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Comparative Analysis of Bacterial RecBCD and SbcC-SbcD Systems and the Yeast
Mre11-Rad50 Complex During DNA Replication

by

Tannos Seyedjavadi

A thesis submitted in partial fulfillment of the
requirements for the degree of

Master of Science
in
Biology

Thesis Committee:
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2026

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Abstract

Accurate completion of DNA replication when forks converge is essential for genome stability. In *Escherichia coli*, several proteins have been identified including RecG, ExoI, RecBCD, and SbcCD, that contribute to completing replication, and loss of these factors causes genetic instability. Eukaryotes encode proteins with homology to several *E. coli* completion enzymes. Mre11–Rad50 complex is structurally and functionally conserved with bacterial SbcCD, and Rrm3 shares homologous motifs with bacterial RecBCD. These proteins are often described as being involved in homologous double strand break repair. Whether they are also conserved and play a similar role as their *E. coli* counterparts in completing DNA replication has not been examined. Using *Saccharomyces cerevisiae*, this study examined whether Mre11–Rad50 and Rrm3 play roles in replication completion and genome maintenance. Mutants lacking Mre11 displayed reduced viability, accumulation of enlarged late G2 populations, and instability of telomeric, plasmid, and mitochondrial DNA. Rrm3 mutants displayed difficulty progressing through S phase and also had abnormalities associated with plasmid and mitochondrial replication. These phenotypes would be consistent with mutations that impair replication completion. However, when I examined the replication profiles of the mutants, neither $\Delta mre11$ or $\Delta rrm3$ strains exhibited pronounced abnormalities at chromosomal replication termination regions as might be expected, and overall replication timing profiles remained broadly similar to wild type.

Aspects of These findings are suggestive of a role for Mre11 and Rrm3 in contributing to completing DNA replication, however it remains inconclusive at this point

as the mutants do not exhibit clear chromosomal abnormalities at these completion loci analogous to the mutants in bacterial systems. I discuss the possibility of how mutations in other gene products may be required to observe the copy number abnormalities at these loci. I also discuss the alternative possibility that the large number of completion loci in eukaryotic organisms, would result in a lethal phenotype in eukaryotic cells, or that other gene products may partially compensate and minimize these defects at in the mutants I examined.

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Chapter 1

Introduction

The process of genome replication in eukaryotic cells is a highly orchestrated, complex phenomenon involving numerous proteins and regulatory mechanisms. Throughout chromosomal replication, cells tightly regulate the stages of initiation, elongation, and completion to guarantee the faithful transmission of genetic information to each daughter cell (Bell et al., 1993; Bell & Stillman, 1992; Dewar et al., 2015; Diffley et al., 1994; Stillman, 1996). Mutations that disrupt genes involved in these mechanisms can lead to genomic instability, aberrant cell proliferation, and contribute to the development of diseases such as cancer in humans (reviewed in Hanawalt et al., 2003). While the mechanisms regulating replication initiation and elongation have been well characterized (reviewed in Bell & Dutta, 2002; Costa et al., 2013), the process of how cells complete replication at the precise point where all sequences have doubled remains a fundamental question that is less understood. Whether this convergence event occurs once per generation as in bacteria, or thousands of times per generation as in human cells, the process must occur with remarkable efficiency and accuracy. Considering the large number of proteins dedicated to ensure the fidelity of replication initiation and elongation (Costa et al., 2013; Méndez & Stillman, 2000; Moyer et al., 2006; Wendel et al., 2014a); it should come as no surprise that this final critical step has also been found to be tightly regulated and controlled enzymatically (Dewar et al., 2015).

In some respects, one could argue that the efficiency of completion is likely to be more critical to genome stability than initiation. In mammalian cells, where multiple origins are found along each linear chromosome, failure of any origin to initiate can be

compensated by continued elongation of replication forks from neighboring origins (Blow & Ge, 2009; Huberman & Riggs, 1968a). Consistent with this view, both prokaryotic and eukaryotic cells have been found to tolerate variations in origin number without severe phenotypic consequences (Aladjem, 2007; Hawkins, Malla, et al., 2013; Hawkins, Retkute, et al., 2013). However, failure to accurately complete any single event where replication forks converge would be expected to compromise genome integrity, resulting in mutations, duplications, deletions, or rearrangements if joined improperly, or cell death if the newly replicated ends fail to join (Wendel et al., 2014a). Previous work in the Courcelle lab and others have begun to dissect this important question in the model organism of *Escherichia coli*, which has a single bidirectional origin of replication (*oriC*), resulting in a single defined terminus region, opposite to the *oriC* on the circular chromosome (Hill, 1992; Prescott & Kuempel, 1972; Wendel et al., 2014a, 2017).

The bacterial model for completing replication

Several genes have now been isolated that are known to compromise the fidelity or efficiency of converging replication forks in *E. coli* when mutated (Hamilton et al., 2019; Rudolph, Upton, & Lloyd, 2009; Wendel et al., 2014). This work has led to a current working model in which converging replication forks transiently bypass each other at the point where they converge, creating an over-replicated region that contains three copies of the genetic information (Fig. 1.1C) (Hiasa & Marians, 1994). RecG is a helicase that acts on branched DNA in order to limit the amount of over-replication by preventing aberrant replication restart at DNA ends (Fukuoh et al., 1997; Rudolph et al., 2009; Whitby et al., 1994). The completion reaction is thought to initiate through the action of SbcCD

and ExoI, structure-specific nucleases which act on a structural intermediate created at the point where forks converge (Hamilton et al., 2019; Wendel et al., 2014, 2018). In the absence of the SbcCD and ExoI nucleases, the over-replicated region persists, leading to genomic instabilities and amplifications at these loci (Hamilton et al., 2019; Wendel et al., 2014, 2018). Following incisions by SbcCD and ExoI, the RecBCD helicase-nuclease complex processes the over-replicated intermediate and is required to catalyze or recruit enzymes that promote joining of the convergent strands (Hamilton et al., 2019; Wendel et al., 2018). In vitro, RecB and RecC interact with RecD to form a dual helicase–nuclease complex that unwinds and degrades double-stranded DNA ends (Taylor and Smith, 1985; Amundsen et al., 1986; Taylor and Smith, 2003). Loss of RecB or -C inactivates the enzyme complex, whereas loss of RecD inactivates the nuclease, but retains the helicase activity and recombination proficiency of the complex (Taylor and Smith, 1985; Amundsen et al., 1986; Taylor and Smith, 2003). In the absence of RecB or C, the nascent ends of convergent replication forks are not joined, leading to excessive degradation, and rendering cells unable to maintain the chromosome region where forks converge (Wendel et al., 2014; 2018). The inability to complete replication or maintain these regions of the genome severely compromises the viability and growth of recBCD cultures (Wendel et al., 2014; 2018; Courcelle et al., 2015). In the absence of *recD*, degradation of the excess sequence is impaired, however, joining appears to occur normally and viability is not compromised (Wendel et al., 2014; 2018; Courcelle et al., 2015.)

Importantly, and perhaps surprisingly, the completion reaction does not require recombination, since *recA* mutants complete replication normally (Courcelle et al., 2015a; N. A. Hamilton et al., 2019a; Wendel et al., 2014a, 2017). As outlined in the sections

below, RecA encodes the primary recombinase of *E. coli*, and is required for essentially all recombination and double strand break repair in this organism (Clark & Margulies, 1965; Willetts & Clark, 1969). Although completion occurs normally in these mutants, RecA is required to resolve aberrant amplifications and duplications that arise when completion is impaired, suggesting that recombination occurring under these conditions is directly responsible for genomic instability (Courcelle et al., 2015a; N. A. Hamilton et al., 2019a; Wendel et al., 2014a, 2017).

Many of the bacterial proteins identified were originally thought to function primarily in double strand break repair.

RecA

As previously mentioned, replication completion can proceed normally, even in the absence of RecA (N. A. Hamilton et al., 2019a; Wendel et al., 2014a, 2017). This observation that DNA replication completion does not require the master recombination gene, RecA, argues that recombination is not required for completion to occur normally (Wendel et al., 2014a). However, many of the proteins identified as participating in the completion reaction were traditionally considered to function primarily as a mechanism for repairing double strand breaks. Below, I review the cellular and biochemical properties of these proteins with respect to their characterization in recombination and double strand break repair.

RecA is a multifunctional protein that acts as the primary recombinase promoting DNA strand exchange, but is also associated with a number of replication processes, including regulation of the SOS response, and direct involvement in translesion synthesis

(Asai et al., 1993; Cox, 2001; Little et al., 1980; McEntee et al., 1979; Tang et al., 1999). The gene was first identified by Clark and Margulies in 1965, during efforts to isolate bacterial mutants that were defective in sexual recombination during conjugation (Clark & Margulies, 1965). Subsequent work revealed that it was also required for survival in the presence of DNA damage during asexual reproduction (Clark et al., 1966; Howard-Flanders & Theriot, 1966).

RecA monomers undergo polymerization upon binding to ssDNA in addition to ATP (Pugh & Cox, 1988). This results in the assembly of RecA into a right-handed helical filament, where three RecA monomers bind per 5 to 6 nucleotides of ssDNA, forming a stable nucleoprotein complex (Egelman & Stasiak, 1986). Biochemical assays using purified RecA have demonstrated its essential role in homologous pairing and the accumulation of strand-exchange complexes (Cox & Lehman, 1981, 1982; T. Shibata et al., 1981; West et al., 1980).

The *recA* protein is central to the homologous recombination mechanism (D. G. Anderson & Kowalczykowski, 1997). Both in vivo and in vitro studies shows RecBCD processes DNA ends and loads RecA onto ssDNA during double-strand break repair (D. G. Anderson & Kowalczykowski, 1997). RecA is essential for survival when DNA replication is disrupted by damage, playing a central role in restoring replication forks that have stalled due to DNA lesions (Courcelle et al., 1997). RecA maintains the integrity of arrested forks and triggers the SOS response, leading to the upregulation of other genes involved in DNA repair and replication restart, which enables resumed replication and cell viability after genotoxic stress (reviewed in Courcelle & Hanawalt, 2003; Crowley & Hanawalt, 1998; Fogliano & Schendel, 1981; Kenyon & Walker, 1981).

RecA-deficient cells fail to recover DNA replication once stalled, leading to extensive genomic degradation and inability to complete replication after damage (Horii & Suzuki, 1968). This is the primary cause of lethality in *recA* mutants under DNA-damaging conditions. Phenotypically, *recA* mutants exhibit sensitivity to DNA-damaging agents such as UV or x-ray because they cannot stabilize or process replication forks that encounter lesions, resulting in persistent breaks and increased frequency of DNA degradation (Chow & Courcelle, 2004; Clark et al., 1966; Courcelle et al., 1997; Howard-Flanders & Theriot, 1966; Kuzminov, 1995).

RecBCD

The RecBCD is a multifunctional ATP-dependent helicase-nuclease heterotrimeric complex that plays a central role in the repair of double-strand breaks and initiation of homologous repair (Amundsen et al., 2000; Spies & Kowalczykowski, 2014). Each subunit has a specialized function: RecB contains a slow helicase motor of 3'→5' polarity and a C-terminal nuclease domain, whereas RecD is a fast helicase motor of opposite (5'→3') polarity (Amundsen et al., 1986, 1990; Taylor & Smith, 2003; Yu et al., 1998); RecC is responsible for recognition of sequence-specific *chi* (cross over hotspot) sites and coordination of the enzymes response to *chi* (Amundsen et al., 2016; Dillingham & Kowalczykowski, 2008; Taylor et al., 2016).

After the initial incision by RecG, the RecBCD complex processes the over-replicated intermediate by binding to the double-strand break ends, and further unwinds and degrades the DNA until it reaches the sequence-specific *chi* site located within RecC (Kowalczykowski et al., 1994; Taylor et al., 1985; Taylor & Smith, 1985). At this point, RecBCD introduces a strand specific nick near the *chi* site, producing a 3' ssDNA tail,

while allowing RecA to load (D. G. Anderson & Kowalczykowski, 1997; Dillingham & Kowalczykowski, 2008; Ponticelli et al., 1985; Taylor et al., 2016; Taylor & Smith, 1985). RecA will then promote strand invasion and initiates recombination or repair (Cox & Lehman, 1982; T. Shibata et al., 1981).

The efficiency of this repair process is influenced by interactions within the RecBCD complex. Loss of either gene product results in pronounced hyper-sensitivity to x-ray exposure and other forms of DNA damage (Chaudhury & Smith, 1984a; Clark, 1973; Kushner, 1974). Mutation of *recB* or *recC* results in the loss of both nuclease and helicase activity, while *recD* mutations result in the loss of nuclease activity and *chi* site recognition, functions crucial for homologous recombination and genomic stability (Amundsen et al., 1986; Myers et al., 1995; Taylor & Smith, 1985; Thaler et al., 1989). However, our lab has identified several enigmatic phenotypes associated with *recBCD* mutants that suggest it may have a broader, more fundamental role in DNA replication completion will be below.

ExoI

Exonuclease I, encoded by the *xonA* gene in *E. coli*, was among several exonucleases identified and characterized in the 1960s as a 3' → 5' exonuclease that preferentially degrades ssDNA and once it encounters a double-strand break, it dissociates (Brutlag & Kornberg, 1972; Lehman, 1960; Lehman & Nussbaum, 1964). The gene *xonA* was originally identified as *sbcB*, named for its ability to resist UV damage in *recBC* mutants (suppressor of *recBC*) (Kushner et al., 1971, 1972.) Null mutations in *xonA*, identified by loss of nuclease activity, had little effect on the suppression of *recBCD*, and both *xonA* (*sbcB*) and *sbcC* mutations are necessary for suppression (Kushner et al.,

1972; Phillips et al., 1988). Additionally, Exol exhibits a binding affinity for single-strand DNA binding protein, which recruits Exol and stimulates the enzymatic hydrolysis of ssDNA (Lu & Keck, 2008). Based on this knowledge, it was later discovered that Exol can stimulate RecA-mediated DNA strand exchange (Bedale et al., 1993).

SbcCD

SbcC and SbcD were identified through studies aimed at understanding the suppressors of *recBC* defects, which highlighted the ability of mutations in these genes to restore viability and recombination functions in *recBC* mutants (Connelly et al., 1998a; Kushner et al., 1972; Lloyd & Buckman, 1985). Together, SbcC (suppressor of *recBC* protein C) and SbcD form a structure-specific endonuclease complex that acts with Exol to cleave DNA hairpins and other aberrant replication fork structures prior to RecBCD processing (Connelly et al., 1999; Connelly & Leach, 1996a; N. A. Hamilton et al., 2019b; Wendel et al., 2017). In particular, SbcCD targets long inverted repeats that form hairpin or cruciform intermediates, generating recombinogenic double-strand breaks that are subsequently repaired by homologous repair pathways (Connelly et al., 1998a; Connelly & Leach, 1996a; Leach et al., 1997a).

Bacterial SbcCD is recognized as an ortholog of eukaryotic Mre11-Rad50 complex, with SbcC corresponding to the Rad50 subunit and SbcD to the Mre11 subunit (Connelly et al., 1998a; Sharples & Leach, 1995; Wendel et al., 2017). Cryo-electron microscopy of purified *E. coli* SbcCD revealed a characteristic “head–rod–tail” morphology consistent with its classification within the structural maintenance of chromosomes (SMC) family of proteins (Connelly et al., 1998a, 1999). Sequencing analysis confirmed canonical Walker A and Walker B nucleotide-binding motifs separated by long α -helical coiled-coils, along

with a conserved CXXC motif -- features typical of Rad50-like SMC proteins involved in recombination and double-strand break repair (Connelly et al., 1998a, 1999; Sharples & Leach, 1995).

Mutations in *sbvC* and *sbvD* are known to accumulate aberrant DNA structures due to unresolved converging replication forks that result in harmful amplifications and duplications and exhibit sensitivity to replication stress (Connelly & Leach, 1996a; Lloyd & Buckman, 1985; Wendel et al., 2014b, 2017). While SbcCD are broadly conserved across evolutionarily diverse organisms, their eukaryotic homologs Rad50 and Mre11, have been identified as core components of double-strand break repair machinery, and mutations in these genes are linked to genomic instability and are essential for normal development (Connelly et al., 1998a; Sharples & Leach, 1995). Although their structural similarities are well characterized, their functional parallels remain poorly understood.

In *E. coli*, DNA replication completion requires RecBCD and involves several other proteins, including SbcC-SbcD, ExoI, and RecG, and occurs independently of homologous repair

Traditional models describe RecBCD as acting solely in homologous recombination. However, a series of enigmatic phenotypes observed previously in our lab indicated that RecBCD may serve a broader and more fundamental role in the DNA replication cycle.

Mutations in the RecBCD complex result in various phenotypes that are not only distinct from those observed in *recA* mutants but also challenge traditional interpretations within the framework of double-strand break repair models. *recA* mutants which are essential for homologous repair (Fig. 1.2A), appeared to be far more sensitive to DNA

damage than *recBC* mutants (Fig. 1.2B), yet in the absence of DNA damage, *recBC* mutants display poor growth and viability issues while *recA* mutants were relatively healthy (Fig. 1.2C), (Capaldo & Barbour, 1975; Capaldo-Kimball & Barbour, 1971; Wendel et al., 2014a). If growth defects seen in *recBC* mutants were solely attributed to homologous repair or double-strand break repair deficiencies, one would anticipate that *recA* mutants would exhibit similar, if not more pronounced, growth issues.

Further compounding these observations, *recBC* and *recD* mutants show difficulties maintaining plasmids, while *recA* mutants display stable plasmid retention (Fig. 1.2D), suggesting that double-strand breaks alone cannot account for issues related to plasmid stability experienced by *recBCD* mutants (Biek & Cohen, 1986; Courcelle et al., 2015b; Kusano et al., 1989; Niki et al., 1988; Wendel et al., 2014a). These phenotypic characteristics suggest the involvement of RecBCD in the normal replication cycle goes beyond initiating recombinational pathways.

Our lab has examined how the RecBCD proteins function in the completion of DNA replication by profiling the genomes of replicating wild-type and mutant cultures using high-throughput sequencing (Wendel et al., 2014). Specifically, *recBC* mutants display a reduced copy number of sequences at the chromosomes terminus region (Fig. 1.3C), where forks converge, suggesting an inability to maintain DNA integrity at this crucial locus (Hamilton et al., 2019; Wendel et al., 2014, 2018.) Conversely, *recD* mutants exhibited an increased copy number, indicative of over-replication (Fig. 1.3D). Interestingly, the completion of DNA replication is seen to occur normally in the absence of *recA*, indicating that homologous repair is not required for this process (Fig. 1.3E) (Wendel et al., 2014b). This suggests alternative pathways or redundancies in the system

that allow for the completion of replication without the intervention of homologous repair-related mechanisms. Other proteins including SbcCD, ExoI (*xonA*) and RecG have been implicated in over-replication at the terminus region, emphasizing their collective role in regulating the replication process (Fig. 1.3F and G),(Wendel et al., 2014, 2018).

Human DNA replication completion

The completion of replication is likely to be highly conserved across diverse organisms, yet far less is known about the genes or mechanisms by which completion occurs in highly complex eukaryotic cells. One significant barrier to this knowledge stems from the large differences in genome size between organisms. For example, humans, whose genomes are 1000 times larger than those of *E. coli*, highlights the complexity of the eukaryotic genome (Blattner et al., 1997; Cooper, 2000; Venter et al., 2001). Eukaryotic cells employ multiple origins of replication across each chromosome that do not activate simultaneously; rather, fire in a temporal pattern set during the G1 phase of the cell cycle that is modulated by developmental cues and environmental factors (Guilbaud et al., 2011; Hayano et al., 2012; Yamashita et al., 1997; Zhao et al., 2020). Given that each chromosome contains thousands of origins, with each origin firing at varying times and efficiencies, identifying and analyzing the specific loci where forks converge can be difficult (Müller & Nieduszynski, 2012; Yamashita et al., 1997; Zhao et al., 2020). Another complication is that replication completion occurs in a small subset of cells in a population at any given time, specifically those in the S phase, making it particularly challenging to detect and study such events against the large background of nonreplicating cells (Eshaghi et al., 2007; Goldar et al., 2009; Zhao et al., 2020). While

eukaryotic replication completion remains poorly understood, identifying and characterizing bacterial homologs offers valuable insights into the shared mechanisms underlie essential cellular processes.

Mre11-Rad50 share sequence and structural similarities to bacterial SbcC-SbcD

In eukaryotic cells, the Mre11-Rad50 complex represents a highly conserved homolog of bacterial SbcC-SbcD, sharing both sequence and structural similarities (Connelly et al., 1998a; Sharples & Leach, 1995). The Mre11-Rad50 complex, which includes Nbs1 in humans (MRN) and Xrs2 in yeast (MRX), are essential for recognizing and processing double-strand breaks, while SbcCD serves similar functions in bacteria (Cromie & Leach, 2001; D'Amours & Jackson, 2001; Dolganov et al., 1996; Lammens et al., 2011; Leach et al., 1997b; Mascarenhas et al., 2006a). These complexes are well-characterized with respect to DNA repair mechanisms, actively coordinating the processing of double-strand breaks through both homologous recombination and non-homologous end joining pathways (Connelly et al., 2003; Cromie & Leach, 2001; Moore & Haber, 1996). Functional assays have demonstrated that mutations affecting the Rad50 and Mre11 proteins can lead to substantial defects in double-strand break repair, parallel to the effects observed when SbcCD is compromised in bacterial models (Ajimura et al., 1993; Bressan et al., 1998; Connelly et al., 1998b; Cromie & Leach, 2001; Johzuka & Ogawa, 1995; Mascarenhas et al., 2006b).

The nature of these proteins is to form dimers with long coiled structures that allow for flexible bridging of DNA ends, promoting efficient recognition and processing of strand breaks (D. E. Anderson et al., 2001; Connelly & Leach, 2002; Hopfner et al., 2001).

Further comparative analysis indicates that both Rad50 and SbcC ATPase activities are crucial for the establishment of the molecular clamp that stabilizes DNA during repair, while Mre11 and SbcD perform nucleolytic processing on DNA ends, including the cleavage of hairpin structures (D. E. Anderson et al., 2001; Connelly et al., 1998b, 1999; Deng et al., 2015; Hopfner et al., 2001; Lammens et al., 2011; Lobachev et al., 2002; Rattray et al., 2005). Specifically, SbcCD has been shown to cleave DNA near blocked ends, activating a series of repair pathways that are similar to those utilized by the Mre11-Rad50 complex in eukaryotic organisms (Connelly et al., 1999, 2003; Cromie & Leach, 2001; Mascarenhas et al., 2006b; Saathoff et al., 2018). This high degree of conservation across evolutionary lineages highlights the fundamental role of these proteins in maintaining genome integrity.

Other important eukaryotic enzymes

Sae2

In *Saccharomyces cerevisiae*, MRX works with Sae2 (CtIP in mammals) to initiate 5' end resection, the preliminary step necessary for homologous repair (Clerici et al., 2006; Lengsfeld et al., 2007; Mimitou & Symington, 2008). The endonuclease activity of MRX/N is stimulated by the phosphorylation of Sae2/CtIP, respectively (Cannavo & Cejka, 2014; Sartori et al., 2007). Sae2 functions primarily as an oligomeric regulator of DNA end resection rather than as a small, independently acting enzyme (Cannavo et al., 2018). In yeast, Sae2 can form oligomeric complexes even in the absence of DNA damage, which is essential for regulating Mre11 nuclease activity and coordinating DNA damage checkpoint signaling (Cannavo et al., 2018; H.-S. Kim et al., 2008). Structural

studies also show that its phosphorylation promotes the formation of an active tetrameric Sae2 complex, enhancing its ability to interact with the MRX complex (Cannavo et al., 2018; H.-S. Kim et al., 2008). Following DNA damage and during cell cycle, phosphorylation of conserved residues within the C-terminal region enables Sae2 to interact directly with Rad50 and stimulates the endonuclease activity of the MRX complex (Cannavo et al., 2018; Huertas et al., 2008).

Deficiency in this collaborative complex impairs both mitotic and meiotic recombination, leading to palindromic amplifications and genetic instabilities (Clerici et al., 2005a; Deng et al., 2015; Lobachev et al., 2002; Mimitou & Symington, 2008; Oh et al., 2016; Rattray et al., 2005). Defective MRX-Sae2 interactions manifest in increased sensitivity to DNA-damaging agents, as the complex is integral to recognizing and processing double-strand breaks (Cannavo & Cejka, 2014; Clerici et al., 2006; Mimitou & Symington, 2008). For instance, mutations that impair activities of either MRX or Sae2 result in diminished double-strand break resection, subsequently reducing the cell's ability to accurately repair broken DNA (Clerici et al., 2005c; Moreau et al., 1999a). In humans, the MRN and CtIP complex serve analogous functions, and hypomorphic mutations in their genes are often associated with severe developmental disorders and cancer predisposition (Kang et al., 2002; Luo et al., 1999; Xiao & Weaver, 1997; Zhu et al., 2001).

Collectively, the structural, biochemical, and phenotypic parallels between SbcC-SbcD, Exo1 and Mre11-Rad50 complexes with Sae2 (yeast) and CtIP (humans) suggest a highly conserved framework for DNA repair mechanisms across different domains of life. Using *S. cerevisiae*, I examined the role of Mre11–Rad50 in replication completion and found that while these factors contribute to viability (in the absence of exogenous

DNA damage,) plasmid maintenance, and broader genomic stability – including telomere and mitochondrial integrity. Unlike its bacterial counterparts, no observable instabilities were seen at convergent fork loci. This suggests that, in eukaryotes, Mre11-Rad50 may promote replication through mechanisms distinct from those seen in bacteria.

Rad51

Eukaryotic Rad51 and bacterial RecA exhibit significant functional and structural similarities, which is evident in their roles in homologous recombination and DNA repair processes (D. G. Anderson & Kowalczykowski, 1997; Courcelle & Hanawalt, 2003; Kuzminov, 1995; Ogawa et al., 1993a; Shinohara et al., 1992a). Both proteins belong to the same family of recombinases, which are essential for DNA strand exchange during these processes (Clark & Margulies, 1965; McEntee et al., 1979; Ogawa et al., 1993b; Shinohara et al., 1992b). Loss of Rad51 causes defective homologous recombination mediated double strand break repair, hypersensitivity to DNA damaging agents, and chromosomal instabilities, and in humans, these defects are often associated with Fanconi Anemia-like disorder as well as cancer predisposition (Ameziane et al., 2015; Shinohara et al., 1992a; Sonoda et al., 1998; P. J. Sung, 1994; Takata et al., 2000, 2001; Wang et al., 2015). Shared core mutant phenotypes with its bacterial counterpart RecA recombination failure and DNA-damage hypersensitivity (Clark et al., 1966; Howard-Flanders & Theriot, 1966; Kuzminov, 1995).

Both Rad51 and RecA share approximately 30% sequence homology, a significant figure considering the evolutionary divergence between prokaryote and eukaryote organisms (Shinohara et al., 1992). This conservation is especially evident within their ATPase core domains (Shinohara et al., 1993; Story et al., 1992). Similar to RecA, Rad51

initiates homologous repair by forming a nucleoprotein filament on ssDNA, which then searches for a complementary dsDNA sequence (Egelman & Stasiak, 1986; Ogawa et al., 1993b; Pugh & Cox, 1988; P. Sung & Robberson, 1995).

Despite these similarities, important differences distinguish Rad51 from bacterial RecA. Rad51 often works in conjunction with mediator proteins such as Rad52 (BRCA2 in mammals) to promote its loading and assembly activity on to single strand DNA, highlighting how this eukaryotic RecA homolog has adapted to a more complex cellular environment (Davies et al., 2001; Jensen et al., 2010; New et al., 1998; Shinohara & Ogawa, 1998; P. Sung, 1997).

Rrm3

Rrm3 is a 5'→3' DNA helicase in *S. cerevisiae* and a member of the conserved Pif1-family helicases (Ivessa et al., 2000a, 2002). It was originally identified through mutations that altered recombination within ribosomal DNA repeats and was later recognized as a critical accessory helicase that promotes replication fork progression through protein–DNA barriers distributed throughout the yeast genome (Azvolinsky et al., 2006b; Ivessa et al., 2000b, 2003; Keil & McWilliams, 1993). Early genetic studies showed that deletion of *Rrm3* causes elevated recombination within repetitive DNA loci, increased replication stress, delayed S-phase progression, and genome instability (Ivessa et al., 2000a; Keil & McWilliams, 1993; Schmidt & Kolodner, 2004; Scholes et al., 2001). Additional genome-wide analyses demonstrated that Rrm3 travels with replication forks and facilitates replisome movement through difficult to replicate regions such as

telomeres, centromeres, and highly transcribed regions (Azvolinsky et al., 2006b; Ivessa et al., 2002, 2003; Torres, Schnakenberg, et al., 2004).

Rrm3 is closely related to the second yeast Pif1-family helicase, Pif1 (Ivessa et al., 2000b). Although both proteins share extensive homology within their helicase domains, they perform partially distinct functions (Ivessa et al., 2000b). Pif1 primarily regulates telomerase activity, mitochondrial DNA maintenance, and G-quadruplex unwinding, whereas Rrm3 specializes in facilitating replisome progression through protein barriers (Foury & Kolodynski, 1983; Ivessa et al., 2000b; Paeschke et al., 2011; Schulz & Zakian, 1994).

Yeast Rrm3 and Pif1, together with human PIF1, belong to the evolutionarily conserved Pif1-like family of helicases, which share a conserved central helicase core containing seven signature motifs characteristic of superfamily I helicases (Bochman et al., 2010; Gorbalenya et al., 1989). The bacterial helicase RecD from *E. coli* has also been proposed to belong to this Pif1-like helicase family based on sequence and biochemical similarities (Zhang, 2006). Sequence analyses revealed that yeast Pif1 and Rrm3 share 18.9% and 16.4% amino acid identity, respectively, with RecD, while Rrm3 alone shares approximately 14% identical and 14% similar residues with RecD (Bochman et al., 2011). Rrm3 and RecD shared biochemical properties, include single-stranded DNA-dependent ATPase activity and 5'→3' DNA helicase activity, further supporting a potential evolutionary relationship between these helicases (Dillingham et al., 2003; Ivessa et al., 2002; Taylor & Smith, 2003).

The proteins and pathways that coordinate DNA replication completion and repair exhibit a remarkable degree of conservation across prokaryotic and eukaryotic organisms. Comparisons between bacterial SbcC-SbcD and RecA systems with their eukaryotic counterparts, including Mre11-Rad50, Sae2, Rad51, and Rrm3, demonstrate that core mechanisms involved in DNA end processing, homologous recombination, and replication fork progression have been preserved throughout evolution despite the increased complexity of eukaryotic genomes. Structural and biochemical similarities among these proteins further support the existence of a conserved framework that safeguards genome stability during replication stress and DNA damage.

Faithful completion of DNA replication is essential for maintaining genome integrity, yet the mechanisms that ensure replication is successfully completed at every genomic locus remain incompletely understood in eukaryotes. Based on the strong evolutionary conservation between bacterial and eukaryotic repair systems discussed, I hypothesized that proteins involved in bacterial replication completion may share functional characteristics with the eukaryotic Mre11-Rad50 complex and associated factors. Although the direct role of these proteins in replication completion has not been fully established, mutants affecting Mre11-Rad50 display several enigmatic phenotypes reminiscent of bacterial systems, including plasmid instability and defects that cannot be fully explained by their established functions in homologous recombination and double-strand break repair alone. To better define the contribution of Mre11-Rad50 to replication completion and genome maintenance, I examined the phenotypic consequences of these mutants in *Saccharomyces cerevisiae*. Our results indicate that the growth defects observed in $\Delta mre11$ mutants cannot be explained solely by the accumulation of double-

strand breaks. While I observed Mre11 to be largely dispensable for chromosomal replication completion, it remains essential for proper telomere maintenance, and loss of Mre11 also gives rise to abnormalities on extrachromosomal DNA substrates that parallel phenotypes previously described in bacterial homolog systems.

Chapter 1 Figures

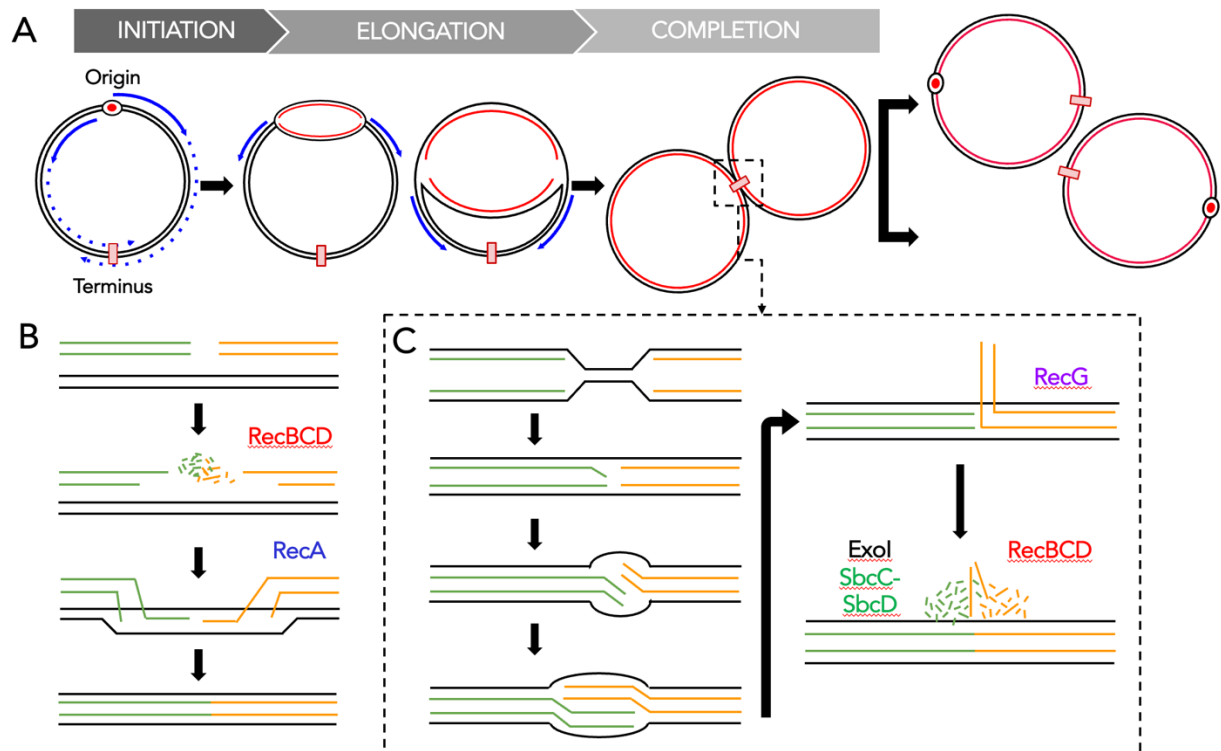


Figure 1.1 Model for bacterial replication completion and processing of over-replicated DNA generated by converging replication forks. (A) Bidirectional replication initiates at a single origin and converging forks meet at the terminus, where aberrant intermediates can form and require processing to ensure accurate chromosome duplication and segregation. (B) RecBCD was thought to only be involved in double strand break repair by processing DNA ends to facilitate RecA loading and initiate homologous recombination through strand invasion into an intact homologous duplex DNA template. (C) At fork convergence, transient fork bypass generates an over-replicated intermediate. RecG remodels aberrant DNA structures, while RecBCD, ExoI, and SbcC–SbcD degrade excess DNA to resolve the intermediate and promote replication completion.

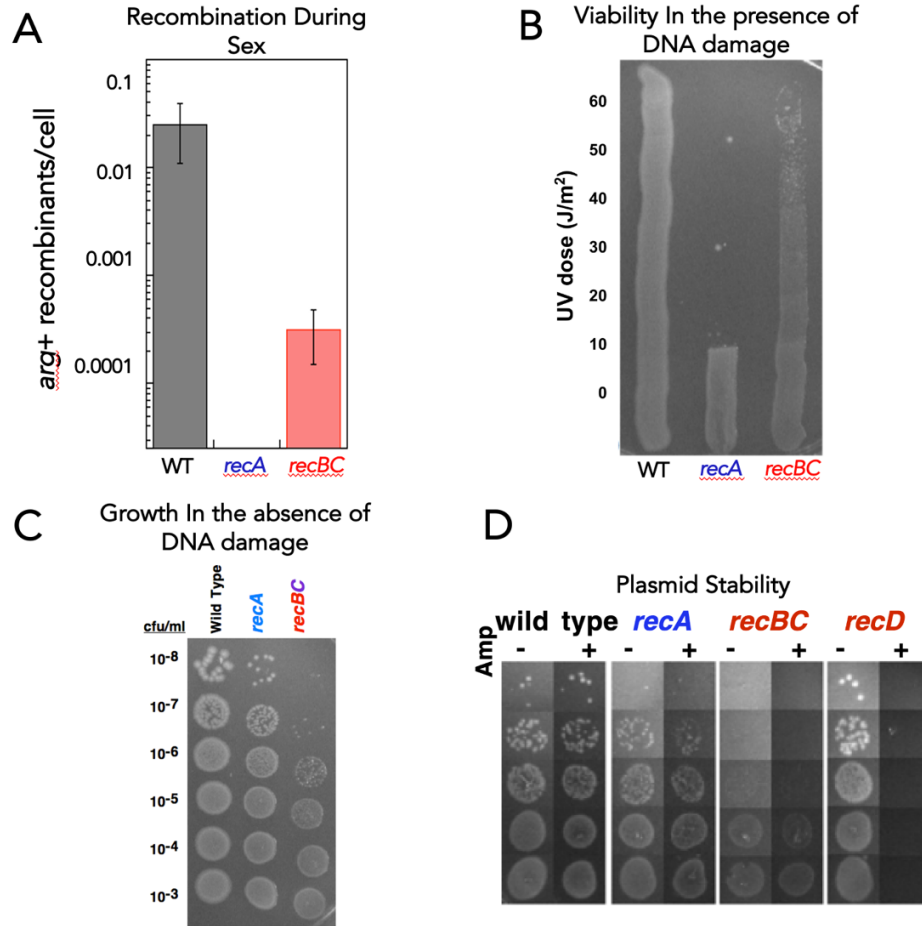


Figure 1.2 Enigmatic phenotypes of *recBC* mutants. (A) Frequency of *arg*⁺ recombinants per cell during conjugation, showing that recombination is absolutely essential in *recA* mutants and significantly decreased in *recBC* mutants relative to wild type. (B) Survival following UV irradiation demonstrates that *recA* mutants are significantly more sensitive to DNA damage than *recBC* mutants. (C) Growth curves (*A*₆₃₀) in the absence of exogenous DNA damage reveal a pronounced growth defect in *recBC* mutants, whereas *recA* mutants grow comparably to wild type. (D) Plasmid stability assay in the presence and absence of ampicillin shows increased plasmid instability in *recBC* and *recD* mutants compared to wild type and *recA* strains (reproduced from Courcelle et al., 2015b; Wendel et al., 2014a).

