolytic process (NEALE *et al.* 2005). The 5' ends of the DNA on either side of the breaks are resected in a process that is dependent on *SAE2/COM1* and the *RAD50/MRE11/XRS2* endonuclease complex of proteins (ALANI *et al.* 1990; MCKEE and KLECKNER 1997; PRINZ *et al.* 1997). Resection results in 3' single strand overhangs (Figure 1-4 C, D). Similar to vegetative cells, strand invasion during meiotic recombination is promoted by the Rad51

recombinase (BISHOP 1994; SCHWACHA and KLECKNER 1997; SHINOHARA *et al.* 1997a). A meiosis-specific recombinase, Dmc1, another RecA ortholog, facilitates interhomolog recombination (BISHOP *et al.* 1992). These two proteins form filaments on the 3' end of the breaks and preferentially catalyze strand invasion of non-sister chromatids (Figure 1-4E). Dmc1's loading onto the DNA is mediated by the accessory proteins, Mei5/Sae3, Hop2/Mnd1 and Tid1/Rdh54 (paralog of Rad54) (HAYASE *et al.* 2004; PETUKHOVA *et al.* 2005; SHINOHARA *et al.* 2000; TSUBOUCHI and ROEDER 2004). After strand invasion, DNA synthesis extends the invading strand. Capture of the second end followed by ligation creates a recombination intermediate containing a double-Holliday junction (dHJ), which is then resolved to produce crossovers (COs) (Figure 1-4F) (ALLERS and LICHTEN 2001). Non-crossover (NCO) chromosomes are generated by synthesis-dependent strand annealing (SDSA), where the extended invading strand is displaced and anneals to the single strand from the other side of the break (Figure 1-4G). In SDSA, there is no reciprocal exchange of DNA. Most CO products originate from the dHJ pathway, which is mediated by the meiosis-specific MutS homolog pair Msh4/Msh5 (HOLLINGSWORTH *et al.* 1995), but an alternative and independent pathway, involving the structure-specific endonuclease, Mus81/Mms4, also produces a subset of crossovers (DE LOS SANTOS *et al.* 2003).

Approximately 160 DSBs are created per meiosis and studies in yeast have shown that about 90 of those breaks are processed into COs, while approximately 20 of them become NCOs (MANCERA *et al.* 2008; CHEN *et al*. 2008) (S. Keeney, personal communication). The remaining breaks may be repaired using the sister chromatid via the Rad51/Rad54 pathway. In organisms that use recombination to promote segregation,

there must be at least one crossover per chromosome and 'crossover homeostasis' ensures that the cell maintains normal levels of COs even in the case where DSB formation might be reduced (MARTINI *et al.* 2006). The number of NCOs decreases to allow for this to occur.

C. Meiotic interhomolog bias

In meiosis, proper segregation of chromosomes requires that crossovers occur between homologs. In meiotic cells, therefore, the bias for which template is used during DSB repair is changed from sister chromatids to non-sister chromatids (SCHWACHA and KLECKNER 1997). Interhomolog bias is created in part by the recombinase activity of Dmc1. In *dmc1Δ* mutants, DSBs form and become resected, but fail to undergo strand invasion of either homologs or sister chromatids (BISHOP *et al.* 1992; HUNTER and KLECKNER 2001). The accumulation of hyperresected breaks triggers the meiotic recombination checkpoint and the cells arrest in prophase (LYDALL *et al.* 1996). Increasing the amount of Rad51 activity in a *dmc1Δ* mutant, either by overexpression of *RAD51* or *RAD54*, or by deletion of *HED1*, a meiosis-specific gene encoding a protein that binds to Rad51 and interferes with Rad54 binding, partially suppresses the interhomolog recombination defect (BISHOP *et al.* 1999; BUSYGINA *et al.* 2008; TSUBOUCHI and ROEDER 2003; TSUBOUCHI and ROEDER 2006). This indicates that Dmc1 is not absolutely required for interhomolog strand invasion and maybe it is just needed to increase the level of recombinase activity. A fact that strengthens this idea is that some organisms such as worms and fruit flies, also have Spo11 catalyzed recombination events, but lack a Dmc1 ortholog, instead they utilize the Rad51 recombinase alone

(VILLENEUVE and HILLERS 2001). In *dmc1Δ* mutants, Rad51 foci assemble normally on the chromosomes, yet there is no DSB repair using the sister chromatid (BISHOP 1994). This observation suggests that interhomolog bias is created in part by actively suppressing Rad51/Rad54 activity from repairing breaks using sister chromatids.

D. *MEK1*-mediated suppression of meiotic intersister DSB repair

The suppression of meiotic intersister DSB repair is dependent on a meiosis-specific protein complex encoded by *RED1*, *HOP1* and *MEK1* (BAILIS and ROEDER 1998; BISHOP *et al.* 1999; DE LOS SANTOS and HOLLINGSWORTH 1999; NIU *et al.* 2005; WAN *et al.* 2004; WOLTERING *et al.* 2000; XU *et al.* 1997). These proteins associate with the axial elements (AEs), a cytological structure that forms by the condensation of pairs of sister chromatids along protein cores during meiotic prophase (BAILIS and ROEDER 1998; HOLLINGSWORTH *et al.* 1990; SMITH and ROEDER 1997). The AEs of homologous chromosomes are connected to form the proteinaceous structure known as the synaptonemal complex (SC) (PAGE and HAWLEY 2004). *RED1* is required for AE formation (ROCKMILL and ROEDER 1990), while in *hop1Δ* mutants, some pieces of the AEs form but the chromosomes fail to synapse (HOLLINGSWORTH *et al.* 1990; LOIDL *et al.* 1994). Although AEs and some regions of the SC have been observed in *mek1*Δ mutants in some strain backgrounds (BAILIS and ROEDER 1998), the synapsis defect is more severe in the SK1 strain background used in my studies (J. Loidl, personal communication). This is consistent with the more severe spore inviability defect observed in SK1 strains (LEEM and OGAWA 1992). Red1, Hop1 and Mek1 form a complex *in vivo*. Hop1 and Red1 interaction has been shown by yeast two-hybrid assays

and co-immunoprecipitation, as well as Red1 and Mek1 interactions (BAILIS and ROEDER 1998; DE LOS SANTOS and HOLLINGSWORTH 1999; WAN *et al.* 2004; WOLTERING *et al.* 2000). Further studies have shown that Mek1, Hop1 and Red1 complex formation is necessary for wild-type levels of Mek1 kinase activity, suggesting that Mek1 acts downstream of the other two proteins to suppress meiotic intersister DSB repair (NIU *et al.* 2007; WAN *et al.* 2004).

Mek1 is a serine/threonine protein kinase whose activation is coupled to the formation of DSBs. Mek1 is activated when DSB dependent phosphorylation of Hop1 promotes Mek1 dimerization and auto-phosphorylation (CARBALLO *et al.* 2008; NIU *et al.* 2005). Several pieces of evidence support the idea that Mek1 suppresses intersister DSB repair during meiosis. First, *mek1Δ* decreases interhomolog recombination and at the same time increasing recombination between sister chromatids (HOLLINGSWORTH *et al.* 1995; NIU *et al.* 2005; THOMPSON and STAHL 1999). Furthermore, Mek1 kinase activity is required in *dmc1Δ* diploids to prevent Rad51-mediated DSB repair using sister chromatids as templates (NIU *et al.* 2005; NIU *et al.* 2009; WAN *et al.* 2004; XU *et al.* 1997). Mek1 phosphorylates Rad54 on threonine 132 both *in vivo* and *in vitro*, thereby reducing its binding affinity and subsequent activity of Rad51 (NIU *et al.* 2009). The *MEK1*-dependent phosphorylation of Rad54 acts in combination with Hed1 to inhibit Rad51-Rad54 complex formation during meiosis (BUSYGINA *et al.* 2008; TSUBOUCHI and ROEDER 2006). Preventing Rad54 phosphorylation in *dmc1*Δ mutants results in interhomolog recombination and the formation of some viable spores. This phenotype differs from that of *mek1*Δ which produces dead spores due to repair by using sister chromatids. There must therefore be a second substrate of Mek1 which inhibits strand

invasion even when Rad51-Rad54 complexes are formed (NIU *et al.* 2009). These data indicate that interhomolog bias during meiosis is created by (1) inhibiting Rad54 activity by preventing interaction with Rad51; it is important to note that Rad54 phosphorylation and Hed1-Rad51 interaction are redundant mechanisms achieving the same result and therefore are not 'independent' processes that promote IH bias; (2) inhibition of Rad51-Rad54 strand invasion of sister chromatids by an as yet unidentified substrate and (3) the activity of Dmc1.

The goal of my thesis research was to determine some of the biological parameters that are required for Mek1's suppression of intersister repair by asking the following questions: (1) Does the Rad51-mediated repair observed in *dmc1*Δ *mek1-as1* diploids occur exclusively between sister chromatids and does it proceed via a JM intermediate? (2) Does the mechanism by which Mek1 suppresses meiotic intersister DSB repair require the presence of homologous chromosomes? (3) What are the roles of Rad51 and Dmc1 in interhomolog and intersister recombination and how does Mek1 regulate the two recombinases? (4) What is the involvement of the meiosis-specific cohesin, Rec8, in the *MEK1*-mediated suppression of intersister DSB repair?

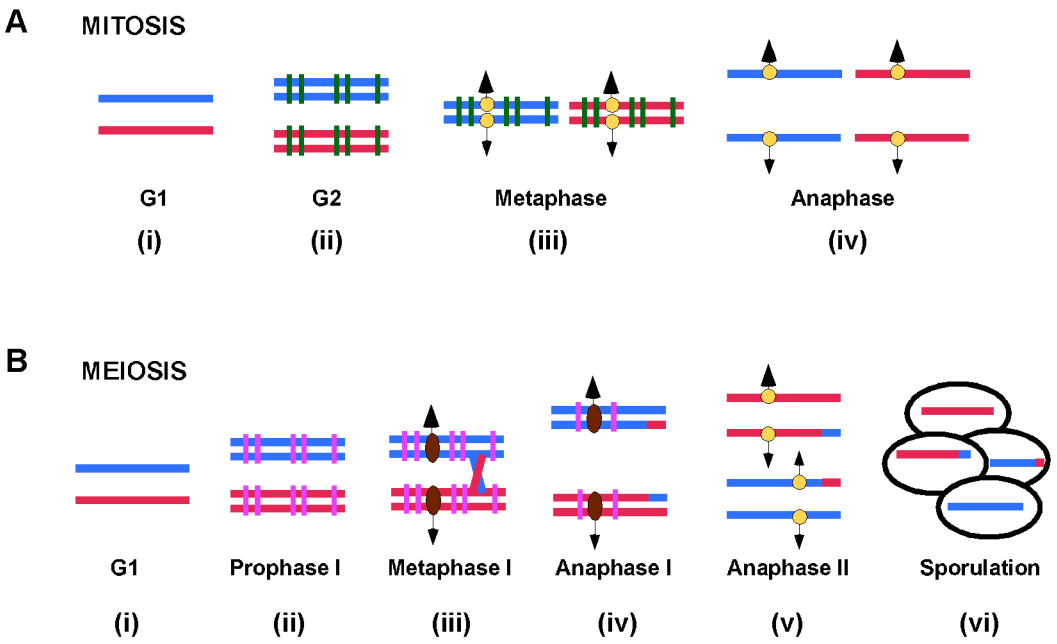

Figure 1-1. Chromosome segregation in mitotic and meiotic cells. Blue and red lines represent two homologous chromosomes, respectively. (A) (i) A diploid cell before entering mitosis. (ii) During DNA replication pairs of sister chromatids are connected by S-phase cohesins containing Mcd1 (green lines). (iii) At metaphase the sister kinetochores are bi-oriented (yellow circles). (iv) The cohesins are cleaved and sister chromatids segregate to opposite poles. (B) (i) A diploid cell before entering meiosis containing a single pair of homologous chromosomes. (ii) During pre-meiotic replication, pairs of sister chromatids are connected by the meiosis-specific cohesins containing Rec8 (purple lines). (iii) A recombination event between the non-sister chromatids physically connects the homologs. (iv) At MI, the sister kinetochores are mono-oriented (brown circles) and arm cohesins are cleaved allowing homologs to segregate to opposite poles. (v) At MII, centromeric cohesins are lost allowing sister chromatids to segregate to opposite poles. (vi) In yeast the four meiotic products are packaged in a sac called an ascus.

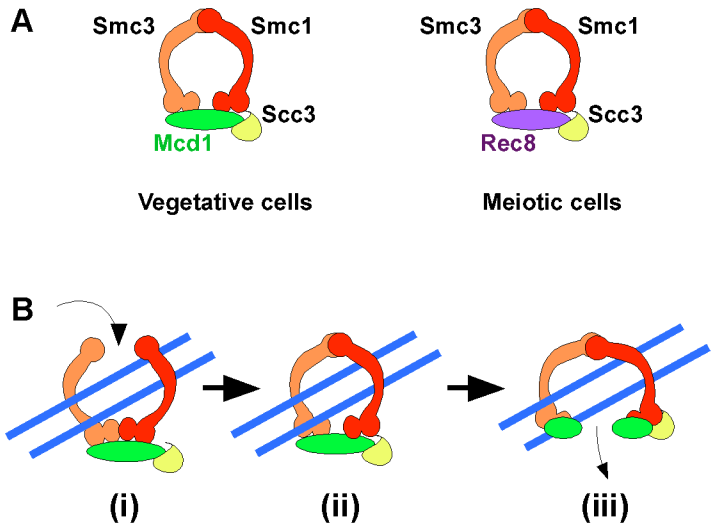

Figure 1-2. Cohesin structure and cleavage. (A) The cohesin complex is comprised of four proteins. In meiotic cells, Mcd1 is replaced with the meiosis-specific subunit, Rec8. (B) (i) In vegetative or meiotic cells, cohesion is established by insertion of sister chromatids (blue lines) into the cohesin ring by opening of the hinge between the Smc1 and Smc3 subunits. (ii) Cohesion is maintained during replication until the onset of anaphase. (iii) Cohesion is removed by cleavage of Mcd1 (or Rec8) by separase, releasing the sister chromatids.

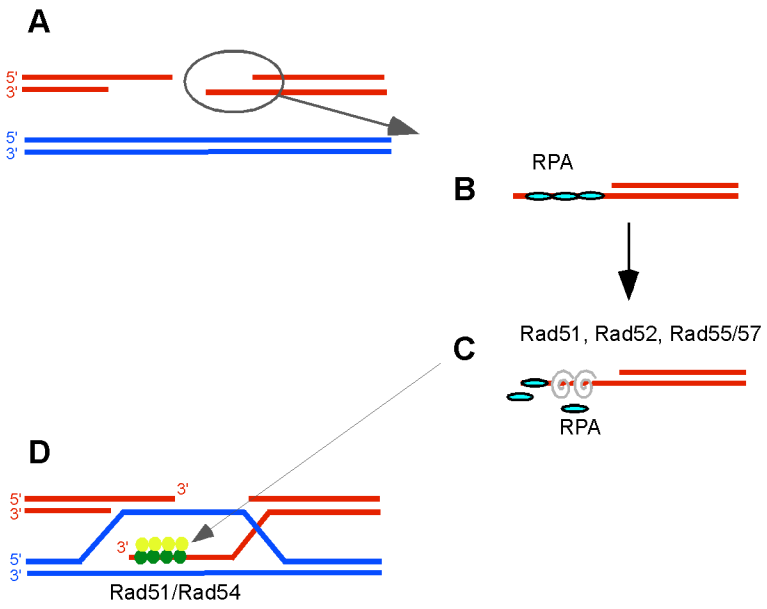

Figure 1-3. Rad51 mediated strand invasion during mitotic recombination. (A) DSBs get resected to produce 3' single strand (ss) tails. (B) ss tails are coated with RPA (blue ovals). (C) The mediator proteins, Rad52, Rad55 and Rad57 promote replacement of RPA with the recombinase Rad51. (D) Rad54 interacts with Rad51 (yellow and green circles) to facilitate strand invasion of the sister chromatid.

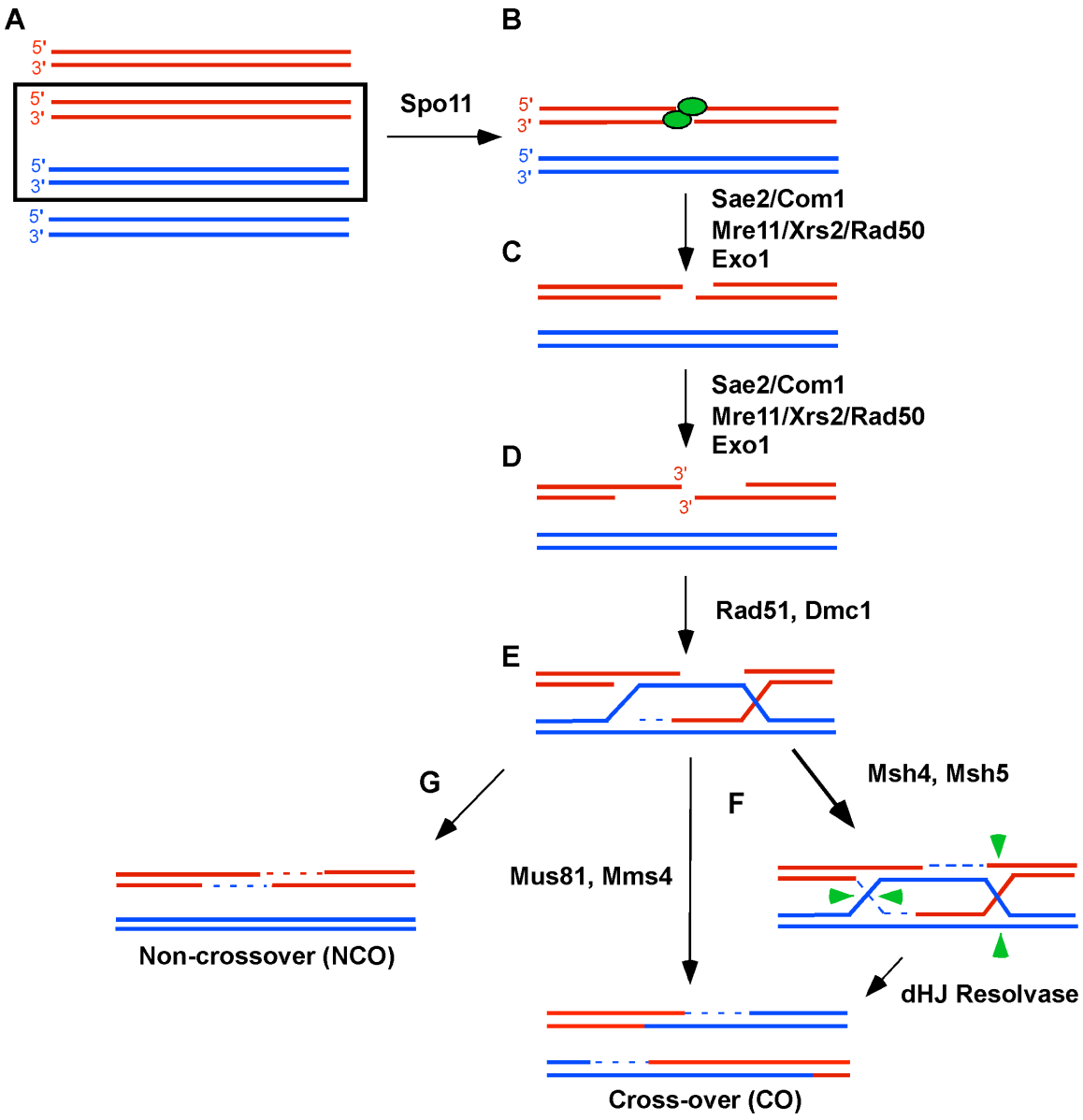

Figure 1-4. A model for meiotic recombination. (A) Pre-meiotic DNA replication of a pair of homologous chromosomes results in four chromatids. The two non-sister chromatids in the black box are going to recombine. Each chromatid is shown as a duplex of DNA. (B) DSB formation is catalyzed by Spo11 (green ovals). (C, D) Spo11 is removed from DSB ends by protein complexes containing Sae2/Com1 and/or Mre11/Xrs2/Rad50 and Exo1. The same complex is involved in the resection of the 5' ends of the DSBs to produce 3' single-strand tails. (E) Rad51 and Dmc1 catalyze strand invasion of non-sister chromatids. (F, G) Recombination intermediates are processed into either non-crossover or cross-over products. Crossover formation can result from either resolving dHJs or through a Mus81-Mms4 mediated pathway.

