Interstitial Telomere Sequences Disrupt Break Induced Replication

Elizabeth Anne Stivison

Submitted in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy
under the Executive Committee
of the Graduate School of Arts and Science

COLUMBIA UNIVERSITY

2019

© 2019
Elizabeth Anne Stivison
All Rights Reserved

ABSTRACT

Interstitial Telomere Sequences Disrupt Break Induced Replication

Elizabeth Anne Stivison

Break Induced Replication (BIR), a mechanism by which cells heal one-ended double-strand breaks, involves the invasion of a broken strand of DNA into a homologous template, and the copying of tens to hundreds of kilobases from the site of invasion to the telomere using a migrating D-loop. Here we show that if BIR encounters an interstitial telomere sequence (ITS) placed in its path, BIR terminates at the ITS 12% of the time, with the formation of a new telomere at this location. We find that the ITS can be converted to a functional telomere by either direct addition of telomeric repeats by telomerase, or by homology-directed repair using natural telomeres. This termination and creation of a new telomere is promoted by Mph1 helicase, which is known to disassemble D-loops. We also show that other sequences that have the potential to form new telomeres, but lack the unique features of a perfect telomere sequence, do not terminate BIR at a significant frequency in wild-type cells. However, these sequences can cause chromosome truncations if BIR is made less processive by loss of Pol32 or Pif1. These findings together indicate that features of the ITS itself, such as secondary structures and telomeric protein binding, pose a challenge to BIR and increase the vulnerability of the D-loop to dissociation by Mph1, promoting telomere formation at the site.

Table of Contents

List of Figures

Glossary of Abbreviations and Terms Used

Term	Meaning
ALT	Alternative lengthening of telomeres. BIR-like method used by telomerase survivors to maintain telomere length.
BIR	Break induced replication
dHJ	Double holiday junction
D-loop	Displacement loop. Created when single-stranded DNA invades a duplex and binds to the homologous sequence, and displacing one strand.
DSB	Double-strand break
DSBR	Double-strand break repair
dsDNA	Double-stranded DNA
G4	G quadruplex. A secondary helical structure formed in some G-rich single-strand DNA. Can be stabilized by hoogsteen base pairing between four guanines.
Gal	Galactose. Used to induce expression of proteins under the control of a galactose-inducible promoter
GC	Gene conversion, DNA repair of two-ended breaks by copying sequence from homologous template
HDR	Homology directed repair
HO	Enzyme used to induce DSB. In this system under control of galactose promoter
HO-cs	Cutsite for HO
HR	Homologous recombination
ITS	Interstitial telomere sequence. A tract of telomeric DNA sequence outside the telomere. The ITS inserted in this thesis is $(TGTGTGGG)_8$.
Mph1	Helicase involved in disassembling D-loops. Human homolog: FANCM
NHEJ	Non-homologous end joining
Pif1	Helicase involved in telomere maintenance, BIR processivity, and DSB repair signaling. Human homolog: PIF1
Pol delta	Polymerase involved in lagging strand synthesis and DNA repair. Primary polymerase used during BIR.
Pol32	Subunit of pol delta required for processivity in BIR. Human homolog: POLD3
SDSA	Synthesis-dependent strand annealing
SSA	Single-strand annealing
SSB	Single-strand break
ssDNA	Single-stranded DNA
Telomerase	The RNA-protein complex that can add telomeric repeats directly to DNA ends.
Telomerase survivor	Cells that maintain their telomeres in the absence of telomerase, typically using a recombination-based method. In yeast, can be type I or II

Telomere	Sequences at the ends of all eukaryotic chromosomes that protect the DNA from the end replication problem, and from being recognized as a DSB.
TG or telomeric repeats	Telomeric sequence. In yeast $TG_{(1-3)}$
TLC1	The RNA template component of telomerase. Human homolog: hTERC
Type I survivors	Telomerase survivors that maintain their telomeres by expansion of Y' elements.
Type II survivors	Telomerase survivors that maintain their telomeres by expansion of telomeric sequences.
Y' element	Sequences on many yeast chromosomes just internal to the telomeric repeats. Expanded in type I telomerase survivors.

Acknowledgements

I owe so much to my mentor and advisor Lorraine. Her broad wealth of knowledge as well as kindness and compassion for the people working in her lab make her an ideal role model and person to work for. She has recognized with honesty both my strengths and weaknesses, and worked with me to help me grow as a scientist and person in the years I spent in her lab.

I'd also like to thank:

- My advisory committee, Richard Baer, Luke Berchowitz, and Max Gottesman. Your enthusiasm about my work, encouragement, and excellent feedback made me look forward to my committee meetings. I am so lucky to have three scientists like you as mentors.
- All the members of the Symington lab: Amr Al-Zain, Roberto Donnianni, Valerie Garcia, Robert Gnügge, Mike Kimble, Lea Marie, Julie Oh, and Tai-Yuan Yu. You made every day a day to look forward to. You're all smart, kind, funny, and supportive. You give me useful, insightful feedback on my work, and you laugh at my dumb jokes. What more could anyone want?
- My roommates Marina Ermakova and Katherine Xu for putting up with me for 7 years and being the absolute best people to come home to at the end of the day.
- The students in my cohort: Morgan Furze, Sam Lee, Diego Scerbo, Katherine Xu, and Changyu Zhu, for being an endless source of food and camaraderie.
- Debra Wolgemuth for always keeping me on track and encouraging me when things were difficult, as well as being part of my defense.
- Theresa Swayne and Mimi Shirasu-Hiza for your support and understanding during my ups and downs, and for your guidance and honesty about both science and life.

- The members of Women in Science at Columbia, for giving me a great community full of smart, motivated, and friendly scientists.
- Liza Pon and the members and friends of the Pon lab: Dana Alessi, Istvan Boldogh, Helen Causton, Alison Cook, Lesley Emtage, Enrique Garcia, Wolfgang Pernice, and Jason Vevea. I'd especially like to thank Ryo Higuchi-Sanabria and Cierra Sing for your constant support through everything.
- Zachary Corter, Leslie De Pena, Alex Sosa, Sara Sternglass, and all the staff at the IHN.
- The Ghosh and Dworkin labs for camaraderie and support (and sometimes reagents).
- Timmy LaRocca, for encouraging me in both science and music, and showing me the joy and creativity that scientists can have.
- My undergraduate mentor, Donald Oliver, and my professors at Wesleyan University for leading me to molecular biology and caring about my education.
- Marianne James who mentored me at MGH, and set a perfect example of dedication to hard work, reliable results, mixed with a sense of humor and kindness.
- All my friends in and out of Columbia who kept me going and comforted me when things went wrong, and celebrated with me when things went well. Besides the science, the biggest thing I learned during my time in grad school was the value and importance of friendship. You make life beautiful!
- My family. It's silly to even try to put into words what I'm thankful for.

Chapter 1: Introduction

1-1 DNA Damage

DNA damage occurs frequently throughout cell growth and division, and can be caused by external factors such as radiation, and internal factors such as free radicals produced by metabolism. Damage can also be caused by DNA replication stress, such as depleted dNTP pools or activation of oncogenes causing cells to proceed into mitosis before the completion of DNA replication. The cell may encounter a wide variety of DNA damage, including damaged bases, abasic sites, protein adducts, crosslinked strands of DNA, single strand breaks, and double-strand breaks (DSBs), each with their own set of risks to the cell. By some estimates, human cells may have to deal with 10^5 DNA lesions every day (Hoeijmakers 2009).

Mishandling of DNA damage by the cell can threaten the integrity of the genome and cause genomic instability. Because of the danger of unrepaired and incorrectly repaired DNA damage, cells must repair all damage quickly and accurately. To achieve this, cells have evolved mechanisms specifically to deal with all types of DNA damage. The importance of these pathways can be seen in human diseases, as defects in DNA repair contribute to many human pathologies. Neurodegenerative diseases like Parkinson's and Huntington's, neurological disorders like Ataxia Telangiectasia and Xeroderma Pigmentosa, developmental disorders like Trichothiodystrophy, most cancers, and even normal and premature aging all have links to DNA repair, or are caused by defects in DNA repair (Pinto et al. 2013; Long et al. 2017; Colnaghi et al. 2011; Welcsh and King 2001; Kulkarni and Wilson 2008; Hoeijmakers 2009). DSBs are particularly toxic to a cell, and will be the focus of this thesis. If DSBs are left unrepaired, they can result in cell death, and if repaired incorrectly, they can result in mutations, from point mutations to genome wide rearrangements that can fundamentally alter the genome and its function.

1-2 Checkpoint Activation

DNA damage checkpoints exists at various stages of the cell cycle, which when activated arrest cell growth and division to allow time for the damage to be corrected (Ciccia and Elledge 2010). During these checkpoint arrests, dedicated DNA damage machinery is activated to heal the damage (Figure 1-2).

Cells can activate checkpoints at three times during their cell cycle depending on when the damage is found and what type of damage is found. The G1/S checkpoint which will prevent the cell from beginning the DNA synthesis of S phase and in yeast, can be activated by loss of mtDNA (Crider et al. 2012) or UV irradiation that occurs in G1 (Gerald, Benjamin, and Kron 2002). The intra S phase checkpoint, which will slow DNA replication during S phase, can be activated in response to replication stress including depleted dNTP pools, polymerase inhibition, and damaged forks (Zou 2013). The G2/M checkpoint will hold cells in mitosis preventing anaphase from occurring until the damage is repaired (Finn, Lowndes, and Grenon 2012). Most of the DNA repair discussed in this thesis occurs during this checkpoint.

In order for the checkpoints to be activated and repair to happen, damage must first be sensed. DSBs in particular can be sensed in two ways: sensing broken ends, or sensing single stranded DNA (ssDNA). The signaling is mediated by the phosphatidylinositol 3-kinase-like protein kinases (PIKKs) family of proteins, which includes Tel1 (ATM in mammals), Mec1 (ATR in mammals) and DNA-PK which only exists in higher eukaryotes, and is involved in regulating end-joining by its interaction with Ku proteins. End joining will be discussed briefly in the next section but will not be the focus of this thesis.

In the first case, sensing DNA ends, Tel1 is recruited to a DSB by the MRX complex, explained in more detail in the next section. Tel1 is then able to set off a cascade of signaling. In the second case, sensing ssDNA, ssDNA is created after the cell has begun processing the

DSB, and this ssDNA is recognized by Mec1 which can in turn set off its signal cascade. Both pathways converge on the activation of Rad53 (CHK2 in mammals) (Oh and Symington 2018). Activation of Rad53 has many effects, including upregulation the dNTP synthesis. It is this effect of Rad53 that makes the protein essential to cells, but Rad53 can be deleted in cells by compensating for this role by also deleting *SML1* which normally down regulates the ribonucleotide reductase pathway required for dNTP synthesis.

The phosphorylation and activation of Pif1 is also a hallmark of Rad53 activation. Pif1 removes telomerase from DSBs so that they can be correctly repaired (Schulz and Zakian 1994). Pif1 has a mitochondrial form as well that functions in maintenance of mtDNA. The two forms of Pif1 use different start codons and can be separated experimentally by mutating one or the other start codons (Wellinger and Zakian 2012; Geronimo and Zakian 2016; Schulz and Zakian 1994),

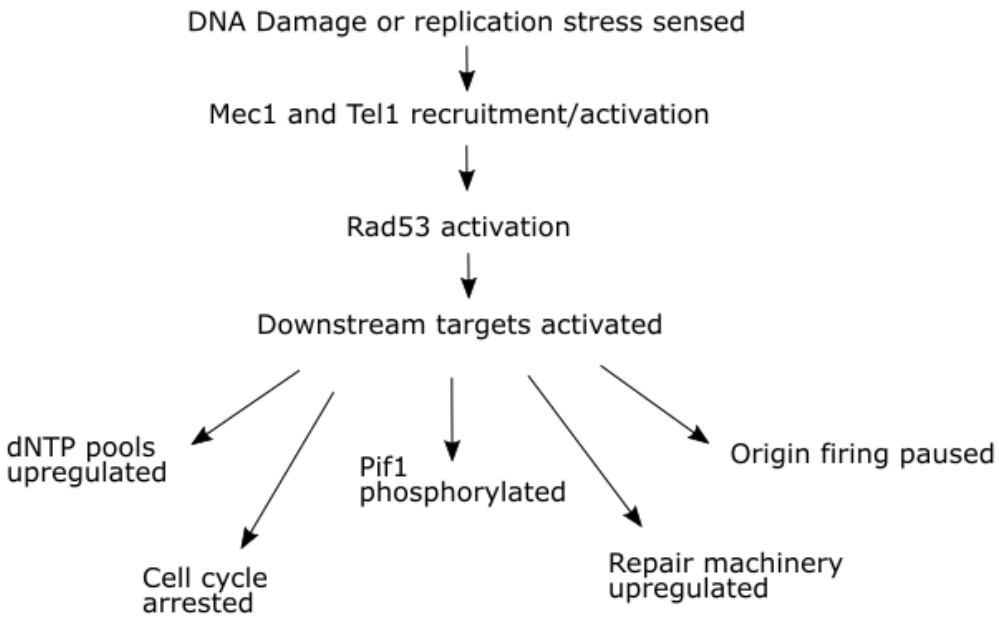


Figure 1-2 Schematic of the DNA Damage Response

Showing cascade of events from sensing damage to downstream effects.

1-3 Homologous Recombination

1-3.1 NHEJ vs HR

Once cells encounter DNA damage, they can use one of two main pathways for repairing DSBs: non-homologous end joining (NHEJ), or homologous recombination (HR). NHEJ involves binding of the ends by Ku proteins, and the direct ligation of broken DNA ends by the DNA ligase IV complex and is more common in G1, and also more common in higher eukaryotes (Chiruvella, Liang, and Wilson 2013; Daley et al. 2005). NHEJ will not be addressed in depth in this thesis.