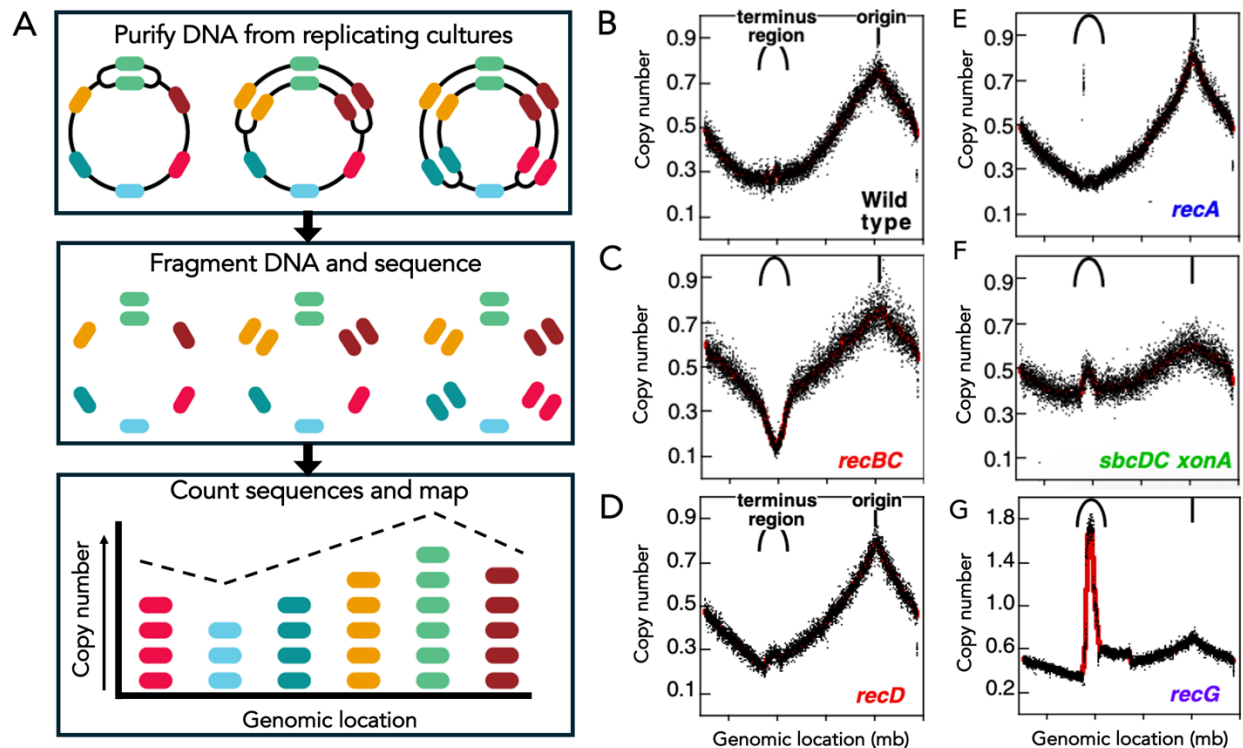


Figure 1.3 Copy number profile analysis reveal altered replication completion in recombination mutants. (A) Illustration of copy number profile methodology, where DNA is purified from a growing culture, and then fragmented, and then subjected to high-throughput sequencing. Read counts are mapped across the chromosome to infer relative copy number by genomic position. (B-G) Wild type (B) shows the expected cycle from origin to terminus. *recA* (E) maintains a profile largely similar to wild type, while *recD* (D) displays slight increase at its terminus. The *sbcCD xonA* double mutant (F) shows a more pronounced over-replication at the terminus and *recG* (G) displays a sharp peak at the terminus, indicating illegitimate reinitiations of replications that may be taking place in its absence (adapted from Wendel et al., 2014a, 2017).

References

- Ajimura, M., Leem, S. H., & Ogawa, H. (1993). Identification of new genes required for meiotic recombination in *Saccharomyces cerevisiae*. *Genetics*, 133(1), 51–66. <https://doi.org/10.1093/genetics/133.1.51>
- Aladjem, M. I. (2007). Replication in context: Dynamic regulation of DNA replication patterns in metazoans. *Nature Reviews Genetics*, 8(8), 588–600. <https://doi.org/10.1038/nrg2143>
- Alani, E., Padmore, R., & Kleckner, N. (1990). Analysis of wild-type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. *Cell*, 61(3), 419–436. [https://doi.org/10.1016/0092-8674\(90\)90524-I](https://doi.org/10.1016/0092-8674(90)90524-I)
- Ameziane, N., May, P., Haitjema, A., van de Vrugt, H. J., van Rossum-Fikkert, S. E., Ristic, D., Williams, G. J., Balk, J., Rockx, D., Li, H., Rooimans, M. A., Oostra, A. B., Velleuer, E., Dietrich, R., Bleijerveld, O. B., Maarten Altelaar, A. F., Meijers-Heijboer, H., Joenje, H., Glusman, G., ... Dorsman, J. C. (2015). A novel Fanconi anaemia subtype associated with a dominant-negative mutation in RAD51. *Nature Communications*, 6, 8829. <https://doi.org/10.1038/ncomms9829>
- Amundsen, S. K., Neiman, A. M., Thibodeaux, S. M., & Smith, G. R. (1990). Genetic Dissection of the Biochemical Activities of Recbcd Enzyme. *Genetics*, 126(1), 25–40. <https://doi.org/10.1093/genetics/126.1.25>
- Amundsen, S. K., Sharp, J. W., & Smith, G. R. (2016). RecBCD Enzyme “Chi Recognition” Mutants Recognize Chi Recombination Hotspots in the Right DNA Context. *Genetics*, 204(1), 139–152. <https://doi.org/10.1534/genetics.116.191056>
- Amundsen, S. K., Taylor, A. F., Chaudhury, A. M., & Smith, G. R. (1986). recD: The gene for an essential third subunit of exonuclease V. *Proceedings of the National Academy of Sciences of the United States of America*, 83(15), 5558–5562.
- Amundsen, S. K., Taylor, A. F., & Smith, G. R. (2000). The RecD subunit of the *Escherichia coli* RecBCD enzyme inhibits RecA loading, homologous recombination, and DNA repair. *Proceedings of the National Academy of Sciences*, 97(13), 7399–7404. <https://doi.org/10.1073/pnas.130192397>

- Anderson, D. E., Trujillo, K. M., Sung, P., & Erickson, H. P. (2001). Structure of the Rad50·Mre11 DNA Repair Complex from *Saccharomyces cerevisiae* by Electron Microscopy *. *Journal of Biological Chemistry*, 276(40), 37027–37033. <https://doi.org/10.1074/jbc.M106179200>
- Anderson, D. G., & Kowalczykowski, S. C. (1997). The Translocating RecBCD Enzyme Stimulates Recombination by Directing RecA Protein onto ssDNA in a χ -Regulated Manner. *Cell*, 90(1), 77–86. [https://doi.org/10.1016/S0092-8674\(00\)80315-3](https://doi.org/10.1016/S0092-8674(00)80315-3)
- A. Rizvi, S. M., Prajapati, H. K., Nag, P., & Ghosh, S. K. (2017). The 2- μ m plasmid encoded protein Raf1 regulates both stability and copy number of the plasmid by blocking the formation of the Rep1–Rep2 repressor complex. *Nucleic Acids Research*, 45(12), 7167–7179. <https://doi.org/10.1093/nar/gkx316>
- Armanios, M., & Blackburn, E. H. (2012). The telomere syndromes. *Nature Reviews. Genetics*, 13(10), 693–704. <https://doi.org/10.1038/nrg3246>
- Asai, T., Sommer, S., Bailone, A., & Kogoma, T. (1993). Homologous recombination-dependent initiation of DNA replication from DNA damage-inducible origins in *Escherichia coli*. *The EMBO Journal*, 12(8), 3287–3295. <https://doi.org/10.1002/j.1460-2075.1993.tb05998.x>
- Azvolinsky, A., Dunaway, S., Torres, J. Z., Bessler, J. B., & Zakian, V. A. (2006a). The *S. cerevisiae* Rrm3p DNA helicase moves with the replication fork and affects replication of all yeast chromosomes. *Genes & Development*, 20(22), 3104–3116. <https://doi.org/10.1101/gad.1478906>
- Azvolinsky, A., Dunaway, S., Torres, J. Z., Bessler, J. B., & Zakian, V. A. (2006b). The *S. cerevisiae* Rrm3p DNA helicase moves with the replication fork and affects replication of all yeast chromosomes. *Genes & Development*, 20(22), 3104–3116. <https://doi.org/10.1101/gad.1478906>
- Baker Brachmann, C., Davies, A., Cost, G. J., Caputo, E., Li, J., Hieter, P., & Boeke, J. D. (1998). Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: A useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast*, 14(2), 115–132. [https://doi.org/10.1002/\(SICI\)1097-0061\(19980130\)14:2%3C115::AID-YEA204%3E3.0.CO;2-2](https://doi.org/10.1002/(SICI)1097-0061(19980130)14:2%3C115::AID-YEA204%3E3.0.CO;2-2)

- Baldacci, G., Chérif-Zahar, B., & Bernardi, G. (1984). The initiation of DNA replication in the mitochondrial genome of yeast. *The EMBO Journal*, 3(9), 2115–2120. <https://doi.org/10.1002/j.1460-2075.1984.tb02099.x>
- Bedale, W. A., Inman, R. B., & Cox, M. M. (1993). A reverse DNA strand exchange mediated by recA protein and exonuclease I. The generation of apparent DNA strand breaks by recA protein is explained. *Journal of Biological Chemistry*, 268(20), 15004–15016. [https://doi.org/10.1016/S0021-9258\(18\)82431-0](https://doi.org/10.1016/S0021-9258(18)82431-0)
- Bell, S. P., & Dutta, A. (2002). DNA Replication In Eukaryotic Cells. *Annual Review of Biochemistry*, 71(1), 333. (6968355). <https://doi.org/10.1146/annurev.biochem.71.110601.135425>
- Bell, S. P., Kobayashi, R., & Stillman, B. (1993). Yeast origin recognition complex functions in transcription silencing and DNA replication. 262(5141), 1844–1849.
- Bell, S. P., & Stillman, B. (1992). ATP-Dependent Recognition of Eukaryotic Origins of DNA Replication by a Multiprotein Complex. *Nature*, 357(6374), 128–134.
- Biek, D. P., & Cohen, S. N. (1986). Identification and characterization of recD, a gene affecting plasmid maintenance and recombination in Escherichia coli. *Journal of Bacteriology*, 167(2), 594–603. <https://doi.org/10.1128/jb.167.2.594-603.1986>
- Blackburn, E. H. (2001). Switching and Signaling at the Telomere. *Cell*, 106(6), 661–673. [https://doi.org/10.1016/S0092-8674\(01\)00492-5](https://doi.org/10.1016/S0092-8674(01)00492-5)
- Blackburn, E. H., & Gall, J. G. (1978). A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in *Tetrahymena*. *Journal of Molecular Biology*, 120(1), 33–53. [https://doi.org/10.1016/0022-2836\(78\)90294-2](https://doi.org/10.1016/0022-2836(78)90294-2)
- Blasco, M. A., Lee, H.-W., Hande, M. P., Samper, E., Lansdorp, P. M., DePinho, R. A., & Greider, C. W. (1997). Telomere Shortening and Tumor Formation by Mouse Cells Lacking Telomerase RNA. *Cell*, 91(1), 25–34. [https://doi.org/10.1016/S0092-8674\(01\)80006-4](https://doi.org/10.1016/S0092-8674(01)80006-4)
- Blattner, F. R., Plunkett, G., Bloch, C. A., Perna, N. T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J. D., Rode, C. K., Mayhew, G. F., Gregor, J., Davis, N. W., Kirkpatrick, H. A., Goeden, M. A., Rose, D. J., Mau, B., & Shao, Y. (1997). The Complete Genome Sequence of Escherichia coli K-12. *Science*, 277(5331), 1453–1462. <https://doi.org/10.1126/science.277.5331.1453>

- Blow, J. J., & Ge, X. Q. (2009). A model for DNA replication showing how dormant origins safeguard against replication fork failure. *EMBO Reports*, 10(4), 406–412. <https://doi.org/10.1038/embor.2009.5>
- Bochman, M. L., Judge, C. P., & Zakian, V. A. (2011). The Pif1 family in prokaryotes: What are our helicases doing in your bacteria? *Molecular Biology of the Cell*, 22(12), 1955–1959. <https://doi.org/10.1091/mbc.E11-01-0045>
- Bochman, M. L., Sabouri, N., & Zakian, V. A. (2010). Unwinding the functions of the Pif1 family helicases. *DNA Repair, Helicase and Translocases Required for the Maintenance of Genome Stability*, 9(3), 237–249. <https://doi.org/10.1016/j.dnarep.2010.01.008>
- Bonetti, D., Clerici, M., Manfrini, N., Lucchini, G., & Longhese, M. P. (2010). The MRX Complex Plays Multiple Functions in Resection of Yku- and Rif2-Protected DNA Ends. *PLOS ONE*, 5(11), e14142. <https://doi.org/10.1371/journal.pone.0014142>
- Borde, V., & de Massy, B. (2013). Programmed induction of DNA double strand breaks during meiosis: Setting up communication between DNA and the chromosome structure. *Current Opinion in Genetics & Development, Genome Architecture and Expression*, 23(2), 147–155. <https://doi.org/10.1016/j.gde.2012.12.002>
- Boulé, J.-B., & Zakian, V. A. (2006). Roles of Pif1-like helicases in the maintenance of genomic stability. *Nucleic Acids Research*, 34(15), 4147–4153. <https://doi.org/10.1093/nar/gkl561>
- Boulton, S. J., & Jackson, S. P. (1998a). Components of the Ku-dependent non-homologous end-joining pathway are involved in telomeric length maintenance and telomeric silencing. *The EMBO Journal*, 17(6), 1819–1828. <https://doi.org/10.1093/emboj/17.6.1819>
- Boulton, S. J., & Jackson, S. P. (1998b). Components of the Ku-dependent non-homologous end-joining pathway are involved in telomeric length maintenance and telomeric silencing. *The EMBO Journal*, 17(6), 1819–1828. <https://doi.org/10.1093/emboj/17.6.1819>
- Bressan, D. A., Baxter, B. K., & Petrini, J. H. J. (1999). The Mre11-Rad50-Xrs2 Protein Complex Facilitates Homologous Recombination-Based Double-Strand Break

- Repair in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology*, 19(11), 7681–7687. <https://doi.org/10.1128/MCB.19.11.7681>
- Bressan, D. A., Olivares, H. A., Nelms, B. E., & Petrini, J. H. J. (1998). *Alteration of N-Terminal Phosphoesterase Signature Motifs Inactivates Saccharomyces cerevisiae Mre11*. 150(2), 591–600.
- Brewer, B. J., & Fangman, W. L. (1967). The Localization of Replication Origins on ARS Plasmids in *S. cerevisiae*. *Cell*, 51(3), 463–471,.
- Broach, J. R., Atkins, J. F., McGill, C., & Chow, L. (1979). Identification and mapping of the transcriptional and translational products of the yeast plasmid, 2 μ circle. *Cell*, 16(4), 827–839. [https://doi.org/10.1016/0092-8674\(79\)90098-9](https://doi.org/10.1016/0092-8674(79)90098-9)
- Broach, J. R., Guarascio, V. R., & Jayaram, M. (1982). Recombination within the yeast plasmid 2 μ circle is site-specific. *Cell*, 29(1), 227–234. [https://doi.org/10.1016/0092-8674\(82\)90107-6](https://doi.org/10.1016/0092-8674(82)90107-6)
- Brutlag, D., & Kornberg, A. (1972). Enzymatic Synthesis of Deoxyribonucleic Acid. *Journal of Biological Chemistry*, 247(1), 241–248. [https://doi.org/10.1016/S0021-9258\(19\)45781-5](https://doi.org/10.1016/S0021-9258(19)45781-5)
- Cannavo, E., & Cejka, P. (2014). Sae2 promotes dsDNA endonuclease activity within Mre11–Rad50–Xrs2 to resect DNA breaks. *Nature*, 514(7520), 122–125. <https://doi.org/10.1038/nature13771>
- Cannavo, E., Johnson, D., Andres, S. N., Kissling, V. M., Reinert, J. K., Garcia, V., Erie, D. A., Hess, D., Thomä, N. H., Enchev, R. I., Peter, M., Williams, R. S., Neale, M. J., & Cejka, P. (2018). Regulatory control of DNA end resection by Sae2 phosphorylation. *Nature Communications*, 9(1), 4016. <https://doi.org/10.1038/s41467-018-06417-5>
- Capaldo, F. N., & Barbour, S. D. (1975). The Role of the rec Genes in the Viability of *Escherichia coli* K12. In P. C. Hanawalt & R. B. Setlow (Eds.), *Molecular Mechanisms for Repair of DNA: Part A* (pp. 405–418). Springer US. https://doi.org/10.1007/978-1-4684-2895-7_56
- Capaldo-Kimball, F., & Barbour, S. D. (1971). *Involvement of Recombination Genes in Growth and Viability of Escherichia coli K-12*. 106(1), 204–212. <https://doi.org/10.1128/jb.106.1.204-212.1971>

- Chamankhah, M., Fontanie, T., & Xiao, W. (2000). The *Saccharomyces cerevisiae mre11(ts)* Allele Confers a Separation of DNA Repair and Telomere Maintenance Functions. *Genetics*, 155(2), 569–576. <https://doi.org/10.1093/genetics/155.2.569>
- Chaudhury, A. M., & Smith, G. R. (1984a). A new class of *Escherichia coli* recBC mutants: Implications for the role of RecBC enzyme in homologous recombination. *Proceedings of the National Academy of Sciences of the United States of America*, 81(24), 7850–7854. <https://doi.org/10.1073/pnas.81.24.7850>
- Chaudhury, A. M., & Smith, G. R. (1984b). *Escherichia coli* recBC deletion mutants. 160(2), 788–791. <https://doi.org/10.1128/jb.160.2.788-791.1984>
- Chow, K.-H., & Courcelle, J. (2004). RecO Acts with RecF and RecR to Protect and Maintain Replication Forks Blocked by UV-induced DNA Damage in *Escherichia coli**. *Journal of Biological Chemistry*, 279(5), 3492–3496. <https://doi.org/10.1074/jbc.M311012200>
- Churchill, J. J., Anderson, D. G., & Kowalczykowski, S. C. (1999). The RecBC enzyme loads RecA protein onto ssDNA asymmetrically and independently of χ , resulting in constitutive recombination activation. *Genes & Development*, 13(7), 901–911. <https://doi.org/10.1101/gad.13.7.901>
- Clark, A. J. (1973). Recombination Deficient Mutants of *E. Coli* and Other Bacteria. *Annual Review of Genetics*, 7(Volume 7, 1973), 67–86. <https://doi.org/10.1146/annurev.ge.07.120173.000435>
- Clark, A. J., Chamberlin, M., Boyce, R. P., & Howard-Flanders, P. (1966). Abnormal metabolic response to ultraviolet light of a recombination deficient mutant of *Escherichia coli* K12. *Journal of Molecular Biology*, 19(2), 442–454. [https://doi.org/10.1016/S0022-2836\(66\)80015-3](https://doi.org/10.1016/S0022-2836(66)80015-3)
- Clark, A. J., & Margulies, A. D. (1965). Isolation and characterization of recombinationdeficient mutants of *Escherichia coli* K12. *Proceedings of the National Academy of Sciences*, 53(2), 451–459. <https://doi.org/10.1073/pnas.53.2.451>
- Clark-Walker, G. D., & Miklos, G. L. G. (1974). Localization and Quantification of Circular DNA in Yeast. *European Journal of Biochemistry*, 41(2), 359–365. <https://doi.org/10.1111/j.1432-1033.1974.tb03278.x>

- Clerici, M., Mantiero, D., Lucchini, G., & Longhese, M. P. (2005a). The *Saccharomyces cerevisiae* Sae2 Protein Promotes Resection and Bridging of Double Strand Break Ends*. *Journal of Biological Chemistry*, 280(46), 38631–38638. <https://doi.org/10.1074/jbc.M508339200>
- Clerici, M., Mantiero, D., Lucchini, G., & Longhese, M. P. (2005b). The *Saccharomyces cerevisiae* Sae2 Protein Promotes Resection and Bridging of Double Strand Break Ends*. *Journal of Biological Chemistry*, 280(46), 38631–38638. <https://doi.org/10.1074/jbc.M508339200>
- Clerici, M., Mantiero, D., Lucchini, G., & Longhese, M. P. (2005c). The *Saccharomyces cerevisiae* Sae2 Protein Promotes Resection and Bridging of Double Strand Break Ends *. *Journal of Biological Chemistry*, 280(46), 38631–38638. <https://doi.org/10.1074/jbc.M508339200>
- Clerici, M., Mantiero, D., Lucchini, G., & Longhese, M. P. (2006). The *Saccharomyces cerevisiae* Sae2 protein negatively regulates DNA damage checkpoint signalling. *EMBO Reports*, 7(2), 212–218. <https://doi.org/10.1038/sj.embor.7400593>
- Connelly, J. C., de Leau, E. S., & Leach, D. R. (1999). DNA cleavage and degradation by the SbcCD protein complex from *Escherichia coli*. *Nucleic Acids Research*, 27(4), 1039–1046.
- Connelly, J. C., de Leau, E. S., & Leach, D. R. F. (2003). Nucleolytic processing of a protein-bound DNA end by the *E. coli* SbcCD (MR) complex. *DNA Repair*, 2(7), 795–807. [https://doi.org/10.1016/S1568-7864\(03\)00063-6](https://doi.org/10.1016/S1568-7864(03)00063-6)
- Connelly, J. C., Kirkham, L. A., & Leach, D. R. F. (1998a). The SbcCD nuclease of *Escherichia coli* is a structural maintenance of chromosomes (SMC) family protein that cleaves hairpin DNA. *Proceedings of the National Academy of Sciences of the United States of America*, 95(14), 7969–7974. <https://doi.org/10.1073/pnas.95.14.7969>
- Connelly, J. C., Kirkham, L. A., & Leach, D. R. F. (1998b). The SbcCD nuclease of *Escherichia coli* is a structural maintenance of chromosomes (SMC) family protein that cleaves hairpin DNA. *Proceedings of the National Academy of Sciences of the United States of America*, 95(14), 7969–7974. <https://doi.org/10.1073/pnas.95.14.7969>

- Connelly, J. C., & Leach, D. R. F. (1996a). The sbcC and sbcD genes of *Escherichia coli* encode a nuclease involved in palindrome inviability and genetic recombination. *Genes to Cells*, 1(3), 285–291. <https://doi.org/10.1046/j.1365-2443.1996.23024.x>
- Connelly, J. C., & Leach, D. R. F. (1996b). The sbcC and sbcD genes of *Escherichia coli* encode a nuclease involved in palindrome inviability and genetic recombination. *Genes to Cells*, 1(3), 285–291. <https://doi.org/10.1046/j.1365-2443.1996.23024.x>
- Connelly, J. C., & Leach, D. R. F. (2002). Tethering on the brink: The evolutionarily conserved Mre11–Rad50 complex. *Trends in Biochemical Sciences*, 27(8), 410–418. [https://doi.org/10.1016/S0968-0004\(02\)02144-8](https://doi.org/10.1016/S0968-0004(02)02144-8)
- Conti, C., Saccà, B., Herrick, J., Lalou, C., Pommier, Y., & Bensimon, A. (2007). Replication Fork Velocities at Adjacent Replication Origins Are Coordinately Modified during DNA Replication in Human Cells. *Molecular Biology of the Cell*, 18(8), 3059–3067. <https://doi.org/10.1091/mbc.E06-08-0689>
- Cooper, G. M. (2000). The Complexity of Eukaryotic Genomes. In *The Cell: A Molecular Approach*. 2nd edition. Sinauer Associates. <https://www.ncbi.nlm.nih.gov/books/NBK9846/>
- Costa, A., Hood, I. V., & Berger, J. M. (2013). Mechanisms for Initiating Cellular DNA Replication. *Annual Review of Biochemistry*, 82(1), 25–54. <https://doi.org/10.1146/annurev-biochem-052610-094414>
- Cottrell, S. F., & Lee, L. H. (1981). Evidence for the synchronous replication of mitochondrial DNA during the yeast cell cycle. *Biochemical and Biophysical Research Communications*, 101(4), 1350–1356. [https://doi.org/10.1016/0006-291X\(81\)91596-5](https://doi.org/10.1016/0006-291X(81)91596-5)
- Courcelle, J., Carswell-Crumpton, C., & Hanawalt, P. C. (1997). recF and recR are required for the resumption of replication at DNA replication forks in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America*, 94(8), 3714–3719. <https://doi.org/10.1073/pnas.94.8.3714>
- Courcelle, J., & Hanawalt, P. C. (2003). RecA-Dependent Recovery of Arrested DNA Replication Forks. *Annual Review of Genetics*, 37(Volume 37, 2003), 611–646. <https://doi.org/10.1146/annurev.genet.37.110801.142616>

- Courcelle, J., Wendel, B. M., Livingstone, D. D., & Courcelle, C. T. (2015a). RecBCD is required to complete chromosomal replication: Implications for double-strand break frequencies and repair mechanisms. *DNA Repair*, 32, 86–95. <https://doi.org/10.1016/j.dnarep.2015.04.018>
- Courcelle, J., Wendel, B. M., Livingstone, D. D., & Courcelle, C. T. (2015b). RecBCD is required to Complete Chromosomal Replication: Implications for Double-Strand Break Frequencies and Repair Mechanisms. *DNA Repair*, 32, 86–95. <https://doi.org/10.1016/j.dnarep.2015.04.018>
- Cox, M. M. (2001). Historical overview: Searching for replication help in all of the rec places. *Proceedings of the National Academy of Sciences*, 98(15), 8173–8180. <https://doi.org/10.1073/pnas.131004998>
- Cox, M. M., & Lehman, I. R. (1981). Directionality and polarity in recA protein-promoted branch migration. *Proceedings of the National Academy of Sciences*, 78(10), 6018–6022. <https://doi.org/10.1073/pnas.78.10.6018>
- Cox, M. M., & Lehman, I. R. (1982). recA protein-promoted DNA strand exchange. Stable complexes of recA protein and single-stranded DNA formed in the presence of ATP and single-stranded DNA binding protein. *Journal of Biological Chemistry*, 257(14), 8523–8532. [https://doi.org/10.1016/S0021-9258\(18\)34363-1](https://doi.org/10.1016/S0021-9258(18)34363-1)
- Cromie, G. A., & Leach, D. R. F. (2001). Recombinational repair of chromosomal DNA double-strand breaks generated by a restriction endonuclease. *Molecular Microbiology*, 41(4), 873–883. <https://doi.org/10.1046/j.1365-2958.2001.02553.x>
- Crowley, D. J., & Hanawalt, P. C. (1998). Induction of the SOS Response Increases the Efficiency of Global Nucleotide Excision Repair of Cyclobutane Pyrimidine Dimers, but Not 6-4 Photoproducts, in UV-Irradiated *Escherichia coli*. *Journal of Bacteriology*, 180(13), 3345–3352. <https://doi.org/10.1128/jb.180.13.3345-3352.1998>
- D'Amours, D., & Jackson, S. P. (2001). The yeast Xrs2 complex functions in S phase checkpoint regulation. *Genes & Development*, 15(17), 2238–2249. <https://doi.org/10.1101/gad.208701>
- Davies, A. A., Masson, J.-Y., McIlwraith, M. J., Stasiak, A. Z., Stasiak, A., Venkitaraman, A. R., & West, S. C. (2001). Role of BRCA2 in Control of the RAD51

- Recombination and DNA Repair Protein. *Molecular Cell*, 7(2), 273–282. [https://doi.org/10.1016/S1097-2765\(01\)00175-7](https://doi.org/10.1016/S1097-2765(01)00175-7)
- Deng, S. K., Yin, Y., Petes, T. D., & Symington, L. S. (2015). Mre11-Sae2 and RPA collaborate to prevent palindromic gene amplification. *Molecular Cell*, 60(3), 500–508. <https://doi.org/10.1016/j.molcel.2015.09.027>
- Dewar, J. M., Budzowska, M., & Walter, J. C. (2015). The mechanism of DNA replication termination in vertebrates. *Nature*, 525(7569), 345–350. <https://doi.org/10.1038/nature14887>
- Diffley, J. F. X. (2010). The Many Faces of Redundancy in DNA Replication Control. *Cold Spring Harbor Symposia on Quantitative Biology*, 75, 135–142. <https://doi.org/10.1101/sqb.2010.75.062>
- Diffley, J. F. X., Cocker, J. H., Dowell, S. J., & Rowley, A. (1994). Two steps in the assembly of complexes at yeast replication origins in vivo. *Cell*, 78(2), 303–316. [https://doi.org/10.1016/0092-8674\(94\)90299-2](https://doi.org/10.1016/0092-8674(94)90299-2)
- Dillingham, M. S., & Kowalczykowski, S. C. (2008). RecBCD Enzyme and the Repair of Double-Stranded DNA Breaks. *Microbiology and Molecular Biology Reviews: MMBR*, 72(4), 642–671. <https://doi.org/10.1128/MMBR.00020-08>
- Dillingham, M. S., Spies, M., & Kowalczykowski, S. C. (2003). RecBCD enzyme is a bipolar DNA helicase. *Nature*, 423(6942), 893–897. <https://doi.org/10.1038/nature01673>
- Dolganov, G. M., Maser, R. S., Novikov, A., Tosto, L., Chong, S., Bressan, D. A., & Petrini, J. H. (1996). Human Rad50 is physically associated with human Mre11: Identification of a conserved multiprotein complex implicated in recombinational DNA repair. *Molecular and Cellular Biology*, 16(9), 4832–4841. <https://doi.org/10.1128/mcb.16.9.4832>
- Egelman, E. H., & Stasiak, A. (1986). Structure of helical RecA-DNA complexes: Complexes formed in the presence of ATP-gamma-S or ATP. *Journal of Molecular Biology*, 191(4), 677–697. [https://doi.org/10.1016/0022-2836\(86\)90453-5](https://doi.org/10.1016/0022-2836(86)90453-5)
- Eshaghi, M., Karuturi, R. K. M., Li, J., Chu, Z., Liu, E. T., & Liu, J. (2007). Global Profiling of DNA Replication Timing and Efficiency Reveals that Efficient Replication/Firing

- Occurs Late during S-Phase in *S. pombe*. *PLOS ONE*, 2(8), e722. <https://doi.org/10.1371/journal.pone.0000722>
- Eykelenboom, J. K., Blackwood, J. K., Okely, E., & Leach, D. R. F. (2008). SbcCD Causes a Double-Strand Break at a DNA Palindrome in the *Escherichia coli* Chromosome. *Molecular Cell*, 29(5), 644–651. <https://doi.org/10.1016/j.molcel.2007.12.020>
- Feng, W., Di Rienzi, S. C., Raghuraman, M. K., & Brewer, B. J. (2011). Replication Stress-Induced Chromosome Breakage Is Correlated with Replication Fork Progression and Is Preceded by Single-Stranded DNA Formation. *G3 Genes|Genomes|Genetics*, 1(5), 327–335. <https://doi.org/10.1534/g3.111.000554>
- Ferguson, B. M., Brewer, B. J., Reynolds, A. E., & Fangman, W. L. (1991). A yeast origin of replication is activated late in S phase. *Cell*, 65(3), 507–515. [https://doi.org/10.1016/0092-8674\(91\)90468-E](https://doi.org/10.1016/0092-8674(91)90468-E)
- Fogliano, M., & Schendel, P. F. (1981). Evidence for the inducibility of the *uvrB* operon. *Nature*, 289(5794), 196–198. <https://doi.org/10.1038/289196a0>
- Foury, F. (1989). Cloning and Sequencing of the Nuclear Gene MIP1 Encoding the Catalytic Subunit of the Yeast Mitochondrial DNA Polymerase. *Journal of Biological Chemistry*, 264(34), 20552–20560. [https://doi.org/10.1016/S0021-9258\(19\)47098-1](https://doi.org/10.1016/S0021-9258(19)47098-1)
- Foury, F., & Kolodnyski, J. (1983). Pif mutation blocks recombination between mitochondrial rho⁺ and rho⁻ genomes having tandemly arrayed repeat units in *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences*, 80(17), 5345–5349. <https://doi.org/10.1073/pnas.80.17.5345>
- Friedman, K. L., Raghuraman, M. K., Fangman, W. L., & Brewer, B. J. (1995). Analysis of the temporal program of replication initiation in yeast chromosomes. *Journal of Cell Science*, 1995(Supplement_19), 51–58. https://doi.org/10.1242/jcs.1995.Supplement_19.7
- Fukuoh, A., Iwasaki, H., Ishioka, K., & Shinagawa, H. (1997). ATP-dependent resolution of R-loops at the ColE1 replication origin by *Escherichia coli* RecG protein, a Holliday junction-specific helicase. *The EMBO Journal*, 16(1), 203–209. <https://doi.org/10.1093/emboj/16.1.203>

- Gerbaud, C., Fournier, P., Blanc, H., Aigle, M., Heslot, H., & Guerineau, M. (1979). High frequency of yeast transformation by plasmids carrying part of entire 2- μ m yeast plasmid. *Gene*, 5(3), 233–253. [https://doi.org/10.1016/0378-1119\(79\)90080-5](https://doi.org/10.1016/0378-1119(79)90080-5)
- Goldar, A., Marsolier-Kergoat, M.-C., & Hyrien, O. (2009). Universal Temporal Profile of Replication Origin Activation in Eukaryotes. *PLOS ONE*, 4(6), e5899. <https://doi.org/10.1371/journal.pone.0005899>
- Gorbalenya, A. E., Koonin, E. V., Donchenko, A. P., & Blinov, V. M. (1989). Two related superfamilies of putative helicases involved in replication, recombination, repair and expression of DNA and RNA genomes. *Nucleic Acids Research*, 17(12), 4713–4730. <https://doi.org/10.1093/nar/17.12.4713>
- Guilbaud, G., Rappailles, A., Baker, A., Chen, C.-L., Arneodo, A., Goldar, A., d'Aubenton-Carafa, Y., Thermes, C., Audit, B., & Hyrien, O. (2011). Evidence for Sequential and Increasing Activation of Replication Origins along Replication Timing Gradients in the Human Genome. *PLOS Computational Biology*, 7(12), e1002322. <https://doi.org/10.1371/journal.pcbi.1002322>
- Haber, J. E. (1998). The Many Interfaces of Mre11. *Cell*, 95(5), 583–586. [https://doi.org/10.1016/S0092-8674\(00\)81626-8](https://doi.org/10.1016/S0092-8674(00)81626-8)
- Hamilton, N. (2000). *Use of Two-replisome Plasmids to Characterize how Chromosome Replication Completes*. <https://doi.org/10.15760/etd.6940>
- Hamilton, N. A., Jehru, A. E., Samples, W. N., Wendel, B. M., Mokhtari, P. D., Courcelle, C. T., & Courcelle, J. (2023). Chi sequences switch the RecBCD helicase–nuclease complex from degradative to replicative modes during the completion of DNA replication. *The Journal of Biological Chemistry*, 299(3), 103013. <https://doi.org/10.1016/j.jbc.2023.103013>
- Hamilton, N. A., Wendel, B. M., Weber, E. A., Courcelle, C. T., & Courcelle, J. (2019a). RecBCD, SbcCD and ExoI process a substrate created by convergent replisomes to complete DNA replication. *Molecular Microbiology*, 111(6), 1638–1651. <https://doi.org/10.1111/mmi.14242>
- Hamilton, N. A., Wendel, B. M., Weber, E. A., Courcelle, C. T., & Courcelle, J. (2019b). RecBCD, SbcCD and ExoI process a substrate created by convergent replisomes

- to complete DNA replication. *Molecular Microbiology*, 111(6), 1638–1651. <https://doi.org/10.1111/mmi.14242>
- Hanawalt, P. C., Ford, J. M., & Lloyd, D. R. (2003). Functional characterization of global genomic DNA repair and its implications for cancer. *Mutation Research/Reviews in Mutation Research, The Fourth International Conference on Environmental Mutagens in Human Populations*, 544(2), 107–114. <https://doi.org/10.1016/j.mrrev.2003.06.002>
- Hardy, J., Churikov, D., Géli, V., & Simon, M.-N. (2014). Sgs1 and Sae2 promote telomere replication by limiting accumulation of ssDNA. *Nature Communications*, 5(1), 5004. <https://doi.org/10.1038/ncomms6004>
- Hawkins, M., Malla, S., Blythe, M. J., Nieduszynski, C. A., & Allers, T. (2013). Accelerated growth in the absence of DNA replication origins. *Nature*, 503(7477), 544–547. <https://doi.org/10.1038/nature12650>
- Hawkins, M., Retkute, R., Müller, C. A., Saner, N., Tanaka, T. U., de Moura, A. P. S., & Nieduszynski, C. A. (2013). High-Resolution Replication Profiles Define the Stochastic Nature of Genome Replication Initiation and Termination. *Cell Reports*, 5(4), 1132–1141. <https://doi.org/10.1016/j.celrep.2013.10.014>
- Hayano, M., Kanoh, Y., Matsumoto, S., Renard-Guillet, C., Shirahige, K., & Masai, H. (2012). Rif1 is a global regulator of timing of replication origin firing in fission yeast. *Genes & Development*, 26(2), 137–150. <https://doi.org/10.1101/gad.178491.111>
- Henderson, E., Hardin, C. C., Walk, S. K., Tinoco, I., & Blackburn, E. H. (1987). Telomeric DNA oligonucleotides form novel intramolecular structures containing guanine·guanine base pairs. *Cell*, 51(6), 899–908. [https://doi.org/10.1016/0092-8674\(87\)90577-0](https://doi.org/10.1016/0092-8674(87)90577-0)
- Heun, P., Laroche, T., Raghuraman, M. K., & Gasser, S. M. (2001). The Positioning and Dynamics of Origins of Replication in the Budding Yeast Nucleus. *Journal of Cell Biology*, 152(2), 385–400. <https://doi.org/10.1083/jcb.152.2.385>
- Hiasa, H., & Marians, K. J. (1994). Tus prevents overreplication of oriC plasmid DNA. *Journal of Biological Chemistry*, 269(43), 26959–26968. [https://doi.org/10.1016/S0021-9258\(18\)47112-8](https://doi.org/10.1016/S0021-9258(18)47112-8)