CHAPTER TWO

Mek1 kinase activity suppresses Rad51-mediated intersister double-strand break repair in a *dmc1*Δ mutant

INTRODUCTION

In meiosis, there is a preference for homologs over sister chromatids in deciding which template will be used for DSB repair (NIU *et al.* 2005; SCHWACHA and KLECKNER 1997; SHERIDAN and BISHOP 2006; WAN *et al.* 2004). When meiotic DSBs are repaired using sister chromatids, failure to physically connect homologs prior to MI causes non-disjunction and inviable spores. Studies of mutations in *HOP1*, *RED1* and *MEK1* have suggested that these genes have important roles in suppressing meiotic intersister DSB repair (HOLLINGSWORTH *et al.* 1995; ROCKMILL and ROEDER 1990; THOMPSON and STAHL 1999). Determining whether *HOP1* plays a role in interhomolog bias has been difficult because in the SK1 strain background *hop1Δ* mutants have a reduction in recombination and DSB formation, more severe phenotypes than those observed in *red1Δ* or *mek1Δ* mutants (PECINA *et al.* 2002; WOLTERING *et al.* 2000). *red1Δ* mutants in the *dmc1Δ* background result in the disappearance of DSBs and the production of dead spores (BISHOP *et al.* 1999; SCHWACHA and KLECKNER 1997; XU *et al.* 1997). However, because of the pleiotropic effects of the *red1* mutation, such as decreased DSBs along with defects in chromosome synapsis, the interpretation of this mutant in understanding meiotic interhomolog bias has also been problematic (ROCKMILL and ROEDER 1990; XU *et al.* 1997).

Inactivation of Mek1 kinase activity after the formation of DSBs in *dmc1Δ* diploids causes DSBs to disappear, thereby suppressing the prophase arrest and producing dead spores (NIU *et al.* 2005; WAN *et al.* 2004). This observation indicated that Mek1 kinase activity in the *dmc1Δ* background may be preventing intersister repair.

The disappearance of DSBs in *dmc1*Δ mutants after inactivation of *MEK1* may therefore be due to DSB repair using the sister chromatid resulting in MI non-disjunction. However, the spore inviability observed could also be as a result of extensive DNA resection. Genetic studies have suggested that *mek1Δ* mutants repair breaks via the sister chromatid and are defective in interhomolog recombination. These studies involved the use of the *spo13Δ* mutant (NIU *et al.* 2005; THOMPSON and STAHL 1999; WAN *et al.* 2004). *SPO13* is a meiosis-specific gene that has been defined as a key regulator of meiotic chromosome segregation. It has been shown to be responsible for the protection of centromeric cohesins in MI, for maintaining monopolin complexes at kinetochores as well as for the reaccumulation of securin (KATIS *et al.* 2004; LEE *et al.* 2004). Cells homozygous for *spo13Δ* undergo a single meiotic division, in which some chromosomes segregate reductionally while others undergo equational segregation. This single division produces diploid spores, thereby eliminating the need for interhomolog crossovers to direct segregation (HOLLINGSWORTH and BYERS 1989; HUGERAT and SIMCHEN 1993; KLAPHOLZ and ESPOSITO 1980).

A chemical genetic strategy to create conditional kinases has been developed by Shokat and colleagues. Mutation of a conserved residue with a bulky side chain in the ATP binding pocket of the kinase of interest to an amino acid with a side chain like glycine or alanine may create more space, without compromising the ability of the kinase to use ATP. Derivatized purine analogs may fit into the enlarged binding pocket in the *analog-sensitive* (*as*) kinase but not the wild-type kinase. This renders the *as* kinase sensitive to small molecule inhibitors (BISHOP *et al.* 1998).

This approach was successfully applied to Mek1. Glutamine 241 (Q241) of the ATP binding site of Mek1 was mutated to glycine (G) to create a conditional allele called *mek1-as1*. Mek1-as1 kinase activity can be inhibited by the addition of 1-NA-PP1 to meiotic cells (WAN *et al.* 2004). In the absence of inhibitor, *mek1-as1* behaves like the wild-type kinase and spores are highly viable. In contrast, addition of inhibitor to a *mek1-as1* diploid produces inviable spores due to intersister DSB repair. The inactivation of Mek1 in a *mek1-as1 dmc1Δ* diploid after DSB formation and prophase arrest results in the repair of DSBs via Rad51/Rad54 activity resulting in inviable spores (WAN *et al.* 2004). The fact that the inactivation of Mek1 in *mek1-as1 dmc1Δ* diploids overexpressing *RAD51* results in intersister repair further supports the idea that Mek1 prevents Rad51/Rad54 from invading sister chromatids (NIU *et al.* 2005).

Mek1 kinase inactivation in *mek1-as1 dmc1Δ spo13Δ* mutants, showed an increase in spore viability when compared to *mek1-as1 dmc1Δ* mutants, 46.7% versus 3% respectively. This increase in spore viability suggests that the disappearance of DSBs in *mek1Δ* mutants is due to repair and not degradation (NIU *et al.* 2005). Measurement of recombination in the viable spores formed in *mek1-as1 dmc1Δ spo13Δ* diploids with 1-NA-PP1 showed that interhomolog recombination was decreased, whereas intersister recombination was increased compared to wild type. THOMPSON and STAHL (1999) also showed by genetic assays that *red1Δ* and *mek1Δ* mutants increase intersister recombination in *spo13*Δ diploids. While these genetic studies provide strong support for the idea that *MEK1* suppresses intersister DSB repair, the caveats are (1) they require the use of viable spores to detect recombinants and therefore the results are based on only a

subset of cells in the sporulated culture and (2) the *spo13* meiosis may have effects on recombination that have not yet been revealed.

In budding yeast, there are well defined physical assays that allow for the identification and monitoring of different intermediates in meiotic recombination (HUNTER and KLECKNER 2001; SCHWACHA and KLECKNER 1995; SUN *et al.* 1989). The *HIS4/LEU2* locus on chromosome III is an engineered meiotic recombination hotspot that allows for the identification of CO and NCO products using Southern blots (CAO *et al.* 1990; XU and KLECKNER 1995). This hotspot was created by insertion of a 2.8 kb *LEU2* segment adjacent to the *HIS4* locus in the SK1 strain background (CAO *et al.* 1990). At this locus, there are XhoI restriction site polymorphisms between the parental homologs, referred to as 'Mom' and 'Dad', that produce the different sized fragments which distinguish the parental and CO fragments. In addition, joint molecules (JMs) both between homologs (IHJMs) and between sister chromatids (ISJMs) can be detected after crosslinking the DNA strands with the chemical psoralen, which stabilizes the double Holliday junction intermediates by covalently joining single DNA strands. This prevents the JMs from branch migrating off the ends of the fragments after restriction digestion (BELL and BYERS 1983; SCHWACHA and KLECKNER 1994). In the first dimension, molecules are separated based on their size by low voltage electrophoresis through low percentage agarose. In the second dimension, the molecules are separated on the basis of size and shape, by increasing both the voltage and agarose concentration (Figure 2-1B) (SCHWACHA and KLECKNER 1997). The linear molecules, such as the parental and CO fragments form an arc in the second dimension, while non-linear species like the single-end invasions and the JMs, are retarded because of their shape and therefore run slower

through the agarose and lie above the arc (Figure 2-1B). To determine the identity of the strands, the DNA molecules from the recombination species were dissociated and their single strands analyzed. It was identified that the single strands were uninterrupted and had parental sequences (SCHWACHA and KLECKNER 1994; 1995). The origin of the strands was also identified by BELL and BYERS (1983), where branched molecules from meiotic DNA were isolated and their features suggested that they were recombination intermediates. A diagram of the various intermediates and meiotic products is shown in Figure 2-1 (HUNTER and KLECKNER 2001).

Assaying intersister recombination by physical analysis of JM formation eliminates the caveats that exist for the genetic studies. I therefore chose to ask whether ISJMs are exclusively formed in a *dmc1Δ* mutant after inactivation of *mek1-as1*. The presence of ISJMs would be consistent with the observations from the genetic assays and confirm that Mek1 kinase activity prevents intersister repair and that *mek1Δ* mutants are utilizing a repair pathway that generates joint molecules.

MATERIALS AND METHODS

Plasmids

The plasmid pJR2, containing the *mek1-as1* allele was constructed by subcloning a 3.2 kb EcoRI/SalI fragment from pB131-Q241G (NIU *et al.* 2009), into EcoRI/SalI digested YIp5. To detect the *HIS4/LEU2* hotspot, a 0.6 kb gel purified AgeI/BglII fragment from pNH90 was used as a probe (HUNTER and KLECKNER 2001).

Yeast Strains and media

All strains are derived from the SK1 background using the NHY1215 and NHY1210 strains provided by Neil Hunter. The genotypes from each strain can be found in Table 2-1. Liquid and solid media were as described previously (DE LOS SANTOS and HOLLINGSWORTH 1999; VERSHON *et al.* 1992). The inhibitor of *mek1-as1*, 4-amino-1-*tert*-butyl-3-(1'naphthyl) pyrazolo [3,4-*d*] pyrimidine (1-NA-PP1), was diluted in dimethyl sulfoxide (DMSO) from a 10 mM stock purchased from Cellular Genomics, Inc. (New Haven, CT). *MEK1* was deleted with *natMX4* (*mek1Δ::natMX4*) using the PCR method of TONG and BOONE (2006) and the second exon of *DMC1* was deleted with *kanMX6* (*dmc1Δ::kanMX6*), using the PCR method of LONGTINE *et al.* (1998). All deletions were confirmed by colony PCR. pJR2 was integrated downstream of the *MEK1* open reading frame by digestion with RsrII.

Time courses and sporulation

Mini liquid sporulation was done at 30ºC in 2% potassium acetate with a final concentration of 1 μM 1-NA-PP1 to check the function of the *mek1-as1* allele. Sporulation was monitored by phase contrast microscopy after 24 hours; 200 cells per condition were counted. Meiotic progression was monitored by staining nuclei with 4'6-diamidino-2-phenylindole (DAPI) and using fluorescence microscopy to score binucleate cells (MI) and tetranucleate cells (MII). For each strain at each time-point, 200 cells were counted.

Sporulation and Psoralen crosslinking

Crosslinking and analysis of the DNA were performed as described in a protocol from Neil Hunter (OH *et al*. 2009). A colony from each strain was inoculated into 5 ml YEPD liquid culture and grown overnight at 30ºC. The 5 ml overnight culture was diluted into 350 ml SPS and shaken overnight at 30ºC. The Optical density 660 (OD_{660}) readings were measured and cultures between 1.0 and 1.4 were selected. The OD_{660} value was converted to cells/ml using the cell density chart (Table 2-2), and the required volume of SPM needed for sporulation was calculated using the following formula:

ml of SPM needed = cells/ml x10^7/ 2 x **1/ 3x10^7** x **vol of SPS (ml)**.

The cells were collected by centrifugation, washed with 25 ml sterile water and resuspended in SPM as calculated above. At each time point, 45.5 ml of cells were taken and mixed with 0.45 ml of 10% sodium azide.

For DAPI staining 0.5 ml of cells were removed from the 45.5 ml of cells collected, pelleted and added to 0.5 ml of 0.1 M Sorbitol/ 40% ethanol and stored at -20ºC until ready to process for microscopy. (The cell suspension will not freeze at -20ºC). Cells were stained by first adding 10 µl of 1X PBS solution to the wells of lysine coated slides (Carlson Scientific, #101204)). The cells were vortexed and 5 µl cell suspension were added to the wells with PBS. The cells were allowed to settle to the bottom of the wells by incubating the slides at room temperature for 2-3 min and then the PBS solution was removed using an aspirator. After washing the cells three times using 1X PBS, the slides

were incubated for 10 min at room temperature to allow them to dry. Four drops of mounting medium containing 1.5 µg/ml DAPI (Vector, #H-1200) were spotted across the center of the slide and a cover slip was placed gently over the cells. The cover slip spreads the DAPI solution into the wells to coat the cells. The cells were then viewed using a fluorescent Zeiss AxioScope microscope.

Gel analysis

The remaining cells were harvested at 5000 rpm for 5 min using a bench-top centrifuge. The pellets were resuspended in 2.5 ml of 1X Psoralen [to make 20 ml of a 5X stock of psoralen: use 10 mg of Trioxalen powder (0.5 mg/ml) (Sigma; #T-6137-100 mg) into 20 ml of 95% ethanol; wrap tube with psoralen in foil and shake at room temperature overnight; dilute to 1X using 50 mM EDTA/50 mM Tris pH8; store on ice or at 4ºC until ready to use] and transferred to a 60 mm x 15 mm culture dish (Corning; # 430166 or Fisher Scientific #08-722-21). Cells were irradiated with 360 nm Long-wave UV light on UV high performance Transilluminator (UVP; #TFL-40) on HIGH setting for 10 min while swirling the dish at least twice. The cells were transferred to 50 ml conical tubes and the culture dish was rinsed with 2 ml of 50 mM EDTA/50 mM Tris pH8. This rinse was pooled with the cells in the 50 ml tube. The cells were then harvested using a bench-top centrifuge and the pellet stored at -20ºC.

Guanidine DNA preparation

The cells were thawed at room temperature and any remaining psoralen solution was removed from the pellet by centrifugation. The pellet was resuspended into 0.9 ml of

Spheroplasting/ β-mercaptoethanol /zymolyase buffer [To prepare 200 ml of stock Spheroplasting buffer: add 36.43 g of 1 M Sorbitol; 10 ml of 1 M Potassium phosphate buffer pH 7* (50 mM); 4 ml 0.5 M EDTA pH 7.5 (10 mM); add distilled water to 200 ml. Filter-sterilize solution and store at 4ºC. To prepare working buffer solution (10 ml): add 9.5 ml of Spheroplasting buffer; 100 µl of β-mercaptoethanol ($1/100^{th}$ volume) and 400 µl of 10 mg/ml zymolyase solution (0.4 mg)] and incubated at 37ºC for 15 min. The cells were mixed gently by swirling twice during this incubation. (Incubations at 37ºC for longer than 15 min will make it difficult to pellet the cells). The spheroplasts were harvested at 2500 rpm for 5 min in a bench-top centrifuge. The supernatant was discarded, 2.5 ml of Guanidine solution (21.5 g Guanidine HCl (4.5M); 10 ml of 0.5 M EDTA pH 7.5 (0.1 M); 0.44 g of sodium chloride (0.15 M); 0.25 ml of 10% sodium lauryl sarkosyl (0.05%); Adjust volume to 50 ml with distilled water; Adjust solution to pH 8) (Guanidine HCl 500g; USB, # G9010) was added to the stringy pellet and the cells were resuspended by finger vortexing. The cells were incubated at 65ºC for 20 min and finger vortexed several times during this period for complete lysis. The cells were incubated on ice until they cooled. 2.5 ml of 95% ethanol was added to the cells and mixed well by inverting the tube. The cells were stored at -20ºC overnight or incubated on ice for 20 min and the protocol continued.

(* To prepare 100 ml of 1 M Potassium phosphate buffer: add 61.5 ml of 1 M dibasic potassium phosphate solution; 38.5 ml of 1 M monobasic potassium phosphate solution; adjust buffer to pH 7).

RNase A treatment

0.7 ml of RNase A solution (For 10 mg/ml stock of RNase A: Dissolve 10 mg of RNaseA into 1 ml of 10X TE pH 8; For working concentration of 50 µg/ml: Add 25 µl of 10 mg/ml stock into 4.75 ml of 10X TE pH 8; RNase A: Sigma #R5000-1G) was added to cells and incubated on a roller drum at 37ºC for 1 hour. The cells were finger vortexed several times during this incubation. (This pellet does not dissolve in this solution).

Proteinase K treatment

After the RNase A treatment, 25 µl of Proteinase K solution (For 1 ml: add 20 mg of Proteinase K (20 mg/ml); 20 µl of 1M Calcium chloride; 10 µl of 1 M Tris-HCl pH 7.5; 470 µl of distilled water; 0.5 ml of 100% glycerol; Proteinase K: Roche # 03 115 852 001) was added directly to the cells/RNase solution and incubated at 65ºC for 1 hour. The cells were finger vortexed several times during this incubation and frozen at -20ºC overnight or the protocol was continued.

DNA extraction and precipitation

The lysates were transferred to a 2 ml tube and the DNA was extracted with 0.7 ml of phenol/chloroform/isoamyl alcohol. The tube was inverted and incubated at room temperature for 3 min. The tube was inverted again and centrifuged for 10 min at 13.2 rpm in a bench-top centrifuge. The top layer was removed and transferred to a clean 2 ml tube and the phenol/chloroform/isoamyl alcohol extraction was repeated. 35 µl of 3M sodium acetate pH 5.2 and 2 volumes of 95% ethanol were added to the top layer and the

tubes were inverted and incubated at room temperature for 20 min. The samples were centrifuged for 5 min at 13.2 rpm using a bench-top centrifuge. The supernatant was discarded and the pellet rinsed with 70% ethanol. The centrifugation was repeated, the pellet was air dried for 10 min at room temperature and resuspended into 100 µl of 1X TE buffer pH 8. The DNA was quantitated using a Nano Drop spectrophotometer (Thermo Scientific; ND-1000 Spectrophotometer).

One-dimensional gel analysis

3-10 µg of DNA was digested with the XhoI restriction enzyme for 2 hours at 37ºC. The digested DNA was precipitated with 5 µl of 3M sodium acetate pH 5.2 and 190 µl of 95% ethanol. The pellet was resuspended into 15 µl of 1X TE pH 8. 5 µl of 6X loading dye (dissolved in 4X NEB3 restriction buffer, instead of water) was added to the samples, and the 1D gel was run as described for the *HIS4/LEU2* hotspot in CALLENDER and HOLLINGSWORTH (2010) and Chapter 3.

Two-dimensional gel analysis

3-10 µg of DNA was digested and precipitated following the same protocol as with the 1D gels.

For the 1st dimension: A 0.4% Seakem GOLD agarose gel (1.4 g of agarose/ 350 ml of 1X TBE) was run in the absence of ethidium bromide for 21 hours at room temperature at 1 V/cm (constant current ~21 amps (33 volts)) using the Owl series buffer recirculating gel system [(dimensions of gel box: 38 cm x 28 cm x 15 cm (L x W x H)); VWR scientific: # 27372-134). This gel box fits a 25 cm x 20 cm gel (L x W)].

For the 2nd dimension: The 1st dimension gel was stained in one liter of 1X TBE with 0.5 μg/ml (final concentration) of ethidium bromide for 30 min, gently shaking at room temperature. A 9.5 cm range of DNA fragments from each lane on the 1st dimension gel (this is usually 1-2 cm from the wells down to the 2.3 kb fragment in λBstEII ladder) was excised. The fragments were laid horizontally in the gel tray. A 0.8% Seakem LE agarose gel (3.2 g of agarose/400 ml of 1X TBE) with 0.5 μg/ml of ethidium bromide (final concentration) was carefully poured around the excised gel fragments and the gel was allowed to solidify at 4ºC. The 2nd dimension gel was run in two liters of pre-chilled 1X TBE with 0.5 μg/ml ethidium bromide at 6 V/cm (170 volts) for 6 hours at 4ºC. To monitor DSBs along with other recombination intermediates and the meiotic products at the *HIS4/LEU2* hotspot in both one and two dimensional gels, southern blotting was done as described in WOLTERING *et al.* (2000).

RESULTS

Inactivation of Mek1 kinase activity in a *dmc1*Δ mutant results exclusively in intersister joint molecules.

To ensure that I would be able to monitor the events occurring at the *HIS4/LEU2* locus, I did a meiotic time course with the wild-type diploid strain, NH716. In this strain, Mek1 kinase is active and the recombinases, Dmc1 and Rad51, required for meiotic recombination are all present, so DSB repair and crossover formation should all be wild type. NH716 was sporulated for 12 hours, resulting in 97.5% sporulation, with the onset of meiotic progression between 4 and 6 hours in Spo medium (Figure 2-2B). Spore viability was 94.7%. Samples were taken every 2 hours and analyzed as described in the

Materials and Methods of this chapter. The samples were first processed on a one-dimensional gel, where DSB formation and repair and the appearance of crossover products were monitored. In this strain, DSBs began to form after 2 hours in Spo medium and were repaired by 8 hours. This repair coincided with the emergence of COs, which were first observed after 4 hours in sporulation, confirming that interhomolog recombination had occurred (Figure 2-2C).