Homologous recombination, aspects of which will be the focus of this thesis, involves the use of a homologous DNA sequence as a template for repair, and is more common in S and G2, when identical sister chromatids are available as homologous templates, and resection machinery is active (Krogh and Symington 2004; Symington and Gautier 2011). Most of the details of these pathways were discovered in yeast, and the yeast proteins will be referred to in this thesis unless specified.

1-3.2 Steps of Homologous Pathways

Homologous recombination can be further divided into sub-pathways, classical double strand break repair (DSBR), synthesis dependent strand annealing (SDSA), single strand annealing (SSA), and break-induced replication (BIR). All of these HR processes can produce different outcomes, but they all begin with end resection, which creates a 3’ overhang on either side the break site that can then be used in the next step to search for a homologous template for repair (Sun, Treco, and Szostak 1991; White and Haber 1990). The creation of the 3’ overhang also commits the cell to HR rather than NHEJ (Symington and Gautier 2011).

Resection begins with recruitment of the MRX/N complex, Mre11, Rad50, and Xrs2 (Nbs1 in humans) to the break. MRX, when activated by Sae2, initiates resection by using the endonuclease activity of Mre11 to create a nick some distance internal to the break and resecting towards the break using the 3' - 5' exonuclease activity of Mre11 (Lisby et al. 2004; Mimitou and Symington 2009; Garcia et al. 2011; Cannavo and Cejka 2014). Exo1 and/or Dna2/Sgs1 continue resecting the 5' terminated strand for several kb away from the break site (Mimitou and Symington 2008; Zhu et al. 2008; Gravel et al. 2008). These single stranded 3' overhangs that are left after resection are then coated in replication protein A (RPA) to protect the strands and prevent secondary structure formation (Symington 2016).

In the absence of Mre11 nuclease, at clean breaks lacking protein adducts, resection is slowed though not abolished, indicating that Exo1 and Dna2/Sgs1 can substitute for Mre11. A more severe delay is observed in the absence of. MRX because of MRX is important to recruit the long range resection machinery (Gobbini et al. 2018; Moreau, Morgan, and Symington 2001; Shim et al. 2010). At dirty breaks, with protein bound ends, such as Spo11 bound breaks in meiosis, loss of any of the MRX components, Mre11 nuclease activity, or Sae2, completely blocks resection (Mimitou and Symington 2009).

After end resection, the three HR pathways begin to diverge (Figure 1-3.2). Here, SSA can only occur if there are repeated sequences on either side of the break that are exposed during resection. In this case Rad52 can mediate the direct annealing of the complementary ssDNA regions corresponding to the repeats. Nonhomologous tails are then removed by the Rad1/Rad10 flap endonuclease complex and the remaining gaps can be filled in in by DNA synthesis and ligation (Fishman-Lobell and Haber 1992; Ivanov and Haber 1995). Because one of the repeats and the intervening sequence are deleted, the SSA mechanism is always mutagenic. SSA is independent of Rad51.

DSBR and SDSA on the other hand proceed into homology search and strand invasion, which requires the loading of Rad51 -the eukaryote homologue of RecA- by Rad52 onto the ssDNA. This DNA/Rad51 filament can then search for a homologous duplex of DNA, using the ability of Rad51 to match triplets of DNA (Lee et al. 2015). In addition to its role in pairing homologous DNA, Rad51 also has strand exchange activity so once matched, the broken 3' end will displace one strand of the homologous duplex creating a displacement loop, or D-loop. This process is known as strand invasion (Haber 2018). DNA synthesis can then be initiated from the newly paired 3' end. Any non-homologous DNA on the 3' end of the invading strand must be removed by Rad1/Rad10, or the proofreading functions of the polymerase before new DNA synthesis can begin (Fishman-Lobell and Haber 1992; Jin et al. 2005; Pâques and Haber 1997).

In DSBR, the other 3' end of the broken DNA molecule finds the corresponding homology in the displaced strand of the D-loop so that both ends of the break are interacting with the homologous duplex (Szostak et al. 1983). This creates a double Holliday junction (dHJ) (Holliday 2007) and can result in crossover or non-crossover products depending on how the junctions are resolved or dissolved. Non-crossovers can be formed by "dissolving" the dHJ, without cleaving, where the two Holliday junctions are moved closer and closer together by the helicase Sgs1, until the crossed strands are released. The dHJ can also be cleaved by resolvases such as Mus81- Mms4 or Yen1, to generate crossover or non-crossover products depending on which strands of the dHJ are cleaved (Wyatt and West 2014). A crossover outcome may be mutagenic, or may be the desired outcome. In meiosis for example, crossovers are favored to create genetic diversity.

In SDSA in contrast to DSBR, only one 3' end of the break is involved in a strand invasion event and DNA synthesis. As the name implies, after the synthesis of DNA, the new strand is dissociated by Mph1 helicase (Prakash et al. 2009) and reannealed to the other side of the break. This reannealing is carried out by the ssDNA annealing activity of Rad52 (Sugiyama,

New, and Kowalczykowski 1998; Shinohara et al. 1998; Morrical 2015). The other end of the break is potentially prevented from also invading and forming a dHJ by the anti-Rad51 role of Srs2 (Ira et al. 2003). This SDSA process can only result in non-crossover products, since the second end is never involved in repair and no crossover cleavage options are available to the cell.

In addition to the DNA damage situations described above, cells may also encounter DSBs that arise with only one end, that still need to be repaired. A method to repair one-ended DSBs is Break Induced Replication, or BIR.

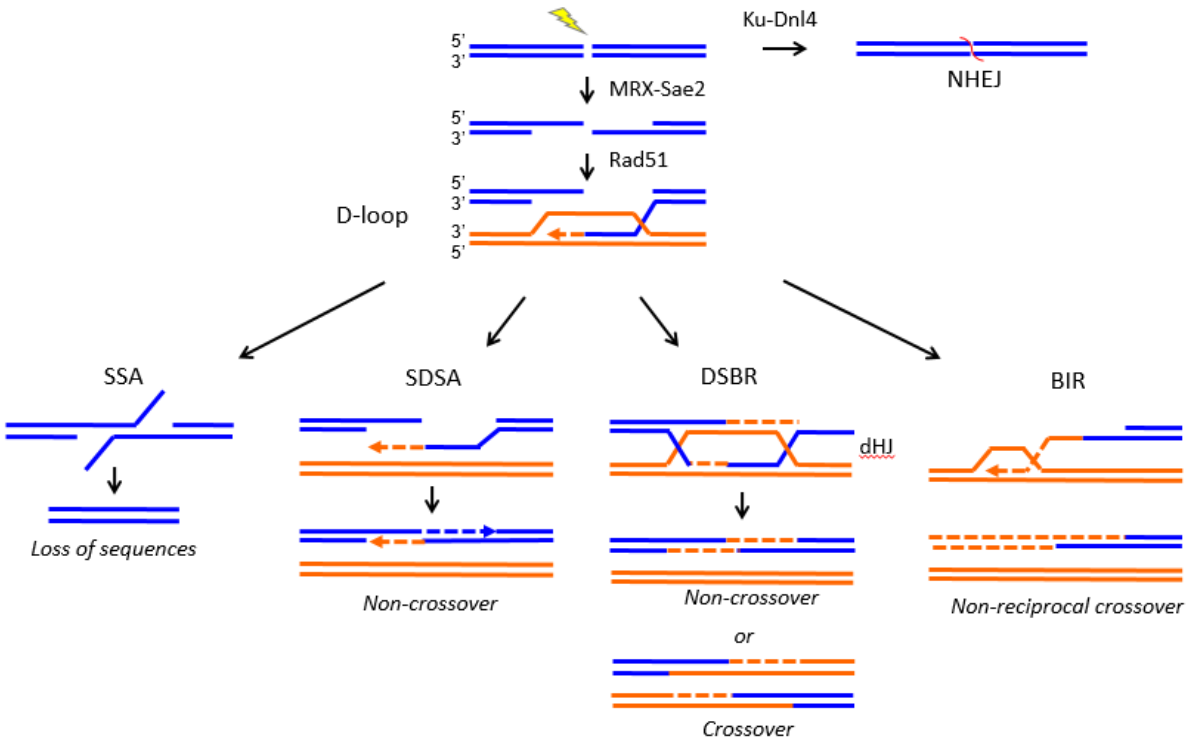


Figure 1-3.2 Outcomes of Homologous Recombination

Shows schematic of the different pathways a DSB may be healed, starting with the creation of a DSB (lightning bolt). Image shows NHEJ as one option (top right) or resection started by MRX-Sae2 (top middle). Following resection, the four different HR pathways with which a break may be healed are shown. Left to right: SSA, showing the annealing of complementary sequences on either side of the break and loss of sequences in between, SDSA showing dissociation of the D-loop and reannealing to the other side of the break, DSBR with formation of dHJ and subsequent crossover or non-crossover outcomes, and BIR showing non-reciprocal crossover produced by copying to the end of the donor chromosome. Image modified from Ho et al. (Ho et al. 2010).

1-4 Break Induced Replication (BIR)

1-4.1 One-Ended Breaks

One-ended DSBs may arise through eroded telomeres, or may be formed during replication by a regressed replication forks or replication through a single stranded nick, or loss of one end of a two-ended DSB (Figure 1-4.1). One-ended DSBs can be repaired similarly to the early steps of HR: resection to create a 3' overhang, strand invasion at a homologous DNA sequence, and DNA synthesis. But because there is no second end of the break to complete the repair process, DNA synthesis can continue through the end of the chromosome, or until an approaching replication fork collides with the D-loop. This extensive DNA synthesis used to repair a one-ended DSB is called Break Induced Replication, or BIR.

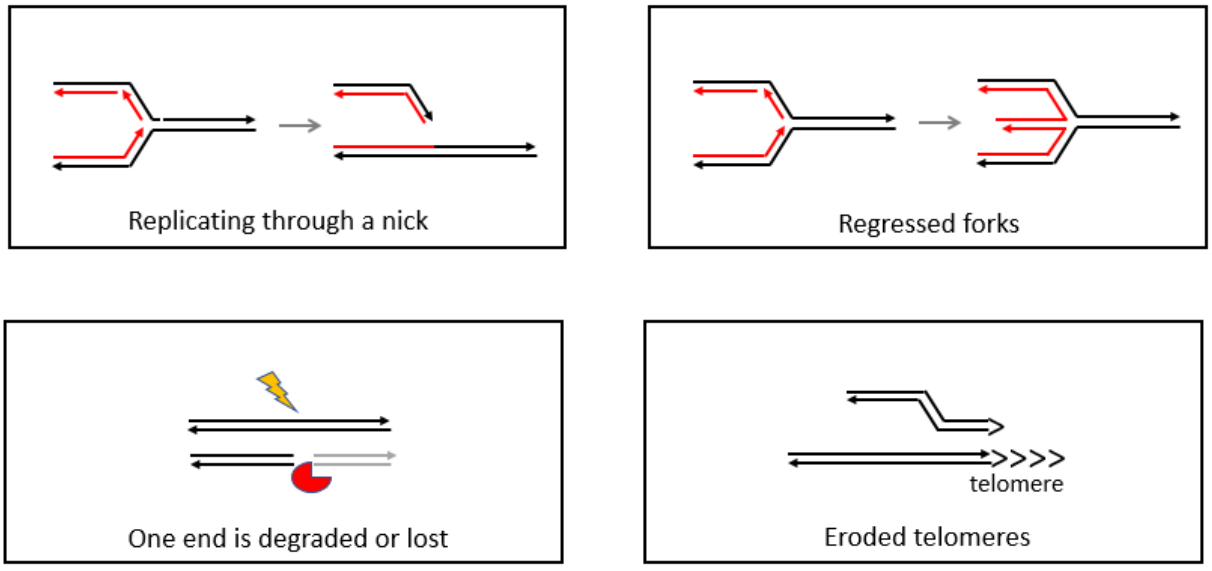


Figure 1-4.1 Schematic of Potential Sources of One-Ended Breaks

Schematic Includes replication through a nick, regressed forks, Degradation or loss of one end of break, and eroded telomeres.

1-4.2 History and Relevance of BIR

BIR was first discovered in phages as recombination dependent DNA duplication where it was seen that a break in the DNA could induce replication of the entire phage genome (Meselson and Weigle 1961; Mosig 1987). It was also recognized as “break-copy” as a mechanism to produce chromosome fragments in yeast (Morrow, Connelly, and Hieter 1997; Kreuzer 2000). It has since been identified as a repair mechanism (Bosco and Haber 1998) and studied most extensively in budding yeast *S. cerevisiae*.

Two hallmarks of the BIR process have been shown to be the dependency on Pol32 (Lydeard et al. 2007; Deem et al. 2008; Smith, Lam, and Symington 2009) -a subunit of polymerase delta that increases its processivity (Burgers and Gerik 1998)- and to a lesser degree, its dependence on the Pif1 helicase (Saini et al. 2013; Wilson et al. 2013; Buzovetsky et al. 2017; Vasianovich, Harrington, and Makovets 2014). Although *POL32* is not essential for viability of budding yeast, its homologues are required for proliferation of fission yeast and for proper development of animals (MacNeill et al. 1996; Murga et al. 2016).

A similar process to BIR has been identified in human cells, where it was shown to be responsible for certain duplications found in cancer cell lines (Costantino et al. 2014), and for replication of fragile sites during early mitosis (Minocherhomji et al. 2015). This mammalian BIR-like process is dependent on the POLD3, the human homologue of Pol32. Recently, Drosophila have also been show to use a BIR-like mechanism dependent on Pol32 and Pif1 to copy more than a megabase of DNA to heal one ended chromosome breaks arising from the breakage of a dicentric chromosome (Bhandari, Karg, and Golic 2019).