- Hill, T. M. (1992). ARREST OF BACTERIAL DNA REPLICATION. *Annual Review of Microbiology*, 46(Volume 46, 1992), 603–633.
<https://doi.org/10.1146/annurev.mi.46.100192.003131>
- Hopfner, K.-P., Craig, L., Moncalian, G., Zinkel, R. A., Usui, T., Owen, B. A. L., Karcher, A., Henderson, B., Bodmer, J.-L., McMurray, C. T., Carney, J. P., Petrini, J. H. J., & Tainer, J. A. (2002). The Rad50 zinc-hook is a structure joining Mre11 complexes in DNA recombination and repair. *Nature*, 418(6897), 562–566.
<https://doi.org/10.1038/nature00922>
- Hopfner, K.-P., Karcher, A., Craig, L., Woo, T. T., Carney, J. P., & Tainer, J. A. (2001). Structural Biochemistry and Interaction Architecture of the DNA Double-Strand Break Repair Mre11 Nuclease and Rad50-ATPase. *Cell*, 105(4), 473–485.
[https://doi.org/10.1016/S0092-8674\(01\)00335-X](https://doi.org/10.1016/S0092-8674(01)00335-X)
- Hopfner, K.-P., Karcher, A., Shin, D. S., Craig, L., Arthur, L. M., Carney, J. P., & Tainer, J. A. (2000). Structural Biology of Rad50 ATPase: ATP-Driven Conformational Control in DNA Double-Strand Break Repair and the ABC-ATPase Superfamily. *Cell*, 101(7), 789–800. [https://doi.org/10.1016/S0092-8674\(00\)80890-9](https://doi.org/10.1016/S0092-8674(00)80890-9)
- Hori, A., Yoshida, M., Shibata, T., & Ling, F. (2009). Reactive oxygen species regulate DNA copy number in isolated yeast mitochondria by triggering recombination-mediated replication. *Nucleic Acids Research*, 37(3), 749–761.
<https://doi.org/10.1093/nar/gkn993>
- Howard-Flanders, P., & Theriot, L. (1966). Mutants of Escherichia coli K-12 defective in DNA repair and in genetic recombination. *Genetics*, 53(6), 1137–1150.
<https://doi.org/10.1093/genetics/53.6.1137>
- Huberman, J. A., & Riggs, A. D. (1968a). On the mechanism of DNA replication in mammalian chromosomes. *Journal of Molecular Biology*, 32(2), 327–341.
[https://doi.org/10.1016/0022-2836\(68\)90013-2](https://doi.org/10.1016/0022-2836(68)90013-2)
- Huberman, J. A., & Riggs, A. D. (1968b). On the mechanism of DNA replication in mammalian chromosomes. *Journal of Molecular Biology*, 32(2), 327–341.
[https://doi.org/10.1016/0022-2836\(68\)90013-2](https://doi.org/10.1016/0022-2836(68)90013-2)

- Huertas, P., Cortés-Ledesma, F., Sartori, A. A., Aguilera, A., & Jackson, S. P. (2008). CDK targets Sae2 to control DNA-end resection and homologous recombination. *Nature*, 455(7213), 689–692. <https://doi.org/10.1038/nature07215>
- Ivessa, A. S., Lenzmeier, B. A., Bessler, J. B., Goudsouzian, L. K., Schnakenberg, S. L., & Zakian, V. A. (2003). The *Saccharomyces cerevisiae* Helicase Rrm3p Facilitates Replication Past Nonhistone Protein-DNA Complexes. *Molecular Cell*, 12(6), 1525–1536. [https://doi.org/10.1016/S1097-2765\(03\)00456-8](https://doi.org/10.1016/S1097-2765(03)00456-8)
- Ivessa, A. S., Zhou, J.-Q., Schulz, V. P., Monson, E. K., & Zakian, V. A. (2002). *Saccharomyces* Rrm3p, a 5' to 3' DNA helicase that promotes replication fork progression through telomeric and subtelomeric DNA. *Genes & Development*, 16(11), 1383–1396. <https://doi.org/10.1101/gad.982902>
- Ivessa, A. S., Zhou, J.-Q., & Zakian, V. A. (2000a). The *Saccharomyces* Pif1p DNA Helicase and the Highly Related Rrm3p Have Opposite Effects on Replication Fork Progression in Ribosomal DNA. *Cell*, 100(4), 479–489. [https://doi.org/10.1016/S0092-8674\(00\)80683-2](https://doi.org/10.1016/S0092-8674(00)80683-2)
- Ivessa, A. S., Zhou, J.-Q., & Zakian, V. A. (2000b). The *Saccharomyces* Pif1p DNA Helicase and the Highly Related Rrm3p Have Opposite Effects on Replication Fork Progression in Ribosomal DNA. *Cell*, 100(4), 479–489. [https://doi.org/10.1016/S0092-8674\(00\)80683-2](https://doi.org/10.1016/S0092-8674(00)80683-2)
- Jayaram, M., Li, Y.-Y., & Broach, J. R. (1983). The yeast plasmid 2 μ circle encodes components required for its high copy propagation. *Cell*, 34(1), 95–104. [https://doi.org/10.1016/0092-8674\(83\)90139-3](https://doi.org/10.1016/0092-8674(83)90139-3)
- Jazayeri, A., Falck, J., Lukas, C., Bartek, J., Smith, G. C. M., Lukas, J., & Jackson, S. P. (2006). ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks. *Nature Cell Biology*, 8(1), 37–45. <https://doi.org/10.1038/ncb1337>
- Jensen, R. B., Carreira, A., & Kowalczykowski, S. C. (2010). Purified human BRCA2 stimulates RAD51-mediated recombination. *Nature*, 467(7316), 678–683. <https://doi.org/10.1038/nature09399>
- Johzuka, K., & Ogawa, H. (1995). Interaction of Mre11 and Rad50: Two Proteins Required for DNA Repair and Meiosis-Specific Double-Strand Break Formation in

- Saccharomyces Cerevisiae. *Genetics*, 139(4), 1521–1532.
<https://doi.org/10.1093/genetics/139.4.1521>
- Kang, J., Bronson, R. T., & Xu, Y. (2002). Targeted disruption of NBS1 reveals its roles in mouse development and DNA repair. *The EMBO Journal*, 21(6), 1447–1455.
<https://doi.org/10.1093/emboj/21.6.1447>
- Kaye, J. A., Melo, J. A., Cheung, S. K., Vaze, M. B., Haber, J. E., & Toczyski, D. P. (2004). DNA Breaks Promote Genomic Instability by Impeding Proper Chromosome Segregation. *Current Biology*, 14(23), 2096–2106.
<https://doi.org/10.1016/j.cub.2004.10.051>
- Keil, R. L., & McWilliams, A. D. (1993). A Gene with Specific and Global Effects on Recombination of Sequences from Tandemly Repeated Gene in *Saccharomyces cerevisiae*. 135, 711–718.
- Kenyon, C. J., & Walker, G. C. (1981). Expression of the *E. coli* *uvrA* gene is inducible. *Nature*, 289(5800), 808–810. <https://doi.org/10.1038/289808a0>
- Kim, H.-S., Vijayakumar, S., Reger, M., Harrison, J. C., Haber, J. E., Weil, C., & Petrini, J. H. J. (2008). Functional Interactions Between Sae2 and the Mre11 Complex. *Genetics*, 178(2), 711–723. <https://doi.org/10.1534/genetics.107.081331>
- Kim, N., & Jinks-Robertson, S. (2012). Transcription as a source of genome instability. *Nature Reviews. Genetics*, 13(3), 204–214. <https://doi.org/10.1038/nrg3152>
- Kim, N., Mieczyslaw, M., Prowse, K., Harley, C., West, M., & Ho, P. (1994). Specific association of human telomerase activity with immortal cells and cancer. *Science*, 266(5193), 2011+.
- Koren, A., Handsaker, R. E., Kamitaki, N., Karlić, R., Ghosh, S., Polak, P., Eggan, K., & McCarroll, S. A. (2014). Genetic Variation in Human DNA Replication Timing. *Cell*, 159(5), 1015–1026. <https://doi.org/10.1016/j.cell.2014.10.025>
- Kowalczykowski, S. C., Dixon, D. A., Eggleston, A. K., Lauder, S. D., & Rehrauer, W. M. (1994). Biochemistry of homologous recombination in *Escherichia coli*. *Microbiological Reviews*, 58(3), 501–465. <https://doi.org/10.1128/mr.58.3.401-465.1994>
- Kusano, K., nakayama, K., & Nakayama, H. (1989). Plasmid-mediated lethality and plasmid multimer formation in an *Escherichia coli* *recBC sbcBC* mutant.

- Involvement of RecF recombination pathway genes. *Journal of Molecular Biology*, 209(4), 623–634. [https://doi.org/10.1016/0022-2836\(89\)90000-4](https://doi.org/10.1016/0022-2836(89)90000-4)
- Kushner, S. R. (1974). In Vivo Studies of Temperature-Sensitive recB and recC Mutants. *Journal of Bacteriology*, 120(3), 1213–1218. <https://doi.org/10.1128/jb.120.3.1213-1218.1974>
- Kushner, S. R., Nagaishi, H., & Clark, A. J. (1972). Indirect Suppression of recB and recC Mutations by Exonuclease I Deficiency. *Proceedings of the National Academy of Sciences of the United States of America*, 69(6), 1366–1370. <https://doi.org/10.1073/pnas.69.6.1366>
- Kushner, S. R., Nagaishi, H., Templin, A., & Clark, A. J. (1971). Genetic Recombination in Escherichia coli: The Role of Exonuclease I. *Proc. Natl. Acad. Sci. U.S.A.*, 68(4), 824–827. <https://doi.org/10.1073/pnas.68.4.824>
- Kuzminov, A. (1995). Collapse and repair of replication forks in Escherichia coli. *Molecular Microbiology*, 16(3), 373–384. <https://doi.org/10.1111/j.1365-2958.1995.tb02403.x>
- Kuzminov, A. (1999). Recombinational Repair of DNA Damage in Escherichia coli and Bacteriophage λ . *Microbiology and Molecular Biology Reviews*, 63(4), 751–813. <https://doi.org/10.1128/mmbr.63.4.751-813.1999>
- Kuzminov, A. (2001). Single-strand interruptions in replicating chromosomes cause double-strand breaks. *Proceedings of the National Academy of Sciences*, 98(15), 8241–8246. <https://doi.org/10.1073/pnas.131009198>
- Kuzminov, A., & Stahl, F. W. (1999). Double-strand end repair via the RecBC pathway in Escherichia coli primes DNA replication. *Genes & Development*, 13(3), 345–356. <https://doi.org/10.1101/gad.13.3.345>
- Lammens, K., Bemeleit, D. J., Möckel, C., Clausing, E., Schele, A., Hartung, S., Schiller, C. B., Lucas, M., Angermüller, C., Söding, J., Sträßer, K., & Hopfner, K.-P. (2011). The Mre11:Rad50 Structure Shows an ATP-Dependent Molecular Clamp in DNA Double-Strand Break Repair. *Cell*, 145(1), 54–66. <https://doi.org/10.1016/j.cell.2011.02.038>
- Le, S., Moore, J. K., Haber, J. E., & Greider, C. W. (1999). RAD50 and RAD51 Define Two Pathways That Collaborate to Maintain Telomeres in the Absence of

- Telomerase. *Genetics*, 152(1), 143–152.
<https://doi.org/10.1093/genetics/152.1.143>
- Leach, D. R. F., Okely, E. A., & Pinder, D. J. (1997a). Repair by recombination of DNA containing a palindromic sequence. *Molecular Microbiology*, 26(3), 597–606.
<https://doi.org/10.1046/j.1365-2958.1997.6071957.x>
- Leach, D. R. F., Okely, E. A., & Pinder, D. J. (1997b). Repair by recombination of DNA containing a palindromic sequence. *Molecular Microbiology*, 26(3), 597–606.
<https://doi.org/10.1046/j.1365-2958.1997.6071957.x>
- Lee, S. E., Moore, J. K., Holmes, A., Umezu, K., Kolodner, R. D., & Haber, J. E. (1998). Saccharomyces Ku70, Mre11/Rad50, and RPA Proteins Regulate Adaptation to G2/M Arrest after DNA Damage. *Cell*, 94(3), 399–409.
[https://doi.org/10.1016/S0092-8674\(00\)81482-8](https://doi.org/10.1016/S0092-8674(00)81482-8)
- Lehman, I. R. (1960). The Deoxyribonucleases of Escherichia coli. *Journal of Biological Chemistry*, 235(5), 1479–1487. [https://doi.org/10.1016/S0021-9258\(18\)69431-1](https://doi.org/10.1016/S0021-9258(18)69431-1)
- Lehman, I. R., & Nussbaum, A. L. (1964). The Deoxyribonucleases of Escherichia coli. *Journal of Biological Chemistry*, 239(8), 2628–2636.
[https://doi.org/10.1016/S0021-9258\(18\)93898-6](https://doi.org/10.1016/S0021-9258(18)93898-6)
- Lengsfeld, B. M., Rattray, A. J., Bhaskara, V., Ghirlando, R., & Paull, T. T. (2007). Sae2 Is an Endonuclease that Processes Hairpin DNA Cooperatively with the Mre11/Rad50/Xrs2 Complex. *Molecular Cell*, 28(4), 638–651.
<https://doi.org/10.1016/j.molcel.2007.11.001>
- Lewis, L. K., Storici, F., Van Komen, S., Calero, S., Sung, P., & Resnick, M. A. (2004a). Role of the Nuclease Activity of Saccharomyces cerevisiae Mre11 in Repair of DNA Double-Strand Breaks in Mitotic Cells. *Genetics*, 166(4), 1701–1713.
<https://doi.org/10.1093/genetics/166.4.1701>
- Lewis, L. K., Storici, F., Van Komen, S., Calero, S., Sung, P., & Resnick, M. A. (2004b). Role of the nuclease activity of Saccharomyces cerevisiae Mre11 in repair of DNA double-strand breaks in mitotic cells. *Genetics*, 166(4), 1701–1713.
<https://doi.org/10.1534/genetics.166.4.1701>
- Ling, F., Hori, A., Yoshitani, A., Niu, R., Yoshida, M., & Shibata, T. (2013). Din7 and Mhr1 expression levels regulate double-strand-break-induced replication and

- recombination of mtDNA at ori5 in yeast. *Nucleic Acids Research*, 41(11), 5799–5816. <https://doi.org/10.1093/nar/gkt273>
- Lisby, M., Barlow, J. H., Burgess, R. C., & Rothstein, R. (2004). Choreography of the DNA Damage Response: Spatiotemporal Relationships among Checkpoint and Repair Proteins. *Cell*, 118(6), 699–713. <https://doi.org/10.1016/j.cell.2004.08.015>
- Little, J. W., Edmiston, S. H., Pacelli, L. Z., & Mount, D. W. (1980). Cleavage of the Escherichia coli lexA protein by the recA protease. *Proceedings of the National Academy of Sciences*, 77(6), 3225–3229. <https://doi.org/10.1073/pnas.77.6.3225>
- Lloyd, R. G., & Buckman, C. (1985). Identification and genetic analysis of sbcC mutations in commonly used recBC sbcB strains of Escherichia coli K-12. *Journal of Bacteriology*, 164(2), 836–844. <https://doi.org/10.1128/jb.164.2.836-844.1985>
- Lobachev, K. S., Gordenin, D. A., & Resnick, M. A. (2002). The Mre11 Complex Is Required for Repair of Hairpin-Capped Double-Strand Breaks and Prevention of Chromosome Rearrangements. *Cell*, 108(2), 183–193. [https://doi.org/10.1016/S0092-8674\(02\)00614-1](https://doi.org/10.1016/S0092-8674(02)00614-1)
- Lopes, M., Cotta-Ramusino, C., Pellicoli, A., Liberi, G., Plevani, P., Muzi-Falconi, M., Newlon, C. S., & Foiani, M. (2001). The DNA replication checkpoint response stabilizes stalled replication forks. *Nature*, 412(6846), 557–561. <https://doi.org/10.1038/35087613>
- Lu, D., & Keck, J. L. (2008). Structural basis of Escherichia coli single-stranded DNA-binding protein stimulation of exonuclease I. *Proceedings of the National Academy of Sciences of the United States of America*, 105(27), 9169–9174. <https://doi.org/10.1073/pnas.0800741105>
- Luo, G., Yao, M. S., Bender, C. F., Mills, M., Bladl, A. R., Bradley, A., & Petrini, J. H. J. (1999). Disruption of mRad50 causes embryonic stem cell lethality, abnormal embryonic development, and sensitivity to ionizing radiation. *Proceedings of the National Academy of Sciences of the United States of America*, 96(13), 7376–7381.
- Makovets, S., Herskowitz, I., & Blackburn, E. H. (2004). Anatomy and Dynamics of DNA Replication Fork Movement in Yeast Telomeric Regions. *Molecular and Cellular Biology*, 24(9), 4019–4031. <https://doi.org/10.1128/MCB.24.9.4019-4031.2004>

- Mascarenhas, J., Sanchez, H., Tadesse, S., Kidane, D., Krisnamurthy, M., Alonso, J. C., & Graumann, P. L. (2006a). *Bacillus subtilis* SbcC protein plays an important role in DNA inter-strand cross-link repair. *BMC Molecular Biology*, 7(1), 20. <https://doi.org/10.1186/1471-2199-7-20>
- Mascarenhas, J., Sanchez, H., Tadesse, S., Kidane, D., Krisnamurthy, M., Alonso, J. C., & Graumann, P. L. (2006b). *Bacillus subtilis* SbcC protein plays an important role in DNA inter-strand cross-link repair. *BMC Molecular Biology*, 7(1), 20. <https://doi.org/10.1186/1471-2199-7-20>
- McEntee, K., Weinstock, G. M., & Lehman, I. R. (1979). Initiation of general recombination catalyzed in vitro by the recA protein of *Escherichia coli*. *Proceedings of the National Academy of Sciences*, 76(6), 2615–2619. <https://doi.org/10.1073/pnas.76.6.2615>
- Méndez, J., & Stillman, B. (2000). Chromatin Association of Human Origin Recognition Complex, Cdc6, and Minichromosome Maintenance Proteins during the Cell Cycle: Assembly of Prereplication Complexes in Late Mitosis. *Molecular and Cellular Biology*, 20(22), 8602–8612. <https://doi.org/10.1128/mcb.20.22.8602-8612.2000>
- Mimitou, E. P., & Symington, L. S. (2008). Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. *Nature*, 455(7214), 10.1038/nature07312. <https://doi.org/10.1038/nature07312>
- Mimitou, E. P., & Symington, L. S. (2010). Ku prevents Exo1 and Sgs1-dependent resection of DNA ends in the absence of a functional MRX complex or Sae2. *The EMBO Journal*, 29(19), 3358–3369. <https://doi.org/10.1038/emboj.2010.193>
- Mitchell, J. R., Wood, E., & Collins, K. (1999). A telomerase component is defective in the human disease dyskeratosis congenita. *Nature*, 402(6761), 551–555. <https://doi.org/10.1038/990141>
- Moore, J. K., & Haber, J. E. (1996). Cell cycle and genetic requirements of two pathways of nonhomologous end-joining repair of double-strand breaks in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology*, 16(5), 2164–2173. <https://doi.org/10.1128/mcb.16.5.2164>

- Moreau, S., Ferguson, J. R., & Symington, L. S. (1999a). The Nuclease Activity of Mre11 Is Required for Meiosis but Not for Mating Type Switching, End Joining, or Telomere Maintenance. *Molecular and Cellular Biology*, 19(1), 556–566. <https://doi.org/10.1128/mcb.19.1.556>
- Moreau, S., Ferguson, J. R., & Symington, L. S. (1999b). The Nuclease Activity of Mre11 Is Required for Meiosis but Not for Mating Type Switching, End Joining, or Telomere Maintenance. *Molecular and Cellular Biology*, 19(1), 556–566. <https://doi.org/10.1128/mcb.19.1.556>
- Moyer, S. E., Lewis, P. W., & Botchan, M. R. (2006). Isolation of the Cdc45/Mcm2–7/GINS (CMG) complex, a candidate for the eukaryotic DNA replication fork helicase. *Proceedings of the National Academy of Sciences*, 103(27), 10236–10241. <https://doi.org/10.1073/pnas.0602400103>
- Müller, C. A., Hawkins, M., Retkute, R., Malla, S., Wilson, R., Blythe, M. J., Nakato, R., Komata, M., Shirahige, K., de Moura, A. P. S., & Nieduszynski, C. A. (2014). The dynamics of genome replication using deep sequencing. *Nucleic Acids Research*, 42(1), e3. <https://doi.org/10.1093/nar/gkt878>
- Müller, C. A., & Nieduszynski, C. A. (2012). Conservation of replication timing reveals global and local regulation of replication origin activity. *Genome Research*, 22(10), 1953–1962. <https://doi.org/10.1101/gr.139477.112>
- Murray, J. A. H., & Cesareni, G. (1986). Functional analysis of the yeast plasmid partition locus STB. *The EMBO Journal*, 5(12), 3391–3399. <https://doi.org/10.1002/j.1460-2075.1986.tb04655.x>
- Murray, J. A., Scarpa, M., Rossi, N., & Cesareni, G. (1987). Antagonistic controls regulate copy number of the yeast 2 mu plasmid. *The EMBO Journal*, 6(13), 4205–4212. <https://doi.org/10.1002/j.1460-2075.1987.tb02768.x>
- Myers, R. S., Kuzminov, A., & Stahl, F. W. (1995). The recombination hot spot chi activates RecBCD recombination by converting Escherichia coli to a recD mutant phenocopy. *Proceedings of the National Academy of Sciences of the United States of America*, 92(14), 6244–6248. <https://doi.org/10.1073/pnas.92.14.6244>

- New, J. H., Sugiyama, T., Zaitseva, E., & Kowalczykowski, S. C. (1998). Rad52 protein stimulates DNA strand exchange by Rad51 and replication protein A. *Nature*, 391(6665), 407–410. <https://doi.org/10.1038/34950>
- Niki, H., Ichinose, C., Ogura, T., Mori, H., Morita, M., Hasegawa, M., Kusukawa, N., & Hiraga, S. (1988). Chromosomal genes essential for stable maintenance of the mini-F plasmid in Escherichia coli. *Journal of Bacteriology*, 170(11), 5272–5278. <https://doi.org/10.1128/jb.170.11.5272-5278.1988>
- O'Donnell, M., Langston, L., & Stillman, B. (2013). Principles and Concepts of DNA Replication in Bacteria, Archaea, and Eukarya. *Cold Spring Harbor Perspectives in Biology*, 5(7), a010108. <https://doi.org/10.1101/cshperspect.a010108>
- Ogawa, T., Yu, X., Shinohara, A., & Egelman, E. (1993a). Similarity of the Yeast RAD51 Filament to the Bacterial RecA Filament. *Science (New York, N.Y.)*, 259, 1896–1899. <https://doi.org/10.1126/science.8456314>
- Ogawa, T., Yu, X., Shinohara, A., & Egelman, E. H. (1993b). Similarity of the Yeast RAD51 Filament to the Bacterial RecA Filament. *Science*, 259(5103), 1896–1899. <https://doi.org/10.1126/science.8456314>
- Oh, J., Al-Zain, A., Cannavo, E., Cejka, P., & Symington, L. S. (2016). Xrs2 Dependent and Independent Functions of the Mre11-Rad50 Complex. *Molecular Cell*, 64(2), 405–415. <https://doi.org/10.1016/j.molcel.2016.09.011>
- Paeschke, K., Capra, J. A., & Zakian, V. A. (2011). DNA Replication through G-Quadruplex Motifs Is Promoted by the *Saccharomyces cerevisiae* Pif1 DNA Helicase. *Cell*, 145(5), 678–691. <https://doi.org/10.1016/j.cell.2011.04.015>
- Paull, T. T., & Gellert, M. (1998). The 3' to 5' Exonuclease Activity of Mre11 Facilitates Repair of DNA Double-Strand Breaks. *Molecular Cell*, 1(7), 969–979. [https://doi.org/10.1016/S1097-2765\(00\)80097-0](https://doi.org/10.1016/S1097-2765(00)80097-0)
- Paull, T. T., & Gellert, M. (1999). Nbs1 potentiates ATP-driven DNA unwinding and endonuclease cleavage by the Mre11/Rad50 complex. *Genes & Development*, 13(10), 1276–1288. <https://doi.org/10.1101/gad.13.10.1276>
- Phillips, G. J., Prasher, D. C., & Kushner, S. R. (1988). Physical and biochemical characterization of cloned sbcB and xonA mutations from Escherichia coli K-12.

- Journal of Bacteriology*, 170(5), 2089–2094. <https://doi.org/10.1128/jb.170.5.2089-2094.1988>
- Ponticelli, A. S., Schultz, D. W., Taylor, A. F., & Smith, G. R. (1985). Chi-dependent DNA strand cleavage by RecBC enzyme. *Cell*, 41(1), 145–151. [https://doi.org/10.1016/0092-8674\(85\)90069-8](https://doi.org/10.1016/0092-8674(85)90069-8)
- Prasai, K., Robinson, L. C., Scott, R. S., Tatchell, K., & Harrison, L. (2017). Evidence for double-strand break mediated mitochondrial DNA replication in *Saccharomyces cerevisiae*. *Nucleic Acids Research*, 45(13), 7760–7773. <https://doi.org/10.1093/nar/gkx443>
- Prescott, D. M., & Kuempel, P. L. (1972). Bidirectional Replication of the Chromosome in *Escherichia coli*. *PNAS*, 69(10), 2842–2845. <https://doi.org/10.1073/pnas.69.10.2842>
- Pugh, B. F., & Cox, M. M. (1988). General mechanism for RecA protein binding to duplex DNA. *Journal of Molecular Biology*, 203(2), 479–493. [https://doi.org/10.1016/0022-2836\(88\)90014-9](https://doi.org/10.1016/0022-2836(88)90014-9)
- Raghuraman, M. K., Winzeler, E. A., Collingwood, D., Hunt, S., Wodicka, L., Conway, A., Lockhart, D. J., Davis, R. W., Brewer, B. J., & Fangman, W. L. (2001). Replication Dynamics of the Yeast Genome. *Science*, 294(5540), 115–121.
- Rattray, A. J., Shafer, B. K., Neelam, B., & Strathern, J. N. (2005). A mechanism of palindromic gene amplification in *Saccharomyces cerevisiae*. *Genes & Development*, 19(11), 1390–1399. <https://doi.org/10.1101/gad.1315805>
- Reynolds, A. E., Murray, A. W., & Szostak, J. W. (1987). Roles of the 2 microns gene products in stable maintenance of the 2 microns plasmid of *Saccharomyces cerevisiae*. *Molecular and Cellular Biology*, 7(10), 3566–3573. <https://doi.org/10.1128/mcb.7.10.3566>
- Rudolph, C. J., Upton, A. L., & Lloyd, R. G. (2009). Replication fork collisions cause pathological chromosomal amplification in cells lacking RecG DNA translocase. *Molecular Microbiology*, 74(4), 940–955. <https://doi.org/10.1111/j.1365-2958.2009.06909.x>
- Saathoff, J.-H., Käshammer, L., Lammens, K., Byrne, R. T., & Hopfner, K.-P. (2018). The bacterial Mre11–Rad50 homolog SbcCD cleaves opposing strands of DNA by two

- chemically distinct nuclease reactions. *Nucleic Acids Research*, 46(21), 11303–11314. <https://doi.org/10.1093/nar/gky878>
- Sandell, L. (1993). Loss of a yeast telomere: Arrest, recovery, and chromosome loss. *Cell*, 75(4), 729–739. [https://doi.org/10.1016/0092-8674\(93\)90493-A](https://doi.org/10.1016/0092-8674(93)90493-A)
- Sartori, A. A., Lukas, C., Coates, J., Mistrik, M., Fu, S., Bartek, J., Baer, R., Lukas, J., & Jackson, S. P. (2007). Human CtIP promotes DNA end resection. *Nature*, 450(7169), 509–514. <https://doi.org/10.1038/nature06337>
- Sazer, S., & Sherwood, S. W. (1990). Mitochondrial growth and DNA synthesis occur in the absence of nuclear DNA replication in fission yeast. *Journal of Cell Science*, 97(3), 509–516. <https://doi.org/10.1242/jcs.97.3.509>
- Schmidt, K. H., & Kolodner, R. D. (2004). Requirement of Rrm3 Helicase for Repair of Spontaneous DNA Lesions in Cells Lacking Srs2 or Sgs1 Helicase. *Molecular and Cellular Biology*, 24(8), 3213–3226. <https://doi.org/10.1128/MCB.24.8.3213-3226.2004>
- Scholes, D. T., Banerjee, M., Bowen, B., & Curcio, M. J. (2001). Multiple Regulators of Ty1 Transposition in *Saccharomyces cerevisiae* Have Conserved Roles in Genome Maintenance. *Genetics*, 159(4), 1449–1465. <https://doi.org/10.1093/genetics/159.4.1449>
- Schulz, V. P., & Zakian, V. A. (1994). The *saccharomyces PIF1* DNA helicase inhibits telomere elongation and de novo telomere formation. *Cell*, 76(1), 145–155. [https://doi.org/10.1016/0092-8674\(94\)90179-1](https://doi.org/10.1016/0092-8674(94)90179-1)
- Seel, A., Padovani, F., Mayer, M., Finster, A., Bureik, D., Thoma, F., Osman, C., Klecker, T., & Schmoller, K. M. (2023). Regulation with cell size ensures mitochondrial DNA homeostasis during cell growth. *Nature Structural & Molecular Biology*, 30(10), 1549–1560. <https://doi.org/10.1038/s41594-023-01091-8>
- Sen, D., & Gilbert, W. (1988). Formation of Parallel Four-Stranded Complexes by Guanine-Rich Motifs in DNA and Its Implications for Meiosis. *Nature*, 334(6180), 364–366.
- Senecoff, J. F., Bruckner, R. C., & Cox, M. M. (1985). The FLP recombinase of the yeast 2-micron plasmid: Characterization of its recombination site. *Proceedings of the*

- National Academy of Sciences of the United States of America*, 82(21), 7270–7274. <https://doi.org/10.1073/pnas.82.21.7270>
- Sharples, G. J., & Leach, D. R. F. (1995). Structural and functional similarities between the SbcCD proteins of *Escherichia coli* and the RAD50 and MRE11 (RAD32) recombination and repair proteins of yeast. *Molecular Microbiology*, 17(6), 1215–1217. https://doi.org/10.1111/j.1365-2958.1995.mmi_17061215_1.x
- Shibata, A., Moiani, D., Arvai, A. S., Perry, J., Harding, S. M., Genois, M.-M., Maity, R., van Rossum-Fikkert, S., Kertokallio, A., Romoli, F., Ismail, A., Ismalaj, E., Petricci, E., Neale, M. J., Bristow, R. G., Masson, J.-Y., Wyman, C., Jeggo, P. A., & Tainer, J. A. (2014). DNA Double-Strand Break Repair Pathway Choice Is Directed by Distinct MRE11 Nuclease Activities. *Molecular Cell*, 53(1), 7–18. <https://doi.org/10.1016/j.molcel.2013.11.003>
- Shibata, T., DasGupta, C., Cunningham, R. P., Williams, J. G., Osber, L., & Radding, C. M. (1981). Homologous pairing in genetic recombination. The pairing reaction catalyzed by *Escherichia coli* recA protein. *Journal of Biological Chemistry*, 256(14), 7565–7572. [https://doi.org/10.1016/S0021-9258\(19\)68999-4](https://doi.org/10.1016/S0021-9258(19)68999-4)
- Shinohara, A., Ogawa, H., Matsuda, Y., Ushio, N., Ikeo, K., & Ogawa, T. (1993). Cloning of human, mouse and fission yeast recombination genes homologous to RAD51 and recA. *Nature Genetics*, 4(3), 239–243. <https://doi.org/10.1038/ng0793-239>
- Shinohara, A., Ogawa, H., & Ogawa, T. (1992a). Rad51 protein involved in repair and recombination in *S. cerevisiae* is a RecA-like protein. *Cell Press*, 69(3), 457–470.
- Shinohara, A., Ogawa, H., & Ogawa, T. (1992b). Rad51 protein involved in repair and recombination in *S. cerevisiae* is a RecA-like protein. *Cell*, 69(3), 457–470. [https://doi.org/10.1016/0092-8674\(92\)90447-K](https://doi.org/10.1016/0092-8674(92)90447-K)
- Shinohara, A., & Ogawa, T. (1998). Stimulation by Rad52 of yeast Rad51-mediated recombination. *Nature*, 391(6665), 404–407. <https://doi.org/10.1038/34943>
- Sonoda, E., Sasaki, M. S., Buerstedde, J., Bezzubova, O., Shinohara, A., Ogawa, H., Takata, M., Yamaguchi-Iwai, Y., & Takeda, S. (1998). Rad51-deficient vertebrate cells accumulate chromosomal breaks prior to cell death. *The EMBO Journal*, 17(2), 598–608. <https://doi.org/10.1093/emboj/17.2.598>

- Spies, M., & Kowalczykowski, S. C. (2014). Homologous Recombination by the RecBCD and RecF Pathways. *The Bacterial Chromosome*, 389–403. <https://doi.org/10.1128/9781555817640.ch21>
- Stillman, B. (1996). Cell cycle control of DNA replication. *Science*, 274(5293).
- Story, R. M., Weber, I. T., & Steitz, T. A. (1992). The Structure of the E. coli recA Protein Monomer and Polymer. *Nature*, 355(6358), 318–325.
- Sundquist, W. I., & Klug, A. (1989). Telomeric DNA Dimerizes by Formation of Guanine Tetrads between Hairpin Loops. *Nature*, 342(6251), 825–829. <https://doi.org/10.1038/342825a0>
- Sung, P. (1997). Function of Yeast Rad52 Protein as a Mediator between Replication Protein A and the Rad51 Recombinase *. *Journal of Biological Chemistry*, 272(45), 28194–28197. <https://doi.org/10.1074/jbc.272.45.28194>
- Sung, P. J. (1994). *Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein*. (5176).
- Sung, P., & Robberson, D. L. (1995). DNA strand exchange mediated by a RAD51-ssDNA nucleoprotein filament with polarity opposite to that of RecA. *Cell*, 82(3), 453–461. [https://doi.org/10.1016/0092-8674\(95\)90434-4](https://doi.org/10.1016/0092-8674(95)90434-4)
- Syed, S., Desler, C., Rasmussen, L. J., & Schmidt, K. H. (2016). A Novel Rrm3 Function in Restricting DNA Replication via an Orc5-Binding Domain Is Genetically Separable from Rrm3 Function as an ATPase/Helicase in Facilitating Fork Progression. *PLOS Genetics*, 12(12), e1006451. <https://doi.org/10.1371/journal.pgen.1006451>
- Symington, L. S., & Gautier, J. (2011). Double-Strand Break End Resection and Repair Pathway Choice. *Annual Review of Genetics*, 45(Volume 45, 2011), 247–271. <https://doi.org/10.1146/annurev-genet-110410-132435>
- Takata, M., Sasaki, M. S., Sonoda, E., Fukushima, T., Morrison, C., Albala, J. S., Swagemakers, S. M. A., Kanaar, R., Thompson, L. H., & Takeda, S. (2000). The Rad51 Paralog Rad51B Promotes Homologous Recombinational Repair. *Molecular and Cellular Biology*, 20(17), 6476–6482. <https://doi.org/10.1128/mcb.20.17.6476-6482.2000>

- Takata, M., Sasaki, M. S., Tachiiri, S., Fukushima, T., Sonoda, E., Schild, D., Thompson, L. H., & Takeda, S. (2001). Chromosome Instability and Defective Recombinational Repair in Knockout Mutants of the Five Rad51 Paralogs. *Molecular and Cellular Biology*, 21(8), 2858–2866. <https://doi.org/10.1128/MCB.21.8.2858-2866.2001>
- Tang, M., Shen, X., Frank, E. G., O'Donnell, M., Woodgate, R., & Goodman, M. F. (1999). UmuD'2C is an error-prone DNA polymerase, Escherichia coli pol V. *Proceedings of the National Academy of Sciences*, 96(16), 8919–8924. <https://doi.org/10.1073/pnas.96.16.8919>
- Taylor, A. F., Amundsen, S. K., & Smith, G. R. (2016). Unexpected DNA context-dependence identifies a new determinant of Chi recombination hotspots. *Nucleic Acids Research*, 44(17), 8216–8228. <https://doi.org/10.1093/nar/gkw541>
- Taylor, A. F., Schultz, D. W., Ponticelli, A. S., & Smith, G. R. (1985). RecBC enzyme nicking at chi sites during DNA unwinding: Location and orientation-dependence of the cutting. *Cell*, 41(1), 153–163. [https://doi.org/10.1016/0092-8674\(85\)90070-4](https://doi.org/10.1016/0092-8674(85)90070-4)
- Taylor, A. F., & Smith, G. R. (1985). Substrate specificity of the DNA unwinding activity of the RecBC enzyme of Escherichia coli. *Journal of Molecular Biology*, 185(2), 431–443. [https://doi.org/10.1016/0022-2836\(85\)90414-0](https://doi.org/10.1016/0022-2836(85)90414-0)
- Taylor, A. F., & Smith, G. R. (2003). RecBCD enzyme is a DNA helicase with fast and slow motors of opposite polarity. *Nature*, 423(6942), 889–893. <https://doi.org/10.1038/nature01674>
- Thaler, D. S., Sampson, E., Siddiqi, I., Rosenberg, S. M., Thomason, L. C., Stahl, F. W., & Stahl, M. M. (1989). Recombination of bacteriophage λ in recD mutants of Escherichia coli. *Genome*, 31(1), 53–67. <https://doi.org/10.1139/g89-013>
- Torres, J. Z., Bessler, J. B., & Zakian, V. A. (2004). Local chromatin structure at the ribosomal DNA causes replication fork pausing and genome instability in the absence of the S. cerevisiae DNA helicase Rrm3p. *Genes & Development*, 18(5), 498–503. <https://doi.org/10.1101/gad.1154704>
- Torres, J. Z., Schnakenberg, S. L., & Zakian, V. A. (2004). Saccharomyces cerevisiae Rrm3p DNA Helicase Promotes Genome Integrity by Preventing Replication Fork Stalling: Viability of rrm3 Cells Requires the Intra-S-Phase Checkpoint and Fork