The DNA samples from three time-points in this experiment were then analyzed on a two-dimensional gel to monitor JMs. During wild-type meiosis, the majority of the DSBs are repaired through interhomolog recombination (SCHWACHA and KLECKNER 1997). The use of the homolog as a template for repair will therefore result in the production of IHJMs, however, some of the repair can also be mediated through the sister chromatid, so some ISJMs are also produced. In the wild-type diploid, after 4 hours in Spo medium, I was able to detect DNA species representing IHJMs as well as two ISJMs (Figure 2-2D). However, after 8 hours, both the IHJMs and the ISJMs decreased significantly, presumably due to the resolution of the Holliday junctions into crossovers.

The JM species are transient during recombination and therefore are sometimes difficult to detect. There is also the issue that the cells are not perfectly synchronized and therefore are not all performing strand invasion simultaneously, so this will also cause the amount of JMs to be reduced. Therefore, one way to increase the JM signal is to synchronize the cells after entry into meiosis. This can be done in *dmc1*Δ mutants because the unrepaired DSBs cause cells to arrest in prophase (LYDALL *et al.* 1996). Using a *dmc1*Δ *mek1-as1* allele allows one to synchronize cells before inactivation of the

kinase by the inhibitor, thereby potentially increasing the signal of the JMs (WAN *et al.* 2004).

Meiotic time-courses were performed using two diploid strains: *mek1-as1*, which behaves like a wild-type diploid in the absence of inhibitor; and *dmc1Δ mek1-as1*, which phenotypically resembles a *dmc1Δ* mutant and accumulates DSBs in the absence of inhibitor (WAN *et al.* 2004). In the *mek1-as1* strain, samples were taken every hour for 10 hours in the absence of inhibitor. In contrast, the *dmc1Δ mek1-as1* strain was arrested in Spo medium at 30ºC for 4 hours, after which a final concentration of 1 μM 1-NA-PP1 was added to the culture. Samples were taken every 20 minutes after addition of inhibitor for a total of 3 hours. For each time point, a subset of cells were either stained with DAPI to monitor meiotic progression or processed for one and two dimensional gel analysis. This experiment was performed twice and similar results were observed both times.

The *mek1-as1* diploid exhibited good synchrony, with the majority of the cells entering MI after 4 hours (Figure 2-3C). This diploid exhibited 95.5% sporulation and produced 94.2% viable spores, indicating that the *mek1-as1* allele behaved like wild type in the absence of inhibitor. The *dmc1Δ mek1-as1* strain arrested in prophase until inhibitor was added to the Spo medium at 4 hours. Forty minutes later, the cells entered MI and went on to produce binucleate and tetranucleate cells and 73% asci formation (Figure 2-3C). Only 1.7% of the spores were viable however, suggesting a lack of interhomolog recombination.

In the *mek1-as1* diploid, DSBs were first observed after approximately 3 hours in Spo medium and disappeared by 6 hours, indicating repair (Figure 2-3A). DNA species

representative of IHJMs were first detected after 4 hours and began to disappear by 7 hours presumably due to resolution (Figure 2-3B). However, in this strain, along with the presence of IHJMs, crossovers were also detected by 4 hours, indicating that these cells were not completely synchronized (Figure 2-3A). In the *dmc1Δ mek1-as1* experiment, species indicative of ISJMs were detected within 20 minutes after the addition of 1-NA-PP1 and were still present after 60 minutes. However, the ISJMs began to decrease after 80 minutes, indicating that resolution had begun which also correlated with the disappearance of DSBs. No IHJMs or COs were observed (Figure 2-3A, B) confirming that no detectable interhomolog recombination occurred after inactivation of Mek1. In the *dmc1Δ mek1-as1* analysis, the DNA species indicative of the 'Mom' ISJM appeared as expected, but the 'Dad' ISJM was variable, where it appeared as a doublet at some time-points and was very faint at other times (Figure 2-3B). This variation could be as a result of differences in DNA loading.

DISCUSSION

Mek1 function is required to suppress Rad51-mediated recombination between sister chromatids.

Although genetic analyses with *spo13Δ* mutants have supported the idea that inactivation of Mek1 kinase activity allows intersister recombination, a caveat of this approach is that it necessarily uses those cells which produced two viable-spore dyads which may not be representative of the population as a whole. I therefore used the physical analysis of joint molecule formation to determine whether inactivation of Mek1

kinase activity does in fact produce intersister and not interhomolog recombination intermediates.

One of the problems I encountered in comparing the *mek1-as1* and the *dmc1Δ mek1-as1* strain with inhibitor was that the latter strain was synchronized due to the *dmc1Δ* mutation, while the *mek1-as1* strain was not. This complicates the interpretation of the data when determining whether the amount of ISJMs in the *dmc1Δ* strain is increased after Mek1 inactivation compared to what is observed during the *mek1-as1* meiosis. One way to address this caveat is to delete the transcription factor, *NDT80*. *NDT80* is required for the expression of several genes, some of which are required for the progression out of prophase, as well as for the resolution of double Holliday junctions (ALLERS and LICHTEN 2001; SOURIRAJAN and LICHTEN 2008; XU *et al.* 1997). The *ndt80Δ* mutant in the presence of the *mek1-as1* allele should cause the JMs to accumulate in the cell making the analysis and subsequent quantitation simpler.

Another explanation for the differences in intensity with the JMs is that the population of JMs that we are examining at a specific time point may not be the same. Until all the DSBs are repaired, there maybe new JMs being made as others are being resolved.

This qualitative analysis of joint molecules indicates that Mek1 kinase activity prevents DSB repair in *dmc1*Δ diploids by suppressing the formation of intersister JMs. Mek1 could potentially be phosphorylating an as yet unidentified target protein, to help with the suppression of sister chromatid repair, similar to its phosphorylation of Rad54 (NIU *et al.* 2009).

Table 2-1. *Saccharomyces cerevisiae* strains

Name	Yeast Genotype	Source
NHY1215	*MATα leu2::hisG his4-X::LEU2-(NgoMIV) ho::hisG ura3(Δpst-sma)*	N. Hunter
NHY1210	*MAT**a** leu2::hisG HIS4::LEU2-(Bam+ori) ho::hisG ura3(Δpst-sma)*	N. Hunter
NH716[a]	*MATα leu2::hisG his4-X::LEU2 (NgoMIV) hoΔ::hisG ura3(Δpst-sma)* *MAT**a** leu2::hisG HIS4::LEU2 (Bam+ori) hoΔ::hisG ura3(Δpst-sma)*	N. Hunter
NH729	NH716 only *mek1Δ::natMX4* *mek1Δ::natMX4*	Callender and Hollingsworth (2010)
NH729::pJR2	NH716 only *mek1Δ::natMX4::URA3::mek1-as1 ura3* *mek1Δ::natMX4 ura3*	This work
NH794[b]	NH716 only *dmc1Δ::kanMX6 mek1Δ::natMX4* *dmc1Δ::kanMX6 mek1Δ::natMX4*	This work
NH794::pJR2	NH716 only *dmc1Δ::kanMX6 mek1Δ::natMX4::URA3::mek1-as1 ura3* *dmc1Δ::kanMX6 mek1Δ::natMX4 ura3*	This work

[a] The strain NH716 is a diploid created from crossing NHY1215 and NHY1210.
[b] In this strain the second exon of *DMC1* is deleted with *kanMX6*.

Table 2-2. The conversion of Optical density 660 (OD_{660}) to haploid cell density.

OD_{660}	[a]Cells/ ml x10^7
0.95	1.67
1.00	1.85
1.15	2.47
1.20	2.71
1.25	2.95
1.30	3.22
1.35	3.50
1.40	3.82
1.45	4.13
1.55	4.85

[a] This value is divided by 2 to give the corresponding diploid cell number.

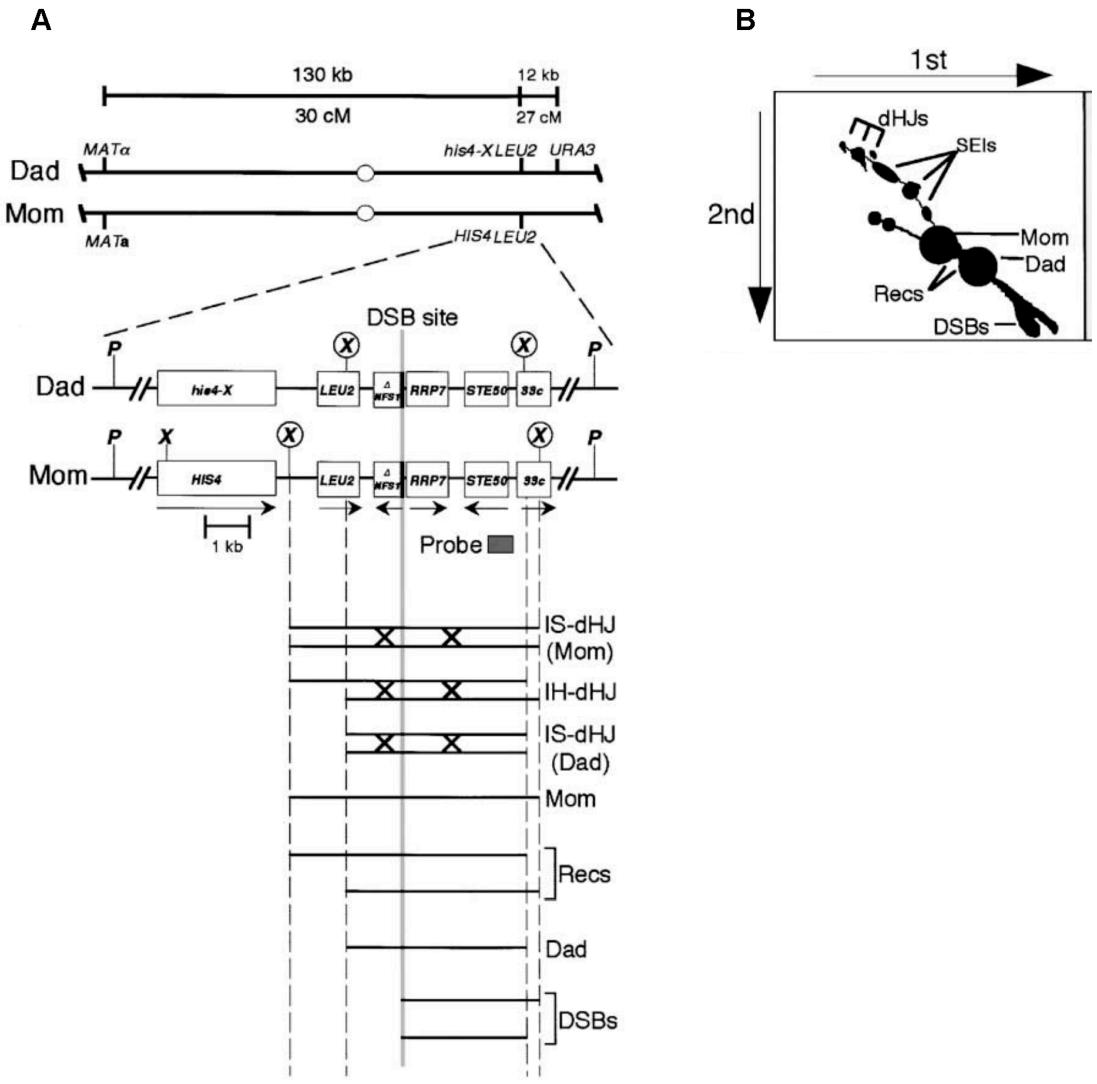

Figure 2-1. Experimental approach using *HIS4/LEU2* hotspot. (A) Physical map of the *HIS4/LEU2* locus (adapted from HUNTER and KLECKNER 2001). The *HIS4/LEU2* regions of the two homologs (Mom and Dad) are distinguished by XhoI restriction digestion sites, marked 'X'. The 'DSB site' is the region where Spo11 will cut. (B) Diagram showing the DNA species observed after XhoI digestion of DNA and analysis via two-dimensional electrophoresis. The 1st dimension separates species by their size, while the 2nd dimension separates species by shape and size. The IHJMs are most pronounced and flanked by two smaller ISJMs (HUNTER and KLECKNER 2001).

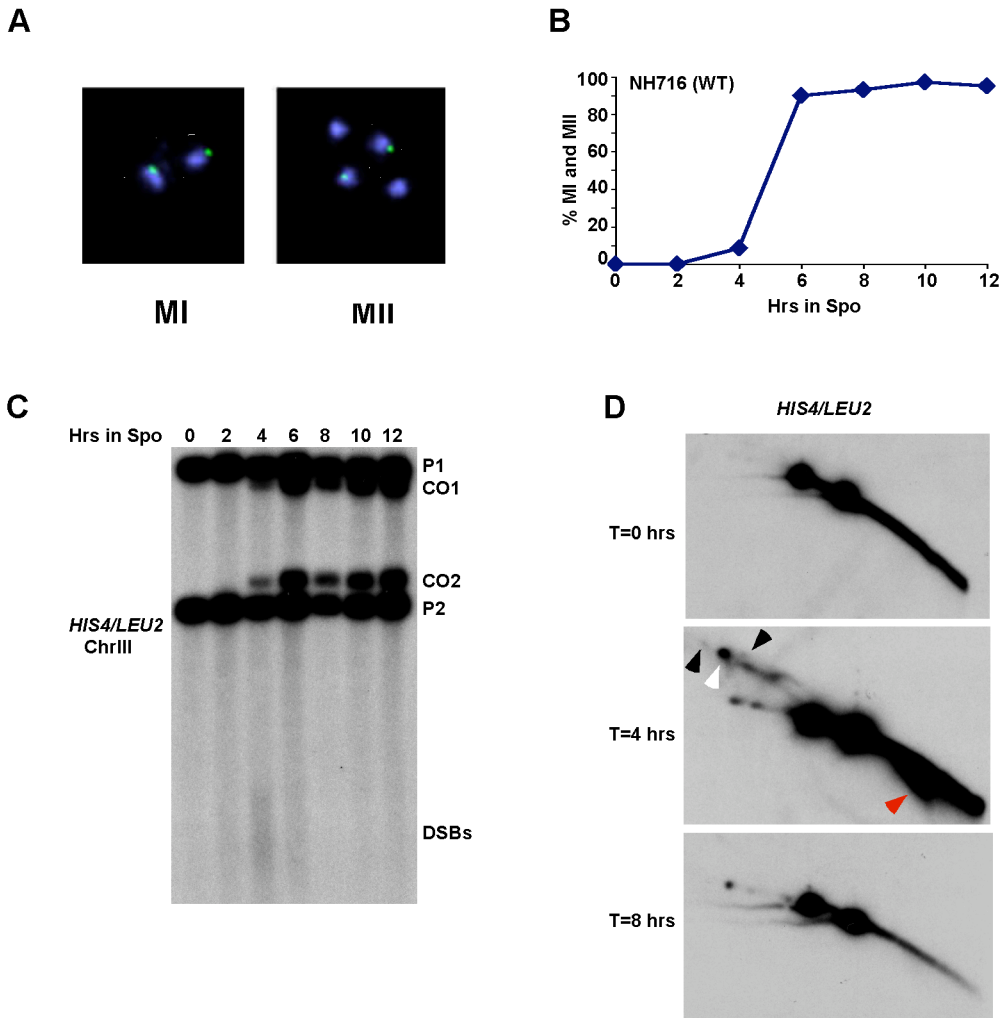

Figure 2-2. Physical analysis of recombination in a wild-type diploid at the *HIS4/LEU2* hotspot on chromosome III. (A) Dapi staining showing meiotic nuclei at both divisions, meiosis I (MI), showing binucleate cells and meiosis II (MII), showing tetranucleate cells [Adapted from MATOS *et al*. (2008)]. (B) Meiotic progression monitored by DAPI staining of nuclei of the NH716 strain. (C) One-dimensional gel showing the fragments corresponding to cross-over products, CO1 and CO2, and parental fragments 'P1 and P2', respectively. (D) Two-dimensional gel showing IHJMs (indicated by the white arrowhead), two ISJMs (indicated by black arrowheads) and DSBs (indicated by the red arrowhead) at the indicated times in sporulation medium.

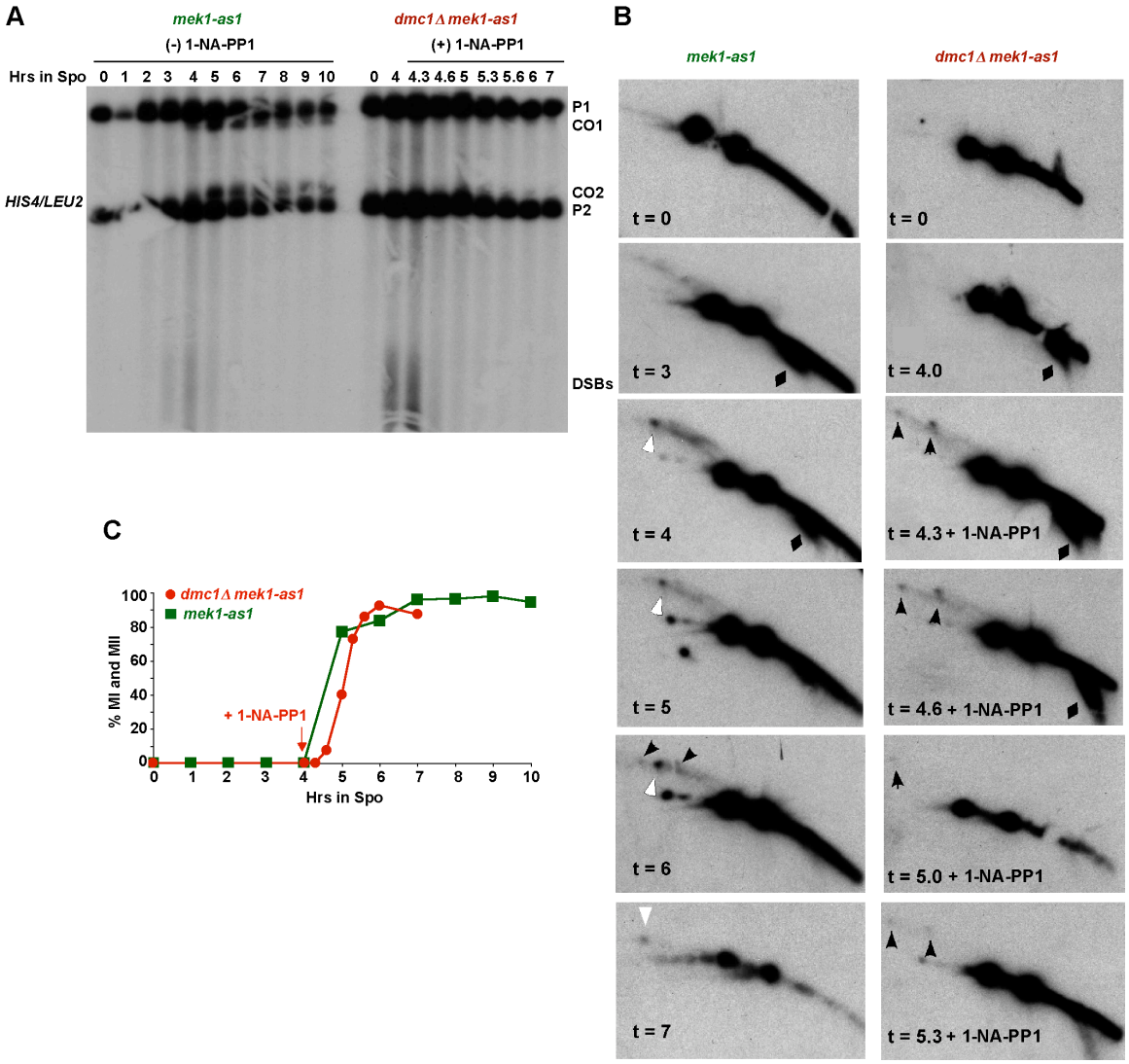

Figure 2-3. Physical analysis of *mek1-as1* and *dmc1Δ mek1-as1* strains at the *HIS4/LEU2* hotspot. (A) One-dimensional gel showing DSBs and CO products at indicated times in Spo medium. (B) 2D gel electrophoresis showing the IHJMs (white arrow head) and ISJMs (black arrow heads) of both strains. 1 µM 1-NA-PP1 was added to the sporulation medium of *dmc1Δ mek1-as1* strain at 4 hours (after the t=4 sample was removed). Black diamonds represent DSBs. 't' represents the hours in Spo medium. (C) DAPI staining of nuclei showing meiotic progression of the two strains.