One particular case of single ended DSB repair via BIR, is maintenance of eroded telomeres by a mechanism known as alternative lengthening of telomeres, or ALT. ALT will be discussed in detail in section 1-6 of this chapter.

1-4.3 Models Used to Study BIR

In order to study BIR, cells need to reliably encounter single-ended DSBs, which means researchers must create situations where single-ended DSBs are formed. Over the years many creative systems have been invented to solve this problem and study BIR. The systems can be categorized as either chromosome fragment and plasmid-based systems, or chromosomal systems (Figure 1-4.3).

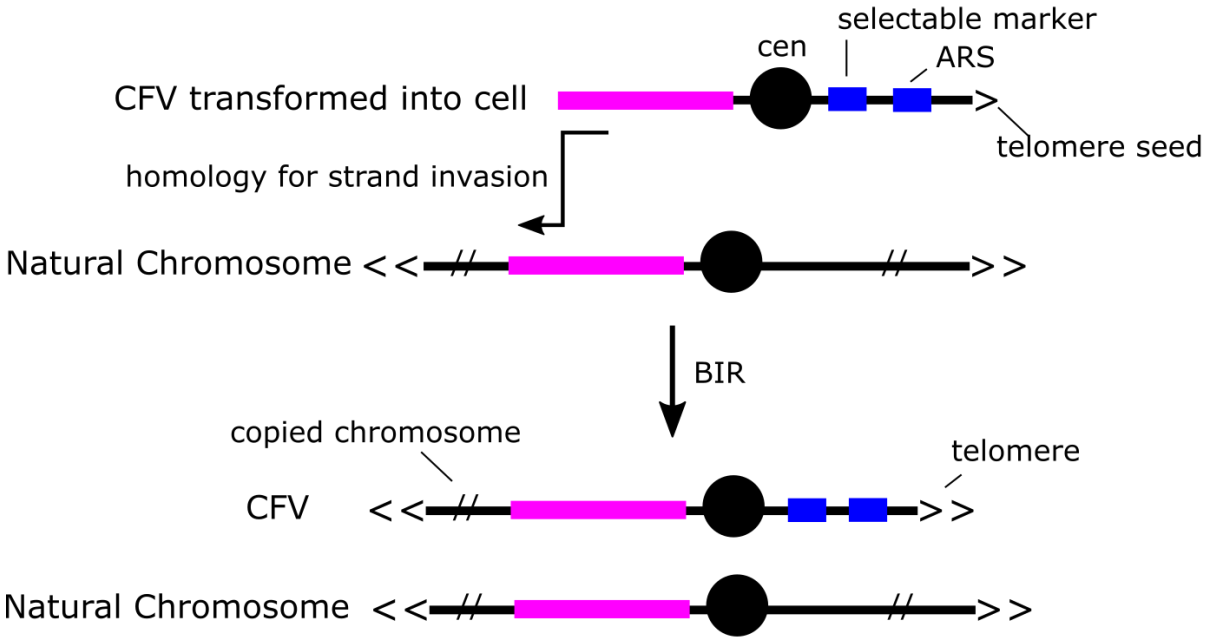
Dunn et al. (1984) were the first to show a BIR like event in eukaryotic cells. They showed that upon transformation, a plasmid fragment containing homology to subtelomeric Y' elements could obtain a full telomere by recombination (Dunn et al. 1984). A similar method was used to specifically study BIR by Morrow et al. in 1997 (Morrow, Connelly, and Hieter 1997). In this study, a chromosome fragment containing a centromere, homology to chromosome III on one end, and homology to a Y' element on the other was transformed into yeast. From this transformation they recovered a stable chromosome product that had duplicated all sequences downstream of the site of homology on chromosome III, as well as formed a telomere on the other end. This product was created by strand invasion and "break-copy" replication, or BIR.

The plasmid/fragment assay was improved upon and used by our lab in 2004 (Davis and Symington 2004). In this case, the chromosome fragment still contains homology to chromosome III, but contains a tract of telomeric repeats, $TG_{(1-3)}$ instead of the subtelomeric Y' element. This can then undergo de novo telomere addition at that end instead of recombination which improves efficiency and specificity of the experiment by requiring recombination at only one end.

Plasmid based assays present some problems, such as transformation efficiency so chromosomal systems were developed. In 1996, Malkova et al. investigated the repair of a DSB

BIR experimental systems

A. Example chromosome fragment vector (CFV) system



B. Example chromosomal system

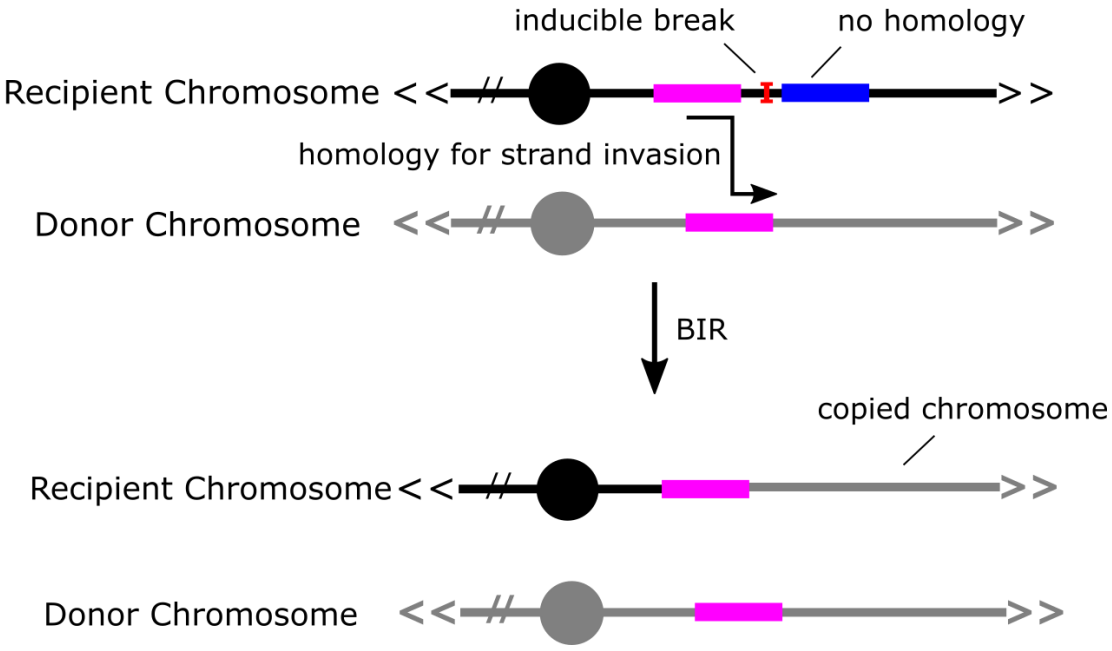


Figure 1-4.3 Schematic of BIR Experimental Systems

A. Shows an example of a chromosome fragment vector system to study BIR. The purple region indicates homology used for strand invasion, the carrots on the other end represent a telomere seeding sequence. The products created by BIR are shown below, and show the copying of the donor chromosome onto the fragment. B. Shows and example chromosomal system to study BIR, showing an inducible break site indicated in red, and homology for strand invasion depicted in purple. The products created by BIR are shown below, and show the copying of the donor chromosome onto the recipient, and the loss of sequences telomeric to the break site on the recipient.

at the mating type (*MAT*) locus (Malkova, Ivanov, and Haber 1996). In this study they initiated a DSB using HO endonuclease, and discovered that although gene conversion happened the majority of the time in WT cells, some different products were found in *rad51* mutants. These mutants showed loss of heterozygosity in the 100kb region downstream of the HO site. They concluded this to be Rad51 independent, Rad52 dependent BIR. However, it was later shown that this Rad51 independent BIR at the *MAT* locus was not classical BIR, and instead depended on homology between inverted repeats of Ty elements, which can allow folding back of the ssDNA (VanHulle et al. 2007).

While this SSA-like Rad51-independent BIR at the *MAT* locus has unique features specific to areas where inverted repeats allow these events to occur, the *MAT* locus does still provide a useful location to study BIR. The Haber lab has conducted many studies using the *MAT* locus. In 2017 for example, Mehta et al. created a system at the *MAT* locus where by modifying the length of homology on either side of the HO cut site, they could tip the balance between GC and BIR one way or the other (Mehta, Beach, and Haber 2017).

Recent studies of BIR in our lab and others have used different chromosomal systems that allow control of the location of both the break site and the donor site. In these systems, the break is induced by HO endonuclease at an ectopic HO cut site placed centromeric to non-essential genes. No homology is made available to the broken segment telomeric to the HOcs so the entire fragment is lost. This allows the centromeric end of the DSB to act single ended. Invasion in these systems is dictated by the placement of genes or parts of genes that share homology. In the systems from our lab, this homology and invasion site specification is achieved with a broken *LYS2* gene, that creates a 2.2kb overlap with *lys* on the broken chromosome and *ys2* on the donor (Donnianni and Symington 2013). Jain et al. created a system reliant on the *LEU2* gene where an HO cut site in the middle of a *LEU2* cassette initiates a break that can

either act as one ended or two ended, based on the different placements of *le* and *u2* donors, and lead to GC or BIR (Jain et al. 2009).

1-4.4 DNA Synthesis During BIR

DNA synthesis during BIR has been shown in yeast to occur via a migrating D-loop in strand displacement synthesis, rather than establishment of a canonical replication fork (Figure 1-4.4) (Donnianni and Symington 2013; Saini et al. 2013). This type of DNA synthesis is conservative, where all the newly synthesized DNA remains on the formerly broken “recipient” chromosome and the template or “donor” chromosome remains unchanged.

This was shown elegantly by Donnianni and Symington, and Saini et al. who both demonstrated that when thymidine analog BrdU is given to cells undergoing BIR and not S phase synthesis, it was incorporated only onto the broken chromosome after BIR and not the donor chromosome. Donnianni showed this by separating intact chromosomes by pulsed field gel electrophoresis and probing with a BrdU antibody, while Saini et al.showed this by excising the recipient and the donor from the pulsed field gel and performing DNA combing to visualize the BrdU incorporation. These results showing BrdU only in the recipient is in contrast to typical semi-conservative replication where the template DNA is split and BrdU would be expected to accumulate in both chromosomes.

Saini et al. provided further evidence for conservative synthesis by studying the accumulation of new mutations. They reasoned that if BIR was semi-conservative, mutations would then accumulate equally on the donor and recipient chromosome. Instead they found the vast majority of mutations in the recipient chromosome, supporting that BIR uses a migrating D-loop rather than a replication fork.

An open question about BIR is how the second strand is synthesized, since BIR is conservative. Recent data from our lab shows that polymerase delta synthesizes the majority of the second strand (Donnianni et al. 2019 in press). What is still unclear is whether the DNA synthesis occurs similar to lagging strand synthesis with several okazaki fragments being laid down with just a moderate delay from the first strand synthesis, or if second strand synthesis does not start until the first strand synthesis is complete, potentially starting from the distal 3' end.

Some data suggest the second model is correct, with second strand synthesis occurring only after the first strand synthesis is complete. These data include the reliance of BIR on RPA which indicates the first strand remains single stranded for an extended period of time (Ruff et al. 2016) and the joint molecules formed by long stretches of ssDNA that can be created during BIR in the absence of Srs2 helicase (Elango et al. 2017), which again would not occur if second strand synthesis occurred closer in time to the first strand.

When regions around the BIR site are digested by restriction enzymes to detect whether the DNA has become double stranded, cutting is delayed in strains undergoing longer distances of BIR, indicating that the region remains single stranded longer in strains undergoing longer stretches of BIR. This again supports that the second strand is not synthesized until the first strand is complete, especially since this product is seen within 30 minutes in gene conversion events (Donnianni and Symington 2013; White and Haber 1990).

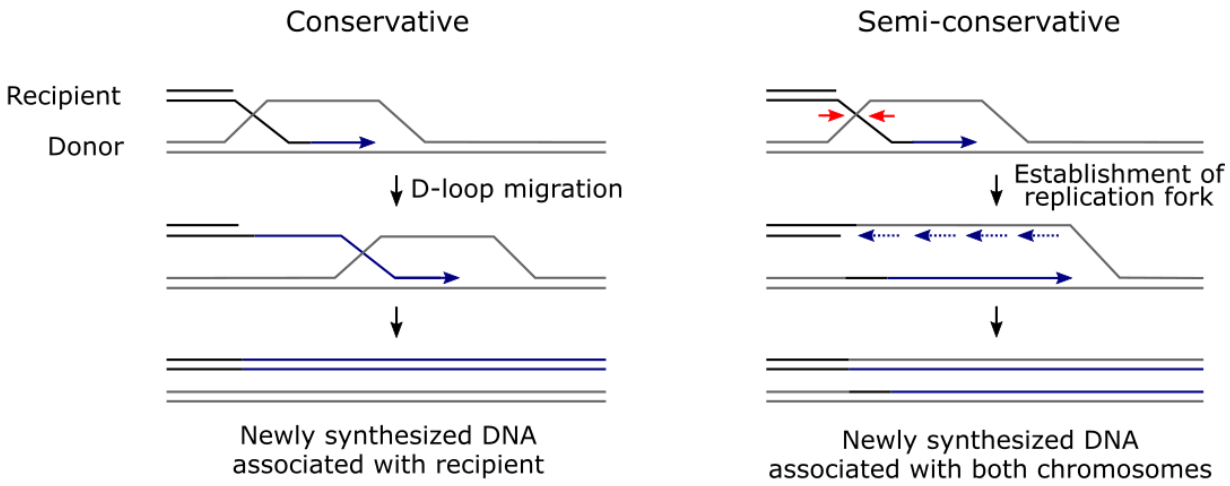


Figure 1-4.4 BIR Occurs by Conservative Synthesis

Schematic showing two possible methods of DNA synthesis during BIR, conservative via migrating D-loop, and semi-conservative via establishment of a canonical replication fork. As Donnianni et al. and Saini et al. demonstrated, BIR occurs by the conservative method of DNA synthesis via migrating D-loop.