- Restart Activities. *Molecular and Cellular Biology*, 24(8), 3198–3212. <https://doi.org/10.1128/MCB.24.8.3198-3212.2004>
- Tsakiri, K. D., Cronkhite, J. T., Kuan, P. J., Xing, C., Raghu, G., Weissler, J. C., Rosenblatt, R. L., Shay, J. W., & Garcia, C. K. (2007). Adult-onset pulmonary fibrosis caused by mutations in telomerase. *Proceedings of the National Academy of Sciences of the United States of America*, 104(18), 7552–7557. <https://doi.org/10.1073/pnas.0701009104>
- Tsubouchi, H., & Ogawa, H. (1998). A Novel mre11 Mutation Impairs Processing of Double-Strand Breaks of DNA during Both Mitosis and Meiosis. *Molecular and Cellular Biology*, 18(1), 260–268. <https://doi.org/10.1128/mcb.18.1.260>
- Van Dyck, E., Foury, F., Stillman, B., & Brill, S. J. (1992). A single-stranded DNA binding protein required for mitochondrial DNA replication in *S. cerevisiae* is homologous to *E. coli* SSB. *The EMBO Journal*, 11(9), 3421–3430. <https://doi.org/10.1002/j.1460-2075.1992.tb05421.x>
- van Gent, D. C., Hoeijmakers, J. H. J., & Kanaar, R. (2001). Chromosomal stability and the DNA double-stranded break connection. *Nature Reviews Genetics*, 2(3), 196–206. <https://doi.org/10.1038/35056049>
- Varon, M., Dovrat, D., Heuzé, J., Tsirkas, I., Singh, S. P., Pasero, P., Galletto, R., & Aharoni, A. (2024). Rrm3 and Pif1 division of labor during replication through leading and lagging strand G-quadruplex. *Nucleic Acids Research*, 52(4), 1753–1762. <https://doi.org/10.1093/nar/gkad1205>
- Veit, B. E., & Fangman, W. L. (1988). Copy number and partition of the *Saccharomyces cerevisiae* 2 micron plasmid controlled by transcription regulators. *Molecular and Cellular Biology*, 8(11), 4949–4957. <https://doi.org/10.1128/mcb.8.11.4949>
- Venter, J. C., Adams, M. D., Myers, E. W., Li, P. W., Mural, R. J., Sutton, G. G., Smith, H. O., Yandell, M., Evans, C. A., Holt, R. A., Gocayne, J. D., Amanatides, P., Ballew, R. M., Huson, D. H., Wortman, J. R., Zhang, Q., Kodira, C. D., Zheng, X. H., Chen, L., ... Zhu, X. (2001). The Sequence of the Human Genome. *Science*, 291(5507), 1304–1351. <https://doi.org/10.1126/science.1058040>

- Volkert, F. C., & Broach, J. R. (1986). Site-specific recombination promotes plasmid amplification in yeast. *Cell*, 46(4), 541–550. [https://doi.org/10.1016/0092-8674\(86\)90879-2](https://doi.org/10.1016/0092-8674(86)90879-2)
- Wach, A., Brachat, A., Pöhlmann, R., & Philippsen, P. (1994). New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. *Yeast*, 10(13), 1793–1808. <https://doi.org/10.1002/yea.320101310>
- Wang, A. T., Kim, T., Wagner, J. E., Conti, B. A., Lach, F. P., Huang, A. L., Molina, H., Sanborn, E. M., Zierhut, H., Cornes, B. K., Abhyankar, A., Sougnez, C., Gabriel, S. B., Auerbach, A. D., Kowalczykowski, S. C., & Smogorzewska, A. (2015). A dominant mutation in human RAD51 reveals its function in DNA interstrand crosslink repair independent of homologous recombination. *Molecular Cell*, 59(3), 478–490. <https://doi.org/10.1016/j.molcel.2015.07.009>
- Wellinger, R. J., & Zakian, V. A. (2012). Everything You Ever Wanted to Know About *Saccharomyces cerevisiae* Telomeres: Beginning to End. *Genetics*, 191(4), 1073–1105. <https://doi.org/10.1534/genetics.111.137851>
- Wendel, B. M., Cole, J. M., Courcelle, C. T., & Courcelle, J. (2017). SbcC-SbcD and ExoI process convergent forks to complete chromosome replication. *Proceedings of the National Academy of Sciences of the United States of America*, 115(2), 349–354. <https://doi.org/10.1073/pnas.1715960114>
- Wendel, B. M., Courcelle, C. T., & Courcelle, J. (2014a). Completion of DNA replication in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America*, 111(46), 16454–16459. <https://doi.org/10.1073/pnas.1415025111>
- Wendel, B. M., Courcelle, C. T., & Courcelle, J. (2014b). Completion of DNA replication in *Escherichia coli*. *Proceedings of the National Academy of Sciences*, 111(46), 16454–16459. <https://doi.org/10.1073/pnas.1415025111>
- West, S. C., Cassuto, E., Mursalim, J., & Howard-Flanders, P. (1980). Recognition of duplex DNA containing single-stranded regions by recA protein. *Proceedings of the National Academy of Sciences*, 77(5), 2569–2573. <https://doi.org/10.1073/pnas.77.5.2569>

- Whitby, M. C., Vincent, S. D., & Lloyd, R. G. (1994). Branch migration of Holliday junctions: Identification of RecG protein as a junction specific DNA helicase. *The EMBO Journal*, 13(21), 5220–5228. <https://doi.org/10.1002/j.1460-2075.1994.tb06853.x>
- Willetts, N. S., & Clark, A. J. (1969). Characteristics of Some Multiply Recombination-Deficient Strains of *Escherichia coli*. *Journal of Bacteriology*, 100(1), 231–239. <https://doi.org/10.1128/jb.100.1.231-239.1969>
- Winzeler, E. A., Shoemaker, D. D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Boeke, J. D., Bussey, H., Chu, A. M., Connelly, C., Davis, K., Dietrich, F., Dow, S. W., El Bakkoury, M., Foury, F., Friend, S. H., Gentalen, E., ... Davis, R. W. (1999). Functional Characterization of the *S. cerevisiae* Genome by Gene Deletion and Parallel Analysis. *Science*, 285(5429), 901–906. <https://doi.org/10.1126/science.285.5429.901>
- Xiao, Y., & Weaver, D. T. (1997). Conditional gene targeted deletion by Cre recombinase demonstrates the requirement for the double-strand break repair Mre11 protein in murine embryonic stem cells. *Nucleic Acids Research*, 25(15), 2985–2991. <https://doi.org/10.1093/nar/25.15.2985>
- Yabuki, N., Terashima, H., & Kitada, K. (2002). Mapping of early firing origins on a replication profile of budding yeast. *Genes to Cells*, 7(8), 781–789. <https://doi.org/10.1046/j.1365-2443.2002.00559.x>
- Yamashita, M., Hori, Y., Shinomiya, T., Obuse, C., Tsurimoto, T., Yoshikawa, H., & Shirahige, K. (1997). The efficiency and timing of initiation of replication of multiple replicons of *Saccharomyces cerevisiae* chromosome VI. *Genes to Cells*, 2(11), 655–665. <https://doi.org/10.1046/j.1365-2443.1997.1530351.x>
- Yu, M., Souaya, J., & Julin, D. A. (1998). Identification of the nuclease active site in the multifunctional RecBCD enzyme by creation of a chimeric enzyme¹. *Journal of Molecular Biology*, 283(4), 797–808. <https://doi.org/10.1006/jmbi.1998.2127>
- Zakian, V. A., Brewer, B. J., & Fangman, W. L. (1979). Replication of each copy of the yeast 2 micron DNA plasmid occurs during the S phase. *Cell*, 17(4), 923–934. [https://doi.org/10.1016/0092-8674\(79\)90332-5](https://doi.org/10.1016/0092-8674(79)90332-5)

- Zhang, D.-H. (2006). The human Pif1 helicase, a potential *Escherichia coli* RecD homologue, inhibits telomerase activity. *Nucleic Acids Research*, 34(5), 1393–1404. <https://doi.org/10.1093/nar/gkl029>
- Zhao, P. A., Sasaki, T., & Gilbert, D. M. (2020). High-resolution Repli-Seq defines the temporal choreography of initiation, elongation and termination of replication in mammalian cells. *Genome Biology*, 21(1), 76. <https://doi.org/10.1186/s13059-020-01983-8>
- Zhu, J., Petersen, S., Tessarollo, L., & Nussenzweig, A. (2001). Targeted disruption of the Nijmegen breakage syndrome gene *NBS1* leads to early embryonic lethality in mice. *Current Biology*, 11(2), 105–109. [https://doi.org/10.1016/S0960-9822\(01\)00019-7](https://doi.org/10.1016/S0960-9822(01)00019-7)

Chapter 2

Materials & Methods

Yeast Strains. All strains used in this work are derived from the BY4741 strain, a *MATa his3 leu2 met15 ura3* descendent of the widely used S288C laboratory strain (Brachmann et al., 1998). Unless otherwise noted, deletion strains were constructed as part of the Saccharomyces Genome Deletion Project (Wach et al., 1994; Winzeler et al., 1999).

Table 1. *S. cerevisiae* strains used in this study

Strain	Genotype	Source or Construction
BY4741	<i>MATa his3 leu2 met15 ura3</i>	R. Rothstein (Brachmann et al., 1998).
Strains isogenic to BY4741		
$\Delta rrm3$	$\Delta rrm3::kanMX$	(Wach et al., 1994; Winzeler et al., 1999).
$\Delta rad50$	$\Delta rad50::kanMX$	(Wach et al., 1994; Winzeler et al., 1999).
CL3551	$\Delta mre11::hygR$	Courcelle lab (unpublished) PCR amplification of <i>hygR</i> cassette with <i>mre11</i> flanking homology using primers mre11- hygF5'AATTGACGCAAGTTGTACCTG CTCAGATCCGATAAACTCGACTCAG ATTGTACTGAGAGTGcmre11-

		hygR5'CTTGGTTATAAATAGGATATAAT ATAATATAGGGATCAAGTACAACCTTA CGCATCTGTGCGG, followed by transformation into BY4741 selecting for hygromycin resistance.
CL3555 <i>Δmre11</i> <i>Δsae2</i>	<i>Δsae2::kanMX</i> <i>mre11Δ::hygR</i>	Courcelle lab (unpublished) PCR amplification of <i>hygR</i> cassette with <i>mre11</i> flanking homology using primers mre11- hygF5'AATTGACGCAAGTTGTACCTG CTCAGATCCGATAAACTCGACTCAG ATTGTACTGAGAGTGCmre11- hygR5'CTTGGTTATAAATAGGATATAAT ATAATATAGGGATCAAGTACAACCTTA CGCATCTGTGCGG, followed by transformation into BY4741 <i>Δsae2::kanMX</i> () selecting for hygromycin resistance.

Growth curve analysis. Fresh overnight cultures were 10-fold serially diluted in rich growth medium (YPD). Aliquots of 100 μ L from each diluted culture were dispensed into a sterile 96-well microtiter plate in duplicate, and wells containing media alone were included as blanks for background correction. Cultures were incubated at 30°C with continuous shaking in a BIO-Whittaker ELx808 plate reader, and absorbance at 600 nm was

measured at 40-minute intervals over a 24-hour period. The number of viable colonies per milliliter in each overnight culture was determined at the start of each experiment to correct for differences in viability when plotting Absorbance over time.

Single-Cell Viability Assay. Fresh overnight yeast cultures were diluted 1:4 in deionized water to reduce cell density. A small volume of the diluted culture was applied onto one side of a YPD agar plate to generate a sparse distribution of cells. Individual cells were isolated using a tetrad dissection manual micromanipulator attachment on a Nikon Ci Brightfield microscope and transferred to defined coordinates on the agar surface to allow for accurate tracking. Plates were incubated at 30°C for 90–120 minutes (depending on strain) to allow each isolated cell to undergo a single round of division. Following incubation, cells that had doubled were carefully separated and repositioned in a matrix on the plate using the micromanipulator. Each cell was assigned a unique identifier to enable subsequent tracking of growth and viability (Lee et al., 1998).

Flow cytometry data analysis and cell cycle phase sorting. *S. cerevisiae* strains were grown overnight in YPD medium at 30 °C in a shaking water bath. The following day, cultures were diluted 1:50 into fresh YPD and grown for approximately 6 hours to mid-log phase ($OD_{600} \approx 0.4\text{--}0.8$) to ensure actively dividing cells. Cells were harvested by centrifugation at 14,000 rpm for 3 minutes and washed once with deionized water. For DNA content analysis, cell pellets were resuspended in 70% ethanol and fixed at 4°C for at least 1 hour (or overnight). Fixed cells were pelleted, resuspended in 50 mM sodium citrate (pH 7.0) supplemented with 0.3 mg/ml RNase A, and incubated at 37°C for 2 hours to remove RNA. Samples were then treated with 0.5 mg/ml Proteinase K and incubated

for an additional 2 hours at 37°C. Following treatment, cells were stored at 4°C for at least 30 minutes prior to staining.

Cells were stained with the DNA-binding dye PicoGreen and incubated in the dark at room temperature for 30–45 minutes. Stained cells were then diluted with deionized water to give an event rate of approximately 2000 events/sec before being analyzed by flow cytometry, using the BD FACSCalibur software. Forward scatter (FSC) and fluorescence intensity were used to gate cells based on size and DNA content. A minimum of 10,000 events were collected per sample for analysis. Data were analyzed using Floreada.io flow cytometry software, and cell cycle (%) distributions were determined from DNA content and cell size plots.

For cell cycle–based sorting by flow cytometry, PicoGreen-stained cells were analyzed and sorted using the FACSsort feature on the BD FACSCalibur flow cytometer. Cells were sorted based on DNA content, using fluorescence intensity to distinguish cell cycle phases. Prior to sorting, populations were gated using forward scatter (FSC) and side scatter (SSC) to exclude debris and non-cellular events using BD CellQuest Software. Gates for G1, S, and G2/M phases were established from DNA content of asynchronous cultures. Cells were then sorted into sterile collection tubes. Approximately 5×10^6 cells were collected for each cell cycle phase and stored at -80°C.

Genomic DNA purification for sequencing. Following sorting, cells were resuspended in 0.15-ml SCE, pH 7.0 (1M sorbitol, 10 mM sodium citrate, 1 mM EDTA, pH 7.0) supplemented with 2 mg/ml zymolyase and incubated at 37°C for 1 hour. Sarkosyl to a final concentration of 1% was then added to the zymolyase-treated cells and incubation

at 37 °C was extended for an additional hour. Samples were extracted with four volumes of 1:1 phenol:chloroform and once with two volumes of chloroform before they were dialyzed against 200-ml TE (2 mM Tris, 1 mM EDTA, pH 8.0) for 30 minutes on floating 47-mm MF Millipore 0.025 μ m pore disks.

Purified genomic DNA was separated and analyzed on a 0.5% alkali agarose gel in 30 mM NaOH, 1 mM EDTA and electrophoresed at 1 V/cm for 15 hours. DNA was visualized by ethidium bromide to determine yields and quality prior to downstream library preparation and sequencing.

Copy-number analysis. Genomic DNA was sequenced using Illumina NextSeq2000 (Illumina) using single-end, 50-bp, bar-coded reads according to the manufacturer's instructions. The original Illumina sequences reads for all subsequent strains were then aligned and assembled using Bowtie 1.0.0 with the S288C parent as reference. The aligned reads were then analyzed for nucleotide frequencies at each position and the copy number of sequences per kilobase was determined using Perl scripts. Relative copy number values for each strain were normalized to values obtained for wild-type G1 phase sorted cells to correct for any differences in genomic DNA purification and read depth, and then plotted against their location on the genome to generate replication profiles for each strain and cell cycle phase (Wendel et al., 2014a, 2017).

Coordinate conversion for genome-wide feature mapping. To generate a genome-wide profile of chromosomal features, including the 2 μ plasmid DNA, mitochondrial DNA, telomeres, centromeres, and replication origins, chromosomal coordinates were converted into a kilobase coordinate system. Feature coordinates were obtained in base

pairs relative to individual chromosomes from the reference genome strain S288C. All coordinates were converted to kb by dividing by 1,000bp. Beginning with Chromosome I and continuing sequentially through Chromosome XVI, the starting position of each chromosome within the genome-wide map was defined based on the cumulative length of the chromosome(s) before. In this way, each chromosome was represented as a continuous, non-overlapping interval along the entire genome. To determine the specific genomic position of each feature within a chromosome, the starting coordinate of the chromosome in kb was added to that feature's chromosomal kb position. The resulting values represent the positions of each feature (telomere, centromere, and origins) on a continuous genome-wide scale. This approach was applied to all chromosomes and all strains.

References

- Baker Brachmann, C., Davies, A., Cost, G. J., Caputo, E., Li, J., Hieter, P., & Boeke, J. D. (1998). Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: A useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast*, 14(2), 115–132. [https://doi.org/10.1002/\(SICI\)1097-0061\(19980130\)14:2%3C115::AID-YEA204%3E3.0.CO;2-2](https://doi.org/10.1002/(SICI)1097-0061(19980130)14:2%3C115::AID-YEA204%3E3.0.CO;2-2)
- Lee, S. E., Moore, J. K., Holmes, A., Umez, K., Kolodner, R. D., & Haber, J. E. (1998). *Saccharomyces* Ku70, Mre11/Rad50, and RPA Proteins Regulate Adaptation to G2/M Arrest after DNA Damage. *Cell*, 94(3), 399–409. [https://doi.org/10.1016/S0092-8674\(00\)81482-8](https://doi.org/10.1016/S0092-8674(00)81482-8)
- Wach, A., Brachat, A., Pöhlmann, R., & Philippsen, P. (1994). New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. *Yeast*, 10(13), 1793–1808. <https://doi.org/10.1002/yea.320101310>
- Wendel, B. M., Cole, J. M., Courcelle, C. T., & Courcelle, J. (2017). SbcC-SbcD and ExoI process convergent forks to complete chromosome replication. *Proceedings of the National Academy of Sciences of the United States of America*, 115(2), 349–354. <https://doi.org/10.1073/pnas.1715960114>
- Wendel, B. M., Courcelle, C. T., & Courcelle, J. (2014). Completion of DNA replication in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America*, 111(46), 16454–16459. <https://doi.org/10.1073/pnas.1415025111>
- Winzler, E. A., Shoemaker, D. D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Boeke, J. D., Bussey, H., Chu, A. M., Connelly, C., Davis, K., Dietrich, F., Dow, S. W., El Bakkoury, M., Foury, F., Friend, S. H., Gentlen, E., ... Davis, R. W. (1999). Functional Characterization of the *S. cerevisiae* Genome by Gene Deletion and Parallel Analysis. *Science*, 285(5429), 901–906. <https://doi.org/10.1126/science.285.5429.901>

Chapter 3

Double stranded breaks alone, fail to account for growth defects in Δ mre11 mutants

Introduction

While double strand breaks are frequently associated with external stressors—such as exposure to chemical mutagens, or ionizing radiation—they can also arise spontaneously as a natural consequence of normal cellular activities in eukaryotic cells. These breaks originate from various intracellular mechanisms, including DNA replication stress, transcription, recombination, and metabolic byproducts (Borde & de Massy, 2013; Feng et al., 2011; N. Kim & Jinks-Robertson, 2012; van Gent et al., 2001). If left unrepaired, double strand breaks can cause chromosome loss, fragmentation, and cell death (Kaye et al., 2004). Replication-associated double strand breaks are postulated to arise when replication forks encounter obstacles such as single-strand breaks in the template DNA, causing fork collapse, generating double strand breaks (Fig 3.2A) (Kuzminov, 1999, 2001). Since these breaks arise during DNA replication—primarily during S and extending into G2—they are primarily repaired through homologous recombination, due to the proximity of the sister chromatid as a template for high-fidelity repair (Jazayeri et al., 2006; Reviewed in Symington & Gautier, 2011).

Similar to its RecA bacterial counterpart, Rad51 is thought to be absolutely essential for the recombinational repair of strand breaks and in eukaryotes due to its role in homology search and strand exchange during repair (Cox & Lehman, 1981; Howard-Flanders & Theriot, 1966; Shinohara et al., 1992a, 1992a). As described in the previous chapter, *E. coli* RecBC and several other proteins were classically characterized as

functioning with RecA in the recombinational repair of double strand breaks (Churchill et al., 1999; Cromie & Leach, 2001; Kuzminov & Stahl, 1999). One piece of evidence suggesting that these proteins have functions outside of double strand break repair (and which helped identify their role in completing chromosome replication) was that *recBC* mutants grew poorly and had reduced viability even in the absence of DNA damage (Capaldo-Kimball & Barbour, 1971; Wendel et al., 2014a). To explain the low viability of these strains, it was speculated that cells must frequently encounter spontaneous strand breaks during the normal replication cycle. Without recombination to repair these breaks, the daughter cell would not inherit an intact copy of the genome, leading to lethality (FIG. 1.2A-C) (Wendel et al., 2014a). However, the argument against this model for RecBC and other classically characterized recombination proteins was that *recA* mutants, which are completely defective in all recombinational repair, grew well and maintained viability at levels approaching those of wild type cultures, arguing that the impaired viability of *recBC* mutants could not be accounted for by a lack of recombination alone (FIG. 1.2C) (Courcelle et al., 2015b; Wendel et al., 2014a).

Yeast mutants contain several enzymes that share structural and functional homology to *E. coli* completion enzymes that have also been classically characterized primarily for their role in the recombinational repair of strand breaks (Connelly & Leach, 1996b; Hopfner et al., 2001, 2002; Saathoff et al., 2018; Sharples & Leach, 1995). The eukaryotic Mre11–Rad50–Nbs1 (MRN) complex in humans and the Mre11–Rad50–Xrs2 (MRX) complex is structurally homologous to the SbcC–SbcD enzymes of *E. coli*, which are thought to initiate the incision of the converged replication fork substrate (Connelly & Leach, 1996b; Eykelenboom et al., 2008; Hopfner et al., 2001, 2002; Sharples & Leach,

1995). In yeast, it is often characterized as being functionally homologous to RecBCD, in that it is thought to process DNA double strand break ends to allow for Rad51-dependent strand invasion, allowing double strand breaks to be repaired (Alani et al., 1990; Bressan et al., 1999; Haber, 1998; Lobachev et al., 2002; Paull & Gellert, 1998). Another protein Rrm3, has motifs that share homology to the RecD protein of *E. coli* (Bochman et al., 2011; Boulé & Zakian, 2006).

Thus, I hypothesized that, similar to their *E. coli* counterparts, these yeast proteins which have been classically characterized for their role in recombination and double strand break repair, may contribute to the completion of chromosome replication. To test this idea, I postulated that if the yeast mutants play an important role in completing replication, then they may similarly exhibit growth and viability problems that go beyond those that could be accounted for by the presence of spontaneous double strand breaks encountered during normal replication. I therefore examine the growth and viability of $\Delta rad51$ mutants, which is essential for all recombinational of double strand breaks, to assess the frequency that these lesions arise in growing cultures. I then measured the growth and viability of $\Delta mre11$, and $\Delta rrm3$ mutants to determine if the growth and viability of these mutants was similar or better than that of $\Delta rad51$, as would be expected if it functioned exclusively in this pathway to promote Rad51-mediated recombinational repair.

Results

Spontaneous double stand breaks, alone, cannot account for the reduced viability of $\Delta mre11$ mutants

To begin to assess whether the absence of these proteins impacts the growth rate cellular fitness in the absence of exogenous DNA damage, the growth of cultures was monitored over time as shown in Figure 3.1. To this end, 10^4 cells were inoculated in YPD growth media, and grown in a 96 well shaking incubator at 30°C, at 40 min intervals the growth of the culture was monitored by following the absorbance at 600nm. By this measure, *Δrad51* mutants grew at a rate that was comparable to that of the wild type culture. Assuming that Rad51 is required for the repair of double strand breaks encountered, the observation indicated that the frequency of spontaneous double strand breaks encountered during normal growth does not noticeably impair the growth of the culture and suggests that they may be fairly rare events. Similarly, *Δrrm3* mutants grew at a rate that was comparable to wild type. However, *Δmre11* mutants, displayed a pronounced growth defect (Fig 3.1). This indicates that loss of Mre11 impairs growth in a manner that is significantly greater than that *Δrad51*, a mutant which is defective in the repair of double strand breaks.

To further evaluate and determine the precise frequency with which spontaneous DNA damage is encountered during normal replication of the cell, I examined single-cell growth outcomes in various haploid yeast mutants lacking these key factors. Single-cell replication viability was assessed by using a micromanipulation-based dissection assay adapted from the Haber et al. (1998) (Fig 3.2A). Yeast cells from a growing overnight culture were streaked on YPD plates. Using a micromanipulator, single cells were then isolated and placed in defined positions across the plate and actively monitored for replication for 90 to 120 minutes (dependent on strain) at 30°C following one cell division. Once doubled, using the manual micromanipulator, mother and daughter were carefully

separated. Plates were then left on the benchtop for approximately 24 hours, and observations were then made. Replication outcomes were quantified as viable when both cells formed colonies of ≥ 10 cells, and not viable when only one cell formed a colony of ≥ 10 cells while the other produced fewer than 9 cells. (Fig. 3.2B). It is important to note that during micromanipulation, individual cells are moved using a glass needle, and accidental mechanical stress from this handling can damage or kill cells, contributing to reduced viability independent of genetic or environmental factors. Statistical analysis on the complete data set indicates that all strains were equally tolerable towards this handling stress (see *appendix Table 3.2 and 3.3* for complete data and analysis).

As shown in Figure 3.2C, when I carried out this analysis in wild type cells, I observed a high level of level of viability, with 95% of mother-daughter pairs successfully forming viable colonies. This outcome is consistent with what would be anticipated for a wild-type strain growing in the absence of exogenous DNA damage. The 5% of non-viable events likely reflects a combination of naturally occurring spontaneous damage due to other genetic or environmental factors or by mechanical stress caused during micromanipulation. Surprisingly, deletion of *rad51*, a core homologous recombination recombinase, did not significantly reduce viability relative to wild type (97% viable), indicating that the frequency which spontaneous double strand breaks arise during replication was below the limits of detection by this assay.

In contrast to wild type cells but consistent with the growth rate analysis, inactivation of Mre11 led to detectable impaired viability, with only 78% of cells forming viable pairs and 22% of cell divisions producing an inviable daughter (Fig. 3.2C). The loss of viability in that absence of Mre11 is greater than that observed in the absence of Rad51,

which is defective in the homologous repair of double strand breaks. Thus, the observation is consistent with the idea that Mre11 has a role that goes beyond that of Rad51-mediated double strand break repair. The results are similar to those observed for mutants lacking *recBC* in *E. coli* (Courcelle et al., 2015b; Wendel et al., 2014a).

Mre11, as part of the MRX(N) complex, functions upstream of Rad51, therefore, loss of Mre11 would disrupt multiple genomic processes, not just homologous recombination repair of double strand breaks (Bressan et al., 1999; Tsubouchi & Ogawa, 1998). If the growth impairment of $\Delta mre11$ cells were primarily due to spontaneous double strand breaks that were unable to be repaired via homologous recombination, one would expect $\Delta rad51$ cells—defective (or severely compromised) in homologous recombination-mediated repair—to exhibit comparable or greater viability loss (Shinohara et al., 1992a). However, $\Delta rad51$ mutants maintained viability comparable to wild type, suggesting that spontaneous double strand breaks alone are insufficient to account for the severe phenotype observed in $\Delta mre11$ cells.

When I examined $\Delta rrm3$ mutants, the viability was similar to that seen in wild type cells (96% viable), indicating that the loss of this protein does detectably affect growth or viability of these cultures. These observations are consistent with the bacterial homolog *recD*, in which mutants do not produce severe defects in cell viability during standard growth conditions (Amundsen et al., 1986; Chaudhury & Smith, 1984b; Wendel et al., 2014a). No phenotypes differing from wild type were noted for $\Delta rrm3$ mutants.

Taken together, the results indicate that the viability defect of $\Delta mre11$ cells cannot be attributed solely to the presence of spontaneous double strand breaks arising during DNA replication. These findings parallel studies in *E. coli*, where loss of RecBC produces

substantially more severe phenotypes than loss of RecD alone, supporting the idea that Mre11, like RecBC, performs essential functions beyond the processing of spontaneous double strand breaks during DNA replication.

***Δmre11* mutants display cell cycle progression defects-- particularly during late G2.**

The above results indicated that *Δmre11* mutants exhibited reduced viability during growth, similar to other completion proteins in *E. coli* such as *recBC*. If the impaired viability in *Δmre11* mutants were due to a role in completing replication, then one might predict that aberrancies might arise in cells during the late stages of S phase or G2 of their cell cycle. To examine this possibility, I quantified the cell-cycle distribution of growing cultures by flow cytometry. To this end, actively growing cultures were collected and stained with SytoxGreen to label the DNA. The cells were then sorted to assess both DNA content (FLH) and cell size (FSC). In Wild type populations, G1 cells have one copy of all chromosomes and therefore migrate as the prominent spot on the lower portion of cells. Cells in G2 have twice as much DNA following replication and have slightly increased in size, thus migrating as the prominent spot above and to the left of the G1 cells on the plot. Cells replicating in S phase will have somewhere between 1 and 2 times as much DNA as their G1 counterparts, appearing as a line connecting the G1 and G2 populations on the plot in Figure 3.3A. When I quantified the cells within these populations, I found that slightly less than half of the cells in the growing culture were in G2, whereas ~ 30% were in G1 while 20% were replicating (Fig 3.3E). When I examined cultures of *Δrad51*, I found that the distribution was statistically similar to wild type (Fig.

3.3 B and E). These observations suggest that the loss of Rad51 does not substantially disturb the overall cell-cycle progression.

In contrast, *Δmre11* cells exhibited a distinct redistribution across cell-cycle phases (Fig. 3.3C and E). Whereas the fraction of replicating S phase and G2 cells were similar to wild type, a new population was observed emerging from the G2 population that corresponded to 13% of the population of cells having a larger size and slightly more DNA content, which I termed G2XL (Fig 3.3C and E). The G2XL population appeared to represent cells that were at the end of their G2 phase, but failing to divide since there was a corresponding reduction in the presence of the newly divided, G1 population.

The observation is consistent with what might be expected to arise if Mre11 played a role in completing DNA replication or has functions at the late stages of DNA replication. The DNA content would have been expected to reach or nearly reach its doubling point, but if the chromosomes were unable to process the DNA intermediates arising where forks converge, cells might not be able to segregate their chromosome properly and arrest cells at the G2 M transition. That this population is not observed in the absence of Rad51 argues that the defect is not due a failure to process DNA breaks arising in the cell. Notably, the proportion of cells in the G2XL population was of approximately half the frequency which cell division produces one of two inviable daughter cells (Fig 3.3C and B), suggesting that this population may not yield viable daughter cells upon division. It is not clear at this stage if all *Δmre11* cells progress through this delayed G2XL phase, or if only a minority of the population enters it.

An abnormal distribution was also observed in replicating cultures in the absence of the RecD-like Rrm3. However, in this case, cells appeared to specifically have difficulty progressing through S-phase, as the population of G2 cells was statistically similar to wild type cultures. The accumulation of cells in S-phase resulted in a corresponding reduction of G1 cells, suggesting that the cells were initiating the replication cycle normally, transitioning from G1 to S, before cells become stalled or slowed throughout the replication phase of the chromosomes (Fig. 3.3D and E). This would argue against a specific role in completing replication, but is consistent with previous reports in yeast suggest that Rrm3 promotes replication fork progression through difficult-to-replicate regions, its absence likely leads to slower fork progression and increased stalling during S-phase, (Ivessa et al., 2000a; Makovets et al., 2004). Perhaps curiously, this slowing of S phase did not impair either cell viability (Figure 3.2C) or its growth rate (Figure 3.1).

Chapter 3 Figures

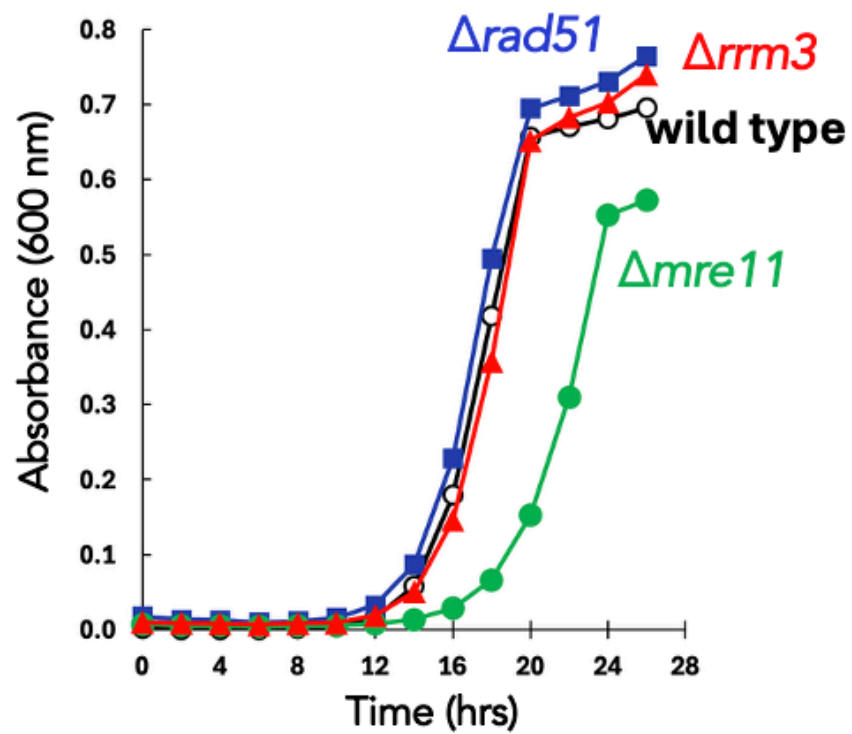


Figure 3.1 The growth rate of $\Delta mre11$, but not $\Delta rrm3$ or $\Delta rad51$, mutants is reduced relative to wild type cultures even in the absence of exogenous DNA damage. The absorbance at 600nm wild type, $\Delta mre11$, $\Delta rad51$, and $\Delta rrm3$ is plotted over time.

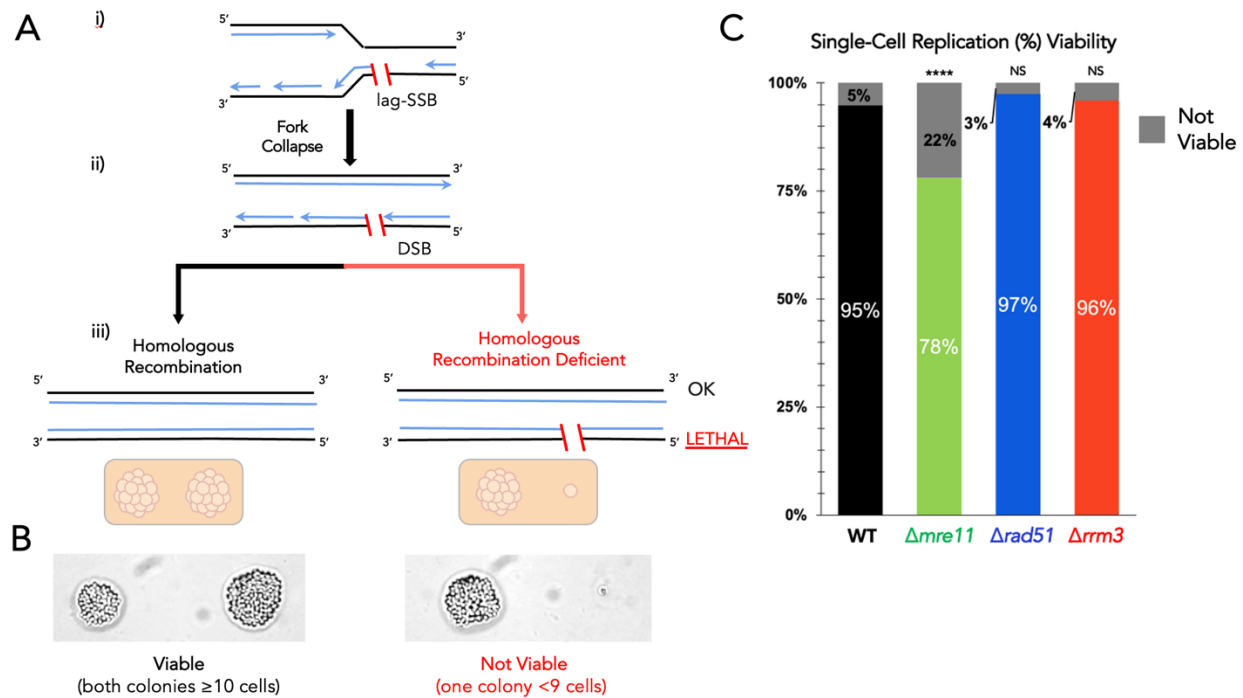


Figure 3.2 Spontaneous double strand breaks, alone, cannot account for the reduced viability of *Mre11* mutants. (A) Schematic for detecting the frequency spontaneous double strand breaks are created during replication. (i) Interruptions in the DNA template encountered during replication lead to the creation of spontaneous double strand breaks in cells. (ii) in the presence of homologous recombination, the double strand breaks can be repaired, using the sister chromatid and a template, and both daughter cells would survive to form colonies after separation. (iii) In mutants lacking enzymes required for homologous recombination, the double strand break remains unrepaired, leading to lethality in the daughter cell that inherits the broken or incomplete chromosome, resulting in at only one cell that can form a colony upon separation. (B) Representative microscopy images of colony growth following separation of divided cells. Single cells were grown YPD plates in and monitored until they divided (90-120 minutes). Following division, cells were placed at separate positions, and their viability, as assessed by their ability to form colonies after 24hrs was assessed. Representative examples of categorization: two viable cells (left) vs one viable and one not viable cell (right) following separation are shown. Separated cells were determined to be viable if ≥ 10 cells were present in the colony after 24 hrs. (C) $\Delta mre11$ mutants have elevated levels of lethality, relative to $\Delta rad51$. The fraction of cells which formed two viable colonies following division is plotted for wild type, $\Delta rad51$, $\Delta mre11$, and $\Delta rrm3$. Total number of successfully replicated and separated pairs represented as N= 230 (WT), 200

(*Δmre11*), 119 (*Δrad51*), 74 (*Δrrm3*). Statistical significance was assessed using a chi-square test comparing viable vs not viable (ns, not significant; **** $p < 0.0001$).