CHAPTER THREE

Mek1 suppression of meiotic double-strand break repair is specific to sister chromatids, chromosome autonomous and independent of Rec8 cohesin complexes

[The text of this chapter is taken from a manuscript published in Genetics 2010 Jul; 185 (3): 771-82 (Callender and Hollingsworth 2010) with some modifications.]

INTRODUCTION

In eukaryotes, meiosis is a specialized type of cell division that produces the gametes required for sexual reproduction. In meiosis, one round of DNA replication is followed by two rounds of chromosome segregation, termed Meiosis I and II. As a result of the two divisions, four haploid cells are produced, each containing half the number of chromosomes as the diploid parent. Proper segregation at Meiosis I requires connections between homologous chromosomes which are created by a combination of sister chromatid cohesion and recombination (PETRONCZKI *et al.* 2003). In vegetative cells, cohesion is mediated by multi-subunit ring-shaped complexes that are removed by proteolysis of the kleisin subunit, Mcd1/Scc1 (ONN *et al.* 2008). In meiotic cells, introduction of a meiosis-specific kleisin subunit, Rec8, allows for a two-step removal of cohesion with loss of arm cohesion at Anaphase I and centromeric cohesion at Anaphase II (KLEIN *et al.* 1999). Missegregation of chromosomes during meiosis causes abnormal chromosome numbers in gametes that may lead to infertility and genetic disorders such as Trisomy 21 or Down syndrome.

In mitotically dividing budding yeast cells, recombination is mediated by an evolutionarily conserved RecA-like recombinase, Rad51, and occurs preferentially between sister chromatids (KADYK and HARTWELL 1992). In contrast, recombination during meiosis is initiated by the deliberate formation of double-strand breaks (DSBs) by an evolutionarily conserved, topoisomerase-like protein, Spo11, and occurs preferentially between homologous chromosomes (JACKSON and FINK 1985; KEENEY 2001; SCHWACHA and KLECKNER 1997). After DSB formation, the 5' ends on either side of the breaks are resected, resulting in 3' single stranded (ss) tails. Rad51, and the meiosis specific

recombinase Dmc1, bind to the 3' ssDNA tails to form protein/DNA filaments that promote strand invasion of homologous chromosomes. DNA synthesis and ligation result in the formation of double Holliday junctions which are then preferentially resolved into crossovers (ALLERS and LICHTEN 2001).

The precise roles that the Rad51 and Dmc1 recombinase activities play in meiotic recombination have been unclear because experiments have indicated both overlapping and distinct functions for the two proteins (HUNTER 2007; SHERIDAN and BISHOP 2006). While both *rad51*Δ and *dmc1*Δ mutants reduce interhomolog recombination, other studies suggest that Rad51, in complex with the accessory protein, Rad54, is involved primarily in intersister DSB repair. In contrast, Dmc1, in conjunction with the accessory protein, Rdh54/Tid1 (a paralog of Rad54), affects DSB repair in diploid meiotic cells by invasion of non-sister chromatids (ARBEL *et al.* 1999; BISHOP *et al.* 1999; DRESSER *et al.* 1997; HAYASE *et al.* 2004; SCHWACHA and KLECKNER 1997; SHERIDAN and BISHOP 2006; SHINOHARA *et al.* 1997a; SHINOHARA *et al.* 1997b).

The preference for recombination to occur between homologous chromosomes during meiosis is created in part by Dmc1. DSBs accumulate in *dmc1*Δ diploids due to a failure in strand invasion (BISHOP *et al.* 1992; HUNTER and KLECKNER 2001). In the efficiently sporulating SK1 strain background, these unrepaired breaks trigger the meiotic recombination checkpoint, resulting in prophase arrest (LYDALL *et al.* 1996; ROEDER and BAILIS 2000). In *dmc1Δ* mutants, Rad51 is present at DSBs, yet there is no strand invasion of sister chromatids (BISHOP 1994; SHINOHARA *et al.* 1997a). These results suggest that in addition to Dmc1 promoting interhomolog strand invasion, Rad51 activity must also be suppressed.

Recent studies have shown that during meiosis Rad51 recombinase activity is inhibited by two different mechanisms that decrease the formation of Rad51/Rad54 complexes: 1) binding of the meiosis-specific Hed1 protein to Rad51, thereby excluding interaction with Rad54 and (2) reduction in the affinity of Rad54 for Rad51 due to phosphorylation of Rad54 by Mek1 (BUSYGINA *et al.* 2008; NIU *et al.* 2009; TSUBOUCHI and ROEDER 2006). Mek1 is a meiosis-specific kinase that is activated in response to DSBs (CARBALLO *et al.* 2008; NIU *et al.* 2007; NIU *et al.* 2005). In addition to phosphorylating Rad54, Mek1 phosphorylation of an as yet undetermined substrate is required to suppress Rad51/Rad54-mediated strand invasion of sister chromatids (NIU *et al.* 2009).

To dissect the mechanism by which Mek1 suppresses meiotic intersister DSB repair, we took advantage of the ability of yeast cells to undergo haploid meiosis. The lack of homologous chromosomes in haploid cells makes it possible to examine sister-chromatid specific events in the absence of interhomolog recombination. DE MASSY *et al.* (1994) previously observed a delay in DSB repair in haploid cells and proposed that this delay was due to a constraint in using sister chromatids. We have shown that this delay is dependent on *MEK1* and utilized the haploid system to determine various biological parameters required to suppress meiotic intersister DSB repair. Our results indicate that Rad51 and Dmc1 recombinase activities have distinct roles during meiosis and that interhomolog bias is established specifically on sister chromatids through regulation of Rad51, not Dmc1. *rec8*Δ diploids exhibit defects in meiotic DSB repair (BRAR *et al.* 2009; KLEIN *et al.* 1999). Given that cohesin complexes are specific for sister chromatids, we investigated the role of *REC8* in intersister DSB repair and found it

is required neither for suppressing intersister DSB repair during meiosis, nor for the repair itself.

MATERIALS AND METHODS

Plasmids and DSB probes

The plasmid, pDT20, contains a 0.6 kb sequence of chromosome VII (coordinates 497700-497759) and was created by amplifying a fragment using genomic DNA and primers that engineered SacI and SphI sites onto the ends. After digestion, the fragment was subcloned into SacI/SphI digested pVZ1 (HOLLINGSWORTH and JOHNSON 1993). Chromosome III hotspot probes were derived from pME1210 (*YCR048w*) (WOLTERING *et al.* 2000) and pNH90 (*HIS4/LEU2*) (HUNTER and KLECKNER 2001). The chromosome VI hotspot (*HIS2*) was detected using pH21 (BULLARD *et al.* 1996) (provided by Bob Malone) and the chromosome VIII hotspot (*ARG4*) used pMJ77 (provided by Michael Lichten). The *mek1-as1* allele in pJR2 was constructed by subcloning a 3.2 kb EcoRI/SalI fragment from pB131-Q241G (NIU *et al.* 2009) into a EcoRI/SalI-digested YIp5 (PARENT *et al.* 1985). pRS306 is a *URA3* integrating plasmid (SIKORSKI and HIETER 1989).

Yeast Strains and media

All strains are derived from the SK1 background, except for NH705-32-1 dmc1, which is from the A364a background. The genotypes of each strain can be found in Table 3-1. Liquid and solid media were as described previously (DE LOS SANTOS and HOLLINGSWORTH 1999; VERSHON *et al.* 1992). *SIR2* and *RME1* were deleted with

natMX4 using the polymerase chain reaction (PCR) method of TONG and BOONE (2006). *MEK1* was mutated using either pTS21 (*mek1*Δ*::URA3*), pTS1 (*mek1*Δ*::LEU2*) (DE LOS SANTOS and HOLLINGSWORTH 1999) or *natMX4*. *REC8* and the second exon of *DMC1* were deleted with *kanMX6* using the PCR method of LONGTINE *et al.* (1998). All deletions were confirmed by yeast colony PCR. pRS306 was targeted to integrate at *ura3* by digestion with StuI while pJR2 was integrated downstream of the *MEK1* open reading frame by digestion with RsrII. NH716 is a diploid resulting from a cross between NHY1215 and NHY1210 (provided by N. Hunter).

The chromosome III disome, Kar-3-WT was constructed using a "kar cross" (DUTCHER 1981). Strains carrying *kar1-1* fail to efficiently undergo karyogamy, creating cells with two nuclei. At low frequency, chromosomes in these dikaryons can be transferred from one nucleus to the other. Disomic III haploids can be obtained by selecting for recessive resistance markers carried by chromosomes in the recipient nucleus as well as for prototrophic markers carried on chromosome III from the donor cell (Figure 3-1). Exceptional cytoductants were selected using recessive resistance markers in the recipient strain. Our recipient strain, NHY1215 Can^R Cyh^R was generated by the sequential selection for *can1* and *cyh2* mutants on SD-arg + 60 mg/ml canavanine and YPDcom + 10 mg/ml cyclohexamide, respectively. For the donor strain, the second exon of *DMC1* was first deleted with *natMX4* to introduce a dominant drug resistance marker. Putative chromosome III disomic haploids were tested for heterozygosity at the *MAT* locus by screening for non-maters. In *kar1-1* crosses, ~10% of the cells are diploid (DUTCHER 1981). The possibility that Kar-3-WT is diploid was ruled out by the following: (1) selecting for two recessive resistance markers. The donor strain is *CAN1*

CYH2 and therefore the diploid should be sensitive to both canavanine and cyclohexamide. (2) Assaying for nourseothricin (NAT) sensitivity: The donor strain was *dmc1*Δ*::natMX4*. Since Nat^R is dominant, the diploid can grow on SD + NAT plates, while the disomic haploid cannot. To make Kar-3-sir2Δ, the *SIR2* gene was deleted with *natMX4* from Kar-3-WT. (3) Quantitation of the number of chromosomes by Southern blot. Plugs were made from 5 ml YEPD stationary cultures of the NHY1215 sir2 haploid, the diploid, NH929, and the disomic haploid, Kar-3-WT, as described by BORDE *et al.* (1999). In addition, a *sir2Δ::natMX4* derivative of Kar-3-WT, Kar-3-sir2, was also examined. The chromosomes were fractionated using a 1.5% contoured-clamp homogeneous electric field (CHEF) gel. After transfer to a nylon membrane, the blot was probed simultaneously with radioactive probes derived from sequences on chromosome III (0.9 kb HindIII fragment from pME1210) and chromosome VII sequences (0.6 kb SacI/SphI fragment from pDT20). The amount of radioactive labeling of each chromosome was quantitated using the Multigauge Software and a Fujifilm FLA 7000 phosphoimager and the ratio of chromosome III/chromosome VII hybridization was calculated. This ratio was the same in the diploid and haploid strains, 0.7 and 0.8, respectively. In contrast, the chromosome III disomic haploids, Kar-3-WT and Kar-3-sir2, exhibited ratios that were approximately two-fold higher (1.4 and 1.5, respectively), as expected if there are two copies of chromosome III to a single copy of chromosome VII.

To construct a diploid that is isogenic with Kar-3-WT, a haploid derivative that had lost the *MAT*α chromosome was isolated by screening for colonies that mated as "**a**"

cells. This *MAT***a** Kar-3-WT derivative was then crossed with NHY1215 Can^R Cyh^R to generate NH929 (Figure 3-1).

DSB analysis

For each time point plugs were prepared and the DNA digested *in situ* as described in BORDE *et al.* (1999). The exception was the experiment shown in Figure 4, in which DNA was crosslinked with psoralen and then isolated from cells prior to restriction enzyme digestion as described in OH *et al.* (2009). The chromosome III hotspot, *YCR048w*, was monitored using a BglII genomic digest and a 0.9 kb HindIII fragment from pME1210 (WU and LICHTEN 1994); for *HIS4/LEU2*, a XhoI digest and a 0.6 kb AgeI/BglII fragment from pNH90, were used (HUNTER and KLECKNER 2001). The chromosome VI *HIS2* hotspot was detected with a BglII digest and a 1 kb BglII/EcoRI fragment from pH21 (BULLARD *et al.* 1996) . The chromosome VIII *ARG4* hotspot also used a BglII genomic digest and a 0.6 kb HpaI/EcoRV fragment from pMJ77. The plugs were loaded onto 0.8% agarose gels that were run for 24 hours in 1 X TBE buffer at 4ºC at 90V for *YCR048w* and *HIS2* and 70V for *ARG4*. To detect the *HIS4/LEU2* DSBs, 0.6% agarose gels were run at 70V at room temperature. DSBs were quantified using the Image Quant 1.1 software and a Molecular Dynamics Phosphoimager or the Multi-Gauge Software with a FujiFilm FLA 7000 Phosphoimager.

Time courses

Liquid sporulation was performed at 30^{o}C in 2% potassium acetate at a density of $3x10^7$ cells/ml. 10 ml samples were taken at the indicated times, mixed with 50 mM

EDTA and 10 ml 95% ethanol and stored at -20ºC. Meiotic progression was monitored by staining nuclei with DAPI (4', 6'-diamidino-2-phenylindole) and using fluorescence microscopy to score binucleate cells (Meiosis I) and tetranucleate cells (Meiosis II). For each strain at each time point, 200 cells were counted. Every time course was performed at least twice.

RESULTS

MEK1-dependent suppression of intersister DSB repair does not require the presence of homologous chromosomes.

To test whether Mek1 suppression of meiotic intersister DSB repair is specific to sister chromatids, DSBs were examined in haploid cells where no homologs are available. If suppression of intersister repair requires homologous chromosomes, then DSBs should be repaired in *dmc1*Δ haploid strains, even though Mek1 is active. Alternatively, if the suppression mechanism is confined to sister chromatids, haploid *dmc1*Δ strains should exhibit unrepaired DSBs.

These two possibilities were distinguished by analyzing meiotic DSB repair in *dmc1*Δ haploids at the *YCR048w*, *HIS2* and *ARG4* hotspots, located on chromosomes III, VI and VIII, respectively. To enable haploid cells to enter meiosis, *SIR2* was deleted, thereby allowing *MAT***a** and *MAT*α information to be expressed from the normally silent mating type loci (RINE and HERSKOWITZ 1987). In the *sir2Δ dmc1Δ* haploid, DSBs appeared by 4 hours at all three hotspots and persisted up to 12 hours (Figure 3-2A). The DSBs in the *dmc1*Δ haploid resemble those in *dmc1Δ* diploids in that they accumulate and become hyperresected (BISHOP *et al.* 1992). Deletion of *MEK1* results in efficient

repair of DSBs at all three locations (Figure 3-2A). The reduced number of DSBs observed in the *mek1*Δ and *mek1*Δ *dmc1*Δ haploids is likely due to rapid repair using sister chromatids, as opposed to a decrease in DSB formation, because *mek1*Δ diploids have previously been shown to exhibit wild-type DSB levels when processing of the breaks is prevented (PECINA *et al.* 2002). These data indicate that the inhibition of DSB repair observed in *dmc1*Δ haploids requires *MEK1*, similar to what is observed in diploid cells (WAN *et al.* 2004; XU *et al.* 1997).

RME1 is a haploid-specific gene that encodes a protein which negatively regulates entry into meiosis by repressing *IME1*, a transcription factor required for the onset of meiosis (KASSIR *et al.* 1988; MITCHELL and HERSKOWITZ 1986). *RME1* is repressed by the a1/α2 transcription factor and this repression is the reason that cells must normally be heterozygous for mating type to sporulate (COVITZ *et al.* 1991). *rme1*Δ mutants bypass the requirement for a1/α2 and therefore this mechanism for inducing haploid meiosis is completely independent of the *sir2*Δ mechanism. Similar to *sir2*Δ *dmc1*Δ, DSBs accumulated in the *rme1*Δ *dmc1*Δ haploid and were repaired in the *rme1*Δ *dmc1*Δ *mek1*Δ strain, indicating that the *MEK1*-dependent suppression of intersister repair is a general property of meiotic haploid cells, and not a function of *sir2*Δ mutants (Figure 3-2B). Therefore the mechanism by which *MEK1* suppresses intersister repair is specific to sister chromatids. There was a reduction in the amount of DSBs formed in the *rme1Δ* haploid versus the *sir2Δ* haploid strain at chromosome III (Figure 3-2 A and B). This may have been observed because they were less cells entering meiosis in the *rme1Δ* experiment as opposed to those experiments done with the *sir2Δ* mutants (Figure 3-2 C and D).

Suppression of meiotic DSB repair in *DMC1* haploid cells is also dependent on *MEK1*.

DSB repair is delayed or absent in the *sir2*Δ and *rme1*Δ haploids [Figure 3-2A and B and DE MASSY *et al.* (1994)]. Elimination of *MEK1* from these strains results in efficient repair of these breaks similar to the *dmc1*Δ *mek1*Δ haploids (Figure 3-2A and B). Therefore Mek1 is able to suppress intersister DSB repair in haploid cells even when Dmc1 is present.

In diploid cells, a failure to repair DSBs triggers the meiotic recombination checkpoint and results in prophase arrest (LYDALL *et al.* 1996; ROEDER and BAILIS 2000). Meiotic progression is delayed or absent in *sir2*Δ *DMC1* and *sir2*Δ *dmc1*Δ haploids, respectively, but not in *sir2*Δ *mek1*Δ or *sir2*Δ *dmc1*Δ *mek1*Δ, indicating unrepaired breaks are effective in activating the recombination checkpoint even in the absence of homologous chromosomes (Figure 3-2C).

The regulation of meiotic DSB repair is chromosome autonomous.

There are a number of possible explanations for the inefficient DSB repair observed in the *sir2*Δ and *rme1*Δ haploids. One possibility is that DSB repair is coordinated between chromosomes. For example, there could be a checkpoint that delays repair until all chromosomes are homologously paired or have initiated strand invasion between homologs. This idea was tested by examining meiotic DSB repair in haploid strains containing two copies of chromosome III. If DSB repair between different chromosomes is coordinated, then the broken haploid chromosomes should inhibit DSB repair between the disomic chromosome III homologs. If, however, meiotic DSB repair

is chromosome autonomous, then DSBs on the disomic chromosome should be fixed by interhomolog recombination, while the breaks on the haploid chromosomes should remain unrepaired.

A chromosome III disomic haploid and isogenic diploid were created as described in the Materials and Methods and also shown in Figure 3-1. The *YCR048w* and *HIS4/LEU2* hotspots on chromosome III were used to look at DSB repair on the disomic chromosome. The *HIS4/LEU2* hotspot has the advantage that interhomolog recombination can be directly monitored by physical assays (HUNTER and KLECKNER 2001). After 12 hours in sporulation medium, DSBs disappeared at both hotspots in the diploid and disomic haploid strains, but not in the haploid (Figure 3-3A and B). Restriction fragments indicative of crossovers were seen in both the wild-type and *sir2*Δ disomic haploids, confirming that interhomolog recombination occurred (Figure 3-3A). The number of crossovers in the disome is delayed and reduced relative to the diploid, however. This delay is not due to *sir2*Δ, since the wild-type disome behaved similarly. The *HIS2* hotspot on chromosome VI is present in only one copy in the disomic haploid. DSBs at this hotspot failed to get efficiently repaired in both the disomic haploid and haploid strains, and both strains were delayed/arrested in meiotic prophase (Figure 3-3C and D). Deletion of *MEK1* relieved the progression defect of these strains and allowed repair of the *HIS2* breaks (data not shown). Therefore repair of DSBs on different chromosomes occurs independently of each other.

Dmc1 is capable of intersister DSB repair in haploid cells.