1-4.5 Protein Requirements for BIR

Since BIR does not take place in a full replication fork, it does not require all the components of traditional DNA replication, but has instead other requirements essential to DNA synthesis outside of a replication fork. It was first suggested that the MCM complex was required for BIR, based on a decrease in PCR product used to detect BIR in MCM temperature sensitive allele strains (Lydeard, Lipkin-Moore, Sheu, et al. 2010) suggesting the BIR DNA synthesis was more like traditional replication. However later, it was shown using a different system that loss of the MCM complex shows only a mild decrease in BIR products, so is in fact not required (Wilson et al. 2013). And as mentioned above, BIR results in conservatively synthesized DNA from a migrating D-loop. This has become the generally accepted view of BIR.

Polymerase alpha is required for its primase activity (Lydeard et al. 2007), and Polymerase delta, which is used preferentially during HR (Maloisel, Fabre, and Gangloff 2008) is also required for BIR. In cells containing a mutant form of Polymerase delta, complete BIR products failed to be recovered. Instead half crossovers resulting from DNA cleavage after strand invasion were found (Smith, Lam, and Symington 2009). One subunit of Polymerase delta in particular, Pol32 as mentioned earlier, is essential for BIR but not other processes such as gene conversion, though it may be essential for long tract gene conversion. Pol32 increases the processivity of Polymerase delta, which is essential in BIR when such long tracts of DNA must be synthesized (Lydeard et al. 2007; Deem et al. 2008; Smith, Lam, and Symington 2009). There is still some controversy over the role Polymerase epsilon may or may not play in BIR, though recent data from our lab show it is not required for BIR and that Polymerase delta in fact carries out the majority of DNA synthesis (Donnianni et al. 2019 in press).

In addition to polymerase requirements, the Pif1 helicase also contributes significantly to BIR completion. In 2013, Wilson et al. showed physical evidence for Pif1 contributing to bubble migration (D-loop migration) in the form of the appearance of D-loop DNA extension products on

a gel that are dependent on the presence of Pif1. Pif1 has a nuclear and a mitochondrial form that can be separated experimentally by mutating its start codons. The *pif1-m2* mutation eliminates the nuclear form of Pif1 while maintaining the mitochondrial form (Schulz and Zakian 1994). Wilson et al. showed the relevance of Pif1 to BIR by showing that when Pif1 was excluded from the nucleus by the *pif1-m2* mutation, BIR events decrease dramatically. Instead, more mutagenic GCRs stemming from half crossovers arise due to failed BIR. They also pointed out that the role of Pif1 in BIR was independent of the role of Pif1 in telomerase inhibition (Wilson et al. 2013). This data was confirmed by a simultaneous paper by Saini et al. where they show a similar increase in half crossovers in *pif1*Δ cells that they attribute to failed BIR (Saini et al. 2013). In 2014, Vasianovich et al. showed by using a phospho mutant *pif1-4A* that the phosphorylation, not the just presence, of Pif1 is required for BIR (Vasianovich, Harrington, and Makovets 2014). More of the mechanism of the role of Pif1 in BIR was elucidated by Buzovetsky et al. in 2017 when they showed how Pif1 interacts via its PIP sequence with PCNA (Buzovetsky et al. 2017), which is also required for processivity in BIR (McVey et al. 2016). In addition to its role in maintaining processivity of the polymerase, Pif1 also unwinds G quadruplexes that may accumulate in single stranded DNA (Dahan et al. 2018).

In addition to the DNA synthesis components described above, HR protein Rad51 is required for BIR. In the absence of Rad51 there can be no strand invasion events and therefore no traditional BIR. Conversely, overexpression of Rad51 can increase BIR frequency (Lydeard, Lipkin-Moore, Jain, et al. 2010). However, there is a BIR-like process that can occur in the absence of Rad51. This process is Rad52 and Rad59 dependent and is less well characterized, but requires less homology than traditional Rad51-dependent BIR, and relies on a single strand annealing mechanism to start DNA synthesis without Rad51 mediated strand invasion (Ira and Haber 2002; Kang and Symington 2000; VanHulle et al. 2007). In some cases, rare BIR-like events that occurred in the absence of Rad51 turned out to be in fact half-crossover events

suggested to be caused by SSA events between the transformed chromosome fragment and randomly broken donor chromosome, instead of BIR (Smith, Lam, and Symington 2009).

1-4.6 Influences on BIR Completion

Many other factors influence BIR completion in addition the protein requirements addressed above. Available homology influences outcomes in many ways. Mehta et al. for example used an assay where they modified available homology on both sides of DSB, and found that when homology of the invading end to the template was decreased to less than 150bp, strand invasion and subsequent creation of DNA repair products was no longer efficient. Similarly, they also showed that when homology on the non-invading end of the two-ended break is less than 150bp the balance between BIR and GC shifted towards BIR, because the second end did not have enough homology to reliably participate in the reaction (Mehta, Beach, and Haber 2017).

Not only the length of homology influences BIR, but the location of the homology also plays a role. Jain et al. showed that by placing two pieces of donor homology at increasing distances apart from 0 kb up to 26kb, they could shift the outcome of the repair from GC to BIR. When the DSB break was induced in cells where the two donors were 26kb apart, nearly fifty percent of recovered cells had performed BIR instead of GC (Jain et al. 2009).

In addition to homology and its role in BIR, is heterology. Many break sites especially in lab-created assays are not completely homologous to the template to be used for repair because they contain the sequence used for initiating the break such as the HO cut site, or sometimes even longer tracts of heterology. These heterologous tails on the invading end must be removed by Rad1/Rad10 endonuclease to initiate homologous recombination (Fishman-Lobell and Haber 1992; Toh et al. 2010).

The amount of DNA that must be synthesized to create a viable product also influences the outcome of BIR. The less DNA the polymerase must synthesize the more cells will be able to complete BIR. Donnianni and Symington showed, for example, that BIR survival could be reduced significantly by moving the site of strand invasion further from the telomere, and conversely BIR survival could be increased by moving the site closer to the telomere, reducing the amount of DNA to be synthesized (Donnianni and Symington 2013).

Protection of the ssDNA created during BIR also influences completion. Ruff et al. showed that susceptibility of the ssDNA to nuclease cleavage decreases BIR efficiency, while protection and stabilization of the ssDNA by RPA promotes BIR completion (Ruff et al. 2016).

1-5 Mutagenicity of Homologous Recombination

1-5.1 Mutagenicity During Repair of Two-Ended Breaks

DSBR and SDSA are often considered to be the “error free” repair pathways, because they rely on a homologous template and therefore ensure that sequences are not lost at the break site. However, several studies have documented increased mutagenesis during HR compared to traditional DNA replication. If strand invasion occurs at a homologous sequence other than the preferred identical sister chromatid, the resulting incorrect homologous recombination can cause mutagenic outcomes including loss or duplications of DNA sequences, loss of heterozygosity, gross chromosomal rearrangements, or potentially other catastrophic mutations where a chromosomes sequence appears shattered and reshuffled (Piazza and Heyer 2018).

These events stem largely from the extensive tracts of ssDNA involved in HR and their ability to anneal to other ssDNA, as well as to invade other DNA templates. These events are

particularly prone to happen when DNA contains repeated sequences or sequences that share homology elsewhere in the genome (Piazza and Heyer 2019). In addition to rearrangements, point or frameshift mutations are also increased in DNA that has been repaired by HR. When measured by testing reversion of a mutant *trp1* gene in yeast, the error rate during DSB repair was estimated at 10^{-5} to 10^{-6} mistakes per base (Strathern, Shafer, and McGill 1995). Other labs using the *URA3* gene found similar outcomes, where DNA having been repaired by HR showed a 1400-fold increase in mutations. These increased rates were attributed largely to errors made by polymerase delta, and the limited proofreading abilities of polymerases outside the typical replication fork, as well as microhomology mediated template switching during synthesis (Hicks, Kim, and Haber 2010).

In 2018, Piazza and Heyer demonstrated that HR is able to induce translocations and combine sequences from different chromosomes. In this study, the authors showed that a broken chromosome could invade two different sequences simultaneously and induce a translocation event. To demonstrate this, they used segments of a *LYS2* gene, where "*YS*" is on the chromosome V, adjacent to an HOcs, while "*LY*" and "*S2*" (sharing no homology with each other, only sharing homology with the broken chromosome) are in different locations on unrelated chromosome II. After inducing HO break near "*YS*" they find that a complete "LYS2" gene, a product of this multi-invasion event, can be found on donor chromosome II, and in the majority of these clones the broken chromosome V has lost the YS-HOcs segment. Further, they show by pulsed field gel analysis that this translocation comes about by a DSB induced by recombination on the donor chromosome II (Piazza and Heyer 2018).

1-5.2 Mutagenicity During BIR

While an increase in mutations occurs in traditional two-ended DSB repair as described above, BIR synthesizes significantly more DNA than what is synthesized during repair of a two-ended DSB, and the newly synthesized ssDNA is released behind the D-loop, without the opportunity of reannealing to the other end of the break where proofreading can occur. In the case of BIR, whatever mutations accumulate during this type of DNA synthesis of the invading strand, are made permanent by the synthesis of the complementary strand.

One outcome of this type of extensive DNA synthesis in a migrating D-loop is increased point and frameshift mutations, creating clusters containing high numbers of mutations (Saini et al. 2013; Deem et al. 2011). When cells perform BIR in the presence of DNA alkylating agents, mutations increase approximately 900 times that of the rest of the genome, to a rate of one mutation every 6.7kb (Sakofsky et al. 2014).

In addition to mutations in the DNA copied during BIR, cells undergoing BIR must work to prevent mutagenic damage caused *by* the ssDNA, not only *to* the ssDNA. These extended tracts of ssDNA must be regulated carefully to avoid improper pairing with other DNA. Elango et al. showed in 2017 that the Srs2 helicase prevents the formation of toxic joint molecules by both removing Rad51 from the ssDNA and by unwinding the joint molecules (Elango et al. 2017). Similarly, the mutagenic potential of BIR on the rest of the genome and not just the chromosome being repaired is demonstrated by the creation of half crossovers and GCRs that can be catastrophic when BIR is unable to complete synthesis, as described earlier in section 1-4.4 “protein requirements of BIR” (Deem et al. 2008; Smith, Lam, and Symington 2009).

1-5.3 Template Switching and D-loop Stability

In addition to the increase of point and frameshift mutations, BIR can also result in deletions, duplications, and loss of heterozygosity if a template other than the identical sister chromatid is chosen for repair. The initial invasion step is not the only opportunity for BIR to choose the wrong template however. BIR products have also been found with DNA from more than one chromosome (Smith, Llorente, and Symington 2007). In this work, Smith et al. showed that when they induced BIR to copy chromosome III in diploid cells where two heterozygous copies of chromosome III were present, the BIR product had copied to the end of chromosome III as expected, but contained sequences from both chromosome III homologs. This indicated that DNA synthesis had switched from one homolog to the other during BIR. Some recovered BIR products when sequenced even showed sequences supporting that the DNA synthesis had switched more than once between the two homologs. This suggests that BIR occurs by successive rounds of strand invasion, synthesis, and dissociation, and at any of these points of invasion, the strand may invade a different site than it was originally copying. This process of reinvading at a different site is known as template switching (distinct from the process during traditional DNA replication that shares the same name) (Figure 1-5.3).

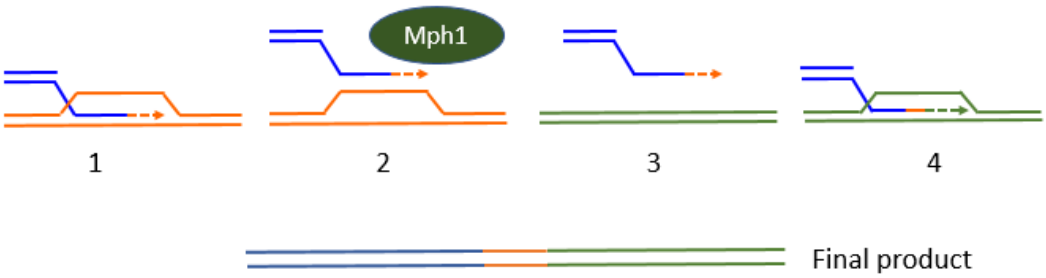


Figure 1-5.3 Schematic of a Template Switch Event

Shows initiation of synthesis (1), disassembly of the D-loop by Mph1 (2), the released strand that can find new homology (3), reinvasion and continuation of synthesis (4), and the final product that is a hybrid of sequences.

These template switch events can result in gross chromosomal rearrangements (GCRs), especially if the reinvasion occurs at the wrong site, but these GCRs are fundamentally different from other spontaneous GCRs. Other GCRs described by the Kolodner lab result from ligation of broken DNA ends with little homology in between (C. Chen and Kolodner 1999). Template switch events during BIR on the other hand are dependent on homology between the two sites, since they depend on strand invasion and DNA synthesis. Because of this, repetitive delta elements especially Ty1 elements which are found in abundance in yeast cells (Lesage and Todeschini 2005) are hot spots for template switching and can lead to rearrangements (Llorente, Smith, and Symington 2008). The role of Ty elements in genomic instability is described in more detail in section 1-7.

Template switching is promoted by the helicase Mph1 (Stafa et al. 2014) which can disassemble D-loops (Prakash et al. 2009). The role of Mph1 in template switching is similar to the role Mph1 plays in SDSA, disassembling the D-loop, potentially in an attempt to finish the repair process. Because of its action on D-loops, Mph1 overexpression not only increases template switch events during BIR, but it significantly decreases the overall number of cells able to complete BIR (Stafa et al. 2014; Luke-Glaser and Luke 2012), providing more evidence that a stable D-loop is necessary for the completion of BIR.