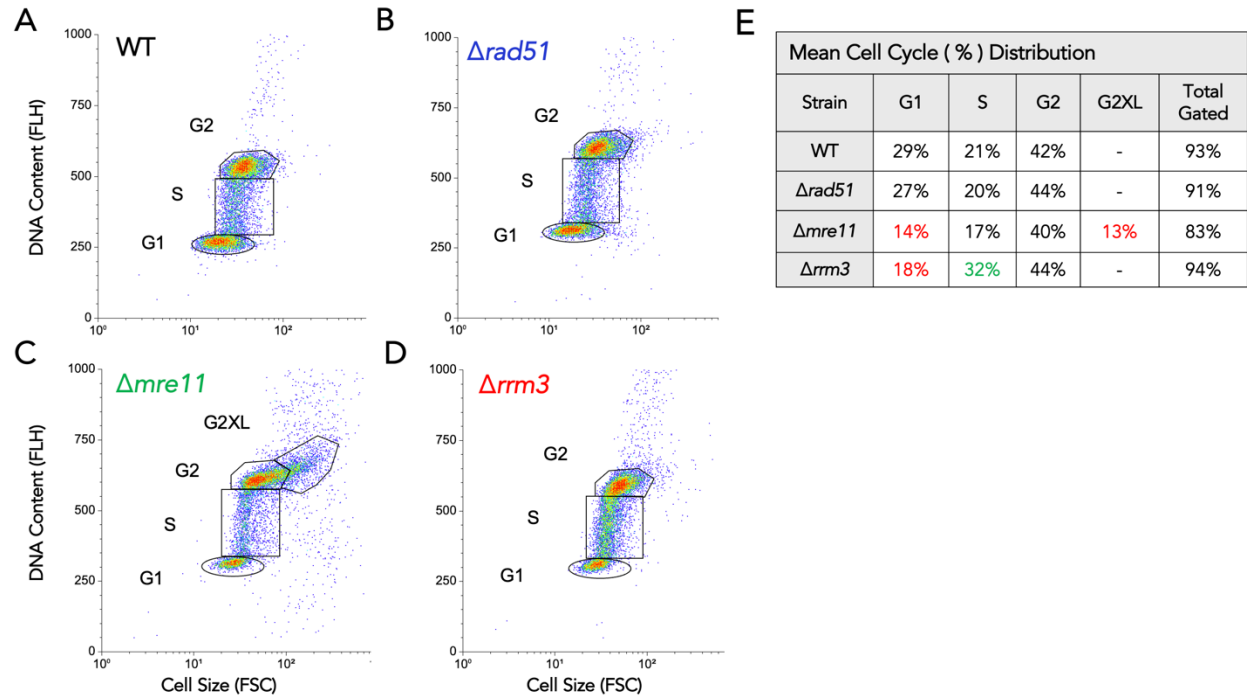


Figure 3.3 Mre11 mutants are impaired in their ability to progress out of G2. (A-D) The x-axis represents cell size measured by forward scatter (FSC) on a logarithmic scale, while the y-axis represents relative DNA content measured by fluorescence intensity (FLH) following DNA staining with SytoxGreen, quantified on a linear scale. Cells were fixed and stained during log-phase growth, and analyzed as asynchronous populations, where each point represents a single cell detected by flow cytometry, with color density indicating regions of higher cell frequency. Cell cycle phase gates (black gates marked G1, S, and G2) were defined using the WT population and applied to all mutant strains for direct comparison. Cells outside of the defined gates were not included in this quantification. put into results. (E) the average percentage of cells in each cell cycle gated phase for each strain calculated across different collection time points (n=6 for all strains). The “Total Gated” column indicates the mean percentage of events included within defined gates.

References

- Alani, E., Padmore, R., & Kleckner, N. (1990). Analysis of wild-type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. *Cell*, 61(3), 419–436. [https://doi.org/10.1016/0092-8674\(90\)90524-I](https://doi.org/10.1016/0092-8674(90)90524-I)
- Amundsen, S. K., Taylor, A. F., Chaudhury, A. M., & Smith, G. R. (1986). *recD*: The gene for an essential third subunit of exonuclease V. *Proceedings of the National Academy of Sciences of the United States of America*, 83(15), 5558–5562.
- Bochman, M. L., Judge, C. P., & Zakian, V. A. (2011). The Pif1 family in prokaryotes: What are our helicases doing in your bacteria? *Molecular Biology of the Cell*, 22(12), 1955–1959. <https://doi.org/10.1091/mbc.E11-01-0045>
- Borde, V., & de Massy, B. (2013). Programmed induction of DNA double strand breaks during meiosis: Setting up communication between DNA and the chromosome structure. *Current Opinion in Genetics & Development, Genome Architecture and Expression*, 23(2), 147–155. <https://doi.org/10.1016/j.gde.2012.12.002>
- Boulé, J.-B., & Zakian, V. A. (2006). Roles of Pif1-like helicases in the maintenance of genomic stability. *Nucleic Acids Research*, 34(15), 4147–4153. <https://doi.org/10.1093/nar/gkl561>
- Bressan, D. A., Baxter, B. K., & Petrini, J. H. J. (1999). The Mre11-Rad50-Xrs2 Protein Complex Facilitates Homologous Recombination-Based Double-Strand Break Repair in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology*, 19(11), 7681–7687. <https://doi.org/10.1128/MCB.19.11.7681>
- Capaldo, F. N., & Barbour, S. D. (1975). The Role of the *rec* Genes in the Viability of *Escherichia coli* K12. In P. C. Hanawalt & R. B. Setlow (Eds.), *Molecular Mechanisms for Repair of DNA: Part A* (pp. 405–418). Springer US. https://doi.org/10.1007/978-1-4684-2895-7_56
- Capaldo-Kimball, F., & Barbour, S. D. (1971). *Involvement of Recombination Genes in Growth and Viability of Escherichia coli K-12*. 106(1), 204–212. <https://doi.org/10.1128/jb.106.1.204-212.1971>
- Chaudhury, A. M., & Smith, G. R. (1984). *Escherichia coli recBC deletion mutants*. 160(2), 788–791. <https://doi.org/10.1128/jb.160.2.788-791.1984>

- Churchill, J. J., Anderson, D. G., & Kowalczykowski, S. C. (1999). The RecBC enzyme loads RecA protein onto ssDNA asymmetrically and independently of χ , resulting in constitutive recombination activation. *Genes & Development*, 13(7), 901–911. <https://doi.org/10.1101/gad.13.7.901>
- Connelly, J. C., & Leach, D. R. F. (1996). The sbcC and sbcD genes of Escherichia coli encode a nuclease involved in palindrome inviability and genetic recombination. *Genes to Cells*, 1(3), 285–291. <https://doi.org/10.1046/j.1365-2443.1996.23024.x>
- Courcelle, J., Wendel, B. M., Livingstone, D. D., & Courcelle, C. T. (2015). RecBCD is required to Complete Chromosomal Replication: Implications for Double-Strand Break Frequencies and Repair Mechanisms. *DNA Repair*, 32, 86–95. <https://doi.org/10.1016/j.dnarep.2015.04.018>
- Cox, M. M., & Lehman, I. R. (1981). Directionality and polarity in recA protein-promoted branch migration. *Proceedings of the National Academy of Sciences*, 78(10), 6018–6022. <https://doi.org/10.1073/pnas.78.10.6018>
- Cromie, G. A., & Leach, D. R. F. (2001). Recombinational repair of chromosomal DNA double-strand breaks generated by a restriction endonuclease. *Molecular Microbiology*, 41(4), 873–883. <https://doi.org/10.1046/j.1365-2958.2001.02553.x>
- Eykelenboom, J. K., Blackwood, J. K., Okely, E., & Leach, D. R. F. (2008). SbcCD Causes a Double-Strand Break at a DNA Palindrome in the Escherichia coli Chromosome. *Molecular Cell*, 29(5), 644–651. <https://doi.org/10.1016/j.molcel.2007.12.020>
- Feng, W., Di Rienzi, S. C., Raghuraman, M. K., & Brewer, B. J. (2011). Replication Stress-Induced Chromosome Breakage Is Correlated with Replication Fork Progression and Is Preceded by Single-Stranded DNA Formation. *G3 Genes|Genomes|Genetics*, 1(5), 327–335. <https://doi.org/10.1534/g3.111.000554>
- Haber, J. E. (1998). The Many Interfaces of Mre11. *Cell*, 95(5), 583–586. [https://doi.org/10.1016/S0092-8674\(00\)81626-8](https://doi.org/10.1016/S0092-8674(00)81626-8)
- Hopfner, K.-P., Craig, L., Moncalian, G., Zinkel, R. A., Usui, T., Owen, B. A. L., Karcher, A., Henderson, B., Bodmer, J.-L., McMurray, C. T., Carney, J. P., Petrini, J. H. J., & Tainer, J. A. (2002). The Rad50 zinc-hook is a structure joining Mre11 complexes in DNA recombination and repair. *Nature*, 418(6897), 562–566. <https://doi.org/10.1038/nature00922>

- Hopfner, K.-P., Karcher, A., Craig, L., Woo, T. T., Carney, J. P., & Tainer, J. A. (2001). Structural Biochemistry and Interaction Architecture of the DNA Double-Strand Break Repair Mre11 Nuclease and Rad50-ATPase. *Cell*, 105(4), 473–485. [https://doi.org/10.1016/S0092-8674\(01\)00335-X](https://doi.org/10.1016/S0092-8674(01)00335-X)
- Howard-Flanders, P., & Theriot, L. (1966). Mutants of Escherichia coli K-12 defective in DNA repair and in genetic recombination. *Genetics*, 53(6), 1137–1150. <https://doi.org/10.1093/genetics/53.6.1137>
- Ivessa, A. S., Zhou, J.-Q., & Zakian, V. A. (2000). The Saccharomyces Pif1p DNA Helicase and the Highly Related Rrm3p Have Opposite Effects on Replication Fork Progression in Ribosomal DNA. *Cell*, 100(4), 479–489. [https://doi.org/10.1016/S0092-8674\(00\)80683-2](https://doi.org/10.1016/S0092-8674(00)80683-2)
- Jazayeri, A., Falck, J., Lukas, C., Bartek, J., Smith, G. C. M., Lukas, J., & Jackson, S. P. (2006). ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks. *Nature Cell Biology*, 8(1), 37–45. <https://doi.org/10.1038/ncb1337>
- Kaye, J. A., Melo, J. A., Cheung, S. K., Vaze, M. B., Haber, J. E., & Toczyski, D. P. (2004). DNA Breaks Promote Genomic Instability by Impeding Proper Chromosome Segregation. *Current Biology*, 14(23), 2096–2106. <https://doi.org/10.1016/j.cub.2004.10.051>
- Kim, N., & Jinks-Robertson, S. (2012). Transcription as a source of genome instability. *Nature Reviews. Genetics*, 13(3), 204–214. <https://doi.org/10.1038/nrg3152>
- Kuzminov, A. (1999). Recombinational Repair of DNA Damage in Escherichia coli and Bacteriophage λ. *Microbiology and Molecular Biology Reviews*, 63(4), 751–813. <https://doi.org/10.1128/mmbr.63.4.751-813.1999>
- Kuzminov, A. (2001). Single-strand interruptions in replicating chromosomes cause double-strand breaks. *Proceedings of the National Academy of Sciences*, 98(15), 8241–8246. <https://doi.org/10.1073/pnas.131009198>
- Kuzminov, A., & Stahl, F. W. (1999). Double-strand end repair via the RecBC pathway in Escherichia coli primes DNA replication. *Genes & Development*, 13(3), 345–356. <https://doi.org/10.1101/gad.13.3.345>

- Lobachev, K. S., Gordenin, D. A., & Resnick, M. A. (2002). The Mre11 Complex Is Required for Repair of Hairpin-Capped Double-Strand Breaks and Prevention of Chromosome Rearrangements. *Cell*, 108(2), 183–193. [https://doi.org/10.1016/S0092-8674\(02\)00614-1](https://doi.org/10.1016/S0092-8674(02)00614-1)
- Makovets, S., Herskowitz, I., & Blackburn, E. H. (2004). Anatomy and Dynamics of DNA Replication Fork Movement in Yeast Telomeric Regions. *Molecular and Cellular Biology*, 24(9), 4019–4031. <https://doi.org/10.1128/MCB.24.9.4019-4031.2004>
- Paull, T. T., & Gellert, M. (1998). The 3' to 5' Exonuclease Activity of Mre11 Facilitates Repair of DNA Double-Strand Breaks. *Molecular Cell*, 1(7), 969–979. [https://doi.org/10.1016/S1097-2765\(00\)80097-0](https://doi.org/10.1016/S1097-2765(00)80097-0)
- Saathoff, J.-H., Käshammer, L., Lammens, K., Byrne, R. T., & Hopfner, K.-P. (2018). The bacterial Mre11–Rad50 homolog SbcCD cleaves opposing strands of DNA by two chemically distinct nuclease reactions. *Nucleic Acids Research*, 46(21), 11303–11314. <https://doi.org/10.1093/nar/gky878>
- Sharples, G. J., & Leach, D. R. F. (1995). Structural and functional similarities between the SbcCD proteins of Escherichia coli and the RAD50 and MRE11 (RAD32) recombination and repair proteins of yeast. *Molecular Microbiology*, 17(6), 1215–1217. https://doi.org/10.1111/j.1365-2958.1995.mmi_17061215_1.x
- Shinohara, A., Ogawa, H., & Ogawa, T. (1992). Rad51 protein involved in repair and recombination in S. cerevisiae is a RecA-like protein. *Cell Press*, 69(3), 457–470.
- Symington, L. S., & Gautier, J. (2011). Double-Strand Break End Resection and Repair Pathway Choice. *Annual Review of Genetics*, 45(Volume 45, 2011), 247–271. <https://doi.org/10.1146/annurev-genet-110410-132435>
- Tsubouchi, H., & Ogawa, H. (1998). A Novel mre11 Mutation Impairs Processing of Double-Strand Breaks of DNA during Both Mitosis and Meiosis. *Molecular and Cellular Biology*, 18(1), 260–268. <https://doi.org/10.1128/mcb.18.1.260>
- van Gent, D. C., Hoeijmakers, J. H. J., & Kanaar, R. (2001). Chromosomal stability and the DNA double-stranded break connection. *Nature Reviews Genetics*, 2(3), 196–206. <https://doi.org/10.1038/35056049>
- Wendel, B. M., Courcelle, C. T., & Courcelle, J. (2014). Completion of DNA replication in Escherichia coli. *Proceedings of the National Academy of Sciences of the United States of America*, 111(12), 4387–4392. <https://doi.org/10.1073/pnas.1319311111>

States of America, 111(46), 16454–16459.
<https://doi.org/10.1073/pnas.1415025111>

Chapter 4

Cell sorting and high throughput sequencing can be used to create replication profiles across the yeast genome

Introduction

I hypothesized that enzymes such as Mre11-Rad50 and Rrm3, which share homology with bacterial enzymes that lead to genetic instabilities at loci where replication completes, may play similar roles in eukaryotes (Connelly et al., 1998a; Sharples & Leach, 1995; Wendel et al., 2014a). In bacteria, the genetic instabilities can be observed as abnormalities in the sequence copy number of the replication profile (Courcelle et al., 2015b; Wendel et al., 2014a). Bacterial replication profiles can be examined by simply looking at the copy number of sequences of genomic DNA purified from a growing culture (Wendel et al., 2014a). However, in eukaryotes, this approach must be modified, since most cells in growing cultures are not replicating (i.e. those in G1 and G2.)

Therefore, to assess the effects on replication timing dynamics—particularly at regions where forks converge, cells from asynchronously growing yeast cultures were first sorted by flow cytometry into G1, S, G2, and the G2-arrested, (termed G2XL) populations based on DNA content and cell size as described in the previous chapter (Fig 3.3). Genomic DNA was isolated from each of these cell cycle specific populations, fragmented, and subjected to high-throughput sequencing. Sequence reads were mapped to the reference genome strain S288C using Bowtie and custom python scripts in 1000 bp bins across the genome. Each bin was then normalized against the average

number of reads per bin over the entire genome to obtain the copy number frequency (Fig 4.1A).

Using this approach. Wild type cells in G1 would be expected display a uniform copy number of ~ 1 across all genomic regions, consistent with an unreplicated genome. In the subsequent S-phase, cells would be expected to exhibit pronounced variation in copy number within each chromosome. These changes should be observed as local peaks and troughs along the map positions of each chromosome. Early firing replication origins would be associated with increased read frequencies, since these sequences replicate first the S-phase population (Koren et al., 2014; Müller et al., 2014). In contrast, late-replicating regions should show reduced copy numbers, with the deepest troughs corresponding to sites where replication forks converge (termination regions) (Koren et al., 2014; Müller et al., 2014). This pattern can be explained by the spatial organization of origin firing (FIG 4.1B), in which bidirectional replication forks firing at adjacent origins converge at a termination region. By G2, DNA replication should have been completed or nearly completed, with each sequence having 2 copies per cell. Replication profiles from G2 phase cells would also be expected to show a uniform relative copy number frequency of ~ 1 across the entire genome when normalized to the average number of sequence reads across the genome.

Copy number frequencies obtained using this experimental approach were plotted in two ways. First, each strain's cell cycle phase was normalized to the wild-type G1 population to enable direct comparison of replication-dependent copy number changes relative to that observed in wild type cells. Second, each strain's cell cycle phase was normalized to its own G1 population to control for within-strain cell cycle variations (see

appendix figure 4.1 for full dataset). These two strategies allowed comparison across strains while also allowing one to observe the pattern of replication within each strain.

Our analysis of copy number frequencies in S phase of wild type cells showed clear replication patterns with peaks typically corresponding with known origins of replication, and troughs along each chromosome, representing replication timing and origin activity (Fig. 4.1C-D; see appendix Fig. 4.2 for chromosome VII). Across chromosome IV (Fig. 4.1) and VII (see appendix Fig. 4.2 for chromosome VII), large peaks correspond to early replicating loci, while smaller peaks marked late replicating regions. Repeating patterns of peaks and troughs can be seen distributed along the chromosome arms. Peaks aligned with marked replication origins (red circles on the x-axis), while localized troughs almost always occurred between adjacent origins. The results clearly demonstrate the approach can be used to map the loci of origins and completion loci across the eukaryotic genome.

Results

Mre11 and Rrm3 do not clearly alter the profiles of chromosomal DNA replication yeast

To examine if the abnormalities in viability and cell cycle progression would be associated with copy number variations in these mutant replication profiles, I compared the S-phase copy number profiles of $\Delta mre11$, $\Delta rrm3$, and $\Delta mre11\Delta sae2$ strains to wild type cultures across chromosomes IV (Fig. 4.3 D-E) and VII (Appendix Fig. 4.2). All profile frequencies are shown normalized to the frequencies observed in wild type G1, enabling direct comparison of relative changes in replication patterns. However, in the case of

Δmre11, the plotted 30 kb- floating average of the profile appears substantially noisier, with less clearly defined peaks and troughs. The observed noise is likely due to the fact that the *Δmre11* data was obtained from a separate sequencing lane, that was performed on a different date than the other mutants. Although this lane also included the set of wild type samples for direct comparison and control, fewer sequencing reads were obtained during this attempt, leading to more noise, or variability, between adjacent mapping bins. Nevertheless, the regions and patterns appear similar to both wild type and the *Δmre11Δsae2* mutant, suggesting that the data remains consistent with the other run, and simply has reduced resolution.

Examination of the *Δmre11* during S- phase shows relatively similar patterns of copy numbers at completion loci across the genome compared to wild type cells (Fig 4.3D and i). These results indicate that, despite the compromised viability and G2 arrest observed in these mutants, the replication timing and fork convergence patterns are broadly maintained in this mutant.

I also examined the *Δmre11* mutants in an *Δsae2* background. Sae2 is predicted to play a regulatory role in activating the nuclease activity of the Mre11–Rad50–Xrs2 complex and has been shown to amplify the levels of amplifications and genetic instabilities arising in yeast (Cannavo & Cejka, 2014; Lengsfeld et al., 2007). It has a similar enzymatic activity and function as Exonuclease I in *E. coli*, and when combined with *sbcCD* mutants, inactivation of exonuclease I leads to elevated amplifications specifically at loci where replication forks converge (Wendel et al., 2014a).

Loss of both *Mre11Sae2* also did not lead to the large aberrant copy number variations observed at termination regions in the *sbcdCD xonA* mutants of their bacterial counterparts (Fig 4.3). In some areas there did appear to be modest variations in copy numbers relative to wild type, either reduced or amplified. However, it remains unclear if these variations are significant or represent variations which would normally be observed between samples.

It is also possible that copy number abnormalities could become apparent in the G2 phase, particularly since $\Delta mre11$ and $\Delta mre11\Delta sae2$ mutants appear to arrest at this stage of the cell cycle. Therefore, I also plotted the G1 and G2 phases of the cell cycle for these mutants (Fig. 4.3A and C for G1 and Fig. 4.3G and I for G2). No obvious copy number abnormalities at completion loci were observed for either mutant during G2 (Fig. 4.3G and I). Similarly, analysis of G2XL populations (Fig. 4.3J and K) showed copy numbers around the expected ~ 1 , similar to that of wild type cultures. Curiously, G1 profiles revealed subtle peaks and troughs in $\Delta mre11\Delta sae2$ cells, whereas the lower resolution of $\Delta mre11$ single mutant cells made it unclear if this was also apparent in this background (Fig. 4.3C). Peaks consistently correlated near origins, while troughs appeared to be between origins, across the genome, suggesting that these regions may have not been resolved appropriately upon division. This observation would be consistent with that expected if these mutants played a role in completing DNA replication on the chromosome.

The $\Delta rrm3$ mutant also closely followed the overall profile pattern of wild type during S-phase. No large copy number differences were observed at the loci where replication completes (FIG 4.3 E and ii). Within the $\Delta rrm3$ S-phase profiles, there does

appear to be a general ‘flattening’ of the regions between origin and completion sites. This is evidenced as peaks with lower maximums than wild type, and troughs modestly elevated relative to wild type. This would be the expected profile if loss of Rrm3 impairs replication fork progression, or results in frequent pausing as has been suggested by other studies (Ivessa et al., 2003; Torres, Bessler, et al., 2004).

(Fig. 4.3B and H). The G2 profile of $\Delta rrm3$ closely resembles that of wild type, with copy number values remaining ~ 1 across all chromosomes (Fig. 4.3H and ii). However, in G1, the $\Delta rrm3$ profile displays numerous localized increases that closely corresponded to known replication origins (Fig. 4.3B and ii).

Thus, although the visual inspection of copy number profiles does not reveal large copy number variations at completion loci as is observed in the bacterial mutants which share homology with Mre11 or Rrm3, the subtle differences suggest that it may be important to repeat this analysis with further sequence read depth to determine if these variations are significant. Further quantitative and statistical analysis across all completion loci may also be useful within the dataset already obtained to perhaps reveal patterns between these mutants and wild type cells. One useful approach may be to employ feature-based averaging, where multiple regions—such as origins or termination regions—are averaged and compared. While this analysis was not done as part for this thesis, it would be an important aspect to explore as this work continues.

Telomeric regions show increased degradation following cell division in $\Delta mre11 \Delta sae2$ cells. $\Delta rrm3$ mutants appear to over-replicate these sequences prior to division

Telomeric replication has several properties that distinguish it replication of the rest of the chromosome in eukaryotes. DNA replication follows a defined temporal program, with telomeres representing some of the latest replicating regions in the yeast genome (Ferguson et al., 1991; Friedman et al., 1995). As illustrated in Figure 4.4A, replication origins (ORI) are distributed along each chromosome, with early firing origins concentrated near centromeres (CEN) and progressively later-firing origins located toward the chromosome ends (Friedman et al., 1995; Heun et al., 2001; Raghuraman et al., 2001; Yabuki et al., 2002). This organization establishes a replication timing gradient, where bidirectional replication forks initiate from inner origins and proceed out toward both chromosome ends (Brewer & Fangman, 1967; Friedman et al., 1995; Huberman & Riggs, 1968b; Raghuraman et al., 2001).

Telomeric regions also contain several other characteristics which make their replication unique. Because of the 'DNA ends' problem associated with leading and lagging strand replication, telomeric DNA also has unique requirements for replication. A unique non-templated polymerase, telomerase, extends these units prior to cell division, thereby preventing their successive shortening (Reviewed in Blackburn, 2001). Since the ends of eukaryotic chromosomes are linear, they also remain susceptible to exonucleolytic degradation throughout the cells cycle. Telomeric regions contain highly G-rich repetitive DNA elements that are capable of folding back onto themselves, forming G-quartet structures that resist unfolding and prevent degradation (Blackburn & Gall, 1978; Henderson et al., 1987; Sandell, 1993; Sen & Gilbert, 1988; Sundquist & Klug, 1989). Additionally, several proteins are known to bind these specialized structures to further enhance their resistance to nucleases (Reviewed in Wellinger & Zakian, 2012).

However, these strongly bound proteins and secondary structures also make it more difficult to replicate these sequences (Reviewed in Wellinger & Zakian, 2012). Several telomere-specific diseases are associated with mutations in the specialized machinery required to protect or maintain these unique sequences, and are associated with shortened lifespan, cell immortality, or cancer (Armanios & Blackburn, 2012; Blasco et al., 1997; N. Kim et al., 1994; Mitchell et al., 1999; Tsakiri et al., 2007).

The profiles also offered an opportunity to examine whether these mutants affected the ability to replicate or maintain telomeric regions of the chromosome. In plots of the copy number profiles, chromosomes are plotted sequentially along a continuous axis. Boundaries between chromosomes therefore correspond to transitions between the right telomere of one chromosome (TEL A right for example in Fig 4.4) and the left telomere of the next (TEL B left in Fig 4.4), denoted by the black “+” symbols in the copy number profiles.

Expanded analysis of copy number profiles of telomeric regions of *Δmre11* mutants revealed that these regions largely maintained similarly to wild type cultures in S phase. Examples of this can be seen in Figure 4.5D (as well as Fig. 4.6D in TEL01R/02L), where the *Δmre11* mutant profile at these specific regions closely resembles that of wild type. Loss of Mre11 appears to also have no effect on these regions during G2, as most telomeric regions have copy numbers comparable wild type (Figure 4.5D as well as Fig. 4.6D in TEL01R/02L). No abnormalities were observed in G2XL in this mutant (Fig. 4.5J and Fig.4.6J).

Additionally, *Δmre11* cells displayed a relatively uniform profile during G1 (Fig. 4.5A and Fig. 4.6A) as well as G2XL (Fig. 4.5J and Fig. 4.6J). This suggests that the loss of Mre11 does not substantially disrupt telomere replication timing, despite the shortened telomere phenotype associated with this mutant (Boulton & Jackson, 1998a; Chamankhah et al., 2000).

However, when I examined the profiles of telomeric DNA during the G1 phase, it was clear that *Δmre11Δsae2* mutants, and likely *Δmre11* mutants, shows distinct reductions in signal at telomeric region (FIG 4.5A and C). The observation suggests that although the telomeric regions appear to replicate and be extended similar to wild type cells, these regions remain susceptible to exonucleolytic degradation following cell division, leading to a loss in copy number of these sequences. It suggests Mre11 contributes to the processing of these sequences in manner that either is required to allow the ends to fold or be protected properly following replication.

In contrast to *Δmre11Δsae2*, the *Δrrm3* strain exhibited a slight increase in copy number at most telomeric regions in the G1 phase (FIG 4.5B and 4.6B). It's important to note that other increases were also observed in some non-telomeric regions interspersed at various loci across the genome. Previous studies have suggested that in the absence of Rrm3, replication stress at protein—DNA barriers can alter chromatin structures in a manner that persists into G1 (Ivessa et al., 2003; Syed et al., 2016; Torres, Bessler, et al., 2004). Because similar increases are also observed at non-telomeric regions across the genome and not just at telomeres, this effect may reflect an aberrant form of replication

initiating at these altered or open loci. However, at present this remains speculative and the reason for the amplified copy numbers at these regions remains unclear.

Previous separation-of-function studies indicate that Mre11 nuclease activity contributes to telomere maintenance but is not strictly required, since nuclease-deficient mutants do not exhibit the severe telomere shortening phenotype observed in *Δmre11* strains (Lewis et al., 2004a; Moreau et al., 1999b). Consistent with this, *Δmre11* cells in this study exhibited relatively modest telomeric abnormalities, supporting the idea that loss of Mre11 alone does not substantially disrupt telomere replication or maintenance under normal conditions. In contrast, *Δmre11Δsae2* mutants displays consistent reductions in telomeric copy number during G1 and S phase that recovers by G2 and G2XL, suggesting that telomeric regions are ultimately replicated but become transiently vulnerable to instability or degradation following cell division. This phenotype is consistent with the established role of Sae2 in MRX-dependent DNA end processing and its functional overlap with Exo1- and Sgs1-mediated pathways (Bonetti et al., 2010; Hardy et al., 2014; Mimitou & Symington, 2010). Sae2 may therefore contribute to the formation or stabilization of protective telomeric end structures, thereby limiting excessive exonucleolytic degradation at chromosome ends.

In contrast, Rrm3 travels with the replication fork and facilitates progression through difficult-to-replicate regions, including G-quadruplex structures on the leading-strand template, while its absence causes replication delays even at loci lacking known Rrm3-dependent pause sites (Azvolinsky et al., 2006a; Ivessa et al., 2002; Varon et al., 2024). This broader role in replication dynamics is consistent with the present findings,

where *Δrrm3* mutants exhibited modest increases in telomeric copy number alongside similar abnormalities at non-telomeric loci, suggesting that loss of Rrm3 alters replication fork progression genome-wide rather than specifically disrupting telomere maintenance. Future analyses quantifying telomeric copy number changes across all chromosome ends could determine whether these effects are globally distributed or preferentially affect specific telomeres, while correlation with telomere length datasets or sequence composition may help identify chromosome ends that are particularly susceptible to instability in *Δmre11Δsae2* and *Δmre11* mutants.

Chapter 4 Figures

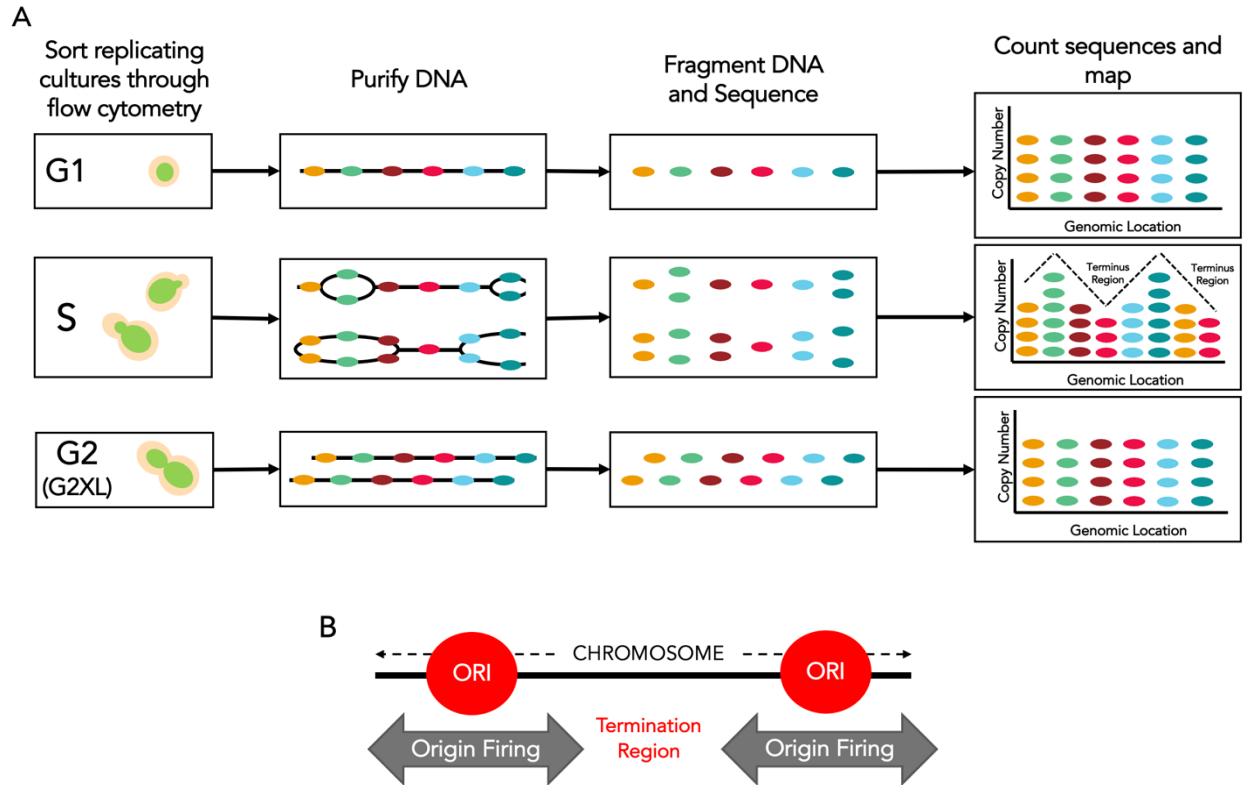


Figure 4.1 Schematic of the approach used to obtain genome-wide DNA copy number profiling and replication timing across cell-cycle stages in yeast. (A) Replicating yeast cultures were first fixed and stained with DNA-binding fluorescent dye and sorted by DNA content using flow cytometry to isolate G1, S, G2 and G2XL populations. Genomic DNA was then purified from each, fragmented, and subjected to high-throughput sequencing. Sequencing reads were mapped to a reference genome (S288C) and quantified in 1kb (1000bp) bins to generate genome-wide copy number profiles. Wild type G1 cells exhibit uniform copy number of 1 across all genomic regions, reflecting unreplicated DNA. S-phase cells display variable copy number, characterized by an increase in copy number in early replicating regions and a reduction at late-replicating regions, including a pronounced decrease at regions where forks converge (termination regions). G2 (and G2XL) cells show uniformly increased copy number across the genome, consistent with the completion of DNA replication. Sequencing reads for each mutant (in each phase) are then aligned to the S288C reference genome using Bowtie, quantified as normalized read counts within 1 kb genomic bins, and plotted according to genomic position to generate genome-wide copy number profiles relative to wild type G1 cells. (B) Model of bi-directional DNA replication from

neighboring origins identified by the red circles on the profiles. Illustrates the formation of termination regions where converging replication forks meet.

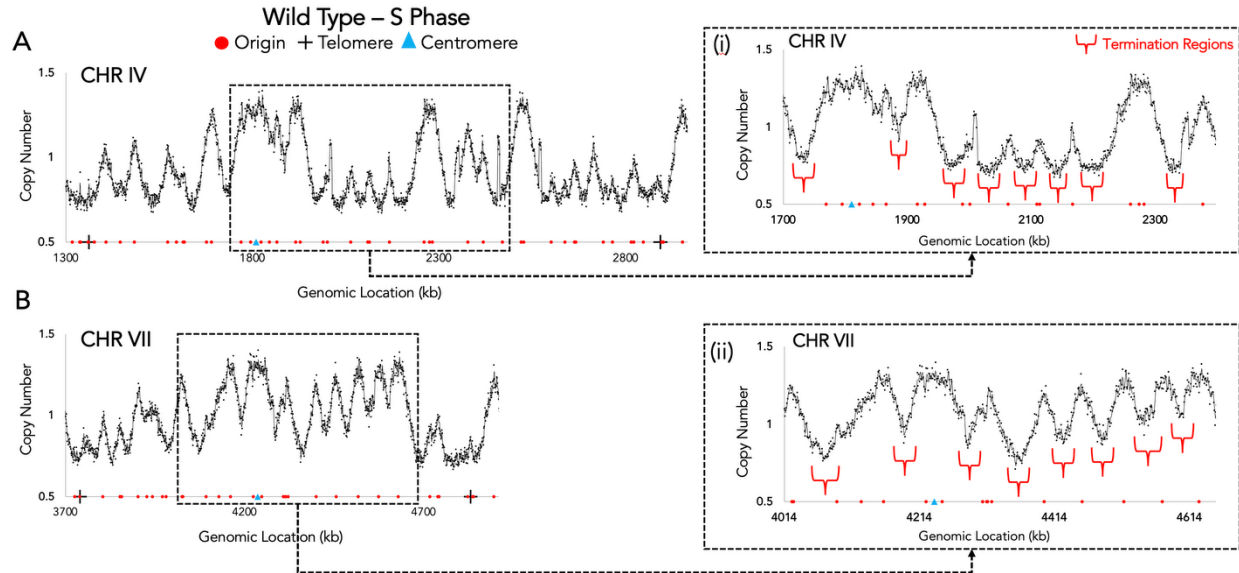
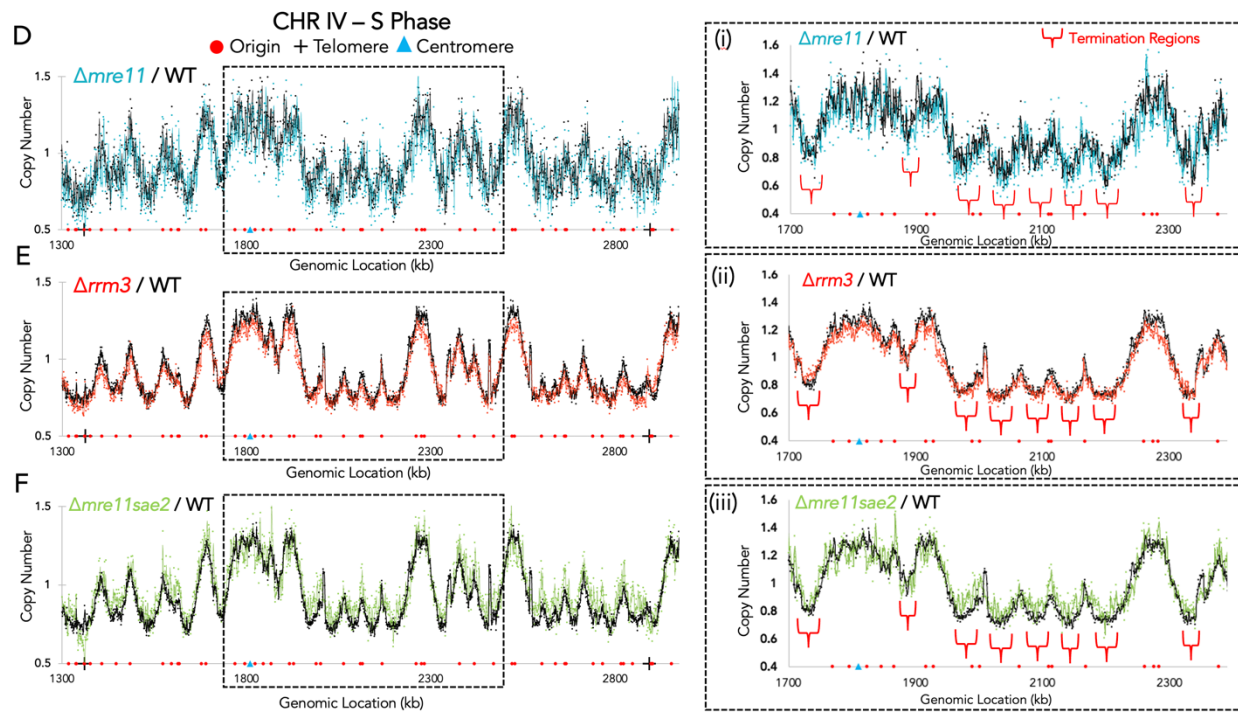
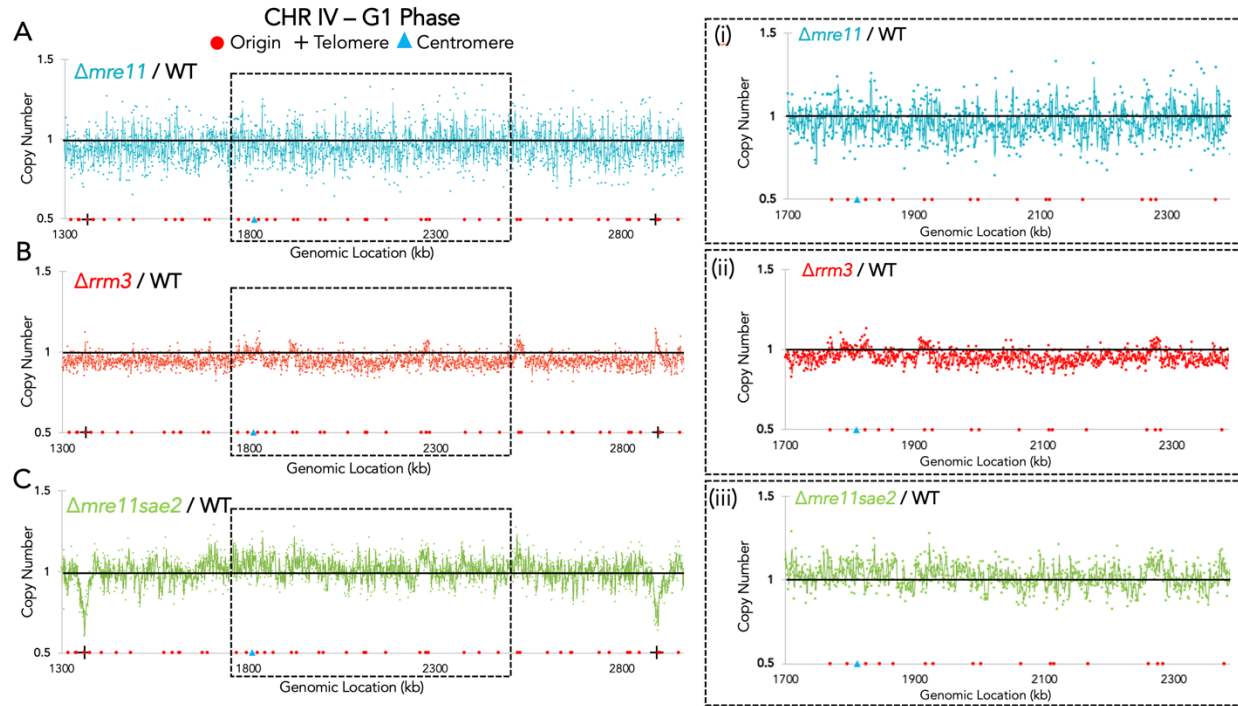


Figure 4.2 Wild type S-phase copy number profiles reveal replication termination regions between adjacent origins can be clearly identified using the replication profiling approach.