Another explanation for the delay/absence of DSB repair in wild-type haploids is that Mek1 acts directly on Dmc1 to suppress strand invasion of sister chromatids. To remove any regulation that might be provided by Rad51, filaments containing only Dmc1 were created by deletion of *RAD52*, a mediator protein that is required for loading Rad51 onto the breaks (LAO *et al.* 2008). [This indirect method of preventing Rad51 from assembling onto breaks is necessary because *rad51*Δ mutants prevent efficient loading of Dmc1 (BISHOP 1994; SHINOHARA *et al.* 1997a)]. In contrast to the DSBs in the wild-type and *dmc1*Δ haploids which persisted up to 10 hours, some of the DSBs in the *rad52*Δ haploid disappeared, suggesting that Dmc1 can mediate strand invasion of sister chromatids (Figure 3-4A). The *rad52*Δ cells failed to enter Meiosis I, however, indicating that a fraction of the DSBs were not repaired (Figure 3-4B). These results suggest that Mek1 does not suppress Dmc1 directly, but rather that it is the presence of Rad51 that constrains Dmc1 from interacting with sister chromatids. Furthermore, it rules out the idea that Dmc1 is activated by the presence of homologous chromosomes. A possible experiment to further strengthen this argument would be to examine DSB repair in the *sir2Δ rad52Δ mek1Δ* strain, where DSB repair should still be observed. It should be noted that the absence of Rad52 maybe preventing Rad51 filament formation, but it may not be affecting the presence of the Rad51 protein in the cell, since Dmc1 can still assemble onto breaks in the absence of *RAD52*.

The experiment shown in Figure 3-3 uses the same *sir2Δ* haploid as used in the experiments in Figure 3-4. However, there seems to be a reduction in the DSBs formed in the *sir2Δ* haploid in the time course shown in Figure 3-4. This could be due to the

different methods of DNA isolation between the two experiments, where the DNA used in the Figure 3-4 was isolated as described in Materials and Methods of Chapter 2 for the two-dimensional gel analysis, whereas in the experiments shown in Figure 3-3 the DNA was digested in whole cells embedded in agarose plugs.

Meiotic intersister DSB repair occurs independently of *REC8*.

To determine whether meiotic cohesin complexes containing Rec8 are necessary for intersister DSB repair, DSBs were compared in *rec8*Δ and *mek1*Δ *rec8*Δ haploids. *rec8*Δ differentially affects the recruitment of Spo11 to chromosomes such that few to no breaks are observed on chromosomes such as VI and VIII (ruling out examination of the *HIS2* and *ARG4* hotspots), while Chromosome III is less affected (KUGOU *et al.* 2009). To see whether *REC8* is required for meiotic intersister recombination, DSB repair was therefore monitored at *YCR048w* and *HIS4/LEU2* in *sir2Δ rec8*Δ and *sir2Δ mek1*Δ *rec8*Δ haploids. DSBs accumulated and became hyperresected in the *sir2*Δ *rec8*Δ haploid at both hotspots, similar to the *sir2*Δ haploid (Figure 3-5A). No meiotic progression was observed in the *sir2*Δ *rec8*Δ strain, indicating that *rec8*Δ is not directly required for the meiotic recombination checkpoint. Deletion of *MEK1* in the *rec8*Δ mutant resulted in repair of the DSBs and the progression of the cells through the meiotic divisions (Figure 3-5A and C). Rec8 cohesin complexes therefore are not required for sister-based repair.

*rec8*Δ exhibits a significant fraction of unrepaired breaks at the *YCR048w* hotspot in diploid cells (BRAR *et al.* 2009; KLEIN *et al.* 1999) (Figure 3-5B). The accumulation of DSBs is not as high as in *dmc1*Δ diploids, perhaps because of less efficient recruitment of Spo11. Consistent with the haploid experiment, DSB repair and meiotic progression

were observed in *mek1*Δ *rec8*Δ and *dmc1*Δ *mek1*Δ *rec8*Δ diploids (Figure 3-5 B and D). Interestingly, DSB repair is less efficient and meiotic progression delayed in *dmc1*Δ *mek1*Δ *rec8*Δ strains relative to *dmc1*Δ *mek1*Δ. Therefore although *REC8* is not required for repair using sister chromatids, it does promote such repair. The *MEK1*-dependent accumulation of DSBs in *rec8*Δ and *dmc1*Δ *rec8*Δ strains rules out Rec8 as the target of Mek1 responsible for suppressing intersister DSB repair.

***REC8* functions with *MEK1* to activate the meiotic recombination checkpoint.**

mek1-as1 is an analog-sensitive version of Mek1 that can be inhibited by addition of purine analogs to the sporulation medium (NIU *et al.* 2005; WAN *et al.* 2004). Genetic experiments monitoring spore viability and meiotic arrest in *dmc1*Δ diploids indicated that *mek1-as1* is as functional as wild-type *MEK1 in vivo*, although kinase assays revealed that *mek1-as1* has a reduced affinity for ATP *in vitro* (NIU *et al.* 2009; WAN *et al.* 2004). A single copy of *mek1-as1* was integrated into a *mek1*Δ *dmc1*Δ diploid isogenic to the *MEK1 dmc1*Δ strain shown in Figure 3-6. In contrast to other *mek1-as1* diploids we have constructed, this diploid exhibited approximately 40% meiotic progression and a reduction in the number of DSBs at 10 hrs at the *YCR048w* hotspot (Figure 3-6). (Note that these experiments were carried out in the absence of inhibitor and *mek1-as1* should therefore be active). This result suggests that in this particular derivative of SK1, a single copy of *mek1-as1* provides less kinase activity *in vivo* than wild-type. DSBs accumulate in the *dmc1*Δ *rec8*Δ diploid, confirming that *REC8* is not required for suppressing intersister DSB repair (Figure 3-6A). When *mek1-as1* was combined with *rec8*Δ *dmc1*Δ, meiotic progression occurred with wild-type kinetics and

efficiency, compared to *dmc1*Δ and *rec8*Δ *dmc1*Δ, even though substantial numbers of DSBs persisted at the *YCR048w* hotspot (Figure 3-6A and B). Progression in the absence of repair is a hallmark of defects in the meiotic recombination checkpoint. Therefore, Mek1 kinase activity and Rec8 work together to promote a robust checkpoint response to unrepaired DSBs.

DISCUSSION

Regulation of meiotic intersister DSB repair occurs at the level of sister chromatids.

An important question is whether suppression of intersister DSB repair during meiosis is a locally regulated process occurring between sister chromatids as we have proposed (NIU *et al.* 2007) or whether the presence of homologous chromosomes somehow acts to channel recombination events away from sister chromatids. To distinguish between these possibilities we exploited the ability of budding yeast to undergo haploid meiosis, thereby creating a situation where the only templates available for repair are sister chromatids. Four different hotspots on three different chromosomes were examined and two completely independent approaches to inducing haploid meiosis were used. Therefore it is likely that our results reflect general properties of meiotic haploid chromosomes. We found that *dmc1*Δ haploids accumulate hyperresected DSBs, similar to *dmc1*Δ diploids, and that these breaks go away in the absence of Mek1. Therefore, Mek1 can inhibit Rad51-mediated strand invasion in the absence of homologous chromosomes, indicating that the mechanism of suppression is specific to sister chromatids.

In vegetative cells, a DSB on one chromosome results in the generation of replication-independent cohesion throughout the genome, indicating that DSBs can have global effects within a cell (STROM *et al.* 2007; UNAL *et al.* 2007). We exploited the haploid meiosis system to determine whether the presence of breaks on unpaired chromosomes impair DSB repair between homologs. Interhomolog recombination was observed between disomic chromosomes in cells where breaks on haploid chromosomes were not repaired, indicating that DSB repair is not coordinated between different pairs of homologous chromosomes. It should be noted, however, that interhomolog recombination was delayed and less efficient on the disomic chromosomes compared to the same homologous pair in a diploid. This maybe because failure to repair breaks on haploid chromosomes results in the accumulation of single stranded DNA, thereby titrating out the recombination proteins that are available for repair. That recombination proteins are limiting in meiotic cells has previously been shown by JOHNSON *et al.* (2007). However, this explanation predicts that the disappearance of DSBs would be delayed on the disomic chromosomes, which was not observed. Therefore while strand invasion appears unaffected on the disome, there may be another step in the recombination pathway that is affected by the unrepaired breaks on the haploid chromosomes. There is a gap between the disappearance of DSBs and the appearance of COs, suggesting that chromosomes are delayed at the dHJ stage. One possible explanation is that DSBs on the haploid chromosomes are triggering the recombination checkpoint which acts by inhibiting *NDT80* (TUNG *et al*. 2000). *NDT80* is required for the expression of the kinase *CDC5*, which is needed for dHJ resolution (SOURIRAJAN and LICHTEN 2008; CLYNE *et al*. 2003).

Rec8 cohesin complexes are not required for suppressing meiotic intersister DSB repair.

Given that Mek1 suppression of intersister DSB repair is specific to sister chromatids, a reasonable hypothesis is that the substrate(s) of Mek1 responsible for this suppression is associated with sister chromatids. One potential target is the multi-subunit cohesin complex that holds sister chromatids together after DNA replication (ONN *et al.* 2008). In mitotic cells, DSBs promote the recruitment of Mcd1-containing cohesin complexes to break sites and the replication-independent establishment of cohesion throughout the genome (GLYNN *et al.* 2004; STROM *et al.* 2007; STROM *et al.* 2004; UNAL *et al.* 2007). DSB-dependent cohesion facilitates, but is not essential, for Rad51-mediated repair of DSBs using sister chromatids as templates. When *REC8* is ectopically expressed in mitotic cells in place of Mcd1, Rec8 does not localize to breaks, suggesting this is a property specific to Mcd1 (HEIDINGER-PAULI *et al.* 2008). During meiosis, however, *rec8*Δ diploids exhibit unrepaired DSBs, raising the possibility that Rec8 cohesin complexes might be required for intersister recombination (BRAR *et al.* 2009; KLEIN *et al.* 1999; KUGOU *et al.* 2009). Our work shows, however, that when suppression of intersister repair is relieved by deletion of *MEK1* in both haploids and diploids, *rec8*Δ DSBs are repaired. Therefore, *REC8* is not necessary for intersister DSB repair and instead specifically promotes interhomolog recombination.

Rec8 cohesin complexes work with Mek1 in the meiotic recombination checkpoint.

Inhibition of Mek1 kinase activity in *dmc1*Δ strains allows meiotic progression because the signal to the meiotic recombination checkpoint—unrepaired DSBs—is

removed by repairing the DSBs using sister chromatids as templates (NIU *et al.* 2005). In mutants that prevent processing of the breaks and their subsequent repair, eliminating Mek1 activity allows meiotic progression, indicating that Mek1 is required for the meiotic recombination checkpoint (XU *et al.* 1997). We found that combining a slightly less active version of *MEK1*, *mek1-as1*, with a deletion of *REC8* eliminated the meiotic recombination checkpoint, whereas checkpoint activity was observed in the single mutant diploids. We propose that the effect of *rec8*Δ on the checkpoint is indirect. Genome wide studies have shown that the distribution of Spo11 on chromosomes is altered in *rec8*Δ mutants, such that fewer breaks occur on chromosomes such as I, V and VI (KUGOU *et al.* 2009). In contrast, little to no reduction in Spo11 localization or DSB formation was observed on chromosome III. Our model is that triggering the meiotic recombination checkpoint requires a threshold number of DSBs. Although the number of breaks generated in *rec8*Δ is reduced relative to wild type, this number is still above the threshold necessary for the checkpoint as cells arrest in meiotic prophase. Some DSB repair and meiotic progression were observed in the *mek1-as1 dmc1*Δ diploid used for these experiments, in contrast to *MEK1 dmc1*Δ, indicating that Mek1 activity is reduced by the analog-sensitive mutation. We propose that the weakened kinase activity of *mek1-as1* raises the threshold of DSBs required to trigger the checkpoint above the number formed in the *rec8*Δ, thereby preventing the checkpoint from detecting unrepaired breaks.

Rad51 and Dmc1 recombinase activities are used differentially for sister chromatid and interhomolog DSB repair.

An unresolved issue in meiotic recombination is the roles that the different recombinases, Rad51 and Dmc1, play. Although several studies have indicated that Rad51 and Dmc1 are primarily involved in intersister and interhomolog recombination, respectively, *rad51*Δ mutants exhibit defects in both interhomolog joint molecule and crossover formation, suggesting that there may be overlapping functions as well (HUNTER 2007; SHERIDAN and BISHOP 2006). However, interpretation of the *rad51*Δ mutant is complicated by the fact that Rad51 is required for efficient loading of Dmc1 onto resected DSB ends (BISHOP 1994; SHINOHARA *et al.* 1997a). Therefore the interhomolog recombination defects of *rad51*Δ could be due in part to an indirect effect from a paucity of Dmc1.

Our studies suggest that the requirements for the recombinase activities of Rad51 and Dmc1 are distinct during meiosis. Wild-type haploid strains exhibit a delay or lack of DSB repair between sister chromatids (this work) (DE MASSY *et al.* 1994). Similar to the *dmc1*Δ diploids, this block to intersister repair is dependent upon *MEK1*, indicating that Dmc1, like Rad51, is constrained in haploid cells from invading sister chromatids by Mek1. However our work shows that suppression of Dmc1-mediated repair between sister chromatids in haploids is indirect and dependent upon Rad51. This is consistent with a lack of homolog bias observed in diploids containing Dmc1-only filaments (LAO *et al.* 2008; SCHWACHA and KLECKNER 1997) .

These results are consistent with previous studies suggesting that the Rad51 protein plays a structural role in proper assembly of Dmc1 onto filaments (HUNTER and

KLECKNER 2001; LAO *et al.* 2008; SCHWACHA and KLECKNER 1997; SHERIDAN and BISHOP 2006). Filaments active for interhomolog recombination that contain only Rad51 can be generated by either over-expressing *RAD51* or *RAD54*, deleting *HED1* or preventing phosphorylation of *RAD54* in *dmc1*Δ strains (BISHOP *et al.* 1999; NIU *et al.* 2009; TSUBOUCHI and ROEDER 2003; TSUBOUCHI and ROEDER 2006). In these cases, inactivation of Mek1 leads to repair off sister chromatids and dead spores. Mek1's ability to suppress Rad51 strand invasion of sister chromatids appears to be independent of Dmc1.

Our data support the proposal that in wild-type cells, Rad51's function in interhomolog recombination is to load Dmc1 onto breaks in a way that directs the filament towards homologous chromosomes instead of sister chromatids. How this actually works is unclear. One intriguing idea is that Rad51 confers different structural properties to the filament compared to Dmc1, but analysis of the biophysical properties of Rad51 and Dmc1 filaments formed *in vitro* have revealed no obvious differences (SHERIDAN and BISHOP 2006; SHERIDAN *et al.* 2008). After Dmc1 is loaded, Rad51 recombinase activity is shut down by Hed1 and Rad54 phosphorylation so that interhomolog recombination is then mediated exclusively by Dmc1. This situation allows Mek1 to act as a switch that controls when intersister DSB repair will occur. Inactivation of Mek1 allows Rad51/Rad54 complex formation and strand invasion of sister chromatids, perhaps to repair any remaining DSBs. The idea that Rad51/Rad54 may be used exclusively for sister recombination is supported by the fact that *rad54*Δ mutants exhibit wild-type levels of interhomolog recombination but still display

reductions in sporulation and spore viability (SCHMUCKLI-MAURER and HEYER 2000; SHINOHARA *et al.* 1997b).

Table 3-1. *Saccharomyces cerevisiae* strains

Name	Yeast Genotype	Source
NHY1215	*MATα leu2::hisG his4-X::LEU2-(NgoMIV) ho::hisG ura3(Δpst-sma)*	N. Hunter
NHY1215 sir2	NHY1215 only *sir2Δ::natMX4*	This work
NHY1215 sir2 mek1	NHY1215 only *mek1Δ::URA3 sir2Δ::natMX4*	This work
NHY1215 sir2 dmc1	NHY1215 only *dmc1Δ::kanMX6 sir2Δ::natMX4*	This work
NHY1215 sir2 dmc1 mek1	NHY1215 only *mek1Δ::URA3 dmc1Δ::kanMX6 sir2Δ::natMX4*	This work
NHY1215 sir2 rec8	NHY1215 only *sir2Δ::natMX4 rec8Δ::kanMX6*	This work
NHY1215 sir2 mek1 rec8	NHY1215 only *sir2Δ::natMX4 rec8Δ::kanMX6 mek1Δ::URA3*	This work
NHY1215 rme1	NHY1215 only *rme1Δ::natMX4*	This work
NHY1215 rme1 mek1	NHY1215 only *mek1Δ::URA3 rme1Δ::natMX4*	This work
NHY1215 rme1 dmc1	NHY1215 only *dmc1Δ::kanMX6 rme1Δ::natMX4*	This work
NHY1215 rme1 dmc1 mek1	NHY1215 only *mek1Δ::URA3 dmc1Δ::kanMX6 rme1Δ::natMX4*	This work
NHY1215 sir2 rad52	NHY1215 only *sir2Δ::natMX4 rad52Δ::kanMX6*	This work
NHY1215 can1 cyh2	NHY1215 only *can1 cyh2*	This work
NH716[a]	*MATα leu2::hisG his4-X::LEU2 (NgoMIV) hoΔ::hisG ura3(Δpst-sma)* *MAT**a** leu2::hisG HIS4::LEU2 hoΔ::hisG ura3(Δpst-sma)*	N. Hunter
NH729	*NH716 only mek1Δ::natMX4* *mek1Δ::natMX4*	This work
NH705-32-1 dmc1	*MAT**a** ura3-52 kar1-1 ade2 dmc1Δ::natMX4*	This work
Kar-3-WT	*MATα leu2::hisG his4-X::LEU2(NgoMIV) ho::hisG ura3(Δpst-sma)can1 cyh2* *MAT**a** LEU2 HIS4*	This work

Table 3-1. *Saccharomyces cerevisiae* strains continued

Name	Yeast Genotype	Source
Kar-3-mek1	Kar-3-WT only *mek1Δ::URA3*	This work
Kar-3-sir2	Kar-3-WT only *sir2Δ::natMX4*	This work
NH929	*MATα leu2::hisG his4-X::LEU2 (NgoMIV) ho::hisG ura3(Δpst-sma) can1 cyh2* *MAT**a** LEU2 HIS4 ho::hisG ura3(Δpst-sma) can1 cyh2*	This work
NH144[a]	*MATα leu2-k HIS4 arg4-Nsp ura3 lys2 hoΔ::LYS2* *MAT**a** leu2::hisG his4-X ARG4 ura3 lys2 hoΔ::LYS2*	Hollingsworth *et al.* (1995)
NH746	NH144 only *rec8Δ::kanMX6* *rec8Δ::kanMX6*	This work
NH748	NH144 only *dmc1Δ::natMX4* *dmc1Δ::natMX4*	This work
NH748::pRS306	NH144 only *ura3::URA3 dmc1Δ::natMX4* *ura3 dmc1Δ::natMX4*	This work
NH749	NH144 only *dmc1Δ::natMX4 mek1Δ::LEU2* *dmc1Δ::natMX4 mek1Δ::LEU2*	This work
NH749::pJR2	NH144 only *dmc1Δ::natMX4 mek1Δ::LEU2::URA3::mek1-as1* *dmc1Δ::natMX4 mek1Δ::LEU2*	This work
NH751	NH144 only *rec8Δ::kanMX6 mek1Δ::LEU2* *rec8Δ::kanMX6 mek1Δ::LEU2*	This work
NH752::pRS306	NH144 only *ura3::URA3 dmc1Δ::natMX4 rec8Δ::kanMX6* *ura3 dmc1Δ::natMX4 rec8Δ::kanMX6*	This work
NH753	NH144 only *dmc1Δ::natMX4 rec8Δ::kanMX6 mek1Δ::LEU2* *dmc1Δ::natMX4 rec8Δ::kanMX6 mek1Δ::LEU2*	This work
NH753::pJR2	NH144 only *dmc1Δ::natMX4 rec8Δ::kanMX6 mek1Δ::LEU2::URA3::mek1-as1* *dmc1Δ::natMX4 rec8Δ::kanMX6 mek1Δ::LEU2*	This work

[a] Although the haploid parents of NH716 and NH144 are derived from the SK1 background, they were obtained from different sources and are not necessarily isogenic with each other.