1-6 Alternative Lengthening of Telomeres

1-6.1 ALT vs Normal Telomere Maintenance

One particular case of single-ended DSB repair, is maintenance of eroded telomeres. In WT cells, telomeres are either allowed to erode with each cell division as a mechanism of regulating lifespan, or they are maintained by telomerase, the specialized ribonucleotide complex that can add telomeric repeats to DNA ends (Blackburn and Collins 2011). However, in

some cases, cells can escape senescence caused by eroded telomeres by maintaining their telomeres in another way, called alternative lengthening of telomeres, or ALT.

1-6.2 Normal Telomere Structure and Function

All eukaryote chromosomes face the end replication problem, where due to the nature of DNA replication the chromosomes lose sequences at the ends every replication. DNA polymerases require a 3' end in order to synthesize DNA. This is not a problem for the leading strand, but the lagging strand must lay down RNA primers -which are eventually removed and replaced with DNA- every time synthesis starts. However, on the very end of the lagging strand, no DNA can fill in this gap at the end, so every time a cell divides, these last few sequences are lost. To save a cell from losing valuable sequences, the cell has telomeres at the ends of all chromosomes. The exact sequence and length of the telomeres vary between species, but they are all repetitive GC-rich sequences. Yeast, in particular, have about 300bp +/- 75bp double stranded telomeres consisting of repeats of $TG_{(1-3)}$ as well as a single stranded 3' tail at the end. (Förstemann and Lingner 2001).

In addition to the $TG_{(1-3)}$ repeats, yeast telomeres have telomere associated sequences, or TAS, as well. About half of all yeast chromosomes contain one or more Y' elements, just internal to the telomeric sequences. The Y' element can be either short or long at 5.2 or 6.7 kb long respectively. The sequence is relatively well conserved between chromosomes. All yeast chromosomes also contain an X element, which is shorter, 0.5kb, and much more heterogeneous in sequence than the Y' element. Both X and Y' elements contain potential ARS and have been shown to be dynamic, participating frequently in recombination events (Zakian, Blanton, and Wetzel 1986).

As mentioned above, cells have the ability to lengthen telomeres by using the enzyme telomerase that can add repeats directly to the telomeres (Blackburn and Collins 2011). Telomerase is a ribonucleotide complex made up of protein subunits Est1-3 and an RNA template for the telomere repeat sequence, *TLC1* (Wellinger and Zakian 2012). Typically If not lengthened by telomerase, a cell's telomeres will erode over a number of cell divisions, reach a critically short length, cause mitotic "catastrophe", and the cells will senesce or die (Sohn et al. 2002). Alternative mechanisms to maintaining telomere length will be discussed later.

In addition to length, telomeres must also be treated and regulated in ways that prevent them from being seen by the cellular machinery as a DSB end. In many ways, telomeres must be treated in the exact opposite way as a DSB. They must not undergo recombination, must not be fused or healed to each other, and they must not activate the cell cycle DNA damage checkpoints. To achieve this special non-DSB existence, telomeres maintain a regulated structure known as telomere capping. Capping is maintained both by folding back of the single stranded 3' overhang into a T loop effectively hiding the end, and by a set of proteins that bind to the telomere and protect and silence it (Wellinger and Zakian 2012).

There are several proteins involved in the capping of telomeres. Progressing from the subtelomeric sites to the very end, the subtelomeric TAS regions are bound by the Sir silencing proteins, Sir2, Sir3, and Sir4. This silencing can spread past TAS subtelomeric genes, in what is called the telomere position effect where subtelomeric genes are silenced. Next, the telomeric double-stranded repeat region itself is also bound by Sir2, Sir3, and Sir4, and in this region they also interact with the essential protein Rap1, that has binding sites (ACACCCACACACC) about every 20bp (Gilson et al. 1993; Wang and Zakian 1990). Rap1 also interacts on the TG repeats with Rif1 and Rif2. Next, the single-stranded 3' overhang can be bound by Cdc13, but can also form secondary structures like G quadruplexes, where a single G rich strand folds back on itself

in a stable quadruplex (Piazza et al. 2015) that then must be unwound by helicases such as Pif1 in order to be replicated (Paeschke, McDonald, and Zakian 2010).

Rap1 is a many faceted protein that in addition to its role at the telomere, also binds many locations outside the telomere and is known to both repress and activate gene expression (Shore and Nasmyth 1987). In telomeres, the abundance of Rap1 binding is part of the mechanism to determine telomere length, and disrupting Rap1 leads to dysregulation of telomere length, while overexpressing it leads to widespread chromosomal instability (Kyrion, Boakye, and Lustig 1992; Conrad et al. 1990). Localization of Rap1 seems to both regulate and be regulated by telomere length; in cells with shortening telomeres, Mec1 induces Rap1 to relocate to promoters throughout the genome, controlling gene expression that regulates senescence (Platt et al. 2013).

Interestingly, DNA damage repair proteins including the MRX complex and Tel1, both known to function in resection, and Ku proteins that function in NHEJ, associate with telomeres, though the roles these proteins have here is less clear. It is thought that Ku as well as the MRX proteins might play a role in recruiting telomerase to telomeres, since the absence of Ku, the MRX complex, or Tel1, telomeres are shorter. Another protein involved in DNA repair, Pif1, also binds at telomeres. Here it is known to remove telomerase from DNA as well as unwinding G quadruplex DNA. Thus, in the absence of Pif1, telomeres are longer than usual. Pif1, as mentioned earlier, has a mitochondrial form that functions in maintenance of mtDNA. The nuclear and mitochondrial forms of Pif1 use different start codons and can be separated experimentally by mutating one or the other start codons (Wellinger and Zakian 2012; Geronimo and Zakian 2016; Schulz and Zakian 1994),

1-6.3 ALT Types and Mechanisms

ALT, the recombination based method of maintaining telomeres in the absence of telomerase mentioned in section 1-6.1, was originally discovered in yeast cells lacking telomerase (Lundblad and Blackburn 1993) and has since been shown to be carried out in human cells as well. Specifically, ALT has been shown to be used by 5-20% of human cancers, including cancers that have become resistant to telomerase inhibitor drugs (Bryan et al. 1995; Cesare and Reddel 2010; Reddel 2014; Bechter et al. 2004). The process in both yeast and humans has been shown to be either very similar to BIR or actually BIR, requiring Pol32 and producing conservatively synthesized DNA (Lydeard et al. 2007; Zhang et al. 2019). It involves the invasion of the eroded telomere end into a full-length telomere or extrachromosomal telomeric DNA circles containing telomere sequences (Cesare and Griffith 2004), and the copying of sequences to restore telomere function.

In yeast, cells can maintain their telomeres in the absence of telomerase in two distinct ways (Le et al. 1999; Q. Chen, Ijpma, and Greider 2001): slow growing type I telomerase survivors, and faster growing type II telomerase survivors (Q. Chen, Ijpma, and Greider 2001). Type I survivors maintain their telomeres by expanding the Y' elements, including an autonomously replicating sequence (ARS), in a tandem array with short tracts of telomeric repeats in between, while type II survivors expand the telomeric repeats themselves to several kb (Teng and Zakian 1999; Lundblad and Blackburn 1993) (Figure 1-6.3). Both have been shown to contain extrachromosomal DNA circles of Y' and telomeric repeats in types I and II respectively (Larrivée and Wellinger 2006; Lin et al. 2005).

Human cancer cells that have survived loss of telomerase most resemble yeast type II survivors, and both have similar genetic requirements: Pol32, and Sgs1, or WRN helicase in humans (Johnson et al. 2001). Interestingly, neither type of survivor require Rad51, requiring instead only Rad52 (Roumelioti et al. 2016) potentially for its strand annealing role.

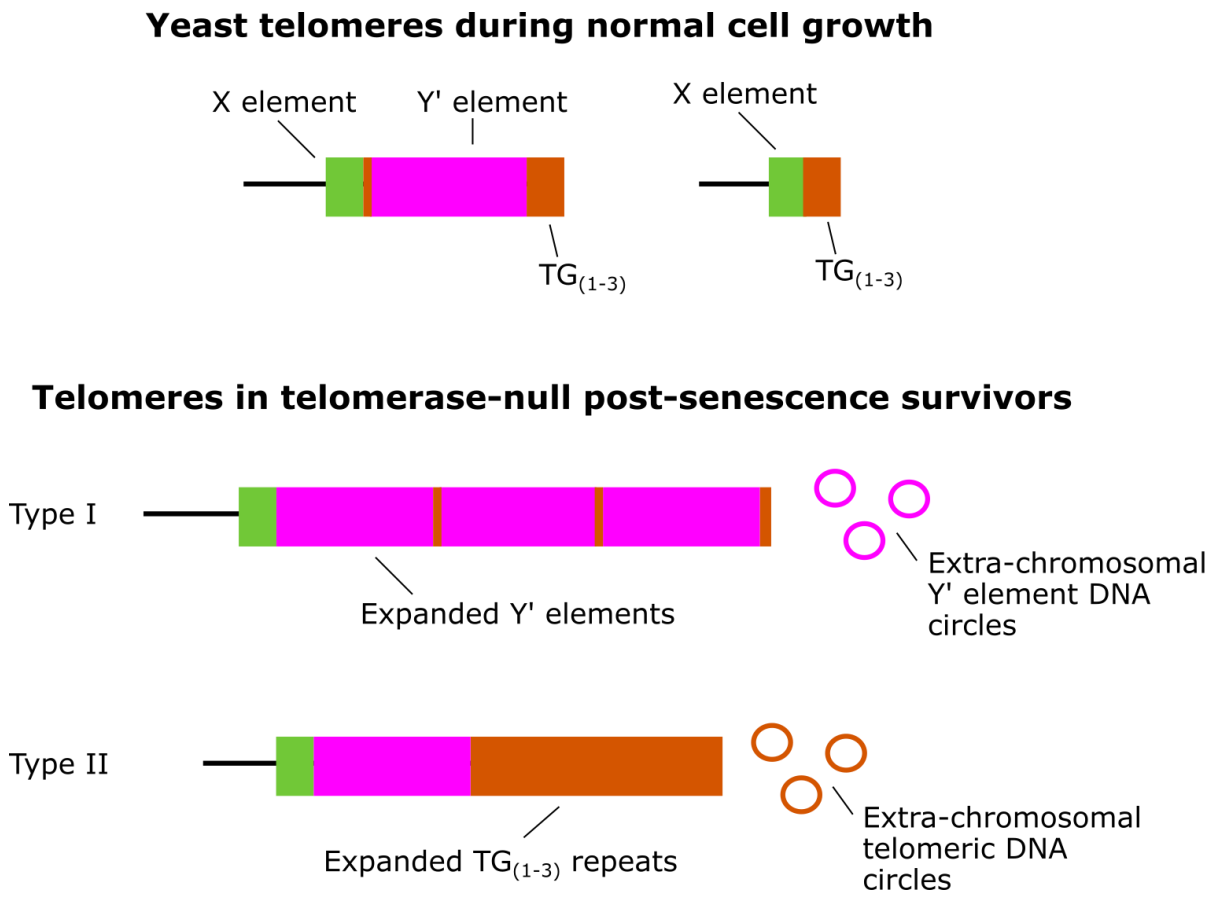


Figure 1-6.3 Characteristics of Yeast Telomeres

Top panel shows telomeres during normal growth, showing that telomeres can contain X elements (green), telomeric $TG_{(1-3)}$ repeat sequences (orange), and sometimes but not always Y' elements (purple). Small tracts of telomeric sequences sometimes found in between X and Y' junctions are represented as small orange bands. Bottom panel shows the two types of survivors. Type I have expanded Y' element tracts and extrachromosomal DNA circles of Y' sequences. Type II have expanded regions of telomeric sequences and extrachromosomal telomeric DNA circles.

1-7 Repeated DNA Sequences and Genomic Instability

1-7.1 Types of Repetitive DNA

Most genomes contain many repetitive sequences in both coding and non-coding regions. 50% of the genome of humans, for example, is made up of repetitive DNA. Repeated sequences can be categorized as either tandem repeats or dispersed repeats. Tandem repeats include tandemly repeated genes including ribosomal DNA, and micro and minisatellites. Dispersed repeats include tRNA genes, transposons like LINEs and SINEs commonly found in human genomes, and retrotransposons like Ty elements commonly found in yeast genomes. These all have been shown to contribute to genomic instability but in different ways (Richard, Kerrest, and Dujon 2008).

1-7.2 Instability of Repetitive DNA

On an evolutionary scale, repetitive DNA may be partially responsible for the evolution of genomes. For example, the junctions of many segmental duplications in the yeast genome are often microsatellites, including telomere sequences (Koszul, Dujon, and Fischer 2006; I. K. Moore, Martin, and Paquin 2000; Koszul et al. 2004), while recombination between SINEs may be responsible for evolutionary changes in the human genome (Chimpanzee Sequencing and Analysis Consortium 2005).

More than 3% of the yeast genome is comprised of Ty elements, sequences that share functionality with retroviruses, and share homology with each other. Ty elements all share similar structure of terminal repeats along with genes similar to viral *gag* and *pol* (Lesage and Todeschini 2005). Ty elements not only are transposons themselves that can act similarly to retroviruses and catalyze their own transpositions via reverse transcriptase, but by sharing

homology with each other they also can stimulate aberrant recombination. The sequences flanking the elements are often duplicated on various chromosomes, supporting the idea that these elements and their propensity for recombination influence the structure and evolution of the genome (J. M. Kim et al. 1998; Fischer et al. 2000).