(A-B) Sequencing reads from S-phase wild type cells were mapped to chromosome IV (A) and VII (B) quantified across the genome. Each data point represents the normalized read depth within a single kb (1000bp). The x-axis shows genomic location along the chromosome, while the y-axis represents relative copy number normalized to wild type G1 phase DNA content. Key genomic features are indicated along the x-axis: red circles denote replication origins, “+” symbols indicate telomeres, and blue triangles mark centromeres. Normalizing all mutants and all phases to wild type G1 establishes a uniform baseline across the entire genome. In S phase, regions that have replicated early show higher read depth and therefore appear as peaks (copy number >1), while regions with lower copy number (closer to 1) represent late replicating regions. A moving average trend line (solid line) is applied to all strains including wild type, to smooth local noise in read depth and highlight the underlying replication timing pattern, to make peaks and troughs more apparent. (i-ii) Expanded view of highlighted region within wild type chromosome IV (A) and VII (B) respectively reveals periodic reductions in copy number that occur between adjacent, bi-directionally firing origins, corresponding to sites where replication forks converge. These regions are regions of replication termination and are characterized by the lowest copy number relative to neighbor peaks and are marked with red brackets.



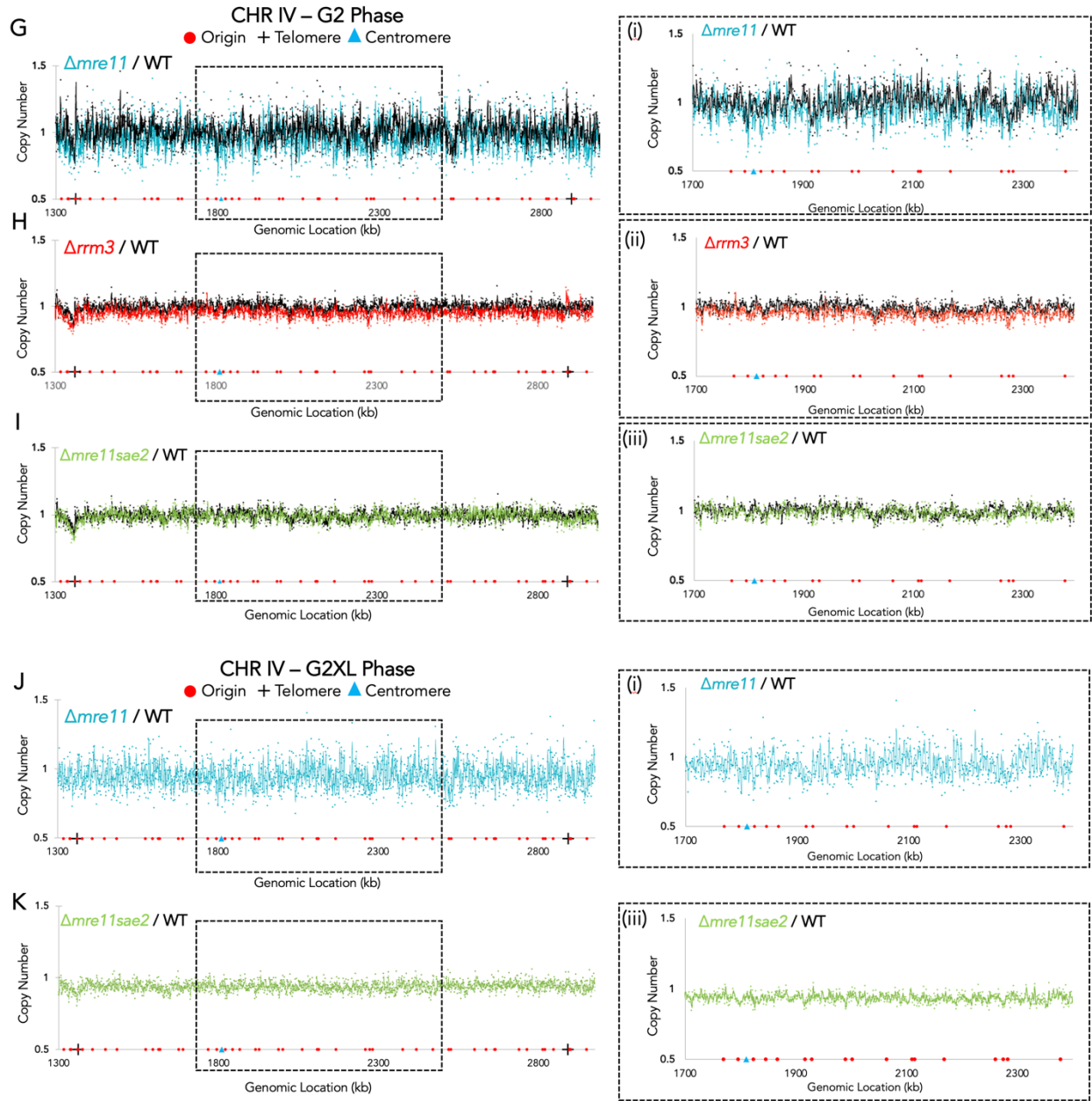


Figure 4.3 Chromosome IV copy number profiles across cell cycle phases in $\Delta mre11$, $\Delta rrm3$, and $\Delta mre11\Delta sae2$ mutants. Copy number profiles across chromosome IV for mutant strains $\Delta mre11$ (blue), $\Delta rrm3$ (pink) and $\Delta mre11\Delta sae2$ overlaid with wild type (black) for G1, S, G2, and G2XL. All profiles are normalized to wild type G1, allowing direct comparison of replication progression across strains. Key genomic features are indicated along the x-axis: red circles denote replication origins, “+” symbols indicate telomeres, and blue triangles mark centromeres. Notably, the $\Delta mre11$ dataset was generated from a different sequencing run than the $\Delta rrm3$ dataset, which accounts for differences in the appearance of the overlaid wild type

profiles. Black boxes identify regions expanded in panels. (i-iii) Expanded views of the highlighted regions corresponding to $\Delta mre11$, $\Delta rrm3$, and $\Delta mre11\Delta sae2$. Red brackets represent termination regions. (A-C) G1 profiles display baseline copy numbers, consistent with non-replicating or fully replicated genomes. The overlaid wild type (black line) profile is normalized to its own G1 and therefore appears as a copy number of 1. (D-F) S phase profiles display characteristic replication timing patterns, including elevated copy number at early replicating regions and reduced copy number between adjacent origins. (G-I) G2 populations display relatively uniform copy number, consistent with the completion of DNA replication. (J-K) G2XL cells exhibit largely uniform copy number profiles across chromosome IV, consistent with fully replicated genomes despite increased cellular DNA content and enlarged cell size.

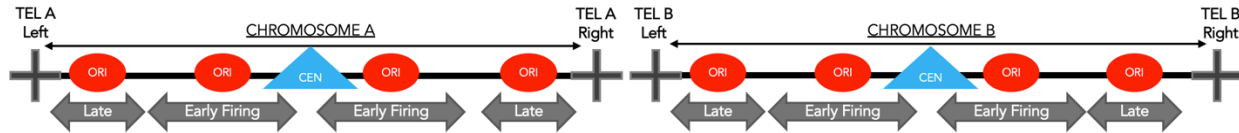
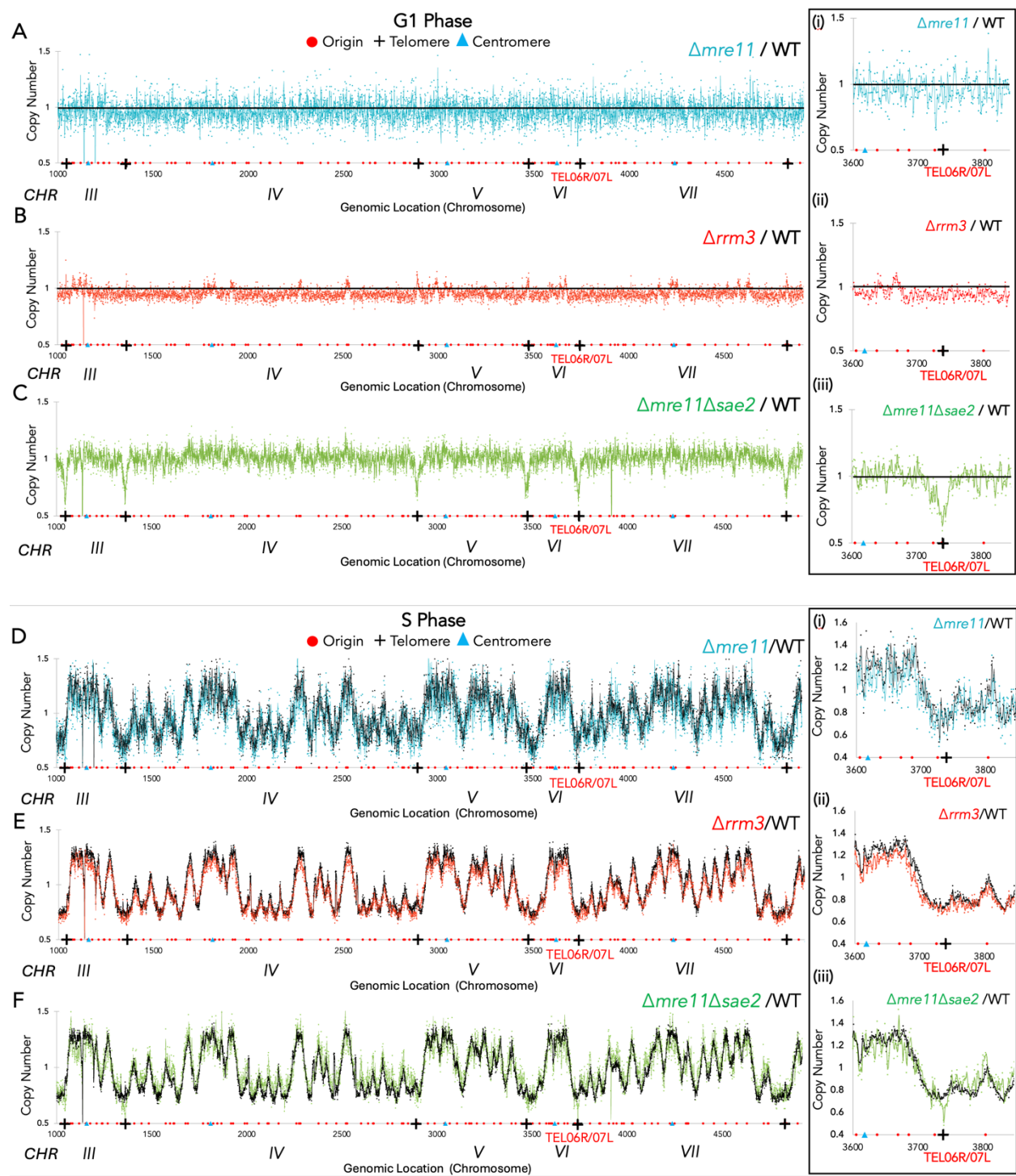


Figure 4.4 Illustration of the spatial relationship between telomeres, centromeres, and replication origin firing patterns along adjacent chromosomes. Model representing organization of chromosomes A and B illustrating the spatial relationship between telomeres. Due to the nature of the continuous alignment of chromosomes along the x-axis, the right telomere of one chromosome aligns with the left telomere of the next. This particular region is represented by a singular black “+” symbol, where the left TEL and chromosome A aligns with the right TEL of chromosome B. Replication origins (red circles labeled ORI) and centromeres (blue triangle labeled CEN) are marked. Early firing origins are located in internal chromosomal regions flanking the centromere, whereas late-firing origins are positioned proximal to telomeres.



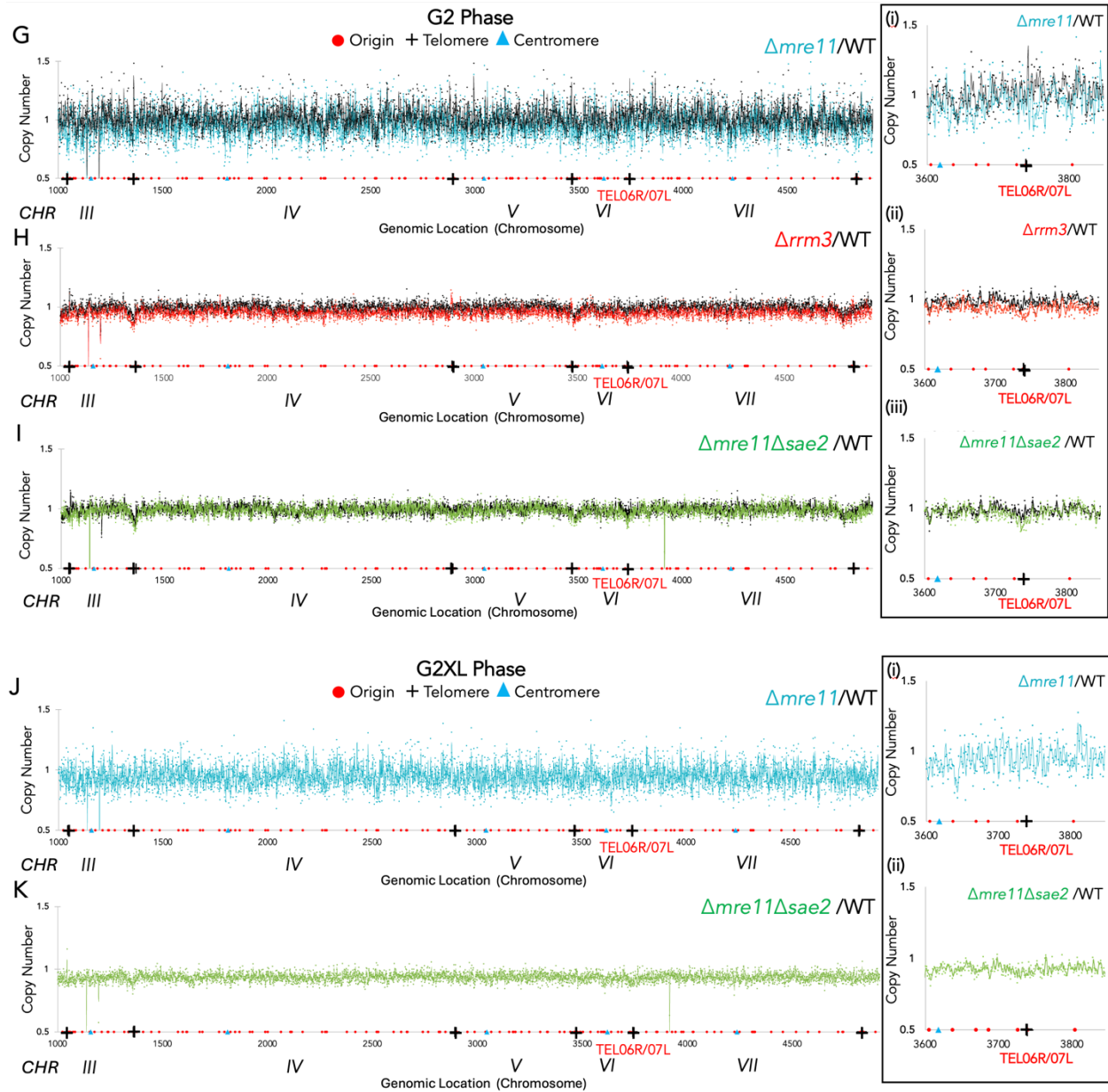


Figure 4.5 Copy number profiles across cell cycle phases and telomeric instabilities in mutant strains. (A-K) Copy number profiles were generated using the same sequencing data sets and analytical parameters as described previously. Representative transition between the right telomere of chromosome VI and the left telomere of chromosome VII (TEL06R/07L) was selected and enlarged in panels (i-iii) for each mutant and phase during the cell cycle. Panels (A-C) show G1 profiles, (D-F) S phase profiles, (G-I) G2 phase profiles, and (J-K) G2XL profiles. In all profiles, black plots represent wild type during that phase normalized to wild type G1. *mre11Δ* (blue), *rrm3Δ* (pink), and *mre11sae2Δ* (yellow) strains across chromosomes III–VII were normalized to wild type G1 (black). Chromosomes are plotted sequentially along a continuous x-

axis, such that chromosome boundaries represent the transition between the right telomere of one chromosome and the left telomere of the next. This is denoted by a black “+” symbol on the x-axis. Red circles denote replication origins (ORI), black crosses indicate telomeres (and chromosome boundaries), and blue triangles mark centromeres. (i-iii) Expanded views highlight the telomeric region spanning between chromosome VII right arm (TEL06R) and chromosome VIII left arm (TEL07L).

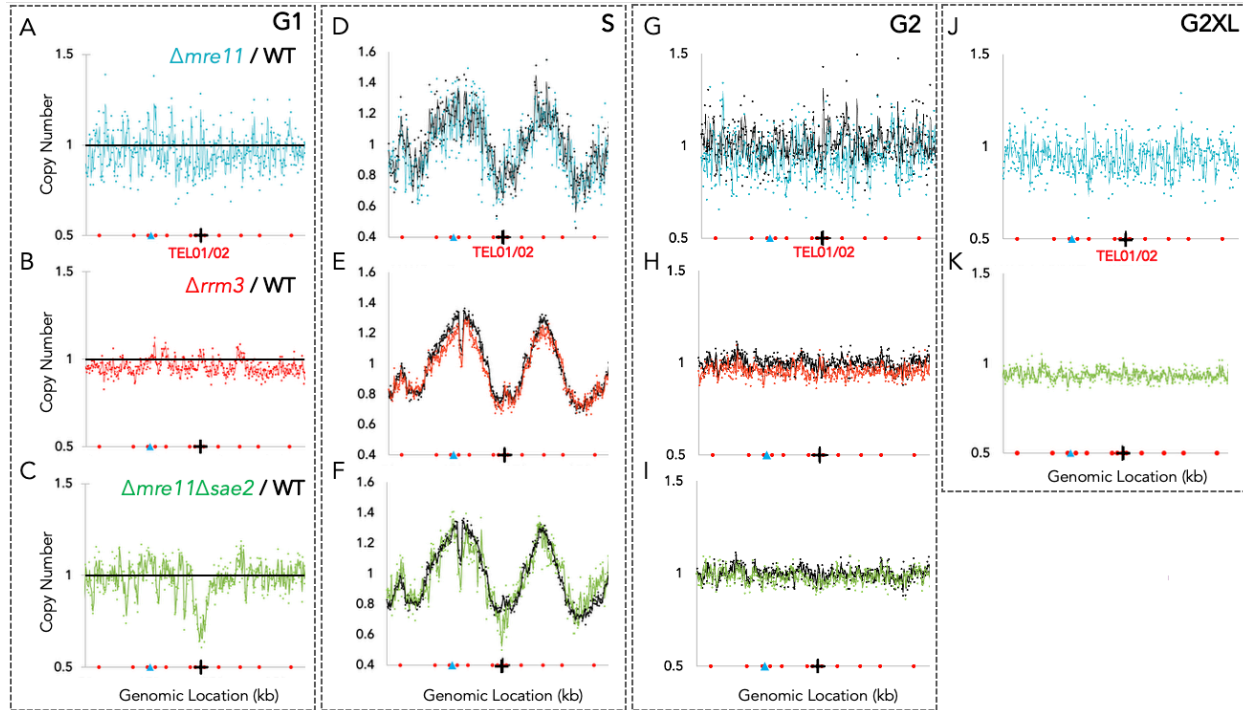


Figure 4.6 Expanded copy number profiles spanning telomeric regions (TEL01R/02L) in $\Delta mre11$, $\Delta rrm3$, and $\Delta mre11\Delta sae2$ cells relative to wild type. (A–C) show G1 phase profiles, (D–F) S phase profiles, (G–I) G2 phase profiles, and (J–K) G2XL phase profiles. While $\Delta mre11$ largely retains wild type copy number patterns across all phases, $\Delta mre11\Delta sae2$ cells exhibited a pronounced reduction in telomeric copy number surrounding the TEL01R/02L boundary during S phase that persisted into the subsequent G1 population, suggesting incomplete replication or instability within these regions in the absence of both Mre11 and Sae2. In contrast, $\Delta rrm3$ mutants displayed modest increases during G1, consistent with abnormalities that may persist following replication stress at protein-DNA barriers in its absence.

References

- Armanios, M., & Blackburn, E. H. (2012). The telomere syndromes. *Nature Reviews. Genetics*, 13(10), 693–704. <https://doi.org/10.1038/nrg3246>
- Blackburn, E. H. (2001). Switching and Signaling at the Telomere. *Cell*, 106(6), 661–673. [https://doi.org/10.1016/S0092-8674\(01\)00492-5](https://doi.org/10.1016/S0092-8674(01)00492-5)
- Blackburn, E. H., & Gall, J. G. (1978). A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in *Tetrahymena*. *Journal of Molecular Biology*, 120(1), 33–53. [https://doi.org/10.1016/0022-2836\(78\)90294-2](https://doi.org/10.1016/0022-2836(78)90294-2)
- Blasco, M. A., Lee, H.-W., Hande, M. P., Samper, E., Lansdorp, P. M., DePinho, R. A., & Greider, C. W. (1997). Telomere Shortening and Tumor Formation by Mouse Cells Lacking Telomerase RNA. *Cell*, 91(1), 25–34. [https://doi.org/10.1016/S0092-8674\(01\)80006-4](https://doi.org/10.1016/S0092-8674(01)80006-4)
- Boulton, S. J., & Jackson, S. P. (1998). Components of the Ku-dependent non-homologous end-joining pathway are involved in telomeric length maintenance and telomeric silencing. *The EMBO Journal*, 17(6), 1819–1828. <https://doi.org/10.1093/emboj/17.6.1819>
- Brewer, B. J., & Fangman, W. L. (1967). The Localization of Replication Origins on ARS Plasmids in *S. cerevisiae*. *Cell*, 51(3), 463–471,.
- Cannavo, E., & Cejka, P. (2014). Sae2 promotes dsDNA endonuclease activity within Mre11–Rad50–Xrs2 to resect DNA breaks. *Nature*, 514(7520), 122–125. <https://doi.org/10.1038/nature13771>
- Chamankhah, M., Fontanie, T., & Xiao, W. (2000). The *Saccharomyces cerevisiae mre11(ts)* Allele Confers a Separation of DNA Repair and Telomere Maintenance Functions. *Genetics*, 155(2), 569–576. <https://doi.org/10.1093/genetics/155.2.569>
- Connelly, J. C., Kirkham, L. A., & Leach, D. R. F. (1998). The SbcCD nuclease of *Escherichia coli* is a structural maintenance of chromosomes (SMC) family protein that cleaves hairpin DNA. *Proceedings of the National Academy of Sciences of the United States of America*, 95(14), 7969–7974. <https://doi.org/10.1073/pnas.95.14.7969>
- Courcelle, J., Wendel, B. M., Livingstone, D. D., & Courcelle, C. T. (2015). RecBCD is required to Complete Chromosomal Replication: Implications for Double-Strand

- Break Frequencies and Repair Mechanisms. *DNA Repair*, 32, 86–95.
<https://doi.org/10.1016/j.dnarep.2015.04.018>
- Ferguson, B. M., Brewer, B. J., Reynolds, A. E., & Fangman, W. L. (1991). A yeast origin of replication is activated late in S phase. *Cell*, 65(3), 507–515.
[https://doi.org/10.1016/0092-8674\(91\)90468-E](https://doi.org/10.1016/0092-8674(91)90468-E)
- Friedman, K. L., Raghuraman, M. K., Fangman, W. L., & Brewer, B. J. (1995). Analysis of the temporal program of replication initiation in yeast chromosomes. *Journal of Cell Science*, 1995(Supplement_19), 51–58.
https://doi.org/10.1242/jcs.1995.Supplement_19.7
- Henderson, E., Hardin, C. C., Walk, S. K., Tinoco, I., & Blackburn, E. H. (1987). Telomeric DNA oligonucleotides form novel intramolecular structures containing guanine·guanine base pairs. *Cell*, 51(6), 899–908. [https://doi.org/10.1016/0092-8674\(87\)90577-0](https://doi.org/10.1016/0092-8674(87)90577-0)
- Heun, P., Laroche, T., Raghuraman, M. K., & Gasser, S. M. (2001). The Positioning and Dynamics of Origins of Replication in the Budding Yeast Nucleus. *Journal of Cell Biology*, 152(2), 385–400. <https://doi.org/10.1083/jcb.152.2.385>
- Huberman, J. A., & Riggs, A. D. (1968). On the mechanism of DNA replication in mammalian chromosomes. *Journal of Molecular Biology*, 32(2), 327–341.
[https://doi.org/10.1016/0022-2836\(68\)90013-2](https://doi.org/10.1016/0022-2836(68)90013-2)
- Ivessa, A. S., Lenzmeier, B. A., Bessler, J. B., Goudsouzian, L. K., Schnakenberg, S. L., & Zakian, V. A. (2003). The *Saccharomyces cerevisiae* Helicase Rrm3p Facilitates Replication Past Nonhistone Protein-DNA Complexes. *Molecular Cell*, 12(6), 1525–1536. [https://doi.org/10.1016/S1097-2765\(03\)00456-8](https://doi.org/10.1016/S1097-2765(03)00456-8)
- Kim, N., Mieczyslaw, M., Prowse, K., Harley, C., West, M., & Ho, P. (1994). Specific association of human telomerase activity with immortal cells and cancer. *Science*, 266(5193), 2011+.
- Koren, A., Handsaker, R. E., Kamitaki, N., Karlić, R., Ghosh, S., Polak, P., Eggan, K., & McCarroll, S. A. (2014). Genetic Variation in Human DNA Replication Timing. *Cell*, 159(5), 1015–1026. <https://doi.org/10.1016/j.cell.2014.10.025>
- Lengsfeld, B. M., Rattray, A. J., Bhaskara, V., Ghirlando, R., & Paull, T. T. (2007). Sae2 Is an Endonuclease that Processes Hairpin DNA Cooperatively with the

- Mre11/Rad50/Xrs2 Complex. *Molecular Cell*, 28(4), 638–651.
<https://doi.org/10.1016/j.molcel.2007.11.001>
- Mitchell, J. R., Wood, E., & Collins, K. (1999). A telomerase component is defective in the human disease dyskeratosis congenita. *Nature*, 402(6761), 551–555.
<https://doi.org/10.1038/990141>
- Müller, C. A., Hawkins, M., Retkute, R., Malla, S., Wilson, R., Blythe, M. J., Nakato, R., Komata, M., Shirahige, K., de Moura, A. P. S., & Nieduszynski, C. A. (2014). The dynamics of genome replication using deep sequencing. *Nucleic Acids Research*, 42(1), e3. <https://doi.org/10.1093/nar/gkt878>
- Raghuraman, M. K., Winzeler, E. A., Collingwood, D., Hunt, S., Wodicka, L., Conway, A., Lockhart, D. J., Davis, R. W., Brewer, B. J., & Fangman, W. L. (2001). Replication Dynamics of the Yeast Genome. *Science*, 294(5540), 115–121.
- Sandell, L. (1993). Loss of a yeast telomere: Arrest, recovery, and chromosome loss. *Cell*, 75(4), 729–739. [https://doi.org/10.1016/0092-8674\(93\)90493-A](https://doi.org/10.1016/0092-8674(93)90493-A)
- Sen, D., & Gilbert, W. (1988). Formation of Parallel Four-Stranded Complexes by Guanine-Rich Motifs in DNA and Its Implications for Meiosis. *Nature*, 334(6180), 364–366.
- Sharples, G. J., & Leach, D. R. F. (1995). Structural and functional similarities between the SbcCD proteins of Escherichia coli and the RAD50 and MRE11 (RAD32) recombination and repair proteins of yeast. *Molecular Microbiology*, 17(6), 1215–1217. https://doi.org/10.1111/j.1365-2958.1995.mmi_17061215_1.x
- Sundquist, W. I., & Klug, A. (1989). Telomeric DNA Dimerizes by Formation of Guanine Tetrads between Hairpin Loops. *Nature*, 342(6251), 825–829.
<https://doi.org/10.1038/342825a0>
- Syed, S., Desler, C., Rasmussen, L. J., & Schmidt, K. H. (2016). A Novel Rrm3 Function in Restricting DNA Replication via an Orc5-Binding Domain Is Genetically Separable from Rrm3 Function as an ATPase/Helicase in Facilitating Fork Progression. *PLOS Genetics*, 12(12), e1006451.
<https://doi.org/10.1371/journal.pgen.1006451>
- Torres, J. Z., Bessler, J. B., & Zakian, V. A. (2004). Local chromatin structure at the ribosomal DNA causes replication fork pausing and genome instability in the

- absence of the *S. cerevisiae* DNA helicase Rrm3p. *Genes & Development*, 18(5), 498–503. <https://doi.org/10.1101/gad.1154704>
- Tsakiri, K. D., Cronkhite, J. T., Kuan, P. J., Xing, C., Raghu, G., Weissler, J. C., Rosenblatt, R. L., Shay, J. W., & Garcia, C. K. (2007). Adult-onset pulmonary fibrosis caused by mutations in telomerase. *Proceedings of the National Academy of Sciences of the United States of America*, 104(18), 7552–7557. <https://doi.org/10.1073/pnas.0701009104>
- Wellinger, R. J., & Zakian, V. A. (2012). Everything You Ever Wanted to Know About *Saccharomyces cerevisiae* Telomeres: Beginning to End. *Genetics*, 191(4), 1073–1105. <https://doi.org/10.1534/genetics.111.137851>
- Wendel, B. M., Courcelle, C. T., & Courcelle, J. (2014). Completion of DNA replication in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America*, 111(46), 16454–16459. <https://doi.org/10.1073/pnas.1415025111>
- Yabuki, N., Terashima, H., & Kitada, K. (2002). Mapping of early firing origins on a replication profile of budding yeast. *Genes to Cells*, 7(8), 781–789. <https://doi.org/10.1046/j.1365-2443.2002.00559.x>

Chapter 5

Abnormal copy numbers of extrachromosomal DNA circles in replicating in mutants lacking Mre11 or Rrm3.

Some genomic replication completion mutants in *E. coli* are also impaired in their abilities to stably replicate plasmids. $\Delta recBC$ mutants show reduced plasmid copy number, $\Delta recD$ mutants over-replicate plasmids, forming unstable linear multimeric species, and loss of SbcCD-ExoI, in a $\Delta recBC$ background, also induces over-replication and unstable multimers (Wendel et al., 2017). Considering that SbcC-SbcD is the bacterial ortholog of the Mre11-Rad50 complex and the RecD motifs found in Rrm3, I considered the possibility that similar genomic instabilities may occur with extrachromosomal circular DNA in eukaryotes. Yeast contains both a 2 μ plasmid as well as a circular mitochondrial genome. In this chapter, I analyzed the large sequencing data set obtained and described in the previous chapter, to assess how the replication of these extrachromosomal DNAs was affected through the cell cycle in mutants lacking either Mre11 or Rrm3.

Introduction: Yeast 2 μ plasmid

The 2 μ plasmid in budding yeast is a circular, multicopy plasmid that is approximately 6.3 kb in size, and on average has a copy number of about 60 (Clark-Walker & Miklos, 1974; Gerbaud et al., 1979). It is non-essential and has been described as a selfish genetic element, wherein its primary function is to ensure its own replication and stability, rather than providing any direct benefits to the host (Broach et al., 1979). It

replicates alongside the normal cell cycle (during S phase) and uses hosts machinery (Zakian et al., 1979).

Copy number of the 2 μ plasmid is tightly regulated through a specific amplification and partitioning systems encoded on the plasmid itself that ensure daughter cells maintain the plasmid. The plasmid contains four major coding regions: FLP, Rep1, Rep2, and Raf1 (Broach et al., 1982; Jayaram et al., 1983; J. A. Murray et al., 1987). Rep1 and Rep2 are essential for plasmid replication and partitioning during cell division, while Raf1 controls copy number of the plasmid by interacting with the Rep1-Rep2 complex (A. Rizvi et al., 2017; Veit & Fangman, 1988). The FLP protein is a site-specific recombinase, facilitating recombination events at FLP recognition targets that are necessary for plasmid stability (Senecoff et al., 1985; Volkert & Broach, 1986). Maintenance and faithful segregation of the plasmid are controlled by the STB (stability) locus, a sequence on the plasmid itself that is critical for binding of Rep1 and Rep2 proteins, to ensure proper plasmid organization and accurate partitioning during cell division (Murray & Cesareni, 1986; Reynolds et al., 1987). Chromosomal genes that influence plasmid copy number and stability are less well characterized.

Results: 2 μ Plasmid

Loss of Mre11 or Rrm3 leads to abnormal 2 μ plasmid copy numbers.

Genomic sequencing datasets from the previous chapter also collected information for both mitochondrial and plasmid DNA. I therefore assessed how the copy number of these sequences varied over the progression of the cell cycle in both wild type and mutants strains. All sequences reads obtained from the 2 μ plasmid in each sequencing dataset were added together and normalized against the total number of

chromosomal DNA reads in the set. In the wildtype strain G1 phase, I set this ratio equal to 1 (normalized to its own G1) and then followed how that ratio changed during over the cell cycle. During S phase, the plasmid copy number increased to approximately 1.5 fold before returning to near baseline levels in G2 (FIG. 5.1A-C solid black bars). This pattern is consistent with plasmid replication occurring early during S phase, when the chromosome copy number is also replicating (Zakian et al., 1979). During G2, one would expect the normalized plasmid/chromosome copy number ratio to return to 1, as observed, since cells contain twice the number of both plasmids and chromosomes.

In the *Δmre11* strain, plasmid copy number, relative to that seen in wild type cells, was reduced by ~50% throughout the phases of the cell cycle (FIG. 5.1A solid blue bars). Notably, the plasmid is not lost, and its copy number remains stable, although reduced, across the cell cycle, arguing against major defects in plasmid segregation or replication timing, where I would expect to see clear phase-specific imbalances. A somewhat similar phenotype was reported in the Courcelle lab using bacterial *recBC* where mutations in these genes destabilized plasmid mini-chromosomes and reduced plasmid retention, while overall viability is also impaired (N. A. Hamilton et al., 2023; Wendel et al., 2017). In both cases, the phenotype presents as difficulty in maintaining plasmids, and in bacterial *recBC* strains, eventually leads to abnormal plasmid forms (N. A. Hamilton et al., 2023; Wendel et al., 2017).

I also examined the effect of *Δmre11* in combination with *Δsae2* on 2μ plasmid circular replication. Sae2 is a critical endonuclease in *S. cerevisiae* that plays a central role in DNA double-strand break repair and homologous recombination (Clerici et al., 2005b; Lengsfeld et al., 2007). It is postulated to work cooperatively with the Mre11-

Rad50 (Cannavo & Cejka, 2014; Clerici et al., 2005b; H.-S. Kim et al., 2008). There is no clear homolog of Sae2 in *E. coli*, however, based on its functional activities, it has been compared with that of Exo I, encoded by *xonA*. The $\Delta mre11sae2$ double mutant appeared to restore the 2 μ plasmid copy number to wild type levels. As shown in Figure 5.1B, relative to wild type (solid green bars,) the plasmid copy number shows little to no differences in the mutant during cell cycle progression (FIG 5.1B).

These results parallel key observations previously described for bacterial *recBC* suppression by *sbcCD xonA* mutations (N. A. Hamilton et al., 2023; Wendel et al., 2017). While mutations in *recBC* cause severe plasmid maintenance defects, the additional mutation of *sbcCD xonA* restores plasmid copy number and partially alleviates DNA damage sensitivity. However, the plasmids remain structurally abnormal, forming unstable linear multimers that fail to segregate properly (N. A. Hamilton et al., 2023; Wendel et al., 2017). Similarly, the reduced copy number observed in yeast $\Delta mre11$ cells is largely restored in the double mutant $\Delta mre11\Delta sae2$, suggesting that loss of Sae2 suppresses the plasmid maintenance defect caused by loss of Mre11. As in the bacterial system, restoration of plasmid copy number may not necessarily reflect complete restoration of normal plasmid maintenance and could instead be consistent with altered processing of replication-associated DNA intermediates. These parallels support the idea that bacterial SbcCD-ExoI and RecBC, together with eukaryotic Mre11-Rad50 perform analogous roles in the processing of extrachromosomal DNA replication intermediates and further indicate that restoration of plasmid copy number can occur independently of faithful plasmid stability and segregation.