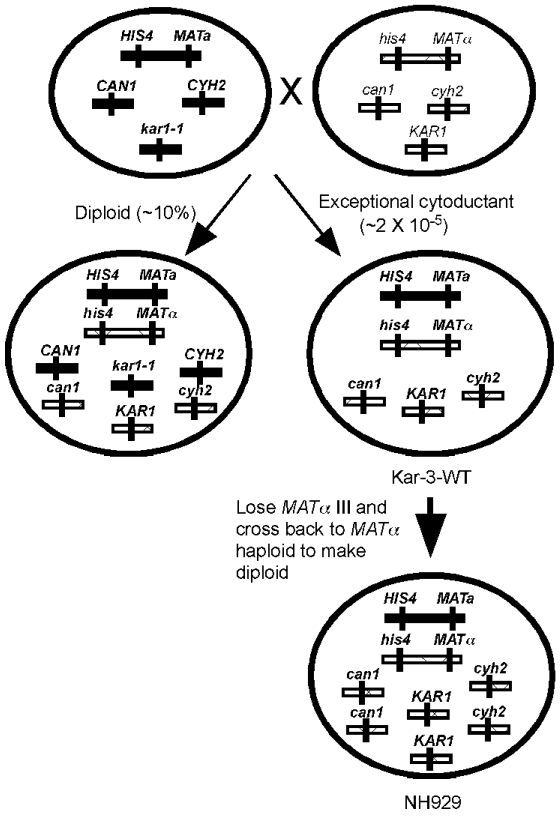

Figure 3-1. Schematic of the construction of the disomic haploid, Kar-3-WT and its isogenic diploid. A *MAT***a** *kar1-1* donor strain was crossed to a *MATα his4* recipient strain and exceptional cytoductants containing the haploid genome of the recipient strain and chromosome III from the donor strain were selected to generate the disomic haploid, Kar-3-WT. A diploid isogenic with Kar-3-WT was created by losing the chromosome carrying *MATα his4* and backcrossing the resulting *MAT***a** *HIS4* haploid to the *MATα his4* parent to make NH929.

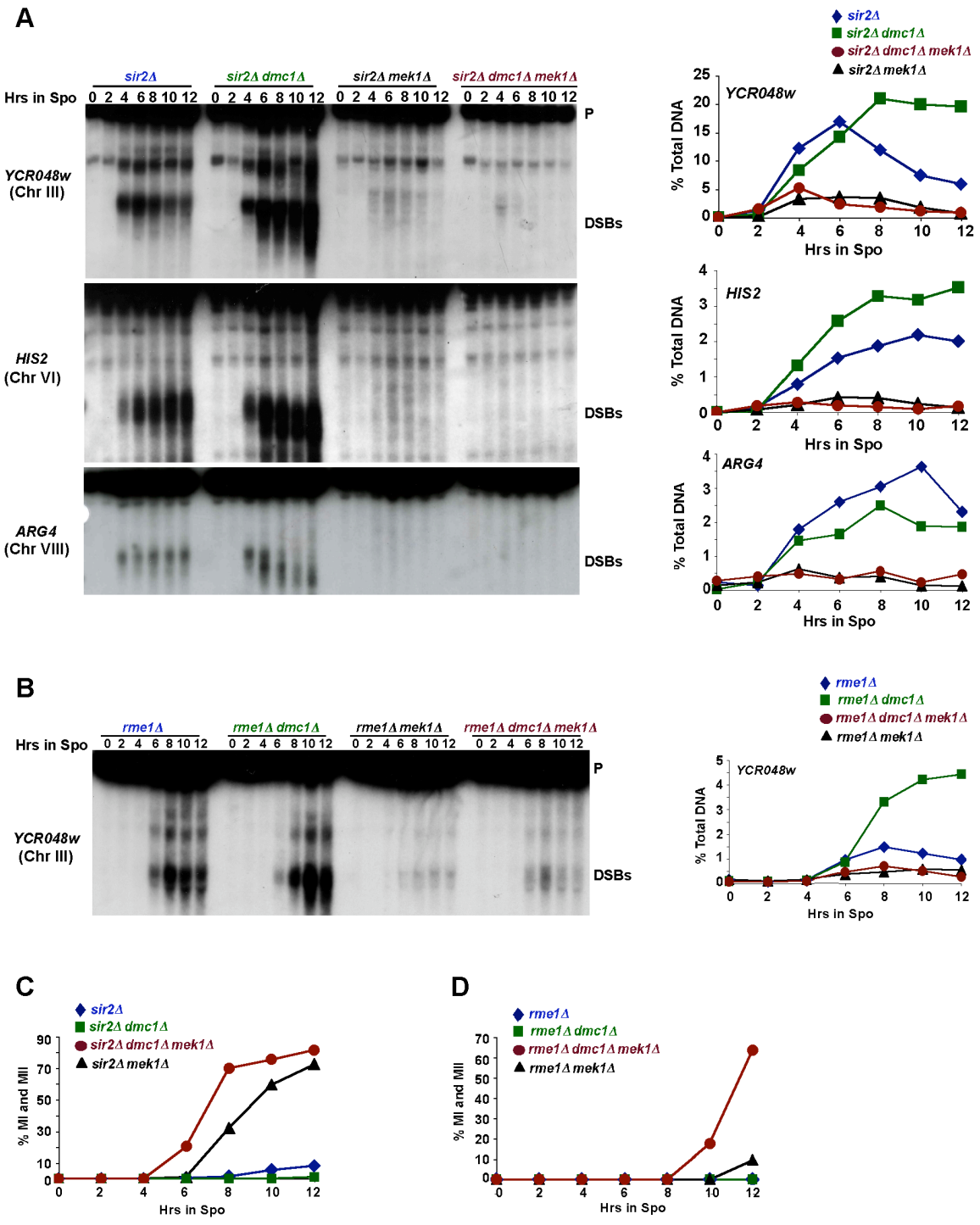

Figure 3-2. Suppression of meiotic intersister DSB repair in various haploids. A. Isogenic derivatives of NHY1215 containing *sir2*Δ, *sir2*Δ *dmc1Δ*, *sir2*Δ *mek1Δ* or *sir2*Δ *dmc1Δ mek1Δ* were sporulated at 30°C. DSBs at three different hotspots were analyzed at various times after transfer to Spo medium. B. Similar experiment to Panel A only the NHY1215 derivatives contain *rme1*Δ instead of *sir2Δ*. Graphs indicate the percent of

total DNA constituted by the DSB fragments. C. Meiotic progression of the time courses shown in Panel A. D. Meiotic progression of the time courses shown in Panel B. % MI and MII refers to the number of bi- and tetra-nucleate cells, respectively.

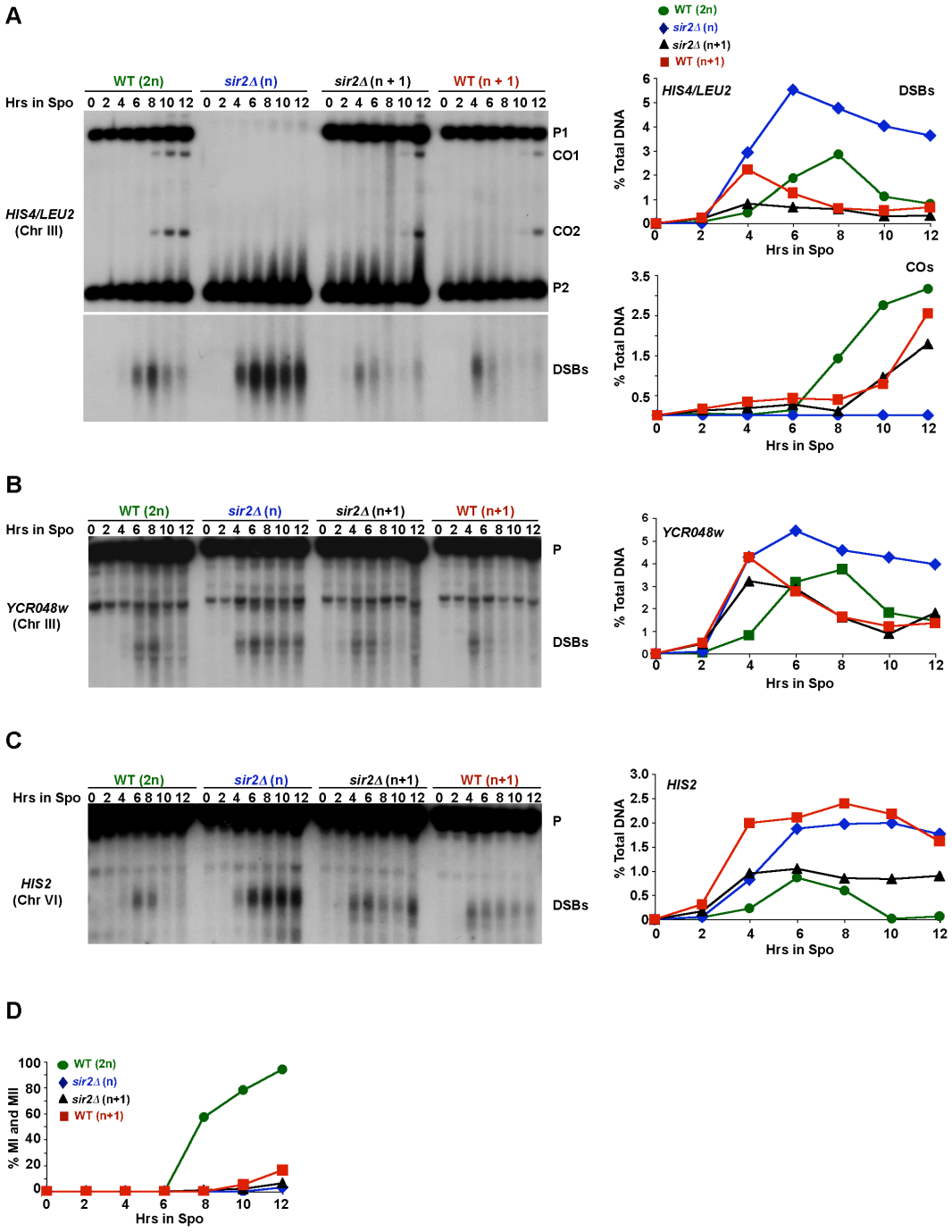

Figure 3-3. Meiotic DSB repair in diploid, haploid and disomic haploid strains. Meiotic time courses of isogenic diploid (2n, NH929), haploid (n, NHY1215 sir2) and chromosome III disomic haploids (n+1, Kar-3-sir2 and Kar-3-WT) strains were performed. A. DSBs and crossovers at the *HIS4/LEU2* hotspot on chromosome III. Parental bands are indicated P1 and P2 and crossover bands are indicated CO1 and CO2. COs and DSBs were detected on the same blot, but for clarity a longer exposure of the DSB portion of the blot is shown. B. DSBs at the *YCR048w* hotspot on chromosome III.

C. DSBs at the *HIS2* hotspot on chromosome VI. Graphs indicate quantitation of the DSB bands. D. Meiotic progression of NH929, NHY1215 sir2, Kar-3-sir2 and Kar-3-WT measured by counting DAPI stained nuclei.

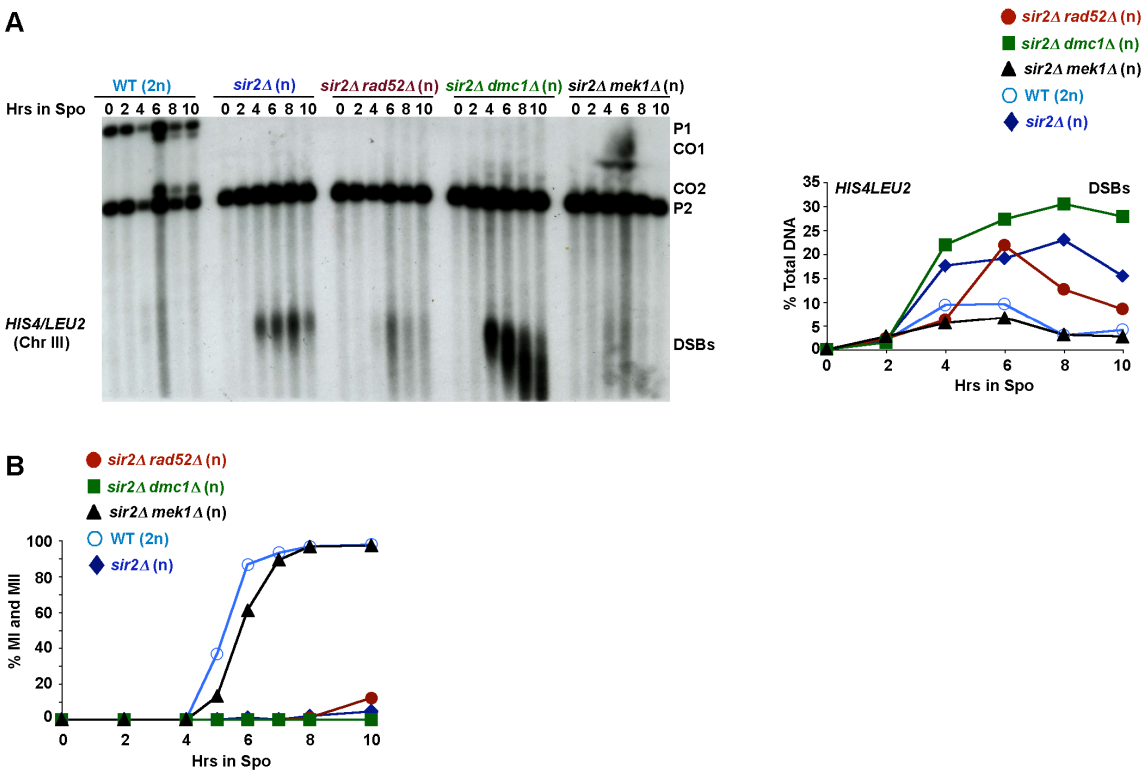

Figure 3-4. Differential patterns of DSB repair in wild-type haploids compared to *dmc1*Δ or *rad52*Δ haploids. A. Diploid wild-type strain NH716 and haploid strains *sir2Δ* (NHY1215 sir2), *sir2*Δ *rad52*Δ (NHY1215 sir2 rad52), *sir2Δ dmc1Δ* (NHY1215 sir2 dmc1) and *sir2*Δ *mek1*Δ (NHY1215 sir2 mek1) were sporulated at 30°C and analyzed at the indicated time-points for DSBs at the *HIS4/LEU2* hotspot. B. Meiotic progression of the time courses shown in Panel A.

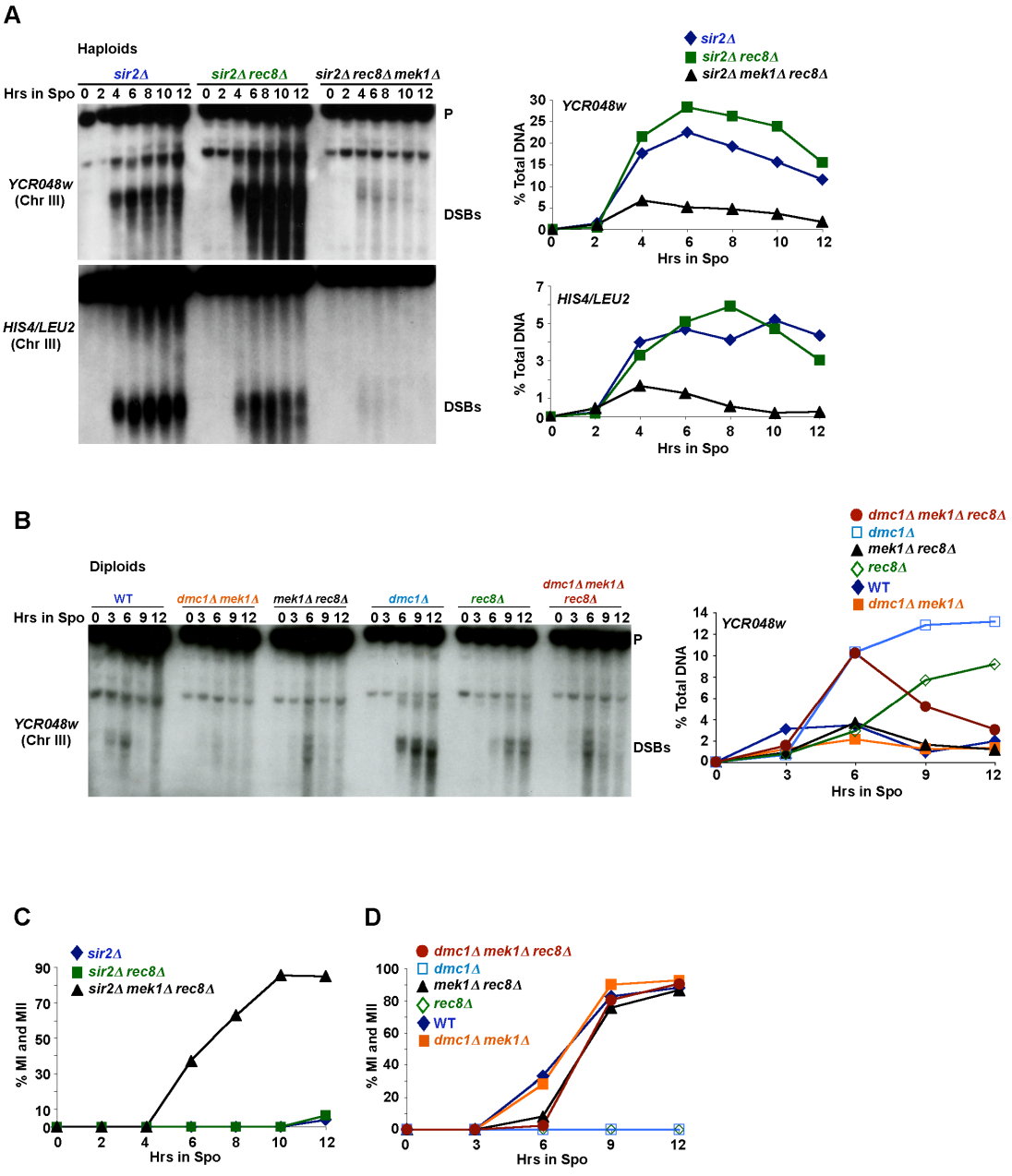

Figure 3-5. DSB repair in various *rec8* strains. A. Haploid strains: DSB repair examined at two different hotspots on chromosome III in *sir2Δ* (NHY1215 sir2), *sir2Δ rec8Δ* (NHY1215 sir2 rec8) and *sir2Δ rec8Δ mek1Δ* (NHY1215 sir2 rec8 mek1). B. Diploid strains: DSB repair at the *YCR048w* hotspot in wild type (NH144), *mek1*Δ *rec8*Δ (NH751), *rec8*Δ (NH746), *dmc1Δ* (NH 748), *dmc1Δ mek1Δ* (NH749) and *dmc1*Δ *mek1Δ rec8*Δ (NH753). Graphs indicate quantitation of the DSB bands. C. Meiotic progression in haploids from the time courses shown in Panel A. D. Meiotic progression in diploids from the time courses shown in Panel B.

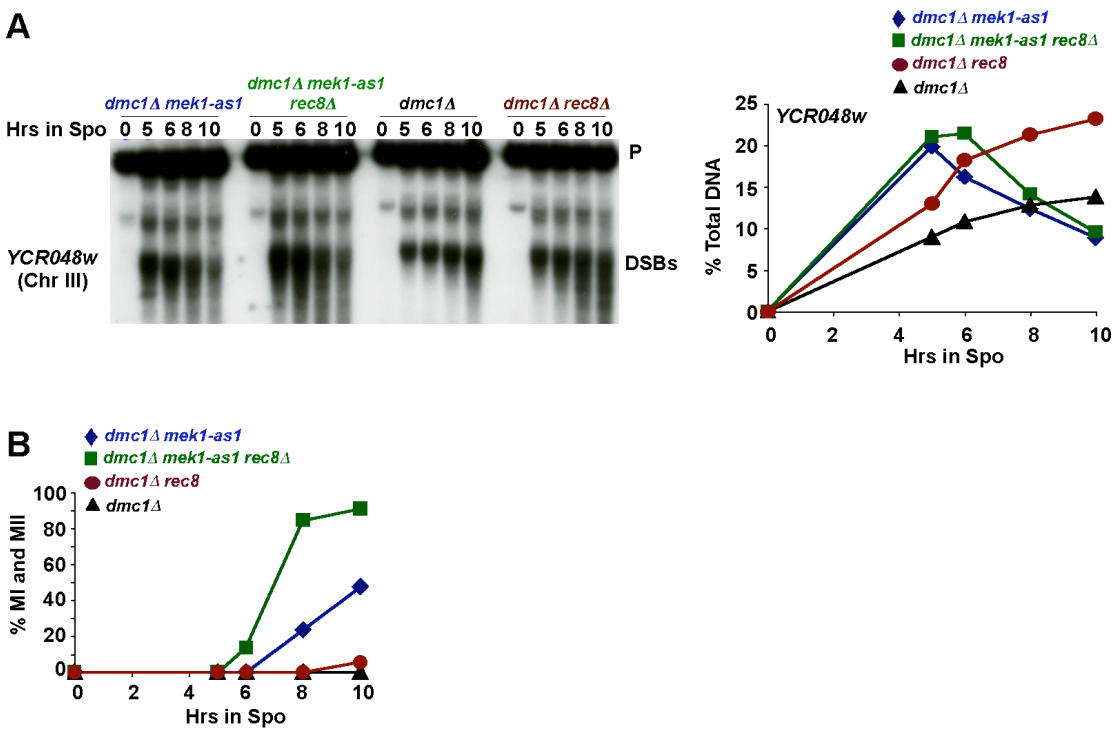

Figure 3-6. Meiotic DSB repair and progression in *dmc1*Δ *mek1-as1* and *dmc1*Δ *mek1-as1 rec8*Δ diploids. Time-courses were performed with *dmc1*Δ *mek1-as1* (NH749::pJR2), *dmc1*Δ *mek1-as1 rec8*Δ (NH 753::pJR2), *dmc1*Δ (NH748::pRS306) and *dmc1Δ rec8*Δ (NH752::pRS306). A. DSBs were analyzed at the *YCR048w* hotspot. Graphs indicate quantitation of DSBs. B. Meiotic progression from the time courses shown in Panel A.