Moving from the scale of evolution to the scale of a lifetime of an organism, LINEs and SINEs gain their mutagenicity from their ability to move or transpose themselves. In humans, LINEs and SINEs including *Alu* elements have been found to be responsible for disrupting or deleting genes predisposing to or causing numerous diseases (J.-M. Chen et al. 2005).

Microsatellite instability also plays a role in disease in addition to the evolutionary role. In addition to being potential sources of large rearrangements and duplications as mentioned above, they are also themselves unstable, expanding and contracting in length. The most famous microsatellites are possibly CAG repeats that are expanded up to hundreds of times in diseases like Huntington's Disease and Myotonic Dystrophy (Podvin et al. 2019), and interstitial telomeric sequences (Aksenova and Mirkin 2019), both known to be unstable. Microsatellite instability, particularly expansions, is found as a hallmark in many cancers and neurodegenerative diseases (Reynolds et al. 2019; Kok, Chalabi, and Haanen 2019; Rehman, Jones, and Poston 2019; Kulkarni and Wilson 2008)

Some, if not the majority, of this instability can be attributed to the unique challenges this DNA poses to homologous recombination: its repetition and its potential for secondary structures including hairpins and G quadruplexes all pose challenges. DNA repair has long been known to contribute to the expansions and contractions in repeat number. Particularly when SDSA has been forced to occur over a microsatellite losing or gaining repeats, or when slippage of the polymerase and hairpin or other secondary structure formation leads to gain of one or few triplets (Kraus, Leung, and Haber 2001; Jeffreys et al. 1994; Balakumaran, Freudenreich, and Zakian 2000; Usdin, House, and Freudenreich 2015; Liu and Wilson 2012). While yeast do not

naturally contain widespread trinucleotide repeats like humans do, researchers have artificially inserted them into yeast in order to study them.

Using yeast in this way, BIR in particular has also been suggested to play a different and significant role in the pathogenic expansions of CAG microsatellites found in Huntington's and other degenerative diseases. Kim et al.show that expansion of repeats by tens or hundreds, much larger than the expansions of just a few repeats seen in other systems, is dependent on Pol32, the hallmark of BIR. Kim et al.propose that these massive expansions can come about if replication forks stall near the repeat tracts and are rescued by BIR (J. C. Kim et al. 2017; Leffak 2017).

1-7.3 Interstitial Telomere Sequences

Another type of challenging microsatellite sequence is the GC rich telomeric sequence found at the ends of chromosomes and described in section 1-6.2. Telomeric sequences also exist elsewhere in the genome, and in this case are referred to as interstitial telomere sequences, or ITS. ITS have been found in most genomes studied (Aksenova and Mirkin 2019) including yeast, some plants, and most vertebrates including humans, who have anywhere from hundreds (Simonet et al. 2011) to thousands (Wood et al. 2014) of interstitial telomeric sequences in our genomes. ITSs, like traditional telomeres, can form secondary structures such as G quadruplexes in the G rich strand (Schaffitzel et al. 2001; Lam et al. 2013), and even certain structures in the C rich strand (Gehring, Leroy, and Guéron 1993; Day, Pavlou, and Waller 2014). They are also found to bind at least some of the proteins that typically bind telomeres such as Rap1 (A. Moore et al. 2018) in yeast and TRF2 in humans (Wood et al. 2014).

ITSs have been established as sites of instability and rearrangements in many organisms including yeast and humans (Samassekou and Yan 2011; Ashley and Ward 1993; Bertoni et al. 1994; Mondello et al. 2000; Kilburn et al. 2001; A. Moore et al. 2018). In experiments where an ITS was inserted into the yeast genome, it was shown to promote breakage at the site and to expand in length (Aksenova et al. 2013). This breakage may be promoted by the binding of telomeric protein Rap1 at the ITS (Goto et al. 2015). ITSs also have the ability to act as seeds for telomere healing events, where a DSB in or near the ITS causes a new telomere to be formed at the site instead of correct repair of the DSB (Diede and Gottschling 1999; Putnam, Pennaneach, and Kolodner 2004; Obodo et al. 2016).

1-8 Rationale for the Current Study

Due to the extreme mutagenic potential of BIR described above, including its role in microsatellite expansions and GCRs, its atypical mode of DNA synthesis, and its use by cells during times of replication stress, it is essential to understand the mechanisms and factors influencing the mutagenicity of BIR. It may be that these characteristics cause BIR to be exceptionally sensitive to difficult-to-replicate DNA sequences, in turn causing BIR to play a central role in the mutagenic potential of these difficult sequences. This becomes relevant to human health in many ways, particularly in times of DNA replication stress and genomic instability, such as cancer. To begin to understand this, we investigate here the role BIR may play in the instability associated with ITSs, a sequence that is found in abundance in most cells.

Chapter 2: Methods

2-1 ITS Strain Creation

All strains described here (Table 1) are derivatives of a previously described W303 yeast strain used to detect BIR (Donnianni and Symington 2013). The original haploid strain, LSY2689-12A, contains a recipient *lys-HO-KanMX* integrated 35kb from the left telomere of Chr V, and the donor *TRP1-ys2* integrated 70 kb from the left telomere of Chr XI and has the *MATa-inc* allele to prevent HO cleavage at the *MAT* locus. In addition, the endogenous *LYS2* gene was replaced with *NatMX* and a *GAL-HO* cassette was integrated at the *ade3* locus. Strain LSY3881 was generated from by replacing *lys2::NatMX with lys2::LEU2*, and the *HML* and *HMR* were replaced with *oripRS*, and *ampR* respectively to prevent aberrant HO cutting (Zierhut and Diffley 2008).

To add the ITS to LSY3881 to create LSY3944 the following PCR method was used:

Primer 1:

20 bp homology to target area of Chr XI + **ITS insert** + forward primer to amplify *k.lactis URA3* cassette

GCTACGTGATTACATTACCA**TGTGTGGGTGTGTGGGTGTGTGGGTGTGTGGGTGTGTGGG TGTGTGGGTGTGTGGGTGTGTGGG**GTGCAGGTCGACAACCCTTAAT

Primer 2:

20 bp homology to target area of Chr XI + reverse primer to amplify *k. lactis URA3*

AAATCAGTATCAGTGGGGACGCAGCGTACGGATATCACCTA

The resulting PCR product contained the desired insert followed by the *URA3* cassette. Homology to the target sequence in Chr XI was extended to 40 bp on both sides by a second round of PCR (Figure 2-1). These fragments were then integrated 2.5 kb centromere distal to

the ys2 donor. Successful integration of the ITS was confirmed by PCR and DNA sequencing. A control (LSY3945) containing the *URA3* cassette but no insert was created the same way. Strains containing Y' element inserts, and GT repeat inserts, were created using the same method, but with different sets of primers (Table 2).

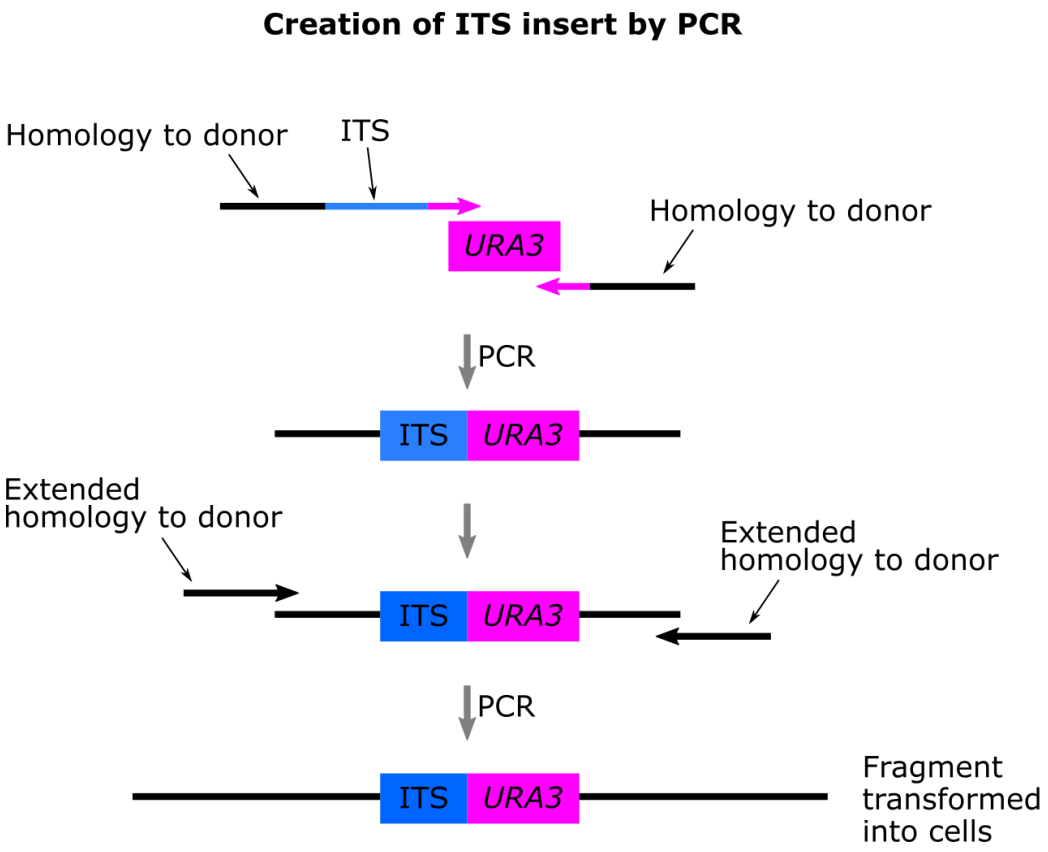


Figure 2-1 Creation of ITS Insert by PCR

Schematic of system used to generate ITS inserts for transformation into BIR strains. The first PCR to amplify the *URA3* gene (purple) shows the ITS in the forward primer (blue segment), which then creates a product containing the ITS and the *URA3* gene. The second PCR shows the extension of the tract of homology to the donor chromosome.

2-2 mph1Δ, tlc1Δ, rad10Δ, and pif1-m2 Strain Creation

To delete *MPH1*, *TLC1,* and *RAD10,* the *HphMX* gene was amplified from pAG32 (Goldstein and McCusker 1999) with primers containing homology upstream and downstream of the gene to be deleted (Table 2).

pif1-m2 mutants were generated by crossing with a MAT-alpha strain containing *pif1-m2* mutation. Colonies were screened using restriction enzyme digestion as the *pif1-m2* mutation creates a Xho1 cut site that does not exist in WT *PIF1* (Schulz and Zakian 1994).

2-3 BIR Assay

Cells were grown to exponential phase in 1% yeast extract, 2% peptone, 2% lactate (YPL), then diluted and plated on rich medium (YP) containing 2% glucose or 2% galactose (wt/vol). Colonies were counted after 3 days and were then replica plated onto synthetic complete (SC) medium lacking lysine or YPD containing geneticin. Cell viability after HO induction was determined by dividing the number of CFUs on YPG by that on YPD. Repair by BIR was confirmed by comparing the number of Lys^+ to the number of YPG CFU. Greater than 99% of cells repaired via BIR, while <1% remained Lys^- $geneticin^r$ following growth on YPG because of repair by nonhomologous end-joining. BIR frequencies shown are averages of at least three independent trials for each strain.

2-4 Chromosome Size Determination by Pulsed Field Gel Electrophoresis

Colonies were suspended in YPD and grown to saturation. Cells from 4mL of each culture were harvested by centrifugation, and pellets were weighed and resuspended in low melting point

agarose and molded in Bio-Rad plug molds. Per 50mg of cell pellet the following amounts were used: 0.5% low melting point agarose in 450uL of 100mM EDTA; and 20uL 25mg/mL 20T zymolyase in 10mM KPO_4 pH7.5. The resulting plugs were incubated overnight in 1mL 500mM EDTA, 10mM Tris at 37 degrees. The next day 400 uL of 5% sarcosyl, 5mg/mL proteinase K in 500mM EDTA was added to the plug buffer, and incubated at 50 degrees for >5 hours with occasional mixing. TE washes (4 washes, >1 hour each, nutating) were then done at 4 degrees. Chromosomes were separated by electrophoresis through 1% agarose at 6 V in 0.5× Tris-borate-EDTA at 14 °C for 36 h (initial switch = 45 s, final switch = 95 s) using a CHEF-DR II Pulsed-Field Electrophoresis system (Bio-Rad).

Truncation frequencies were determined by counting the number of lanes of a PFG showing the truncated size chromosome and dividing by the total number of samples tested by PFG. Significance was determined by chi square analysis.

Southern blots were performed by transferring DNA from the PFGE overnight to nitrocellulose membrane. The membrane was then hybridized with a P^{32} labeled probe generated by amplification of a region the *COS9* gene, and visualized on a Typhoon machine (Amersham Biosciences).

2-5 Telomere Sequencing

To sequence the region near the ITS and the potential new telomere, DNA was extracted from colonies tested on PFGE. Three colonies showing truncated chromosomes were selected. C-tailing was performed on each according to the protocol by New England Biolabs. C tailed DNA was used in a PCR reaction with a poly-G primer and a primer just upstream of the ITS insert (“screen insert F” primer). This PCR product was gel purified and sequenced by Genewiz.

2-6 Telomerase Survivor Creation

To create type II telomerase survivors *tlc1* cells were grown in 25mL YPD to saturation followed by a dilution of 1:200 or greater. This was repeated at least four times. Cells were then struck on YPD plates to confirm that they had recovered the faster growth rate indicating type II survivors were created.

2-7 *MPH1* Overexpression Plasmid Creation

An *MPH1* overexpressing plasmid was received as a gift from the lab of Brian Luke (Luke-Glaser and Luke 2012). This plasmid contained MPH1 under the control of a galactose inducible promoter, and a KanMX selectable marker. This marker was switched to a *HphMX* marker by PCR amplifying from pAG32 (Goldstein and McCusker 1999) with primers overlapping the site of the *KanMX* marker. The full plasmid was then PCR amplified without the *KanMX* gene, and the full plasmid + *HphMX* gene was assembled using Gibson Assembly and sequenced to confirm correct assembly.