In the *Δrrm3* strain, I observed the opposite effect of that seen in *Δmre11*. The plasmid copy number was 2-fold higher at all stages of the cell cycle (FIG 5.1C). Rrm3 helps replication forks move smoothly, particularly at protein-DNA complexes or hard-to-replicate regions; when absent, forks may stall and break (Ivessa et al., 2002, 2003). While Rrm3 can influence plasmid replication dynamics indirectly (by reducing fork stalling,) loss of this helicase appears to dysregulate the plasmids tightly regulated feedback course to a higher constant (Ivessa et al., 2000b, 2002; Torres, Schnakenberg, et al., 2004). Similar phenotypes have been described in *E. coli recD* mutants, in which loss of the fast-moving helicase subunit of RecBCD appears to promotes aberrant replication and recombination events of plasmids, leading to accumulation of multimeric DNA molecules (Biek & Cohen, 1986; N. A. Hamilton et al., 2023; Wendel et al., 2017). Thus, loss of Rrm3 may promote abnormal plasmid replication structures to persist, similar to the over-replicated plasmid DNA structures observed in *recD* mutants in bacteria.

These findings show that although plasmid partitioning and replication timing stay consistent, the proper maintenance of the yeast 2μ plasmid copy number may also depend on factors that share homology to the bacterial completion enzymes and have homologous activities in regulating the ability to maintain extrachromosomal, circular plasmids.

Introduction: Mitochondrial DNA

Mitochondria can also be considered a form of extrachromosomal circular DNA in the cell. Unlike DNA replication on the chromosomes, mitochondrial DNA replication in

yeast is a specialized process that does not follow a strict cell cycle schedule and instead maintains its copy number through the cells volume and growth state (Cottrell & Lee, 1981; Seel et al., 2023). Mitochondrial DNA replication occurs continuously throughout the cell cycle and is largely independent of the nuclear replication machinery (Sazer & Sherwood, 1990.) In *S. cerevisiae*, mitochondrial DNA (mtDNA) contains eight origin-like sequences, of which at least three are thought to function as active replication origins (Baldacci et al., 1984.) Although several models have been proposed to explain mtDNA replication in yeast, most remain largely hypothetical and are supported by limited experimental evidence. The most extensively studied and best-supported model is recombination-dependent replication (RDR), in which homologous recombination intermediates, rather than defined origins alone, initiate mtDNA synthesis (Prasai et al., 2017.) RDR occurs at site-specific double strand breaks within these mitochondrial origin-like sequences and employs specialized replication proteins that function independently of chromosomal DNA replication (Foury, 1989; Hori et al., 2009; Ling et al., 2013; Prasai et al., 2017; Van Dyck et al., 1992.). Here I followed how Mre11, Mre11Sae2, and Rrm3 affected the copy number and replication of this extrachromosomal circular DNA.

Results: Mitochondrial DNA

Cell cycle-dependent mtDNA copy number changes in $\Delta mre11$, $\Delta mre11\Delta sae2$, and $\Delta rrm3$ mutants

I assessed how the copy number of mitochondria DNA varied over the progression of the cell cycle in both wild type and mutants strains using a similar approach to that for the 2 μ plasmid. All sequences reads obtained for mitochondrial DNA were added together and normalized against the total number of chromosomal sequence reads in the set. In

the wildtype strain G1 phase, I set this ratio equal to 1 (normalized to its own G1) and then followed how that ratio changed over the cell cycle. In wild type cultures, the mitochondrial copy number remained relative constant throughout the cell cycle, relative the quantity of chromosomal DNA in the cell (FIG. 5.2 solid black bars). This pattern is consistent with previous report indicating that the mitochondria are replicating throughout the cell cycle.

In the $\Delta mre11$ mutant, mtDNA copy number differed from wild type across the cell cycle when normalized to wild type G1 (Figure 5.2A dark blue bars). Relative to wild type, $\Delta mre11$ cells exhibited elevated mtDNA levels in G1 (~1.5), S (~1.2), and G2 (~1.4). However, in the unique larger cell G2XL population that accumulated in $\Delta mre11$ mutants— (Figure 5.2E—also, *see flow cytometry profile Figure 3.1 for more detailed data*), the mtDNA copy number increased dramatically (~2.4), indicating that some cellular signal in this population of cells induces mtDNA replication. Curiously, increased mtDNA are not found in the G1 cell population. The absence suggests that a large portion of these cells fail to survive upon division. This lethal population would be consistent with the elevated levels of lethality that were observed in this population as described in Chapter 3.

The $\Delta mre11\Delta sae2$ double mutant displayed a similar mtDNA copy number profile (Fig. 5.2B) to the $\Delta mre11$ single mutant (Fig. 5.2A), including a pronounced induction and accumulation of mtDNA within the G2XL population. This suggests that the additional loss of Sae2 does not substantially alter the mtDNA accumulation phenotype associated with loss of Mre11.

Inactivation of Rrm3 also altered the mtDNA copy number. In the $\Delta rrm3$ strain, mtDNA copy number was elevated relative wild type throughout the cell cycle (Fig. 5.2C). Loss of Rrm3 resulted in a 1.6 to 1.9-fold increase in both S and G2 phases of the cell cycle the cell cycle. mtDNA replication appeared to be induced during the period of cellular stress in both $\Delta mre11$ single mutant as well as $\Delta rrm3$ mutant cells. In the case of $\Delta mre11$ and $\Delta mre11\Delta sae2$, mitochondrial copy numbers increased specifically in the G2 arrested population (Fig. 5.2 A-B). However, a significant portion of this stressed population is rendered inviable upon division, consistent with the return to near normal mtDNA copy number levels in surviving G1 population. In contrast in $\Delta rrm3$, the elevated mtDNA copy number was may still be triggered by the stress associated with impaired replication progression or S phase replication stress response, a known role for Rrm3 (Ivessa et al., 2002; Torres, Schnakenberg, et al., 2004). However, despite this stress, the cell population remains fully viable, and thus the cells carrying the higher mtDNA copy numbers are retained and persist in the population.

Chapter 5 Figures

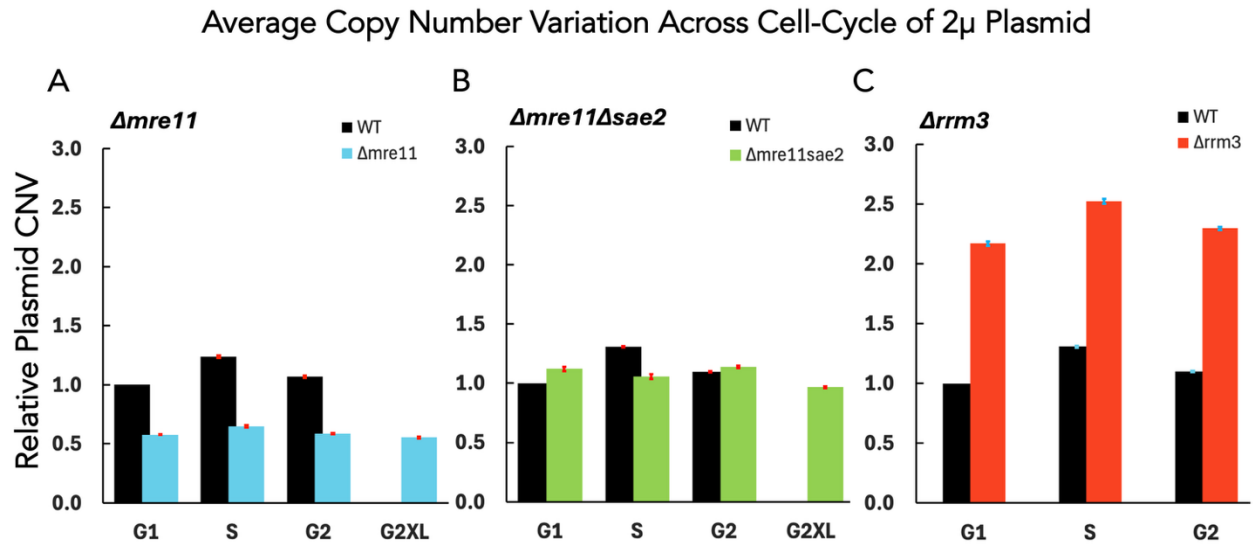


Figure 5.1 Mre11 and Rrm3 differentially regulate 2 μ plasmid copy number across the cell cycle. (A–C) Relative 2 μ plasmid copy number variation was calculated as total plasmid reads normalized to total chromosomal reads. Data are presented relative to wild type G1 and shown for wild type (black bars) and the indicated mutant strains: (A) $\Delta mre11$, (B) $\Delta mre11\Delta sae2$, and (C) $\Delta rrm3$. Copy number was measured across G1, S, and G2 phases of the cell cycle, with G2XL population shown for $\Delta mre11$ and $\Delta mre11\Delta sae2$ mutants. Data are presented as mean $\pm$ error (n = 7).

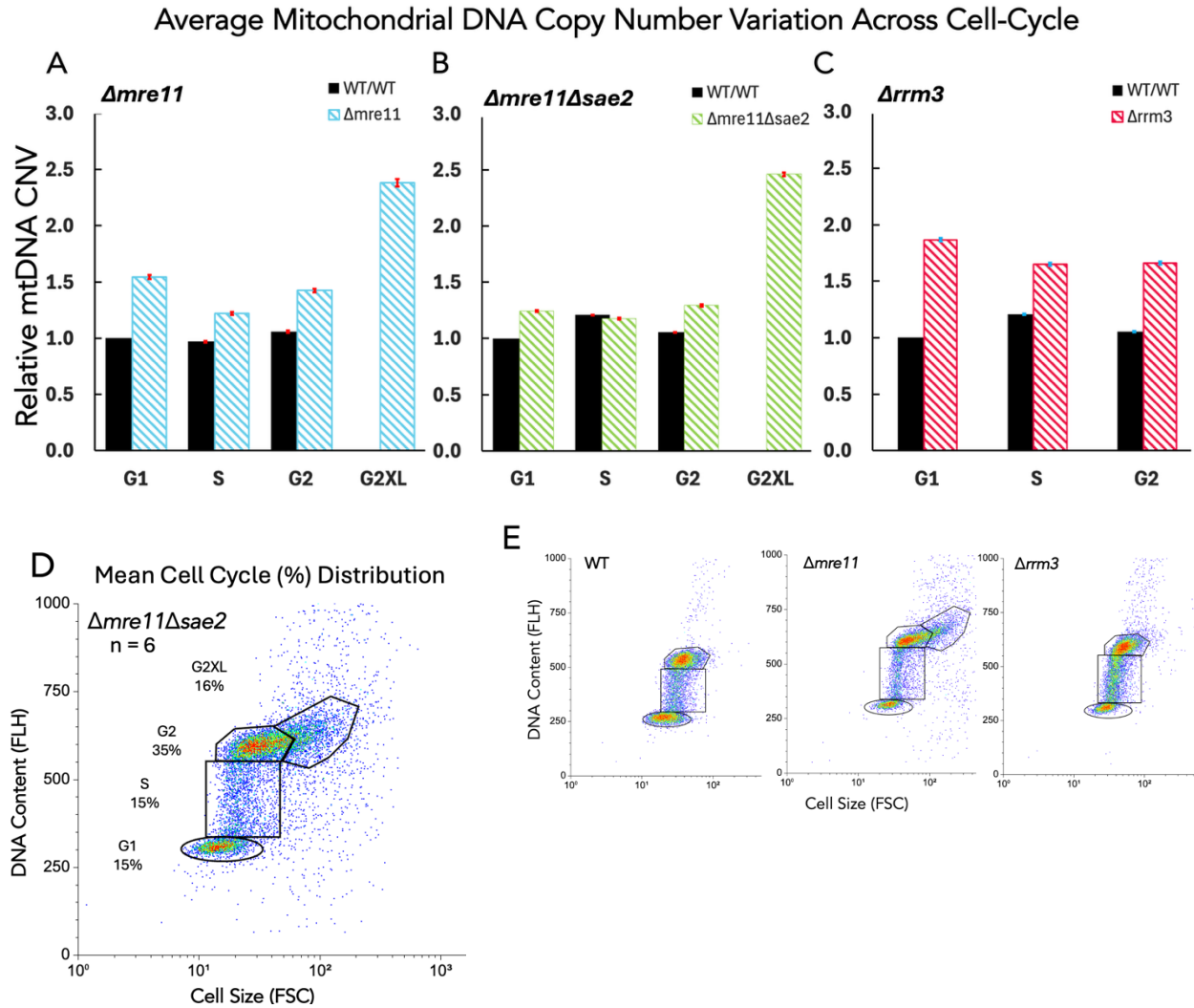


Figure 5.2 Mitochondrial DNA copy number is elevated across the cell cycle and accumulates in G2XL in *Δmre11* and *Δmre11Δsae2*. (A–C) Relative mitochondrial DNA copy number variation was calculated as total mtDNA reads normalized to total chromosomal reads and expressed relative to wild type G1. Data are shown for wild type (black bars) and the indicated mutant strains: (A) *Δmre11*, (B) *Δmre11Δsae2*, and (C) *Δrrm3*. Mitochondrial DNA copy number variation was measured across G1, S, and G2 phases of the cell cycle, with G2XL shown for *Δmre11* and *Δmre11Δsae2* strains. Data are presented as mean $\pm$ error (n = 86). (D–E) Representative flow cytometry profile of asynchronous *Δmre11*, *Δrrm3*, and *Δmre11Δsae2* populations. Cell size (FSC, log scale) and DNA content (FLH) distributions were used to define G1, S, G2, and G2XL populations. The *Δmre11Δsae2* profile (D) includes the mean percentage of cells within each gated cell cycle population (n = 6; see chapter 3 for data collection methods).

Complete cell cycle distribution data for wild type, $\Delta mre11$, and $\Delta rrm3$ can be found in chapter 3 (n=6 for all strains).

References

- A. Rizvi, S. M., Prajapati, H. K., Nag, P., & Ghosh, S. K. (2017). The 2- μ m plasmid encoded protein Raf1 regulates both stability and copy number of the plasmid by blocking the formation of the Rep1–Rep2 repressor complex. *Nucleic Acids Research*, 45(12), 7167–7179. <https://doi.org/10.1093/nar/gkx316>
- Baldacci, G., Chérif-Zahar, B., & Bernardi, G. (1984). The initiation of DNA replication in the mitochondrial genome of yeast. *The EMBO Journal*, 3(9), 2115–2120. <https://doi.org/10.1002/j.1460-2075.1984.tb02099.x>
- Biek, D. P., & Cohen, S. N. (1986). Identification and characterization of recD, a gene affecting plasmid maintenance and recombination in Escherichia coli. *Journal of Bacteriology*, 167(2), 594–603. <https://doi.org/10.1128/jb.167.2.594-603.1986>
- Broach, J. R., Atkins, J. F., McGill, C., & Chow, L. (1979). Identification and mapping of the transcriptional and translational products of the yeast plasmid, 2 μ circle. *Cell*, 16(4), 827–839. [https://doi.org/10.1016/0092-8674\(79\)90098-9](https://doi.org/10.1016/0092-8674(79)90098-9)
- Broach, J. R., Guarascio, V. R., & Jayaram, M. (1982). Recombination within the yeast plasmid 2 μ circle is site-specific. *Cell*, 29(1), 227–234. [https://doi.org/10.1016/0092-8674\(82\)90107-6](https://doi.org/10.1016/0092-8674(82)90107-6)
- Cannavo, E., & Cejka, P. (2014). Sae2 promotes dsDNA endonuclease activity within Mre11–Rad50–Xrs2 to resect DNA breaks. *Nature*, 514(7520), 122–125. <https://doi.org/10.1038/nature13771>
- Clark-Walker, G. D., & Miklos, G. L. G. (1974). Localization and Quantification of Circular DNA in Yeast. *European Journal of Biochemistry*, 41(2), 359–365. <https://doi.org/10.1111/j.1432-1033.1974.tb03278.x>
- Clerici, M., Mantiero, D., Lucchini, G., & Longhese, M. P. (2005). The Saccharomyces cerevisiae Sae2 Protein Promotes Resection and Bridging of Double Strand Break Ends*. *Journal of Biological Chemistry*, 280(46), 38631–38638. <https://doi.org/10.1074/jbc.M508339200>
- Cottrell, S. F., & Lee, L. H. (1981). Evidence for the synchronous replication of mitochondrial DNA during the yeast cell cycle. *Biochemical and Biophysical Research Communications*, 101(4), 1350–1356. [https://doi.org/10.1016/0006-291X\(81\)91596-5](https://doi.org/10.1016/0006-291X(81)91596-5)

- Foury, F. (1989). Cloning and Sequencing of the Nuclear Gene MIP1 Encoding the Catalytic Subunit of the Yeast Mitochondrial DNA Polymerase. *Journal of Biological Chemistry*, 264(34), 20552–20560. [https://doi.org/10.1016/S0021-9258\(19\)47098-1](https://doi.org/10.1016/S0021-9258(19)47098-1)
- Gerbaud, C., Fournier, P., Blanc, H., Aigle, M., Heslot, H., & Guerineau, M. (1979). High frequency of yeast transformation by plasmids carrying part of entire 2- μ m yeast plasmid. *Gene*, 5(3), 233–253. [https://doi.org/10.1016/0378-1119\(79\)90080-5](https://doi.org/10.1016/0378-1119(79)90080-5)
- Hamilton, N. A., Jehru, A. E., Samples, W. N., Wendel, B. M., Mokhtari, P. D., Courcelle, C. T., & Courcelle, J. (2023). Chi sequences switch the RecBCD helicase–nuclease complex from degradative to replicative modes during the completion of DNA replication. *The Journal of Biological Chemistry*, 299(3), 103013. <https://doi.org/10.1016/j.jbc.2023.103013>
- Hori, A., Yoshida, M., Shibata, T., & Ling, F. (2009). Reactive oxygen species regulate DNA copy number in isolated yeast mitochondria by triggering recombination-mediated replication. *Nucleic Acids Research*, 37(3), 749–761. <https://doi.org/10.1093/nar/gkn993>
- Ivessa, A. S., Lenzmeier, B. A., Bessler, J. B., Goudsouzian, L. K., Schnakenberg, S. L., & Zakian, V. A. (2003). The *Saccharomyces cerevisiae* Helicase Rrm3p Facilitates Replication Past Nonhistone Protein-DNA Complexes. *Molecular Cell*, 12(6), 1525–1536. [https://doi.org/10.1016/S1097-2765\(03\)00456-8](https://doi.org/10.1016/S1097-2765(03)00456-8)
- Ivessa, A. S., Zhou, J.-Q., Schulz, V. P., Monson, E. K., & Zakian, V. A. (2002). *Saccharomyces* Rrm3p, a 5' to 3' DNA helicase that promotes replication fork progression through telomeric and subtelomeric DNA. *Genes & Development*, 16(11), 1383–1396. <https://doi.org/10.1101/gad.982902>
- Ivessa, A. S., Zhou, J.-Q., & Zakian, V. A. (2000). The *Saccharomyces* Pif1p DNA Helicase and the Highly Related Rrm3p Have Opposite Effects on Replication Fork Progression in Ribosomal DNA. *Cell*, 100(4), 479–489. [https://doi.org/10.1016/S0092-8674\(00\)80683-2](https://doi.org/10.1016/S0092-8674(00)80683-2)
- Jayaram, M., Li, Y.-Y., & Broach, J. R. (1983). The yeast plasmid 2 μ circle encodes components required for its high copy propagation. *Cell*, 34(1), 95–104. [https://doi.org/10.1016/0092-8674\(83\)90139-3](https://doi.org/10.1016/0092-8674(83)90139-3)

- Kim, H.-S., Vijayakumar, S., Reger, M., Harrison, J. C., Haber, J. E., Weil, C., & Petrini, J. H. J. (2008). Functional Interactions Between Sae2 and the Mre11 Complex. *Genetics*, 178(2), 711–723. <https://doi.org/10.1534/genetics.107.081331>
- Lengsfeld, B. M., Rattray, A. J., Bhaskara, V., Ghirlando, R., & Paull, T. T. (2007). Sae2 Is an Endonuclease that Processes Hairpin DNA Cooperatively with the Mre11/Rad50/Xrs2 Complex. *Molecular Cell*, 28(4), 638–651. <https://doi.org/10.1016/j.molcel.2007.11.001>
- Ling, F., Hori, A., Yoshitani, A., Niu, R., Yoshida, M., & Shibata, T. (2013). Din7 and Mhr1 expression levels regulate double-strand-break–induced replication and recombination of mtDNA at ori5 in yeast. *Nucleic Acids Research*, 41(11), 5799–5816. <https://doi.org/10.1093/nar/gkt273>
- Murray, J. A. H., & Cesareni, G. (1986). Functional analysis of the yeast plasmid partition locus STB. *The EMBO Journal*, 5(12), 3391–3399. <https://doi.org/10.1002/j.1460-2075.1986.tb04655.x>
- Murray, J. A., Scarpa, M., Rossi, N., & Cesareni, G. (1987). Antagonistic controls regulate copy number of the yeast 2 mu plasmid. *The EMBO Journal*, 6(13), 4205–4212. <https://doi.org/10.1002/j.1460-2075.1987.tb02768.x>
- Prasai, K., Robinson, L. C., Scott, R. S., Tatchell, K., & Harrison, L. (2017). Evidence for double-strand break mediated mitochondrial DNA replication in *Saccharomyces cerevisiae*. *Nucleic Acids Research*, 45(13), 7760–7773. <https://doi.org/10.1093/nar/gkx443>
- Reynolds, A. E., Murray, A. W., & Szostak, J. W. (1987). Roles of the 2 microns gene products in stable maintenance of the 2 microns plasmid of *Saccharomyces cerevisiae*. *Molecular and Cellular Biology*, 7(10), 3566–3573. <https://doi.org/10.1128/mcb.7.10.3566>
- Sazer, S., & Sherwood, S. W. (1990). Mitochondrial growth and DNA synthesis occur in the absence of nuclear DNA replication in fission yeast. *Journal of Cell Science*, 97(3), 509–516. <https://doi.org/10.1242/jcs.97.3.509>
- Seel, A., Padovani, F., Mayer, M., Finster, A., Bureik, D., Thoma, F., Osman, C., Klecker, T., & Schmoller, K. M. (2023). Regulation with cell size ensures mitochondrial DNA

- homeostasis during cell growth. *Nature Structural & Molecular Biology*, 30(10), 1549–1560. <https://doi.org/10.1038/s41594-023-01091-8>
- Senecoff, J. F., Bruckner, R. C., & Cox, M. M. (1985). The FLP recombinase of the yeast 2-micron plasmid: Characterization of its recombination site. *Proceedings of the National Academy of Sciences of the United States of America*, 82(21), 7270–7274. <https://doi.org/10.1073/pnas.82.21.7270>
- Torres, J. Z., Schnakenberg, S. L., & Zakian, V. A. (2004). Saccharomyces cerevisiae Rrm3p DNA Helicase Promotes Genome Integrity by Preventing Replication Fork Stalling: Viability of rrm3 Cells Requires the Intra-S-Phase Checkpoint and Fork Restart Activities. *Molecular and Cellular Biology*, 24(8), 3198–3212. <https://doi.org/10.1128/MCB.24.8.3198-3212.2004>
- Van Dyck, E., Foury, F., Stillman, B., & Brill, S. J. (1992). A single-stranded DNA binding protein required for mitochondrial DNA replication in S. cerevisiae is homologous to E. coli SSB. *The EMBO Journal*, 11(9), 3421–3430. <https://doi.org/10.1002/j.1460-2075.1992.tb05421.x>
- Veit, B. E., & Fangman, W. L. (1988). Copy number and partition of the Saccharomyces cerevisiae 2 micron plasmid controlled by transcription regulators. *Molecular and Cellular Biology*, 8(11), 4949–4957. <https://doi.org/10.1128/mcb.8.11.4949>
- Volkert, F. C., & Broach, J. R. (1986). Site-specific recombination promotes plasmid amplification in yeast. *Cell*, 46(4), 541–550. [https://doi.org/10.1016/0092-8674\(86\)90879-2](https://doi.org/10.1016/0092-8674(86)90879-2)
- Wendel, B. M., Cole, J. M., Courcelle, C. T., & Courcelle, J. (2017). SbcC-SbcD and ExoI process convergent forks to complete chromosome replication. *Proceedings of the National Academy of Sciences of the United States of America*, 115(2), 349–354. <https://doi.org/10.1073/pnas.1715960114>
- Zakian, V. A., Brewer, B. J., & Fangman, W. L. (1979). Replication of each copy of the yeast 2 micron DNA plasmid occurs during the S phase. *Cell*, 17(4), 923–934. [https://doi.org/10.1016/0092-8674\(79\)90332-5](https://doi.org/10.1016/0092-8674(79)90332-5)

Chapter 6

Discussion

The work presented in this thesis investigated whether the eukaryotic Mre11-Rad50 complex contributes to DNA replication completion through mechanisms analogous to those previously described for the bacterial SbcCD and RecBCD systems (Wendel et al., 2014a, 2017). Previous studies in our lab using *E. coli* demonstrated that proteins traditionally associated with homologous recombination and double-strand break repair also function directly during the completion of chromosome replication at converging replication forks (Wendel et al., 2014a, 2017). Importantly, many of the phenotypes associated with bacterial *recBC* and *sbcCD* mutants—including poor viability, genomic instability, and plasmid maintenance defects—could not be fully explained by their role in homologous recombination (Capaldo-Kimball & Barbour, 1971; N. A. Hamilton et al., 2023; Leach et al., 1997a; Wendel et al., 2014a, 2017). Given the strong structural and biochemical conservation between bacterial SbcCD and the eukaryotic Mre11-Rad50 complex, my research sought to determine whether similar functional relationships exist in *S. cerevisiae* (Connelly et al., 1998a; Connelly & Leach, 2002; Hopfner et al., 2001; Sharples & Leach, 1995).

The findings presented here demonstrate that loss of Mre11 results in phenotypes that parallel several key characteristics observed in homologous bacterial systems. As described in chapter 3, the severe growth and viability defects observed in $\Delta mre11$ mutants in the absence of exogenous DNA damage cannot be attributed solely to defects in homologous recombination or the accumulation of spontaneous double-strand breaks.

Mre11 is typically thought to function upstream of Rad51 in this pathway (A. Shibata et al., 2014). However, $\Delta rad51$ mutants appeared to maintain viability and cell-cycle progression largely comparable to wild type, whereas $\Delta mre11$ exhibited reduced single-cell viability and accumulation of abnormal late G2 populations (G2XL). These observations indicate similarity to the relationship previously described between bacterial *recA* and *recBC* mutants, where *recA* mutants remain relatively healthy during normal growth despite being defective in homologous recombination. By comparison, *recBC* mutants exhibit substantial viability defects independent of exogenous DNA damage (Wendel et al., 2014a, 2017). Collectively, these findings argue that the essential functions of Mre11 extend beyond canonical double strand break repair and instead support a broader role in replication-associated genome maintenance.

The accumulation an enlarged G2XL population in $\Delta mre11$ cells further supports this interpretation. These cells accumulate with increased DNA content and cell size, consistent with defects in late cell-cycle progression. Such defects were not observed in $\Delta rad51$ mutants.

The structural similarities between Mre11-Rad50 and bacterial SbcCD provide a useful framework for interpreting functional similarities. SbcC and SbcD form a conserved ATP-dependent nuclease complex structurally analogous to Rad50 and Mre11, respectively (D. E. Anderson et al., 2001; Connelly et al., 1998a; Dolganov et al., 1996; Paull & Gellert, 1999; Sharples & Leach, 1995). Both complexes contain long coiled-coil ATPase subunits (Rad50/SbcC) and nuclease subunits (Mre11/SbcD) capable of processing DNA ends and structure intermediates (D. E. Anderson et al., 2001; Connelly et al., 1998a, 1999; Dolganov et al., 1996; Hopfner et al., 2000; Sharples & Leach, 1995).

Functionally, both complexes are essential for the processing of double strand break repair through homologous repair and end joining pathways and are essential for resolving replication-associated intermediates such as converging forks and stalled replication structures (Ajimura et al., 1993; Connelly et al., 2003; Cromie & Leach, 2001; Johzuka & Ogawa, 1995; Moore & Haber, 1996; Wendel et al., 2014a).

However, despite the conserved structure and biochemical properties, the genome-wide replication profiling done in chapter 5 revealed that the bulk of chromosomal replication completion, identified at regions where forks converge, remains largely intact in *Δmre11* mutant. Replication timing profiles across all chromosomes retained the characteristic patterns of origin firing and fork convergence observed in wild type cells, suggesting that Mre11 is not required for chromosomal replication completion to the same extent as bacterial SbcCD or even RecBC. This contrasts with bacterial *recBC* and *sbcCD* mutants, which fail to properly maintain the chromosomal terminus region where replication forks converge (Wendel et al., 2014a, 2017). The S phase replication profiles of *Δmre11* cells largely retained normal organization, indicating that origin firing patterns, fork progression, and replication termination remained broadly intact.

However, subtle abnormalities were apparent at many termination regions during S phase when both Mre11 and Sae2 were lost. Sae2 stimulates the MRX complex endonuclease activity, therefore, loss of Mre11 alone would still leave Sae2 and the downstream pathways available to complete fork convergence and broader repair pathways (Clerici et al., 2005b; Lewis et al., 2004b, 2004b; Mimitou & Symington, 2008). This may justify why no abnormalities were observed in *Δmre11* mutants. Losing both

Mre11 and Sae2 appears to disrupt a redundant end-processing or fork-stabilization pathway, where termination defects become more visible at regions where forks converge (Cannavo & Cejka, 2014). Nevertheless, even in the double mutant, the overall replication program remained largely functional throughout the cell cycle. These results suggest that although aspects of replication-associated processing may be conserved between bacteria and eukaryotes, eukaryotic cells possess additional redundant pathways capable of compensating for loss of MRX functioning during chromosomal replication completion.

Although chromosomal replication completion appeared largely unaffected in the *Δmre11* mutant, telomeric regions displayed greater sensitivity to the combined loss of Mre11 and Sae2. The *Δmre1Δsae2* double mutant exhibited variable reductions in telomeric copy number across multiple chromosome ends during S phase and G1, whereas *Δmre11* cells showed relatively normal profiles despite known telomere shortening phenotypes (Boulton & Jackson, 1998b; Le et al., 1999). Thus, the additional loss of Sae2 likely leads to defective processing intermediates at chromosome ends.

A strong parallel between bacterial and eukaryotic systems did emerge through the analysis of our sequencing profiles of extrachromosomal DNA elements. In bacteria, *recBCD* and *sbcCD* mutants are known to display profound plasmid maintenance defects (Biek & Cohen, 1986; N. Hamilton, 2000; Kusano et al., 1989; Lloyd & Buckman, 1985; Niki et al., 1988; Wendel et al., 2014a). Similarly, *Δmre11* mutant in this study exhibited substantial reductions in its 2μ plasmid copy number. In addition, the loss of Rrm3, a DNA helicase involved in fork progression at difficult-to-replicate regions (Azvolinsky et al., 2006b; Ivessa et al., 2000b, 2003; Keil & McWilliams, 1993), showed dysregulated copy

number control, resembling the plasmid over-replication phenotypes on bacterial counterpart, *recD* (Biek & Cohen, 1986; N. A. Hamilton et al., 2023; Wendel et al., 2017). Importantly, these defects occurred despite relatively normal chromosomal replication profiles, suggesting that extrachromosomal DNA elements are particularly sensitive to disruptions in replication-associated processing pathways.

The $\Delta mre11\Delta sae2$ double mutant is especially informative because it partially suppresses the single mutant phenotype. The interaction mirrors the suppression relationships observed in the bacterial replication completion process. Sae2 together with Mre11-Rad50 perform conserved roles in processing intermediates on extrachromosomal DNA. A model is also supported in which successful 2 μ plasmid copy number is not only regulated by its own control system, but also by host replication and DNA-processing factors.

Cell cycle copy number profiles of mitochondrial DNA maintenance also revealed defects in $\Delta mre11$, $\Delta mre11\Delta sae2$, and $\Delta rrm3$, suggesting that mitochondrial genome maintenance is sensitive to defects in nuclear genome processing pathways that influence replication stress and cell-cycle progression. Loss of Mre11 and both Mre11 and Sae2 resulted in identical abnormalities, as seen in elevated mitochondrial DNA copy number across the whole cell cycle, with a pronounced increase in the G2XL population. However, the substantial reduction observed during G1 suggests that many of these enlarged late-stage cells fail to successfully complete division or produce viable progeny. This interpretation is consistent with the reduced single-cell viability phenotypes described in chapter 3, where $\Delta mre11$ cells displayed elevated lethality in the absence of exogenous DNA damage.

An important limitation of this study is that genome-wide replication profiling measures average signals across large populations of cells and may therefore miss rare or short-lived replication intermediates. As a result, subtle replication defects may not be detectable if they occur only briefly or within a small fraction of cells. In addition, the relatively mild chromosomal replication phenotypes observed in these mutants may reflect the activity of parallel pathways that compensate for loss of MRX function. Detecting these defects is likely more difficult in eukaryotic organisms than in bacteria due to eukaryotic cells containing more complex and overlapping replication, repair, and checkpoint pathways that can mask stronger phenotypes (Conti et al., 2007; Lisby et al., 2004; Lopes et al., 2001). Furthermore, eukaryotic chromosomes are larger, linear, and organized into structurally specialized regions such as telomeres and centromeres, which may further distribute or suppress localized replication-associated abnormalities (Reviewed in O'Donnell et al., 2013).

Replication completion defects may also arise only transiently before being resolved by compensatory mechanisms, making them difficult to capture using bulk sequencing approaches (Reviewed in Diffley, 2010). Therefore, although the genome-wide copy number analyses performed here revealed broadly normal chromosomal replication patterns in the *Δmre11* mutant, additional higher-resolution approaches may be required to better define the replication-associated roles of the Mre11-Rad50 complex in budding yeast. Future studies involving direct analysis of replication intermediates, deeper sequencing approaches, or single-molecule methods may help determine whether transient defects accumulate at specific genomic regions during replication completion.

Future work aimed at identifying the pathways that compensate for loss of MRX function during chromosomal replication will help clarify how replication-associated intermediates are processed in eukaryotic cells. Further studies examining the roles of additional helicases, nucleases, checkpoint proteins, and recombination factors may help define how these pathways contribute to replication fork progression, fork restart, end processing, and resolution of recombination intermediates. Additional characterization of the DNA structures that accumulate in the absence of Mre11-Rad50 and associated enzymes function will also help clarify how genome stability is maintained across different genomic contexts.

The findings presented in this thesis suggest that Mre11 is largely dispensable for efficient bulk chromosomal DNA replication completion in budding yeast under normal growth conditions. Genome-wide replication profiles of both $\Delta mre11$ and $\Delta mre11\Delta sae2$ mutants retained overall replication timing patterns and termination-region organization similar to wild type, indicating that chromosomal replication can proceed largely normally in the absence of Mre11. However, defects became more apparent at specialized genomic regions and extrachromosomal DNA substrates, including telomeres, the 2 μ plasmid, and mitochondrial DNA.

Several observations from this work parallel phenotypes previously described in bacterial *recBCD* and *sbcCD* mutants, particularly with respect to instability of extrachromosomal DNA elements. These similarities support the idea that the eukaryotic Mre11-Rad50 complex shares conserved mechanistic features with bacterial chromosome-processing systems (Connelly et al., 1998b; Hopfner et al., 2001; Sharples

& Leach, 1995). At the same time, the relatively mild chromosomal replication defects observed in yeast highlight important differences between bacterial and eukaryotic replication organization. Unlike bacteria, eukaryotic chromosomes replicate from multiple origins and are supported by interconnected replication and repair pathways, which may reduce dependence on any single completion factor during chromosomal replication (Brewer & Fangman, 1967; Conti et al., 2007; Lopes et al., 2001; Raghuraman et al., 2001).

Overall, these findings support a model in which the Mre11-Rad50 complex contributes primarily to the maintenance and processing of difficult-to-replicate DNA regions and specialized DNA substrates rather than functioning as an essential chromosomal replication completion factor similar to bacterial systems. The stronger phenotypes observed at telomeres, plasmids, and mitochondrial genomes suggest that these substrates may be particularly sensitive to defects in replication-associated DNA processing pathways.