CHAPTER FOUR

DISCUSSION

Understanding interhomolog bias in meiosis is fundamental in determining how meiotic DNA repair is regulated. Studies have shown that the axial element proteins Mek1, Hop1 and Red1, bias the cell to use the homologous chromosomes for DSB repair (BAILIS and ROEDER 1998; DE LOS SANTOS and HOLLINGSWORTH 1999; SCHWACHA and KLECKNER 1997; WAN *et al.* 2004; XU *et al.* 1997).

Mek1 has been shown to have similarities to members of the conserved family of ForkHead Associated (FHA) domain containing protein kinases, which includes the *S. cerevisiae* checkpoint kinase, Rad53. Rad53 has been shown to have two FHA domains, which are phosphoprotein regulation motifs that mediate phosphorylation dependent protein-protein interactions, versus Mek1's single FHA domain (HOFFMAN and BUCHER 1995). Like budding yeast, there has been identification of a *S. pombe* ortholog of the Mek1 kinase. Studies have shown that the fission yeast Mek1 also has one FHA domain and the *mek1Δ* mutation causes a reduction in spore viability and recombination similar to that seen in budding yeast, however there has not been a role for SC formation, as *S. pombe* lack SCs (LORENZ *et al*. 2004). Despite the differences between the two yeasts, it appears that Mek1 plays a common role in both systems, in that it appears to be essential for the meiotic recombination checkpoint (BIALIS and ROEDER 2000; MACQUEEN and VILLENEUVE, 2001; PÉREZ-HIDALGO *et al*. 2002).

Although the identification of Mek1 kinase and Red1 in non-fungal species has been difficult, orthologs of Hop1 have been identified in several other species beyond yeasts. The ortholog Him-3, a component of the chromosome axes in *C. elegans*, has been shown to be required for homolog pairing and recombination (ZETKA *et al.* 1999) and some phenotypes in *him-3* mutants have implicated its requirement in the suppression

of meiotic intersister DSB repair (COUTEAU *et al.* 2004). Similarly, the *HTP* genes, also closely related to *HIM3*, have been functionally linked to SC assembly coordination, homolog alignment and pairing in worms (COUTEAU and ZETKA 2005; GOODYER *et al.* 2008; MARTINEZ-PEREZ and VILLENEUVE 2005). In plants, *ASY1* exhibits homology with Hop1 and Him3 (CARYL *et al.* 2000), where it has been shown that the presence of this protein is required to promote *AtDMC1*-mediated recombination (SANCHEZ-MORAN *et al.* 2007). The studies done in mice with *HORMAD1*, a putative mammalian ortholog of Hop1, have suggested that it is an important part of a surveillance system that ensures and monitors homologous chromosome synapsis (FUKUDA *et al.* 2010; WOJTASZ *et al.* 2009). These studies using these organisms suggest that the regulation of interhomolog interaction during meiosis is evolutionarily conserved.

Studies using *dmc1*Δ mutants as well as wild-type haploid strains clearly show that Mek1 suppresses intersister DSB repair under these conditions (CALLENDER and HOLLINGSWORTH 2010; NIU *et al.* 2005; WAN *et al.* 2004). In both of these situations, the accumulation of DSBs leads to induction of the meiotic recombination checkpoint, raising the possibility that Mek1 functions differently in wild-type meioses. For example, Goldfarb and Lichten (submitted) have recently shown that Mek1 induces a three-fold delay in the repair of DSBs in hemizygous or monosomic chromosomes, in contrast to what I observed in haploid cells where DSB repair was greatly delayed or non-existent. Goldfarb and Lichten propose, similar to NIU *et al.* (2007), that Mek1 is activated locally in the vicinity of a DSB. Rather than proposing there is a second substrate other than Rad54 whose phosphorylation prevents strand invasion of the sister, Goldfarb and

Lichten suggest that Mek1 activity creates a kinetic barrier to intersister repair that promotes strand invasion of the homologs.

My studies using haploid strains induced to undergo meiosis reveal that DSBs are not repaired in the absence of homologs, also shown by DE MASSY *et al.* (1994), and that this repair is dependent on the presence and activity of Mek1. One explanation to reconcile the different phenotypes observed between haploid and hemizygous strains is that in haploid cells there are increased levels of ssDNA, due to the hyperresected breaks, in the cell. This may activate Mec1/Tel1 responses ultimately leading to a hyperactive Mek1, which then imposes an overall suppression of intersister repair (Goldfarb and Lichten 2010, submitted). Another perspective on Mek1's role in intersister repair has been identified by TERENTYEV *et al.* (2010). Using a system where DSBs can be repaired either using homologs or by single strand annealing with the same chromatid or between sister chromatids, Mek1 was observed to promote interhomolog strand invasion. It should be noted that the fact that Mek1 functions to suppress intersister strand invasion does not rule out that it may have a role in actively promoting interhomolog strand invasion as well.

My thesis research focused on determining some of the biological parameters that were required for the suppression of meiotic intersister DSB repair in budding yeast. Previous genetic studies in the lab have shown that in the absence of Mek1 kinase activity in a *dmc1Δ* diploid mutant, DSBs are repaired using sister chromatids (NIU *et al.* 2005). However, my studies confirmed, through taking advantage of the physical assays available to detect recombination intermediates and products, that the Rad51-mediated repair observed after the inactivation of Mek1 kinase activity in *dmc1Δ* mutants, occurs

via a joint molecule pathway exclusively between sister chromatids, indicated by the absence of IHJMs. This also reinforces that the suppression is dependent on Mek1 kinase activity.

Previous studies with the recombinases Rad51 and Dmc1 have revealed that they have distinct roles, in that they are responsible for mediating meiotic intersister strand invasion and interhomolog strand invasion respectively (BISHOP *et al.* 1992; PAQUES and HABER 1999). However, the two recombinases also work in concert with each other during interhomolog strand invasion in meiosis, where Rad51 is required to efficiently load Dmc1 onto single stranded filaments. It is also known that over-expressing *RAD51* in a *dmc1Δ* diploid can partially restore the interhomolog strand invasion defect indicating that they have overlapping roles as well (SHINOHARA *et al.* 1997a; TSUBOUCHI and ROEDER 2003). My studies revealed that Dmc1 is capable of intersister DSB repair in haploid cells when the option of homolog strand invasion and Rad51 are absent. This result can therefore be interpreted to mean that the presence of Rad51 prevents Dmc1 from functioning in intersister repair. It is possible to speculate that Rad51 confers a structural property to Dmc1 which directs it to homologous chromosomes. The *DMC1* gene is absent from both worms and flies, which also do not rely on recombination events to synapse their homologs. However, both organisms still have the conserved Rad51 recombinase indicating that whatever Dmc1's function is to mediate interhomolog strand invasion, may not be necessary (VILLENEUVE and HILLERS 2001).

Understanding how Mek1 kinase functions in creating the suppression of intersister DSB repair, raises the question of whether one of its substrates is closely associated with sister chromatids. I examined the possibility of whether the meiotic

cohesin complex, Rec8, was involved in meiotic intersister DSB repair similar to what has been previously published in vegetative cells with Mcd1 (STROM *et al.* 2004; UNAL *et al.* 2004). However, my studies revealed that Rec8 is specifically required for interhomolog recombination, indicating that there may be other complexes needed to mediate intersister repair.

REFERENCES

ALANI, E., R. PADMORE and N. KLECKNER, 1990 Analysis of wild-type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. Cell **61:** 419-436.

ALLERS, T., and M. LICHTEN, 2001 Differential timing and control of noncrossover and crossover recombination during meiosis. Cell **106:** 47-57.

ARBEL, A., D. ZENVIRTH and G. SIMCHEN, 1999 Sister chromatid-based DNA repair is mediated by *RAD54*, not by *DMC1* or *TID1*. Embo J **18:** 2648-2658.

BAILIS, J. M., and G. S. ROEDER, 1998 Synaptonemal complex morphogenesis and sister-chromatid cohesion require Mek1-dependent phosphorylation of a meiotic chromosomal protein. Genes Dev **12:** 3551-3563.

BAILIS, J. M., and G. S. ROEDER, 2000 Pachytene exit controlled by reversal of Mek1 dependent phosphorylation. Cell **101:** 211-221.

BASCOM-SLACK, C. A., L. O. ROSS and D. S. DAWSON, 1997 Chiasmata, crossovers, and meiotic chromosome segregation. Adv Genet **35:** 253-284.

BELL, L. R., and B. BYERS, 1983 Homologous association of chromosomal DNA during yeast meiosis. Cold Spring Harb Symp Quant Biol **47 Pt 2:** 829-840.

BISHOP, A. C., K. SHAH, Y. LIU, L. WITUCKI, C. KUNG and K. M. SHOKAT, 1998 Design of allele-specific inhibitors to probe protein kinase signaling. Curr Biol **8:** 257-266.

BISHOP, D. K., 1994 RecA homologs Dmc1 and Rad51 interact to form multiple nuclear complexes prior to meiotic chromosome synapsis. Cell **79:** 1081-1092.

BISHOP, D. K., Y. NIKOLSKI, J. OSHIRO, J. CHON, M. SHINOHARA and X. CHEN, 1999 High copy number suppression of the meiotic arrest caused by a *dmc1* mutation: *REC114* imposes an early recombination block and *RAD54* promotes a *DMC1*-independent DSB repair pathway. Genes Cells **4:** 425-444.

BISHOP, D. K., D. PARK, L. XU and N. KLECKNER, 1992 *DMC1*: a meiosis-specific yeast homolog of E. coli recA required for recombination, synaptonemal complex formation, and cell cycle progression. Cell **69:** 439-456.

BLITZBLAU, H. G., G. W. BELL, J. RODRIGUEZ, S. P. BELL and A. HOCHWAGEN, 2007 Mapping of meiotic single-stranded DNA reveals double-stranded break hotspots near centromeres and telomeres. Curr Biol **17:** 2003-2012.

BORDE, V., T. C. WU and M. LICHTEN, 1999 Use of a recombination reporter insert to define meiotic recombination domains on chromosome III of *Saccharomyces cerevisiae*. Mol Cell Biol **19:** 4832-4842.

BRAR, G. A., A. HOCHWAGEN, L. S. EE and A. AMON, 2009 The multiple roles of cohesin in meiotic chromosome morphogenesis and pairing. Mol Biol Cell **20:** 1030-1047.

BUHLER, C., V. BORDE and M. LICHTEN, 2007 Mapping meiotic single-strand DNA reveals a new landscape of DNA double-strand breaks in *Saccharomyces cerevisiae*. PLoS Biol **5:** e324.

BULLARD, S. A., S. KIM, A. M. GALBRAITH and R. E. MALONE, 1996 Double strand breaks at the *HIS2* recombination hot spot in *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A **93:** 13054-13059.

BUONOMO, S. B., R. K. CLYNE, J. FUCHS, J. LOIDL, F. UHLMANN and K. NASMYTH, 2000 Disjunction of homologous chromosomes in meiosis I depends on proteolytic cleavage of the meiotic cohesin Rec8 by separin. Cell **103:** 387-398.

BUSYGINA, V., M. G. SEHORN, I. Y. SHI, H. TSUBOUCHI, G. S. ROEDER and P. SUNG, 2008 Hed1 regulates Rad51-mediated recombination via a novel mechanism. Genes Dev **22:** 786-795.

BZYMEK, M., N. H. THAYER, S. D. OH, N. KLECKNER and N. HUNTER, 2010 Double Holliday junctions are intermediates of DNA break repair. Nature **464:** 937-941.

CALLENDER, T. L., and N. M. HOLLINGSWORTH, 2010 Mek1 suppression of meiotic double-strand break repair is specific to sister chromatids, chromosome autonomous and independent of Rec8 cohesion complexes. Genetics **185:** 771-782.

CAO, L., E. ALANI and N. KLECKNER, 1990 A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. Cell **61:** 1089-1101.

CARBALLO, J. A., A. L. JOHNSON, S. G. SEDGWICK and R. S. CHA, 2008 Phosphorylation of the axial element protein Hop1 by Mec1/Tel1 ensures meiotic interhomolog recombination. Cell **132:** 758-770.

CARYL, A. P., S. J. ARMSTRONG, G. H. JONES and F. C. FRANKLIN, 2000 A homologue of the yeast *HOP1* gene is inactivated in the *Arabidopsis* meiotic mutant *asy1*. Chromosoma **109:** 62-71.

CHAMPION, M. D., and R. S. HAWLEY, 2002 Playing for half the deck: the molecular biology of meiosis. Nat Cell Biol **4 Suppl:** s50-56.

CHEN, S., T. TSUBOUCHI, B. ROCKMILL, J. S. SANDLER, D. R RICHARDS, G. VADER, A. HOCHWAGEN, G. ROEDER and J. C. FUNG, 2008 Global analysis of the meiotic crossover landscape. Dev Cell **15:** 401-415.

CIOSK, R., W. ZACHARIAE, C. MICHAELIS, A. SHEVCHENKO, M. MANN and K. NASMYTH, 1998 An ESP1/PDS1 complex regulates loss of sister chromatid cohesion at the metaphase to anaphase transition in yeast. Cell **93:** 1067-1076.

CYLNE, R., V. KATIS, L. JESSOP, K. BENJAMIN, I. HERSKOWITZ, M. LICHTEN and K. NASMYTH, 2003 Polo like kinase Cdc5 promotes chiasmata formation and cosegregation of sister centromeres at meiosis I. Nat Cell Biol **5:** 480-485.

COHEN-FIX, O., J. M. PETERS, M. W. KIRSCHNER and D. KOSHLAND, 1996 Anaphase initiation in *Saccharomyces cerevisiae* is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p. Genes Dev **10:** 3081-3093.

COUTEAU, F., K. NABESHIMA, A. VILLENEUVE and M. ZETKA, 2004 A component of *C. elegans* meiotic chromosome axes at the interface of homolog alignment, synapsis, nuclear reorganization, and recombination. Curr Biol **14:** 585-592.

COUTEAU, F., and M. ZETKA, 2005 HTP-1 coordinates synaptonemal complex assembly with homolog alignment during meiosis in *C. elegans*. Genes Dev **19:** 2744-2756.

COVITZ, P. A., I. HERSKOWITZ and A. P. MITCHELL, 1991 The yeast *RME1* gene encodes a putative zinc finger protein that is directly repressed by a1-alpha 2. Genes Dev **5:** 1982-1989.

DE LOS SANTOS, T., and N. M. HOLLINGSWORTH, 1999 Red1p, a *MEK1*-dependent phosphoprotein that physically interacts with Hop1p during meiosis in yeast. J Biol Chem **274:** 1783-1790.

DE LOS SANTOS, T., N. HUNTER, C. LEE, B. LARKIN, J. LOIDL and N. M. HOLLINGSWORTH, 2003 The Mus81/Mms4 endonuclease acts independently of double-Holliday junction resolution to promote a distinct subset of crossovers during meiosis in budding yeast. Genetics **164:** 81-94.

DE MASSY, B., F. BAUDAT and A. NICOLAS, 1994 Initiation of recombination in *Saccharomyces cerevisiae* haploid meiosis. Proc Natl Acad Sci U S A **91:** 11929-11933.

DE MASSY, B., and A. NICOLAS, 1993 The control in cis of the position and the amount of the *ARG4* meiotic double-strand break of *Saccharomyces cerevisiae*. Embo J **12:** 1459-1466.

DRESSER, M. E., D. J. EWING, M. N. CONRAD, A. M. DOMINGUEZ, R. BARSTEAD, H. JIANG and T. KODADEK, 1997 *DMC1* functions in a *Saccharomyces cerevisiae* meiotic

pathway that is largely independent of the *RAD51* pathway. Genetics **147:** 533-544.

DUTCHER, S. K., 1981 Internuclear transfer of genetic information in *kar1-1/KAR1* heterokaryons in *Saccharomyces cerevisiae*. Mol Cell Biol **1:** 245-253.

FUKUDA, T., K. DANIEL, L. WOJTASZ, A. TOTH and C. HOOG, 2010 A novel mammalian HORMA domain-containing protein, HORMAD1, preferentially associates with unsynapsed meiotic chromosomes. Exp Cell Res **316:** 158-171.

GASIOR, S. L., A. K. WONG, Y. KORA, A. SHINOHARA and D. K. BISHOP, 1998 Rad52 associates with RPA and functions with Rad55 and Rad57 to assemble meiotic recombination complexes. Genes Dev **12:** 2208-2221.

GLYNN, E. F., P. C. MEGEE, H. G. YU, C. MISTROT, E. UNAL, D. E. KOSHLAND, J. L. DERISI and J. L. GERTON, 2004 Genome-wide mapping of the cohesin complex in the yeast *Saccharomyces cerevisiae.* PLoS Biol **2:** E259.

GOODYER, W., S. KAITNA, F. COUTEAU, J. D. WARD, S. J. BOULTON and M. ZETKA, 2008 HTP-3 links DSB formation with homolog pairing and crossing over during *C. elegans* meiosis. Dev Cell **14:** 263-274.

GRUBER, S., P. ARUMUGAM, Y. KATOU, D. KUGLITSCH, W. HELMHART, K. SHIRAHIGE and K. NASMYTH, 2006 Evidence that loading of cohesin onto chromosomes involves opening of its SMC hinge. Cell **127:** 523-537.

GRUBER, S., C. H. HAERING and K. NASMYTH, 2003 Chromosomal cohesin forms a ring. Cell **112:** 765-777.

GUACCI, V., D. KOSHLAND and A. STRUNNIKOV, 1997 A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of *MCD1* in *S. cerevisiae*. Cell **91:** 47-57.

HAYASE, A., M. TAKAGI, T. MIYAZAKI, H. OSHIUMI, M. SHINOHARA and A. SHINOHARA, 2004 A protein complex containing Mei5 and Sae3 promotes the assembly of the meiosis-specific RecA homolog Dmc1. Cell **119:** 927-940.

HAWLEY, R. S., 2002 Meiosis: How male flies do meiosis. Curr Biol **12:** 660-662.

HEIDINGER-PAULI, J. M., E. UNAL, V. GUACCI and D. KOSHLAND, 2008 The kleisin subunit of cohesin dictates damage-induced cohesion. Mol Cell **31:** 47-56.

HOFFMAN, K. and P. BUCHER, 1995 The FHA domain: a putative nuclear signaling domain found in protein kinases and transcription factors. Trends Biochem Sci **20:** 347-349.

HOLLINGSWORTH, N. M., and B. BYERS, 1989 *HOP1*: a yeast meiotic pairing gene. Genetics **121:** 445-462.

HOLLINGSWORTH, N. M., L. GOETSCH and B. BYERS, 1990 The *HOP1* gene encodes a meiosis-specific component of yeast chromosomes. Cell **61:** 73-84.

HOLLINGSWORTH, N. M., and A. D. JOHNSON, 1993 A conditional allele of the *Saccharomyces cerevisiae HOP1* gene is suppressed by overexpression of two other meiosis-specific genes: *RED1* and *REC104*. Genetics **133:** 785-797.

HOLLINGSWORTH, N. M., L. PONTE and C. HALSEY, 1995 *MSH5*, a novel MutS homolog, facilitates meiotic reciprocal recombination between homologs in *Saccharomyces cerevisiae* but not mismatch repair. Genes Dev **9:** 1728-1739.