Table 1 Strain list

Description	Number	Genotype
Parent BIR strain	LSY3881	*MATa-inc lys2::LEU2, hml::oripPRS, hmr::ampR, AVT2::lys-HOcs::KanMX6 ade3::GAL-HO,TRP1-ys2* Ch XI 70 kb donor
BIR strain with ITS	LSY3944	*MATa-inc lys2::LEU2, hml::oripPRS, hmr::ampR, AVT2::lys-HOcs::KanMX6 ade3::GAL-HO, TRP1-ys2* Ch XI 70 kb donor, *ITS::URA3* on ChXI
BIR strain without ITS	LSY3945	*MATa-inc lys2::LEU2, hml::oripPRS, hmr::ampR, AVT2::lys-HOcs::KanMX6 ade3::GAL-HO, TRP1-ys2* Ch XI 70 kb donor, *URA3* on ChXI
BIR strain with pif1-m2 + ITS	LSY3989-42	*MATa-inc lys2::LEU2, hml::oripPRS, hmr::ampR, AVT2::lys-HOcs::KanMX6 ade3::GAL-HO, TRP1-ys2* Ch XI 70 kb donor, *ITS::URA3* on ChXI, *pif1-m2*
BIR strain with pif1-m2 - ITS	LSY3989-52	*MATa-inc lys2::LEU2, hml::oripPRS, hmr::ampR, AVT2::lys-HOcs::KanMX6 ade3::GAL-HO, TRP1-ys2* Ch XI 70 kb donor, URA3 on ChXI, *pif1-m2*
BIR strain with mph1::hyg + ITS	LSY4146	*MATa-inc lys2::LEU2, hml::oripPRS, hmr::ampR, AVT2::lys-HOcs::KanMX6 ade3::GAL-HO, TRP1-ys2* Ch XI 70 kb donor, *ITS::URA3* on ChXI, *mph1::HphMX*
BIR strain with mph1::hyg - ITS	LSY4145	*MATa-inc lys2::LEU2, hml::oripPRS, hmr::ampR, AVT2::lys-HOcs::KanMX6 ade3::GAL-HO, TRP1-ys2* Ch XI 70 kb donor, *URA3* on ChXI, *mph1::HphMX*
BIR strain with tlc1::hyg + ITS	LSY4155	*MATa-inc lys2::LEU2, hml::oripPRS, hmr::ampR, AVT2::lys-HOcs::KanMX6 ade3::GAL-HO, TRP1-ys2* Ch XI 70 kb donor, *ITS::URA3* on ChXI, *tlc1::HphMX*
BIR strain with tlc1::hyg - ITS	LSY4156	*MATa-inc lys2::LEU2, hml::oripPRS, hmr::ampR, AVT2::lys-HOcs::KanMX6 ade3::GAL-HO, TRP1-ys2* Ch XI 70 kb donor, *URA3* on ChXI, *tlc1::HphMX*
BIR strain with Y' element	LSY4318	*MATa-inc lys2::LEU2, hml::oripPRS, hmr::ampR, AVT2::lys-HOcs:: KanMX6 ade3::GAL-HO, TRP1-ys2* Ch XI 70 kb donor, *Y'element4::URA3* on ChXI
BIR strain with (GT)32	LSY4425	*MATa-inc lys2::LEU2, hml::oripPRS, hmr::ampR, AVT2::lys-HOcs:: KanMX6 ade3::GAL-HO, TRP1-ys2* Ch XI 70 kb donor, *(GT)32::URA3* on ChXI
BIR strain with pol32::hyg + ITS	LSY4314	*MATa-inc lys2::LEU2, hml::oripPRS, hmr::ampR, AVT2::lys-HOcs:: KanMX6 ade3::GAL-HO, TRP1-ys2* Ch XI 70 kb donor, *ITS::URA3* on ChXI, p*ol32::HphMX*
BIR strain with pol32::hyg - ITS	LSY4315	*MATa-inc lys2::LEU2, hml::oripPRS, hmr::ampR, AVT2::lys-HOcs:: KanMX6 ade3::GAL-HO, TRP1-ys2* Ch XI 70 kb donor, *URA3* on ChXI, *pol32::HphMX*
BIR strain with pol32::hyg + (GT)32	LSY4452	*MATa-inc lys2::LEU2, hml::oripPRS, hmr::ampR, AVT2::lys-HOcs:: KanMX6 ade3::GAL-HO, TRP1-ys2* Ch XI 70 kb donor, *(GT)32::URA3* on ChXI, *pol32::HphMX*
BIR strain with pol32::hyg + Y' element	LSY4453	*MATa-inc lys2::LEU2, hml::oripPRS, hmr::ampR, AVT2::lys-HOcs:: KanMX6 ade3::GAL-HO, TRP1-ys2* Ch XI 70 kb donor, *Y' element::URA3* on ChXI, *pol32::HphMX*

BIR strain with pif1-m2, tlc1::hyg and ITS	LSY4426	*MATa-inc lys2::LEU2, hml::oripPRS, hmr::ampR, AVT2::lys-HOcs:: KanMX6 ade3::GAL-HO, TRP1-ys2* Ch XI 70 kb donor, *ITS::URA3* on ChXI, *tlc1::HphMX, pif1-m2*
BIR strain 48kb + ITS	LSY4096	*MATa-inc lys2::LEU2, hml::oripPRS, hmr::ampR, AVT2::lys-HOcs:: KanMX6 ade3::GAL-HO, TRP1-ys2* Ch XI 70 kb donor, *ITS::URA3* 48kb from *ys2* on ChXI,
BIR strain 48kb - ITS	LSY4097	*MATa-inc lys2::LEU2, hml::oripPRS, hmr::ampR, AVT2::lys-HOcs:: KanMX6 ade3::GAL-HO, TRP1-ys2* Ch XI 70 kb donor, *URA3* 48kb from *ys2* on ChXI,
BIR strain with *HphMX* cassette 55kb on donor	LSY4143	*MATa-inc lys2::LEU2, hml::oripPRS, hmr::ampR, AVT2::lys-HOcs:: KanMX6 ade3::GAL-HO, TRP1-ys2* Ch XI 70 kb donor, *HphMX* on ChXI,
BIR strain with NatMX cassette 55kb on donor	LSY4134	*MATa-inc lys2::LEU2, hml::oripPRS, hmr::ampR, AVT2::lys-HOcs:: KanMX6 ade3::GAL-HO, TRP1-ys2* Ch XI 70 kb donor, *NatMX* on ChXI,
BIR strain with *HphMX* on donor and *NatMX* on ChXII	LSY4144	*MATa-inc lys2::LEU2, hml::oripPRS, hmr::ampR, AVT2::lys-HOcs:: KanMX6 ade3::GAL-HO, TRP1-ys2* Ch XI 70 kb donor, *HphMX* on ChXI, *NatMX* on ChXII

Table 2 Primer List

Primers used to insert ITS, Y' elements, $(GT)_{32}$, or markers

F to insert ITS 2.5	GCTACGTGATTACATTACCATGTGTGGGTGTGTGGGTGTGTGGGTGTGTGGGTG TGTGGGTGTGTGGGTGTGTGGGTGTGTGGGGTGCAGGTCGACAACCCTTAAT
R to insert ITS 2.5	AAATCAGTATCAGTGGGGACGCAGCGTACGGATATCACCTA
F to insert *URA3* only 2.5	GCTACGTGATTACATTACCAGTGCAGGTCGACAACCCTTAAT
R Extend homology	TGGTCTTAGACTTTGGTAACTCTTCCAGTTCCTTTTTCAAAAGATCTTTCAAATCAG TATCAGTGGGGAC
F extend homology	GACAAAGTAGATGATGGGTTAGTCAATATGGCTACACTAAACATCACTGAGCTAC GTGATTACATTACCA
Screen insert F	CAAGATTCATTGACGTCGACAC
Screen Insert R	CCGTCATAGTCGAACCAAAATC
F insert from Y' element	GCTACGTGATTACATTACCAGCGTTCCATGACGAGCGCTGGACTGCAGGGTCCG CAGTACGTCAAGCTGCAGTTTAGCAGGCATCATCGACAGTTGAGGTGCAGGTCG ACAACCCTTAAT
F insert $(GT)_{32}$	GCTACGTGATTACATTACCATGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT GTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGCAGGTCGACAACCCTTAAT
F to insert ITS 48	CGCGCTCAATAACTTCCTCGTGTGTGGGTGTGTGGGTGTGTGGGTGTGTGGGTG TGTGGGTGTGTGGGTGTGTGGGTGTGTGGGTGCAGGTCGACAACCCTTAAT
R to insert ITS 48	ATGAAGACTATTCGCCGTACGCAGCGTACGGATATCACCTA
F to insert URA3 only 48	CGCGCTCAATAACTTCCTCGTGCAGGTCGACAACCCTTAAT
R extend homology 48	TCCTGGTGGAGAATAATGGTAGGCTTGGCGGCTCTGAGATGGAGAAATAGTTTCA AGCCGCGCGCTCAATAACTTCCTCG
F extend homology 48	GCAGCACCTGCGTCATTCACGCTGAAGCGGCAGCAAGCATTTTCGATCAGCTCC AATTAAATGAAGACTATTCGCCGTAC
F screen 48	CTCAACAATGATGTGCAATG
R screen 48	GCAATTTACTCCACTAGACC
Add HphMX chXI 55kb R	CCAGAGGGGAATAGGAAAAAGAATAGTTGTAATAATAATAATAAGGCTGCAGGTC GACGGATCCC
Add HphMX chXI 55kb R	TTTCAATTTATAGATCGTGTAGTTTATTAGATGCGAATAGCCTAAGAATTCGAGCT CGTTTTCGAC
Add NatMX ChXII F	CATCAAAAAATACGTGTACCCCCTTGCTGTGGCATGCTAGAATGCGCTGCAGGTC GACGGATCCC
Add NatMX chXII R	ATTTGTATATAAACCATGAGATTAAGTGGTCTGAAATTATGTATCGAATTCGAGCT CGTTTTCGAC

Primers used to delete genes

F mph1	TTCCGGTTCTGTTTTATTTTAGTGTCCTTTTTTCTCTCTGGCTGCAGGTCGACGGATCCC
R mph1	CGTTATTTTTGTATAGACGCCGACGTATAAGAGTCTCCTAGAATTCGAGCTCGTTTTCGAC
F mph1 screen	GTGATTCAAGATATAGTAGCTCAC
R mph1 screen	GTTGATACTAAAAATATAATG
F tlc1	ACCTTCTTTGTAGCTTTTAGTGTGATTTTTCTGGTTTGAGGCTGCAGGTCGACGGATCCC
R tlc1	TATATTCTAAAAAGAAGAAGCCATTTGGTGGGCTTTATTAGAATTCGAGCTCGTTTTCGAC
F tlc1 screen	GGTGACATATAGATCTCAAGG
R tlc1 screen	CAAGACAATTACTAGGATGTTC
F rad10	ACACAAAAAAGGGCATAAACAAAGTTGGTTATCCTAGAAGGCTGCAGGTCGACGGATCCC
R Rad10	TAAGCATGGAACAGATTTATTAAAAGAAAATAGGAATTGTGAATTCGAGCTCGTTTTCGAC
F rad10 screen	GACATGGCTTGATTTTTACAGTG
R rad10 screen	CAGCAGAAATTCCCATTAATTTC
F pol32	ACAACCAGAAATAGGCTTTAGTTAACTCAATCGGTAATTAGCTGCAGGTCGACGGATCCC
R pol32	TCACAATTAGTAATGGAAAGTGTTTGGAAAAAAAAGAAGAGAATTCGAGCTCGTTTTCGAC
F pol32 screen	GAAGTTCGTTACATCGCAATC
R pol32 screen	CTATCACGTAAGTTGACATTTG

Primers for screening GC/BIR events

Screen F	CAAGATTCATTGACGTCGACAC
HPH R	GCCCTCCGAGAGCTGCATCAG
NAT R	GTAAGCCGTGTCGTCAAGAGTG
KAN R	ACGTGAGTCTTTTCCTTACCCAT

Chapter 3: An Interstitial Telomere Sequence Can Disrupt BIR

3-1 Introduction

Single-ended double strand DNA breaks can arise in the cell at regressed forks, if a replication fork collapses by passage of replication through a single stranded DNA nick, if one end of a two ended break is lost, or at eroded telomeres (see Figure 1-4.1). Single-ended breaks pose a unique problem for the cell, as they must be repaired to prevent cell death, but the traditional double strand break repair method (DSBR) and synthesis-dependent strand annealing (SDSA) cannot occur without two ends of a break being available for repair. These breaks can however be repaired by break-induced replication (BIR).

BIR, like DSBR and SDSA, involves resection to create a single stranded 3' overhang, Rad51-mediated search for homology, strand invasion, and DNA synthesis primed be the 3' end of the break (see section 1-3). Here it diverges from the other pathways and continues synthesizing DNA until it either reaches the end of the chromosome, is stopped by an approaching replication fork, or for various reasons fails to complete synthesis resulting in cell death. DNA synthesis during BIR occurs outside of a stable replication fork, instead occurring by a migrating D-loop mechanism. This leads to DNA replication that is far more mutagenic that typical S phase replication.

This unique method of DNA synthesis during BIR implicates BIR in mutagenesis including point and frameshift mutations (Deem et al. 2011), half-crossovers (Smith, Lam, and Symington 2009; Deem et al. 2008), duplications (Costantino et al. 2014), and microsatellite expansions (Leffak 2017). Given this mutagenic potential of BIR, as well as the abundance and mutagenicity of ITSs in cell (Aksenova and Mirkin 2019), we wanted to investigate the potential contribution of BIR to the observed mutagenicity of ITSs. This would increase understanding of both the role ITSs play in genome instability, as well as the influence different DNA sequences have on the accuracy and outcome of BIR.