References

- Ajimura, M., Leem, S. H., & Ogawa, H. (1993). Identification of new genes required for meiotic recombination in *Saccharomyces cerevisiae*. *Genetics*, 133(1), 51–66. <https://doi.org/10.1093/genetics/133.1.51>
- Anderson, D. E., Trujillo, K. M., Sung, P., & Erickson, H. P. (2001). Structure of the Rad50·Mre11 DNA Repair Complex from *Saccharomyces cerevisiae* by Electron Microscopy *. *Journal of Biological Chemistry*, 276(40), 37027–37033. <https://doi.org/10.1074/jbc.M106179200>
- Azvolinsky, A., Dunaway, S., Torres, J. Z., Bessler, J. B., & Zakian, V. A. (2006). The *S. cerevisiae* Rrm3p DNA helicase moves with the replication fork and affects replication of all yeast chromosomes. *Genes & Development*, 20(22), 3104–3116. <https://doi.org/10.1101/gad.1478906>
- Biek, D. P., & Cohen, S. N. (1986). Identification and characterization of recD, a gene affecting plasmid maintenance and recombination in *Escherichia coli*. *Journal of Bacteriology*, 167(2), 594–603. <https://doi.org/10.1128/jb.167.2.594-603.1986>
- Boulton, S. J., & Jackson, S. P. (1998). Components of the Ku-dependent non-homologous end-joining pathway are involved in telomeric length maintenance and telomeric silencing. *The EMBO Journal*, 17(6), 1819–1828. <https://doi.org/10.1093/emboj/17.6.1819>
- Cannavo, E., & Cejka, P. (2014). Sae2 promotes dsDNA endonuclease activity within Mre11–Rad50–Xrs2 to resect DNA breaks. *Nature*, 514(7520), 122–125. <https://doi.org/10.1038/nature13771>
- Capaldo-Kimball, F., & Barbour, S. D. (1971). *Involvement of Recombination Genes in Growth and Viability of Escherichia coli K-12*. 106(1), 204–212. <https://doi.org/10.1128/jb.106.1.204-212.1971>
- Clerici, M., Mantiero, D., Lucchini, G., & Longhese, M. P. (2005). The *Saccharomyces cerevisiae* Sae2 Protein Promotes Resection and Bridging of Double Strand Break Ends*. *Journal of Biological Chemistry*, 280(46), 38631–38638. <https://doi.org/10.1074/jbc.M508339200>

- Connelly, J. C., de Leau, E. S., & Leach, D. R. (1999). DNA cleavage and degradation by the SbcCD protein complex from *Escherichia coli*. *Nucleic Acids Research*, 27(4), 1039–1046.
- Connelly, J. C., de Leau, E. S., & Leach, D. R. F. (2003). Nucleolytic processing of a protein-bound DNA end by the *E. coli* SbcCD (MR) complex. *DNA Repair*, 2(7), 795–807. [https://doi.org/10.1016/S1568-7864\(03\)00063-6](https://doi.org/10.1016/S1568-7864(03)00063-6)
- Connelly, J. C., Kirkham, L. A., & Leach, D. R. F. (1998). The SbcCD nuclease of *Escherichia coli* is a structural maintenance of chromosomes (SMC) family protein that cleaves hairpin DNA. *Proceedings of the National Academy of Sciences of the United States of America*, 95(14), 7969–7974. <https://doi.org/10.1073/pnas.95.14.7969>
- Connelly, J. C., & Leach, D. R. F. (2002). Tethering on the brink: The evolutionarily conserved Mre11–Rad50 complex. *Trends in Biochemical Sciences*, 27(8), 410–418. [https://doi.org/10.1016/S0968-0004\(02\)02144-8](https://doi.org/10.1016/S0968-0004(02)02144-8)
- Cromie, G. A., & Leach, D. R. F. (2001). Recombinational repair of chromosomal DNA double-strand breaks generated by a restriction endonuclease. *Molecular Microbiology*, 41(4), 873–883. <https://doi.org/10.1046/j.1365-2958.2001.02553.x>
- Dolganov, G. M., Maser, R. S., Novikov, A., Tosto, L., Chong, S., Bressan, D. A., & Petrini, J. H. (1996). Human Rad50 is physically associated with human Mre11: Identification of a conserved multiprotein complex implicated in recombinational DNA repair. *Molecular and Cellular Biology*, 16(9), 4832–4841. <https://doi.org/10.1128/mcb.16.9.4832>
- Hamilton, N. (2000). *Use of Two-replisome Plasmids to Characterize how Chromosome Replication Completes*. <https://doi.org/10.15760/etd.6940>
- Hamilton, N. A., Jehru, A. E., Samples, W. N., Wendel, B. M., Mokhtari, P. D., Courcelle, C. T., & Courcelle, J. (2023). Chi sequences switch the RecBCD helicase–nuclease complex from degradative to replicative modes during the completion of DNA replication. *The Journal of Biological Chemistry*, 299(3), 103013. <https://doi.org/10.1016/j.jbc.2023.103013>
- Hopfner, K.-P., Karcher, A., Craig, L., Woo, T. T., Carney, J. P., & Tainer, J. A. (2001). Structural Biochemistry and Interaction Architecture of the DNA Double-Strand

- Break Repair Mre11 Nuclease and Rad50-ATPase. *Cell*, 105(4), 473–485.
[https://doi.org/10.1016/S0092-8674\(01\)00335-X](https://doi.org/10.1016/S0092-8674(01)00335-X)
- Hopfner, K.-P., Karcher, A., Shin, D. S., Craig, L., Arthur, L. M., Carney, J. P., & Tainer, J. A. (2000). Structural Biology of Rad50 ATPase: ATP-Driven Conformational Control in DNA Double-Strand Break Repair and the ABC-ATPase Superfamily. *Cell*, 101(7), 789–800. [https://doi.org/10.1016/S0092-8674\(00\)80890-9](https://doi.org/10.1016/S0092-8674(00)80890-9)
- Ivessa, A. S., Lenzmeier, B. A., Bessler, J. B., Goudsouzian, L. K., Schnakenberg, S. L., & Zakian, V. A. (2003). The *Saccharomyces cerevisiae* Helicase Rrm3p Facilitates Replication Past Nonhistone Protein-DNA Complexes. *Molecular Cell*, 12(6), 1525–1536. [https://doi.org/10.1016/S1097-2765\(03\)00456-8](https://doi.org/10.1016/S1097-2765(03)00456-8)
- Ivessa, A. S., Zhou, J.-Q., & Zakian, V. A. (2000). The *Saccharomyces* Pif1p DNA Helicase and the Highly Related Rrm3p Have Opposite Effects on Replication Fork Progression in Ribosomal DNA. *Cell*, 100(4), 479–489. [https://doi.org/10.1016/S0092-8674\(00\)80683-2](https://doi.org/10.1016/S0092-8674(00)80683-2)
- Johzuka, K., & Ogawa, H. (1995). Interaction of Mre11 and Rad50: Two Proteins Required for DNA Repair and Meiosis-Specific Double-Strand Break Formation in *Saccharomyces Cerevisiae*. *Genetics*, 139(4), 1521–1532. <https://doi.org/10.1093/genetics/139.4.1521>
- Keil, R. L., & McWilliams, A. D. (1993). A Gene with Specific and Global Effects on Recombination of Sequences from Tandemly Repeated Genes in *Saccharomyces cerevisiae*. 135, 711–718.
- Kusano, K., nakayama, K., & Nakayama, H. (1989). Plasmid-mediated lethality and plasmid multimer formation in an *Escherichia coli* recBC sbcBC mutant. Involvement of RecF recombination pathway genes. *Journal of Molecular Biology*, 209(4), 623–634. [https://doi.org/10.1016/0022-2836\(89\)90000-4](https://doi.org/10.1016/0022-2836(89)90000-4)
- Le, S., Moore, J. K., Haber, J. E., & Greider, C. W. (1999). RAD50 and RAD51 Define Two Pathways That Collaborate to Maintain Telomeres in the Absence of Telomerase. *Genetics*, 152(1), 143–152. <https://doi.org/10.1093/genetics/152.1.143>

- Leach, D. R. F., Okely, E. A., & Pinder, D. J. (1997). Repair by recombination of DNA containing a palindromic sequence. *Molecular Microbiology*, 26(3), 597–606. <https://doi.org/10.1046/j.1365-2958.1997.6071957.x>
- Lewis, L. K., Storici, F., Van Komen, S., Calero, S., Sung, P., & Resnick, M. A. (2004). Role of the nuclease activity of *Saccharomyces cerevisiae* Mre11 in repair of DNA double-strand breaks in mitotic cells. *Genetics*, 166(4), 1701–1713. <https://doi.org/10.1534/genetics.166.4.1701>
- Lloyd, R. G., & Buckman, C. (1985). Identification and genetic analysis of sbcC mutations in commonly used recBC sbcB strains of *Escherichia coli* K-12. *Journal of Bacteriology*, 164(2), 836–844. <https://doi.org/10.1128/jb.164.2.836-844.1985>
- Mimitou, E. P., & Symington, L. S. (2008). Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. *Nature*, 455(7214), 10.1038/nature07312. <https://doi.org/10.1038/nature07312>
- Moore, J. K., & Haber, J. E. (1996). Cell cycle and genetic requirements of two pathways of nonhomologous end-joining repair of double-strand breaks in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology*, 16(5), 2164–2173. <https://doi.org/10.1128/mcb.16.5.2164>
- Niki, H., Ichinose, C., Ogura, T., Mori, H., Morita, M., Hasegawa, M., Kusakawa, N., & Hiraga, S. (1988). Chromosomal genes essential for stable maintenance of the mini-F plasmid in *Escherichia coli*. *Journal of Bacteriology*, 170(11), 5272–5278. <https://doi.org/10.1128/jb.170.11.5272-5278.1988>
- O'Donnell, M., Langston, L., & Stillman, B. (2013). Principles and Concepts of DNA Replication in Bacteria, Archaea, and Eukarya. *Cold Spring Harbor Perspectives in Biology*, 5(7), a010108. <https://doi.org/10.1101/cshperspect.a010108>
- Paull, T. T., & Gellert, M. (1999). Nbs1 potentiates ATP-driven DNA unwinding and endonuclease cleavage by the Mre11/Rad50 complex. *Genes & Development*, 13(10), 1276–1288. <https://doi.org/10.1101/gad.13.10.1276>
- Sharples, G. J., & Leach, D. R. F. (1995). Structural and functional similarities between the SbcCD proteins of *Escherichia coli* and the RAD50 and MRE11 (RAD32) recombination and repair proteins of yeast. *Molecular Microbiology*, 17(6), 1215–1217. https://doi.org/10.1111/j.1365-2958.1995.mmi_17061215_1.x

- Shibata, A., Moiani, D., Arvai, A. S., Perry, J., Harding, S. M., Genois, M.-M., Maity, R., van Rossum-Fikkert, S., Kertokallio, A., Romoli, F., Ismail, A., Ismalaj, E., Petricci, E., Neale, M. J., Bristow, R. G., Masson, J.-Y., Wyman, C., Jeggo, P. A., & Tainer, J. A. (2014). DNA Double-Strand Break Repair Pathway Choice Is Directed by Distinct MRE11 Nuclease Activities. *Molecular Cell*, 53(1), 7–18. <https://doi.org/10.1016/j.molcel.2013.11.003>
- Wendel, B. M., Cole, J. M., Courcelle, C. T., & Courcelle, J. (2017). SbcC-SbcD and ExoI process convergent forks to complete chromosome replication. *Proceedings of the National Academy of Sciences of the United States of America*, 115(2), 349–354. <https://doi.org/10.1073/pnas.1715960114>
- Wendel, B. M., Courcelle, C. T., & Courcelle, J. (2014). Completion of DNA replication in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America*, 111(46), 16454–16459. <https://doi.org/10.1073/pnas.1415025111>

Appendix: Supplementary Tables and Figures

Observed	WT	$\Delta rad51$	$\Delta mre11$	$\Delta rrm3$
Viable (both grew)	218	114	156	71
Not Viable (Only One Grew)	12	3	44	3
Expected (to WT proportion)	WT	$\Delta rad51$	$\Delta mre11$	$\Delta rrm3$
Viable (both grew)	0.95	110.90	189.57	70.14
Not Viable (Only One Grew)	0.05	6.10	10.43	3.86
n	230	117	200	74
χ^2		0.1968	1.36×10^{-26}	0.6527

Table 3.1 Chi-square analysis for statistical significance for single-cell viability assay.

Observed counts of cell division outcomes using 2 categories (both grew vs only one grew) are shown for all strains from the single-cell viability assay. Expected values were calculated based on the proportional distribution observed in WT and scaled to the total number of events for each strain. Chi-square analysis was used to compare observed and expected distributions, with corresponding values indicating deviation from WT expectations. *Indicates data used for Figure 3.3E

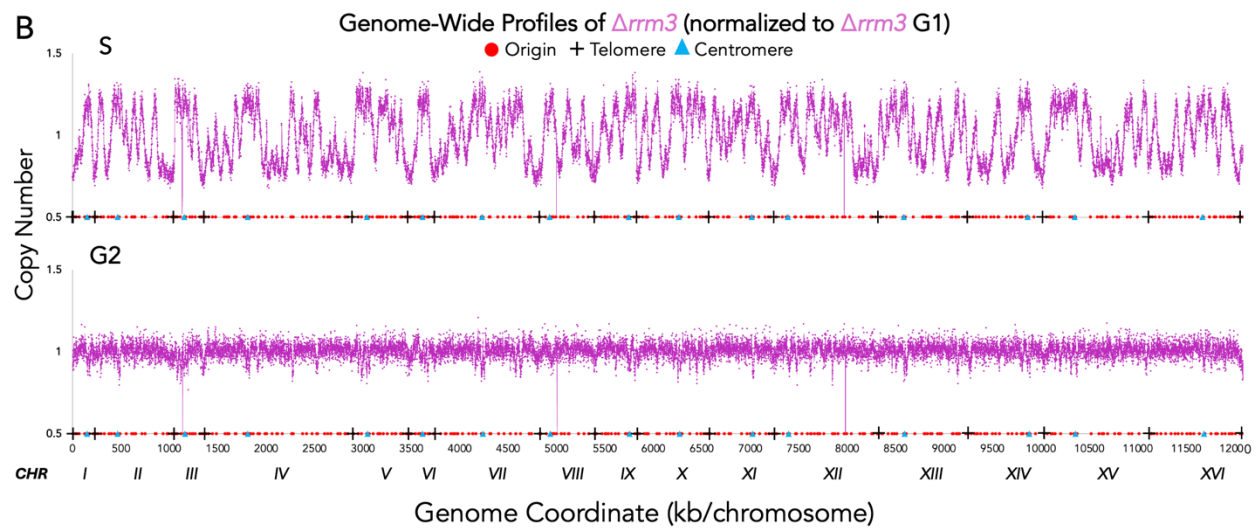
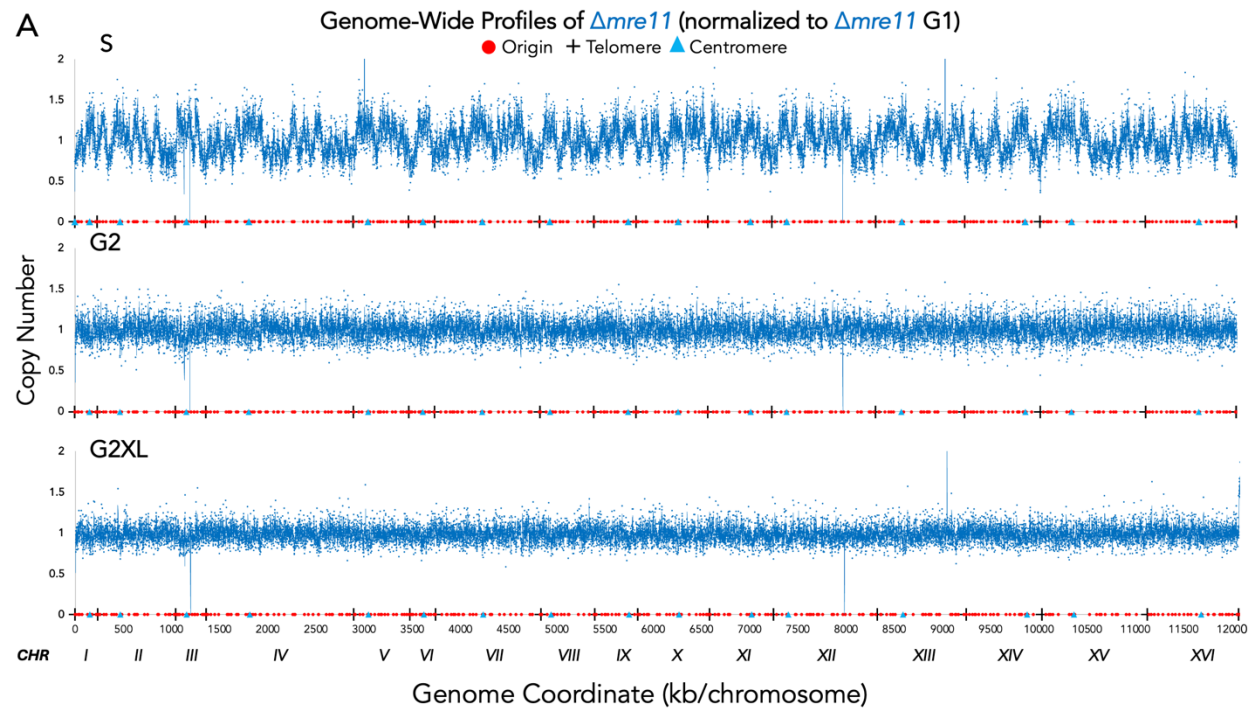
Appendix Table 3.2

Outcome (after 24hrs)	WT	$\Delta rad51$	$\Delta mre11$	$\Delta rrm3$
*Both (viable)	218	114	156	71
*Only One (not viable)	12	3	44	3
Neither (both dead)	5	5	19	0
Isolated cells that never replicated for separation				
Single eventually grew	23	41	45	41
Single never grew	57	37	39	22
n	315	200	303	137

Appendix Table 3.3

Observed	WT	$\Delta rad51$	$\Delta mre11$	$\Delta rrm3$
Growth (both + single grew)	241	155	201	112
Dead (single never grew + neither grew)	62	42	58	22
Expected (to WT proportion)	WT	$\Delta rad51$	$\Delta mre11$	$\Delta rrm3$
Growth (both + single grew)	0.86	169.56	222.93	115.34
Dead (single never grew + neither grew)	0.22	43.62	57.35	29.67
n	303	197	259	134
X ²		0.2523	0.1413	0.1493

Table 3.2 and 3.3. Complete data set for single-cell viability assay and chi-square analysis for statistical significance for mechanical sensitivity during assay.



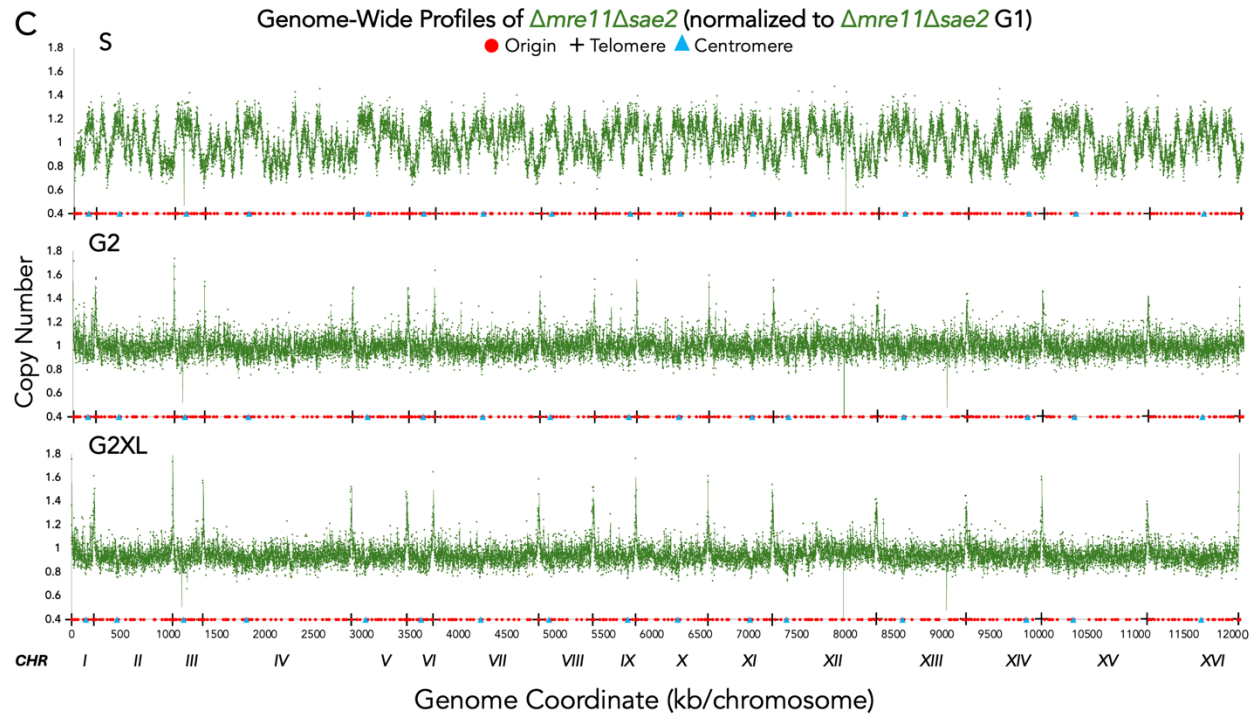
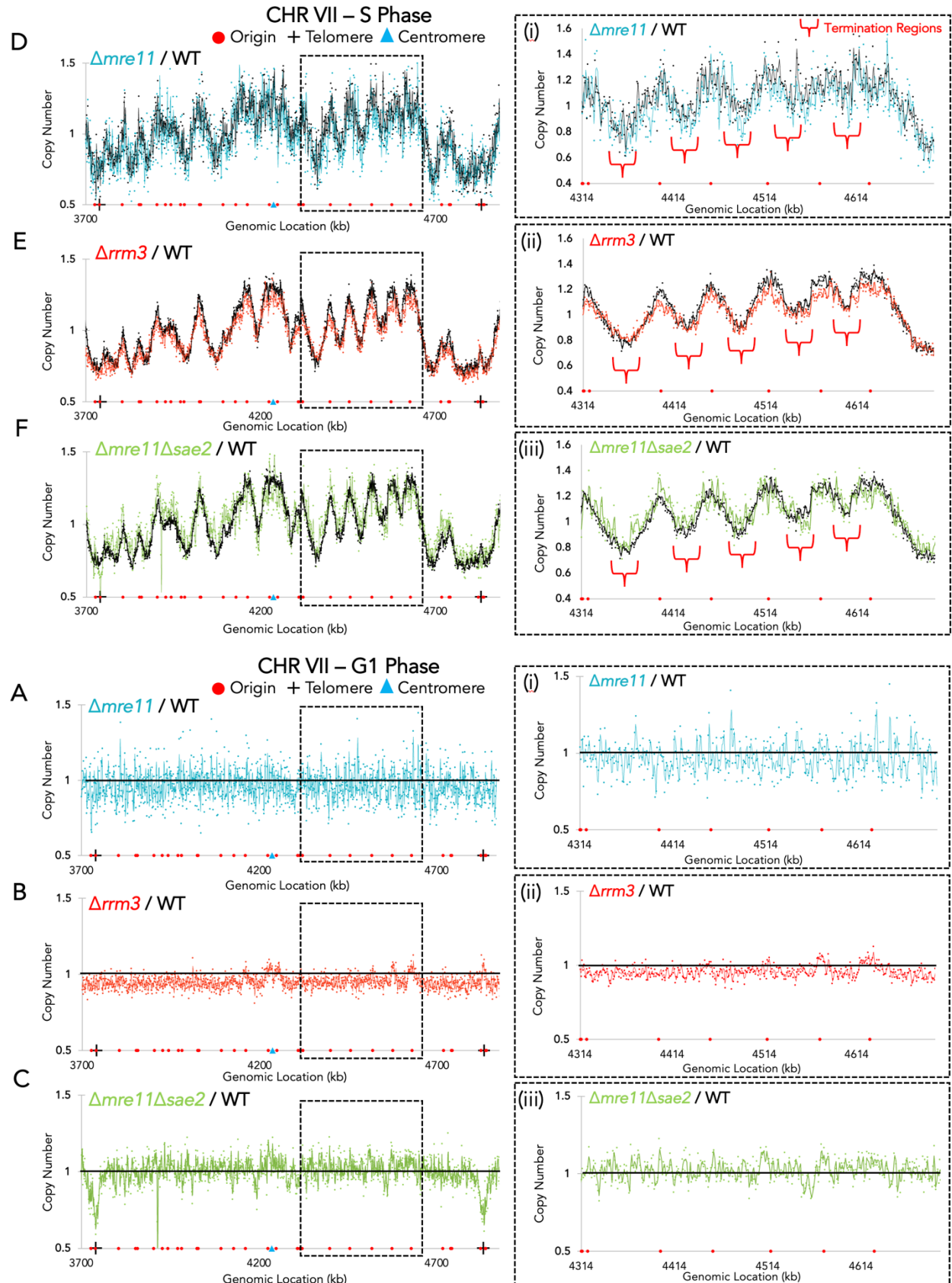


Figure 4.1 Genome-wide replication profile of mutants normalized to its own G1. Whole genome copy number profiles of (A) $\Delta mre11$, (B) $\Delta rrm3$ and (C) $\Delta mre11\Delta sae2$ cells. For each strain, S, G2, and G2XL copy number profiles were normalized to the corresponding G1 population from the same mutant background to control for within-strain cell cycle variation. Sharp peaks or troughs consistently observed across all phases likely reflect mapping or sequence-related artifacts, including deletions at the LEU, URA, and HIS loci introduced during strain construction.



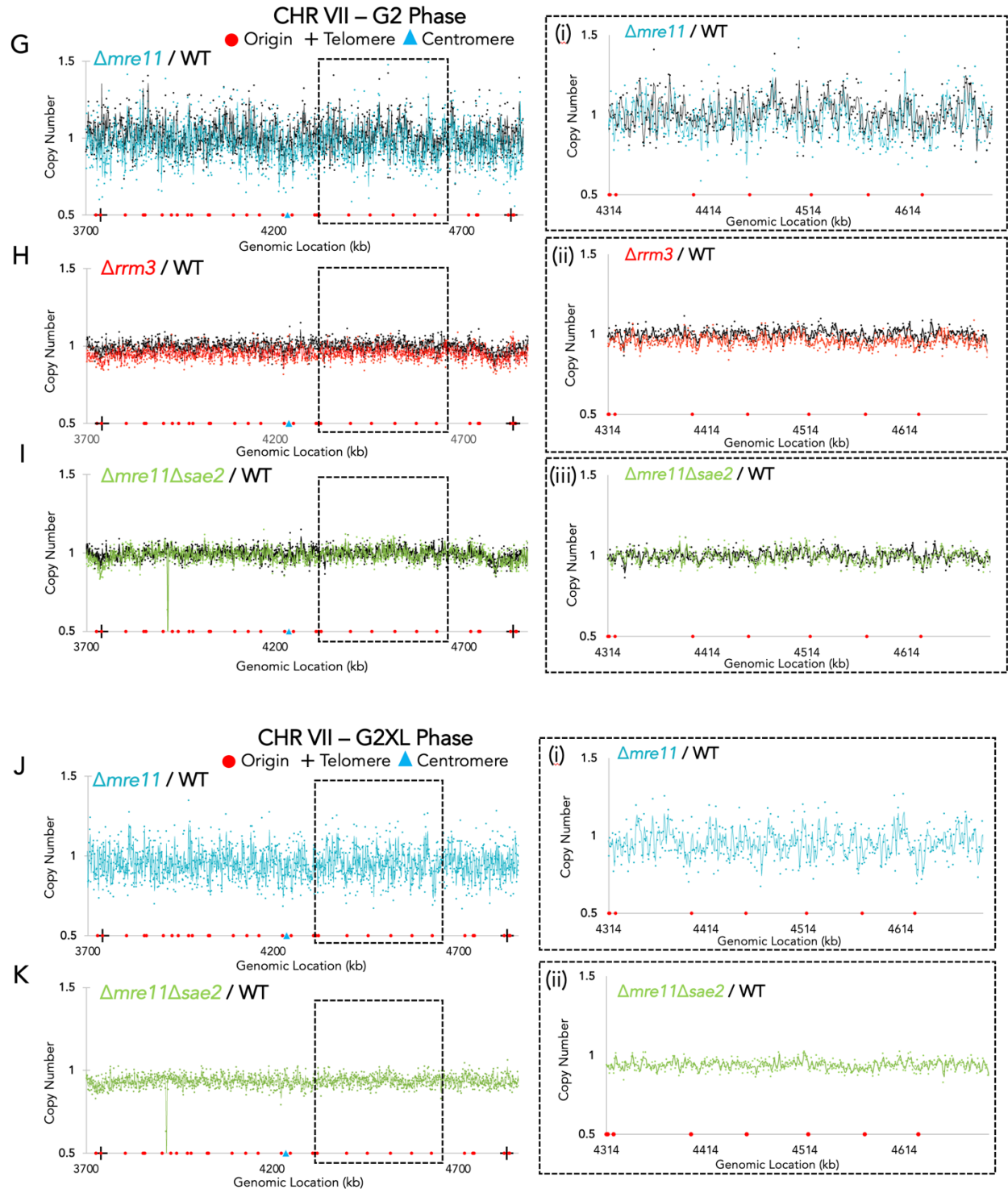
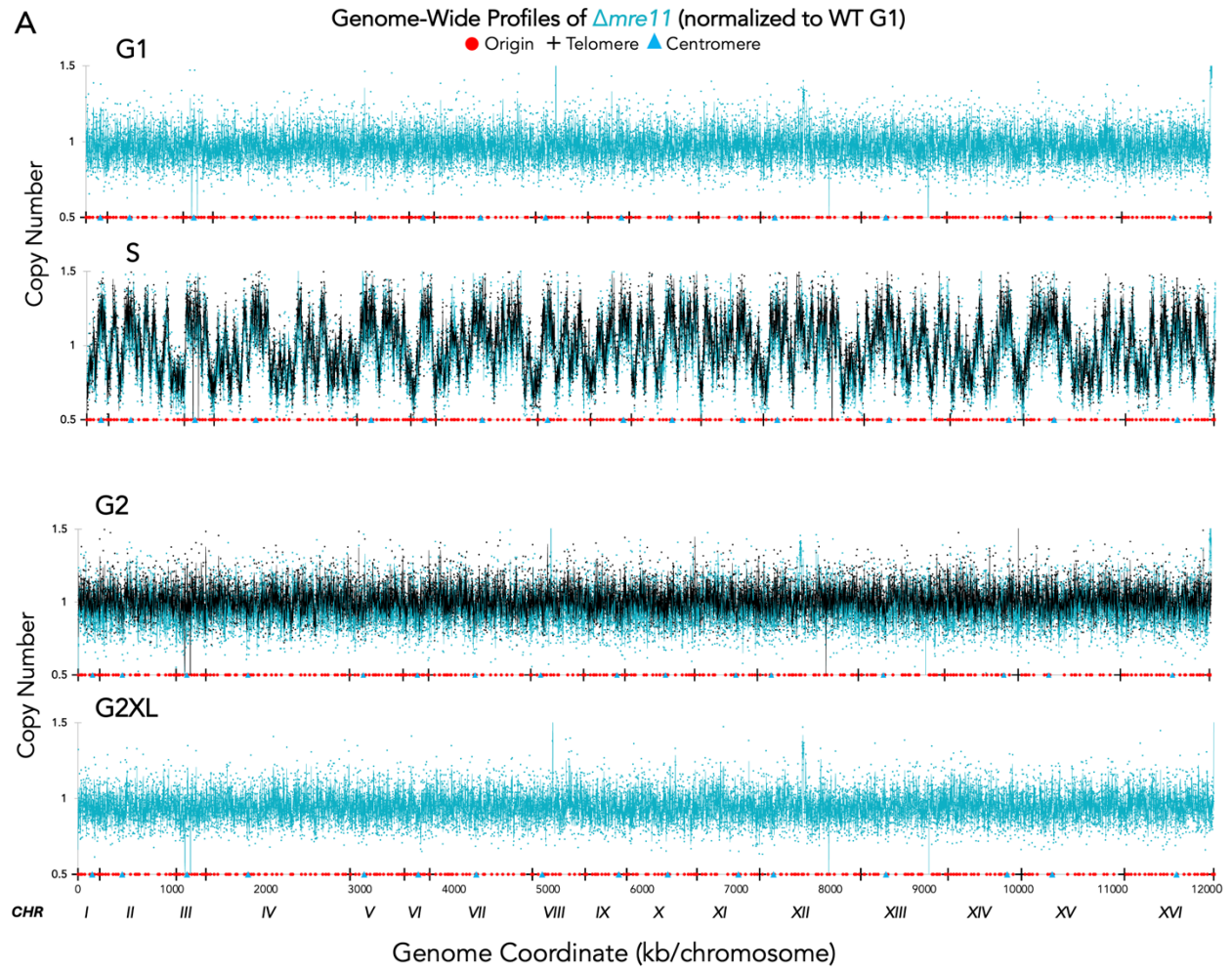
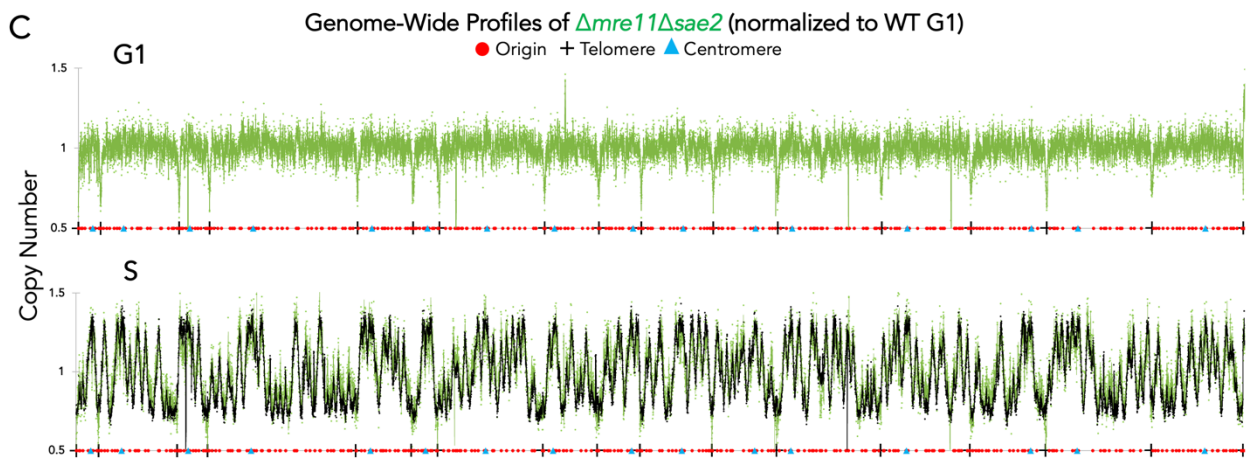
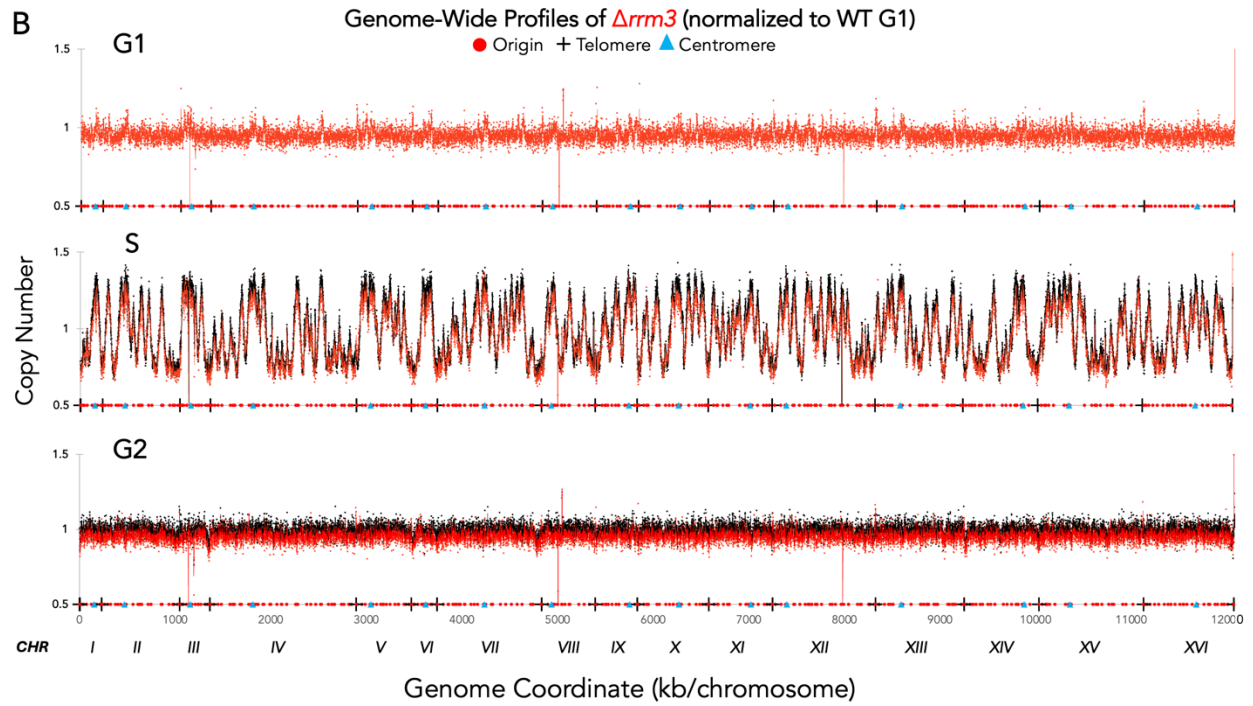


Figure 4.2 Chromosome VII copy number profiles across cell cycle phases in $\Delta mre11$, $\Delta rrm3$, and $\Delta mre11\Delta sae2$ mutants. (A-C) G1 profiles display baseline copy numbers, consistent with non-replicating or fully replicated genomes. The overlaid wild type (black line) profile is

normalized to its own G1 and therefore appears as a copy number of 1. (D-F) S phase profiles display characteristic replication timing patterns, including elevated copy number at early replicating regions and reduced copy number between adjacent origins. (G-I) G2 populations display relatively uniform copy number, consistent with the completion of DNA replication. (J-K) G2XL cells exhibit largely uniform copy number profiles across chromosome IV, consistent with fully replicated genomes despite increased cellular DNA content and enlarged cell size. (i-iii) Expanded views of the highlighted regions corresponding to *Δmre11*, *Δrrm3*, and *Δmre11Δsae2*. Red brackets represent termination regions.





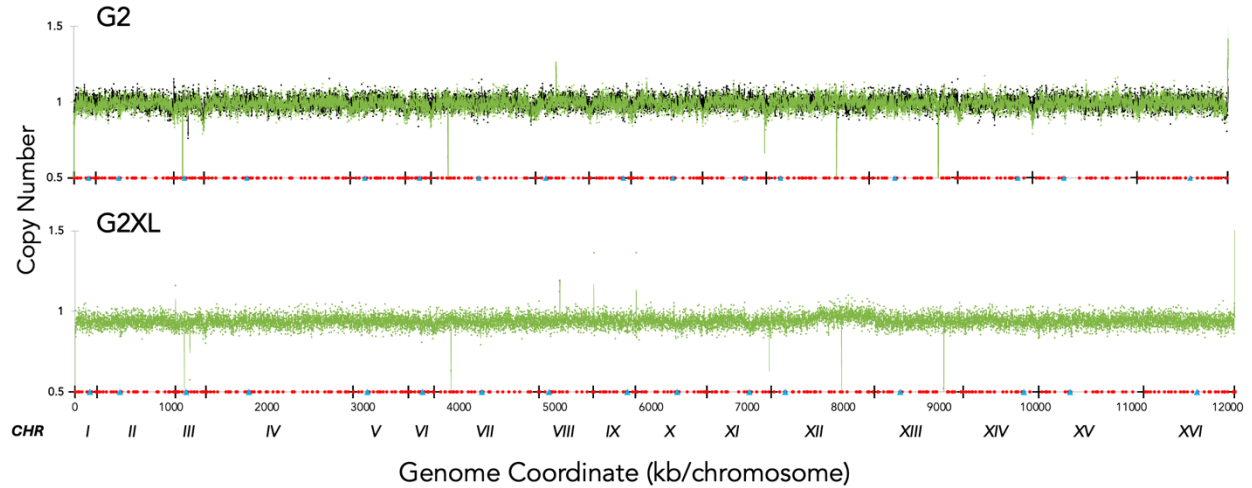


Figure 4.3 Genome-wide replication profile of mutants normalized to wild type G1. Whole genome copy number profiles of (A) $\Delta mre11$, (B) $\Delta rrm3$ and (C) $\Delta mre11\Delta sae2$ cells. Copy number profiles from G1, S, G2, and G2XL populations for each mutant, normalized to wild type G1 allowed for direct comparison with the wild type replication profile. Sharp peaks or troughs consistently observed across all phases likely reflect mapping or sequence-related artifacts, including deletions at the LEU, URA, and HIS loci introduced during strain construction.