HUGERAT, Y., and G. SIMCHEN, 1993 Mixed segregation and recombination of chromosomes and YACs during single-division meiosis in *spo13* strains of *Saccharomyces cerevisiae*. Genetics **135:** 297-308.

HUNTER, N., 2007 Meiotic recombination Springer-Verlag, Heidelberg, Germany.

HUNTER, N., and N. KLECKNER, 2001 The single-end invasion: an asymmetric intermediate at the double-strand break to double-holliday junction transition of meiotic recombination. Cell **106:** 59-70.

JACKSON, J. A., and G. R. FINK, 1985 Meiotic recombination between duplicated genetic elements in *Saccharomyces cerevisiae*. Genetics **109:** 303-332.

JOHNSON, R., V. BORDE, M. J. NEALE, A. BISHOP-BAILEY, M. NORTH, S. HARRIS, A. NICOLAS and A. S. GOLDMAN, 2007 Excess single-stranded DNA inhibits meiotic double-strand break repair. PLoS Genet **3:** e223.

KADYK, L. C., and L. H. HARTWELL, 1992 Sister chromatids are preferred over homologs as substrates for recombinational repair in *Saccharomyces cerevisiae*. Genetics **132:** 387-402.

KASSIR, Y., D. GRANOT and G. SIMCHEN, 1988 *IME1*, a positive regulator gene of meiosis in *S. cerevisiae*. Cell **52:** 853-862.

KATIS, V. L., J. MATOS, S. MORI, K. SHIRAHIGE, W. ZACHARIAE and K. NASMYTH, 2004 Spo13 facilitates monopolin recruitment to kinetochores and regulates maintenance of centromeric cohesion during yeast meiosis. Curr Biol **14:** 2183-2196.

KEENEY, S., 2001 Mechanism and control of meiotic recombination initiation. Curr Top Dev Biol **52:** 1-53.

KEENEY, S., C. N. GIROUX and N. KLECKNER, 1997 Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell **88:** 375-384.

KITAJIMA, T. S., S. A. KAWASHIMA and Y. WATANABE, 2004 The conserved kinetochore protein shugoshin protects centromeric cohesion during meiosis. Nature **427:** 510-517.

KLAPHOLZ, S., and R. E. ESPOSITO, 1980 Isolation of *SPO12-1* and *SPO13-1* from a natural variant of yeast that undergoes a single meiotic division. Genetics **96:** 567-588.

KLEIN, F., P. MAHR, M. GALOVA, S. B. BUONOMO, C. MICHAELIS, K. NAIRZ and K. NASMYTH, 1999 A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. Cell **98:** 91-103.

KUGOU, K., T. FUKUDA, S. YAMADA, M. ITO, H. SASANUMA, S. MORI, Y. KATOU, T. ITOH, K. MATSUMOTO, T. SHIBATA, K. SHIRAHIGE and K. OHTA, 2009 Rec8 guides canonical Spo11 distribution along yeast meiotic chromosomes. Mol Biol Cell **20:** 3064-3076.

LAO, J. P., S. D. OH, M. SHINOHARA, A. SHINOHARA and N. HUNTER, 2008 Rad52 promotes postinvasion steps of meiotic double-strand break repair. Mol Cell **29:** 517-524.

LEE, B. H., B. M. KIBURZ and A. AMON, 2004 Spo13 maintains centromeric cohesion and kinetochore coorientation during meiosis I. Curr Biol **14:** 2168-2182.

LEEM, S. H., and H. OGAWA, 1992 The *MRE4* gene encodes a novel protein kinase homologue required for meiotic recombination in *Saccharomyces cerevisiae*. Nucleic Acids Res **20:** 449-457.

LI, X., and R. B. NICKLAS, 1995 Mitotic forces control a cell-cycle checkpoint. Nature **373:** 630-632.

LOIDL, J., F. KLEIN and H. SCHERTHAN, 1994 Homologous pairing is reduced but not abolished in asynaptic mutants of yeast. J Cell Biol **125:** 1191-1200.

LONGTINE, M. S., A. MCKENZIE, 3RD, D. J. DEMARINI, N. G. SHAH, A. WACH, A. BRACHAT, P. PHILIPPSEN and J. R. PRINGLE, 1998 Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. Yeast **14:** 953-961.

LORENZ, A., J. L. WELLS, D. W. PRYCE, M. NOVATCHKOVA, F. EISENHABER, R. J. MCFARLANE and J. LOIDL, 2004 *S. pombe* meiotic linear elements contain

proteins related to synaptonemal complex components. J Cell Sci **117:** 3343-3351.

LOSADA, A., M. HIRANO and T. HIRANO, 1998 Identification of *Xenopus* SMC protein complexes required for sister chromatid cohesion. Genes Dev **12:** 1986-1997.

LYDALL, D., Y. NIKOLSKY, D. K. BISHOP and T. WEINERT, 1996 A meiotic recombination checkpoint controlled by mitotic checkpoint genes. Nature **383:** 840-843.

MACQUEEN, A. J. and A. M. VILLENEUVE, 2001 Nuclear reorganization and homologous chromosome pairing during meiotic prophase require *C. elegans chk-2* Genes Dev **15:** 1674-1687.

MANCERA, E., R. BOURGON, A. BROZZI, W. HUBER and L. M. STEINMETZ, 2008 High-resolution mapping of meiotic crossovers and non-crossovers in yeast. Nature **454:** 479-485.

MARTINEZ-PEREZ, E., and A. M. VILLENEUVE, 2005 HTP-1-dependent constraints coordinate homolog pairing and synapsis and promote chiasma formation during *C. elegans* meiosis. Genes Dev **19:** 2727-2743.

MARTINI, E., R. L. DIAZ, N. HUNTER and S. KEENEY, 2006 Crossover homeostasis in yeast meiosis. Cell **126:** 285-295.

MATOS, J., J. J. LIPP, A. BOGDANOVA, S. GUILLOT, E. OKAZ, M. JUNQUEIRA, A. SHEVCHENKO and W. ZACHARIAE, 2008 Dbf4 dependent *CDC7* kinase links DNA replication to the segregation of homologous chromosomes in meiosis I. Cell **135:** 662-678.

MCKEE, A. H., and N. KLECKNER, 1997 A general method for identifying recessive diploid-specific mutations in *Saccharomyces cerevisiae*, its application to the isolation of mutants blocked at intermediate stages of meiotic prophase and characterization of a new gene *SAE2*. Genetics **146:** 797-816.

MICHAELIS, C., R. CIOSK and K. NASMYTH, 1997 Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. Cell **91:** 35-45.

MITCHELL, A. P., and I. HERSKOWITZ, 1986 Activation of meiosis and sporulation by repression of the *RME1* product in yeast. Nature **319:** 738-742.

NEALE, M. J., J. PAN and S. KEENEY, 2005 Endonucleolytic processing of covalent protein-linked DNA double-strand breaks. Nature **436:** 1053-1057.

NIU, H., X. LI, E. JOB, C. PARK, D. MOAZED, S. P. GYGI and N. M. HOLLINGSWORTH, 2007 Mek1 kinase is regulated to suppress double-strand break repair between sister chromatids during budding yeast meiosis. Mol Cell Biol **27:** 5456-5467.

NIU, H., L. WAN, B. BAUMGARTNER, D. SCHAEFER, J. LOIDL and N. M. HOLLINGSWORTH, 2005 Partner choice during meiosis is regulated by Hop1-promoted dimerization of Mek1. Mol Biol Cell **16:** 5804-5818.

NIU, H., L. WAN, V. BUSYGINA, Y. KWON, J. A. ALLEN, X. LI, R. C. KUNZ, K. KUBOTA, B. WANG, P. SUNG, K. M. SHOKAT, S. P. GYGI and N. M. HOLLINGSWORTH, 2009 Regulation of meiotic recombination via Mek1-mediated Rad54 phosphorylation. Mol Cell **36:** 393-404.

OH, S. D., L. JESSOP, J. P. LAO, T. ALLERS, M. LICHTEN and N. HUNTER, 2009 Stabilization and electrophoretic analysis of meiotic recombination intermediates in *Saccharomyces cerevisiae*. Methods Mol Biol **557:** 209-234.

ONN, I., J. M. HEIDINGER-PAULI, V. GUACCI, E. UNAL and D. E. KOSHLAND, 2008 Sister chromatid cohesion: a simple concept with a complex reality. Annu Rev Cell Dev Biol **24:** 105-129.

PAGE, S. L., and R. S. HAWLEY, 2004 The genetics and molecular biology of the synaptonemal complex. Annu Rev Cell Dev Biol **20:** 525-558.

PAQUES, F., and J. E. HABER, 1999 Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev **63:** 349-404.

PARENT, S. A., C. M. FENIMORE and K. A. BOSTIAN, 1985 Vector systems for the expression, analysis and cloning of DNA sequences in *S. cerevisiae*. Yeast **1:** 83-138.

PECINA, A., K. N. SMITH, C. MEZARD, H. MURAKAMI, K. OHTA and A. NICOLAS, 2002 Targeted stimulation of meiotic recombination. Cell **111:** 173-184.

PEREZ-HIDALGO, L., S. MORENO and P. A. SAN-SEGUNDO, 2003 Regulation of meiotic progression by the meiosis-specific checkpoint kinase Mek1 in fission yeast. J Cell Sci **116:** 259-271.

PETRONCZKI, M., M. F. SIOMOS and K. NASMYTH, 2003 *Un menage a quatre*: the molecular biology of chromosome segregation in meiosis. Cell **112:** 423-440.

PETUKHOVA, G., S. STRATTON and P. SUNG, 1998 Catalysis of homologous DNA pairing by yeast Rad51 and Rad54 proteins. Nature **393:** 91-94.

PETUKHOVA, G. V., R. J. PEZZA, F. VANEVSKI, M. PLOQUIN, J. Y. MASSON and R. D. CAMERINI-OTERO, 2005 The Hop2 and Mnd1 proteins act in concert with Rad51 and Dmc1 in meiotic recombination. Nat Struct Mol Biol **12:** 449-453.

PRINZ, S., A. AMON and F. KLEIN, 1997 Isolation of *COM1*, a new gene required to complete meiotic double-strand break-induced recombination in *Saccharomyces cerevisiae*. Genetics **146:** 781-795.

QI, J., A. J. WIJERATNE, L. P. TOMSHO, Y. HU, S. C. SCHUSTER and H. MA, 2009 Characterization of meiotic crossovers and gene conversion by whole-genome sequencing in *Saccharomyces cerevisiae*. BMC Genomics **10:** 475.

RINE, J., and I. HERSKOWITZ, 1987 Four genes responsible for a position effect on expression from *HML* and *HMR* in *Saccharomyces cerevisiae*. Genetics **116:** 9-22.

ROCKMILL, B., and G. S. ROEDER, 1990 Meiosis in asynaptic yeast. Genetics **126:** 563-574.

ROEDER, G. S., 1997 Meiotic chromosomes: it takes two to tango. Genes Dev **11:** 2600-2621.

ROEDER, G. S., and J. M. BAILIS, 2000 The pachytene checkpoint. Trends Genet **16:** 395-403.

SANCHEZ-MORAN, E., J. L. SANTOS, G. H. JONES and F. C. FRANKLIN, 2007 *ASY1* mediates *AtDMC1*-dependent interhomolog recombination during meiosis in *Arabidopsis*. Genes Dev **21:** 2220-2233.

SCHMUCKLI-MAURER, J., and W. D. HEYER, 2000 Meiotic recombination in *RAD54* mutants of *Saccharomyces cerevisiae*. Chromosoma **109:** 86-93.

SCHWACHA, A., and N. KLECKNER, 1994 Identification of joint molecules that form frequently between homologs but rarely between sister chromatids during yeast meiosis. Cell **76:** 51-63.

SCHWACHA, A., and N. KLECKNER, 1995 Identification of double Holliday junctions as intermediates in meiotic recombination. Cell **83:** 783-791.

SCHWACHA, A., and N. KLECKNER, 1997 Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog-only pathway. Cell **90:** 1123-1135.

SHERIDAN, S., and D. K. BISHOP, 2006 Red-Hed regulation: recombinase Rad51, though capable of playing the leading role, may be relegated to supporting Dmc1 in budding yeast meiosis. Genes Dev **20:** 1685-1691.

SHERIDAN, S. D., X. YU, R. ROTH, J. E. HEUSER, M. G. SEHORN, P. SUNG, E. H. EGELMAN and D. K. BISHOP, 2008 A comparative analysis of Dmc1 and Rad51 nucleoprotein filaments. Nucleic Acids Res **36:** 4057-4066.

SHINOHARA, A., S. GASIOR, T. OGAWA, N. KLECKNER and D. K. BISHOP, 1997a *Saccharomyces cerevisiae* recA homologues *RAD51* and *DMC1* have both distinct and overlapping roles in meiotic recombination. Genes Cells **2:** 615-629.

SHINOHARA, M., S. L. GASIOR, D. K. BISHOP and A. SHINOHARA, 2000 Tid1/Rdh54 promotes colocalization of Rad51 and Dmc1 during meiotic recombination. Proc Natl Acad Sci U S A **97:** 10814-10819.

SHINOHARA, M., E. SHITA-YAMAGUCHI, J. M. BUERSTEDDE, H. SHINAGAWA, H. OGAWA and A. SHINOHARA, 1997b Characterization of the roles of the *Saccharomyces cerevisiae RAD54* gene and a homologue of *RAD54, RDH54/TID1*, in mitosis and meiosis. Genetics **147:** 1545-1556.

SIKORSKI, R. S., and P. HIETER, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:** 19-27.

SMITH, A. V., and G. S. ROEDER, 1997 The yeast Red1 protein localizes to the cores of meiotic chromosomes. J Cell Biol **136:** 957-967.

SOLINGER, J. A., K. KIIANITSA and W. D. HEYER, 2002 Rad54, a Swi2/Snf2-like recombinational repair protein, disassembles Rad51:dsDNA filaments. Mol Cell **10:** 1175-1188.

SOURIRAJAN, A., and M. LICHTEN, 2008 Polo-like kinase Cdc5 drives exit from pachytene during budding yeast meiosis. Genes Dev **22:** 2627-2632.

STROM, L., C. KARLSSON, H. B. LINDROOS, S. WEDAHL, Y. KATOU, K. SHIRAHIGE and C. SJOGREN, 2007 Postreplicative formation of cohesion is required for repair and induced by a single DNA break. Science **317:** 242-245.

STROM, L., H. B. LINDROOS, K. SHIRAHIGE and C. SJOGREN, 2004 Postreplicative recruitment of cohesin to double-strand breaks is required for DNA repair. Mol Cell **16:** 1003-1015.

SUGAWARA, N., X. WANG and J. E. HABER, 2003 In vivo roles of Rad52, Rad54, and Rad55 proteins in Rad51-mediated recombination. Mol Cell **12:** 209-219.

SUN, H., D. TRECO, N. P. SCHULTES and J. W. SZOSTAK, 1989 Double-strand breaks at an initiation site for meiotic gene conversion. Nature **338:** 87-90.

TERENTYEV, Y., R. JOHNSON, M. J. NEALE, M. KHISROON, A. BISHOP-BAILEY and A. S. GOLDMAN, 2010 Evidence that *MEK1* positively promotes interhomologue double-strand break repair. Nucleic Acids Res **13:** 4349-4360.

THOMPSON, D. A., and F. W. STAHL, 1999 Genetic control of recombination partner preference in yeast meiosis. Isolation and characterization of mutants elevated for meiotic unequal sister-chromatid recombination. Genetics **153:** 621-641.

TONG, A. H., and C. BOONE, 2006 Synthetic genetic array analysis in *Saccharomyces cerevisiae*. Methods Mol Biol **313:** 171-192.

TOTH, A., R. CIOSK, F. UHLMANN, M. GALOVA, A. SCHLEIFFER and K. NASMYTH, 1999 Yeast cohesin complex requires a conserved protein, Eco1p(Ctf7), to establish cohesion between sister chromatids during DNA replication. Genes Dev **13:** 320-333.

TOTH, A., K. P. RABITSCH, M. GALOVA, A. SCHLEIFFER, S. B. BUONOMO and K. NASMYTH, 2000 Functional genomics identifies monopolin: a kinetochore protein required for segregation of homologs during meiosis I. Cell **103:** 1155-1168.

TSUBOUCHI, H., and G. S. ROEDER, 2003 The importance of genetic recombination for fidelity of chromosome pairing in meiosis. Dev Cell **5:** 915-925.

TSUBOUCHI, H., and G. S. ROEDER, 2004 The budding yeast Mei5 and Sae3 proteins act together with Dmc1 during meiotic recombination. Genetics **168:** 1219-1230.

TSUBOUCHI, H., and G. S. ROEDER, 2006 Budding yeast Hed1 down-regulates the mitotic recombination machinery when meiotic recombination is impaired. Genes Dev **20:** 1766-1775.

TUNG, K. S., E. J. HONG, and G. S. ROEDER, 2000 The pachytene checkpoint prevents accumulation and phosphorylation of the meiosis-specific transcription factor, Ndt80. Proc Natl Acad Sci U S A **97:** 12187-12192

UHLMANN, F., F. LOTTSPEICH and K. NASMYTH, 1999 Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. Nature **400:** 37-42.

UHLMANN, F., and K. NASMYTH, 1998 Cohesion between sister chromatids must be established during DNA replication. Curr Biol **8:** 1095-1101.

UHLMANN, F., D. WERNIC, M. A. POUPART, E. V. KOONIN and K. NASMYTH, 2000 Cleavage of cohesin by the CD clan protease separin triggers anaphase in yeast. Cell **103:** 375-386.

UNAL, E., A. ARBEL-EDEN, U. SATTLER, R. SHROFF, M. LICHTEN, J. E. HABER and D. KOSHLAND, 2004 DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain. Mol Cell **16:** 991-1002.

UNAL, E., J. M. HEIDINGER-PAULI and D. KOSHLAND, 2007 DNA double-strand breaks trigger genome-wide sister-chromatid cohesion through Eco1 (Ctf7). Science **317:** 245-248.

VERSHON, A. K., N. M. HOLLINGSWORTH and A. D. JOHNSON, 1992 Meiotic induction of the yeast *HOP1* gene is controlled by positive and negative regulatory sites. Mol Cell Biol **12:** 3706-3714.

VILLENEUVE, A. M., and K. J. HILLERS, 2001 Whence meiosis? Cell **106:** 647-650.

WAN, L., T. DE LOS SANTOS, C. ZHANG, K. SHOKAT and N. M. HOLLINGSWORTH, 2004 Mek1 kinase activity functions downstream of *RED1* in the regulation of meiotic double strand break repair in budding yeast. Mol Biol Cell **15:** 11-23.

WOJTASZ, L., K. DANIEL, I. ROIG, E. BOLCUN-FILAS, H. XU, V. BOONSANAY, C. R. ECKMANN, H. J. COOKE, M. JASIN, S. KEENEY, M. J. MCKAY and A. TOTH, 2009 Mouse HORMAD1 and HORMAD2, two conserved meiotic chromosomal proteins, are depleted from synapsed chromosome axes with the help of TRIP13 AAA-ATPase. PLoS Genet **5:** e1000702.

WOLTERING, D., B. BAUMGARTNER, S. BAGCHI, B. LARKIN, J. LOIDL, T. DE LOS SANTOS and N. M. HOLLINGSWORTH, 2000 Meiotic segregation, synapsis, and recombination checkpoint functions require physical interaction between the chromosomal proteins Red1p and Hop1p. Mol Cell Biol **20:** 6646-6658.

WU, T. C., and M. LICHTEN, 1994 Meiosis induced double-strand break sites determined by yeast chromatin structure. Science **263:** 515-518.

WU, T. C., and M. LICHTEN, 1995 Factors that affect the location and frequency of meiosis-induced double-strand breaks in *Saccharomyces cerevisiae*. Genetics **140:** 55-66.

XU, L., and N. KLECKNER, 1995 Sequence non-specific double-strand breaks and interhomolog interactions prior to double-strand break formation at a meiotic recombination hot spot in yeast. Embo J **14:** 5115-5128.

XU, L., B. M. WEINER and N. KLECKNER, 1997 Meiotic cells monitor the status of the interhomolog recombination complex. Genes Dev **11:** 106-118.

ZETKA, M. C., I. KAWASAKI, S. STROME and F. MULLER, 1999 Synapsis and chiasma formation in *Caenorhabditis elegans* require *HIM-3*, a meiotic chromosome core component that functions in chromosome segregation. Genes Dev **13:** 2258-227.