3-2 Experimental Design

To investigate whether completion of BIR may be affected by ITSs, we created a variation of a previously described system (Donnianni and Symington 2013) to monitor BIR. In this system, an HO endonuclease recognition site (HOcs) is integrated 35 kb from the left telomere of chromosome V ("recipient") in a strain expressing a galactose-inducible HO gene. The HOcs is directly adjacent to a partial *LYS2* gene ("*lys*") on the centromeric side, and a *KanMX* gene providing resistance to G418 on the telomeric side. Homology for repair is provided on chromosome XI ("donor") by another partial *LYS2* gene ("*ys2*") inserted 70kb from the left telomere. These two *lys2* fragments share 2.2 kb homology, and are non-functional before recombination since they are partial genes (Figure 3-2).

After the DSB is formed by transferring cells to galactose-containing medium, the distal end of chromosome V (Chr V) containing the *KanMX* gene is lost due to lack of available homology, while the centromeric side of the break containing "*lys*", invades the homologous "*ys2*" site and copies to the end of donor Chr XI by BIR. This heals the break and creates a functional *LYS2* gene on the recipient chromosome. More than 99% of cells that grow on galactose-containing medium are Lys^+ and have lost the Kan resistance marker located centromere-proximal to the DSB on the recipient chromosome. Thus, the BIR frequency is derived from the ratio of colony-forming units on galactose and glucose-containing media.

To this strain we inserted a 64 bp tract of telomere sequence, $(TGTGTGGG)_8$ and a *K. lactis URA3* marker on the donor chromosome, 2.5kb centromere distal to the site of strand invasion ("+ITS" strain). As a control, we generated a strain with just the *URA3* marker inserted at the same site as the +ITS strain ("-ITS" strain). Following strand invasion, DNA synthesis in the +ITS strain must traverse the ITS to complete BIR. If cells complete BIR fully, the resulting BIR product would be approximately 605kb. If the cells terminate BIR at the ITS the resulting BIR product would be approximately 538kb (Figure 3-2).

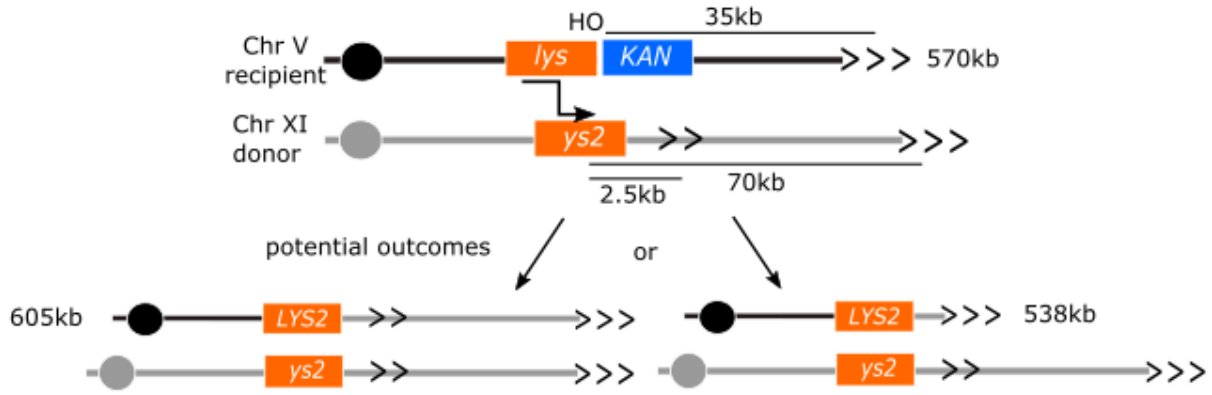


Figure 3-2 Experimental Design

Schematic of experimental BIR system showing site of DSB break (gap between *lys* and *KAN* labeled “HO”) invasion (black bent arrow), and telomere tracts (carrots). Bottom chromosomes show possible outcomes of BIR and the predicted sizes of the repaired recipient chromosome. For clarity, the *URA3* gene used as a marker to insert the ITS, is not shown in this image. Control strains containing only an inserted *URA3* gene were also generated, and not shown here.

3-3 An Interstitial Telomere Sequence Can Disrupt BIR

We first investigated whether an ITS would affect BIR completion. The rates of BIR for the two strains, with or without the ITS, were the not significantly different: 31.5% and 32.7% respectively (Figure 3-3A), suggesting that BIR synthesis is not impaired by the ITS. Next we asked whether the ITS changes the outcome of BIR by causing a telomere to be formed at the ITS, effectively terminating BIR DNA synthesis and generating a truncated chromosome. To address this question, chromosome sizes from independent colonies that completed BIR (Lys^+ Kan^- colonies after HO induction) were analyzed by pulsed field gel electrophoresis (PFGE), which allows separation and visualization of intact chromosomes. In a strain without the ITS, all colonies screened contained the expected size Chr V:XI translocation chromosome (Figure 3-3C [left panel for whole gel image], and 3D [top panel for zoomed in portion. Portion of gel shown in the zoom in D is highlighted with brackets in C]).

We found that 12% of colonies derived from the strain containing the ITS had BIR products approximately 68kb shorter than full length BIR (Figure 3-3B, 3C [right panel for whole gel], and 3D [bottom panel for zoomed in portion]). Since this is the size of product that would be created by termination of BIR at the ITS, we PCR amplified and sequenced the region from 3 colonies containing this size BIR product and found a full length (200-300bp) telomere to have been added (Figure 3-3E).

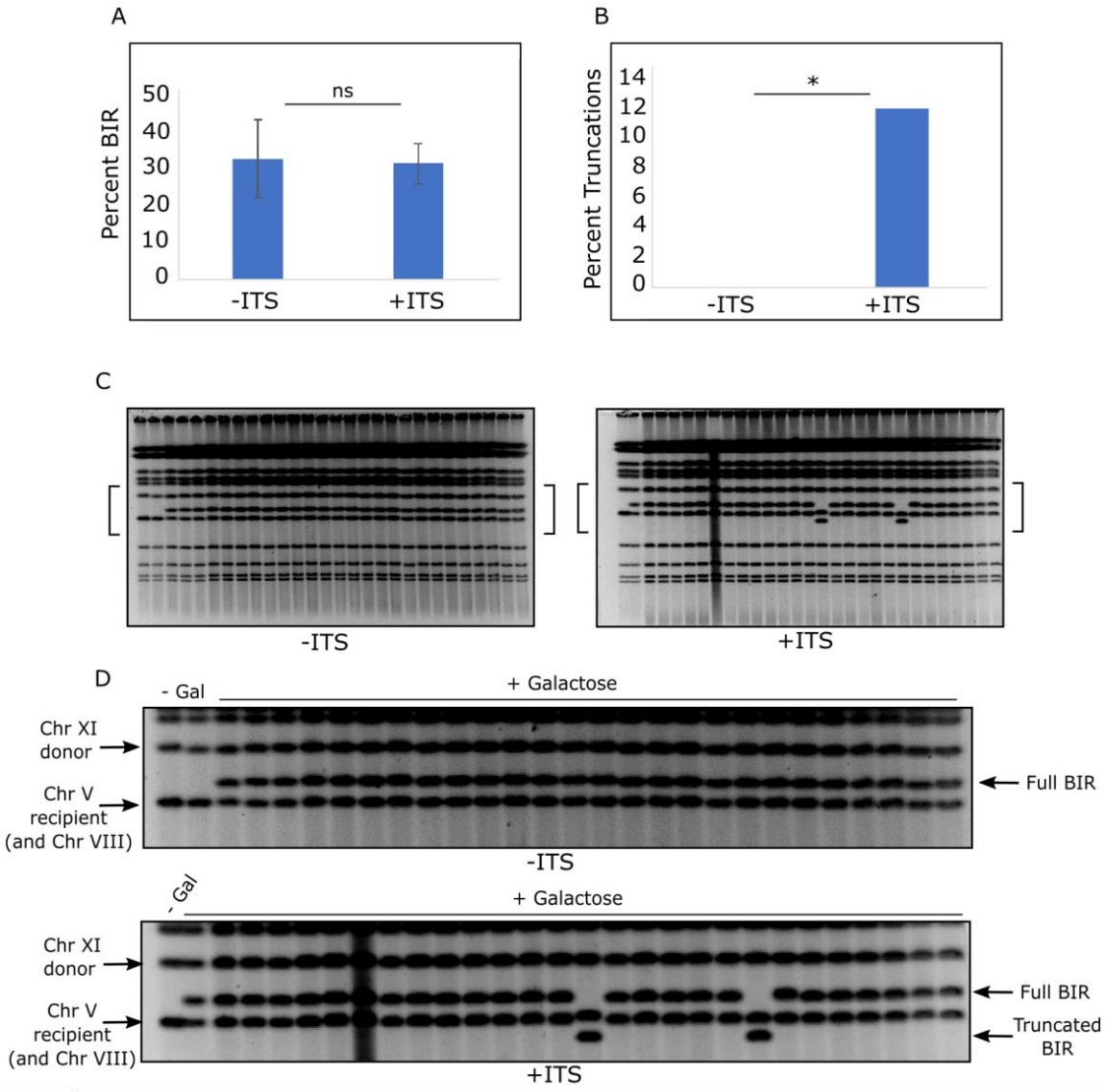

A
Percent BIR
50
40
30
20
10
0
ns
-ITS
+ITS
B
Percent Truncations
14
12
10
8
6
4
2
0
*
-ITS
+ITS
C
-ITS
+ITS
D
- Gal
+ Galactose
Chr XI donor
Chr V recipient (and Chr VIII)
Full BIR
-ITS
- Gal
+ Galactose
Chr XI donor
Chr V recipient (and Chr VIII)
Full BIR
Truncated BIR
+ITS
E
Inserted ITS
Telomere added by cell

Figure 3-3 Interstitial Telomere Sequences Cause Truncations During Break Induced Replication

A. BIR completion by strains with or without the inserted ITS, 31.5% and 32.7% respectively. Error bars show standard deviations. B. Percent of colonies recovered from BIR assay found to have truncations. No truncations were seen in >50 -ITS colonies screened, 14 out of 115 +ITS colonies screened. p-value 0.011 using chi square analysis. C. Representative pulsed field gels showing chromosome sizes before and after induction of HO with galactose. Left and right panels show strains -ITS and +ITS respectively. Brackets indicating area shown in panel D. D. Zoomed in portion from panel C. Gel of +ITS shows two colonies with truncated BIR products. PFGE of -ITS colonies shows no truncated chromosome repair products (0 out of 60 screened). E. Example trace from sequencing one of the truncated chromosome products. Sequences of the inserted ITS are highlighted in grey, and telomeric sequences added by the cell are highlighted in red. The short string of cytosines at the end is an artifact of the C-tailing used to PCR amplify the region for sequencing.

3-4 Telomere Synthesis is Driven by Homology Directed Repair or by De Novo Synthesis by Telomerase

We envisioned two mechanisms by which the new telomeres could be synthesized. First, telomerase could act directly at the ITS to synthesize a telomere; and second, the ssDNA intermediate formed when the migrating D-loop traverses the ITS could invade a natural telomere and complete synthesis by homology-directed repair (Figure 3-4A). To distinguish between these two possibilities, we tested the ability of cells lacking telomerase (*tlc1Δ*) to form truncated BIR products. Haploid *tlc1Δ* cells were first allowed to adapt in liquid culture to select for the faster growing type II telomerase survivors that maintain their telomeres by using a BIR-like mechanism to expand telomere sequences (Q. Chen, Ijpma, and Greider 2001). BIR efficiency was measured in these survivors and was not significantly different from strains containing *TLC1* (Figure 3-4B). Notably, truncated chromosomes were formed in the *tlc1Δ* strain at the same frequency as the *TLC1* strain (Figure 3-4C), indicating that telomerase is not essential for telomere formation at the ITS.

To address whether telomerase *could* be used to form truncations, we analyzed cells containing the *pif1-m2* mutation which ablates nuclear Pif1, an inhibitor of telomerase activity at DSBs (Boulé and Zakian 2006), while retaining mitochondrial Pif1 function (Schulz and Zakian 1994). We reasoned that since Pif1 inhibits telomerase at DSB, if the frequency of truncated chromosomes increased in the absence of Pif1, this would indicate that telomerase can act at the ITS. BIR efficiency decreased significantly in the *pif1-m2* strains (Figure 3-4B) while the frequency of chromosome truncations increased to 58% (Figure 3-4C). While this result supports the idea that telomerase can act directly at the ITS, it is important to keep in mind that Pif1 is partially required for BIR, and the increase in chromosome truncations could be due to BIR decreased processivity during BIR resulting in premature termination at the ITS.

To determine the mechanism used for telomere addition in the absence of Pif1, we tested strains lacking both nuclear Pif1 and Tlc1. A previous study reported an increase in type II-like survivors with expanded telomere tracts in the *pif1-m2 tlc1Δ* double mutant (Hu et al. 2013). We reasoned that if the increase in truncations seen in *pif1-m2* is dependent on the activity of telomerase, and fewer truncations are seen in *pif1-m2 tlc1*∆ cells compared to *pif1-m2* alone, then telomerase may be acting at the ITS in addition to homology-directed repair. The frequency of chromosome truncations in the double mutant was 10%, not significantly different from WT or *tlc1Δ* alone, compared to 58% in the *pif1-m2* single mutant (Figure 3-4C), indicating that homology-directed repair is not the only mechanism acting to form a telomere at the ITS, and the increased frequency seen in *pif1-m2* strains is due at least in part to the activity of telomerase.

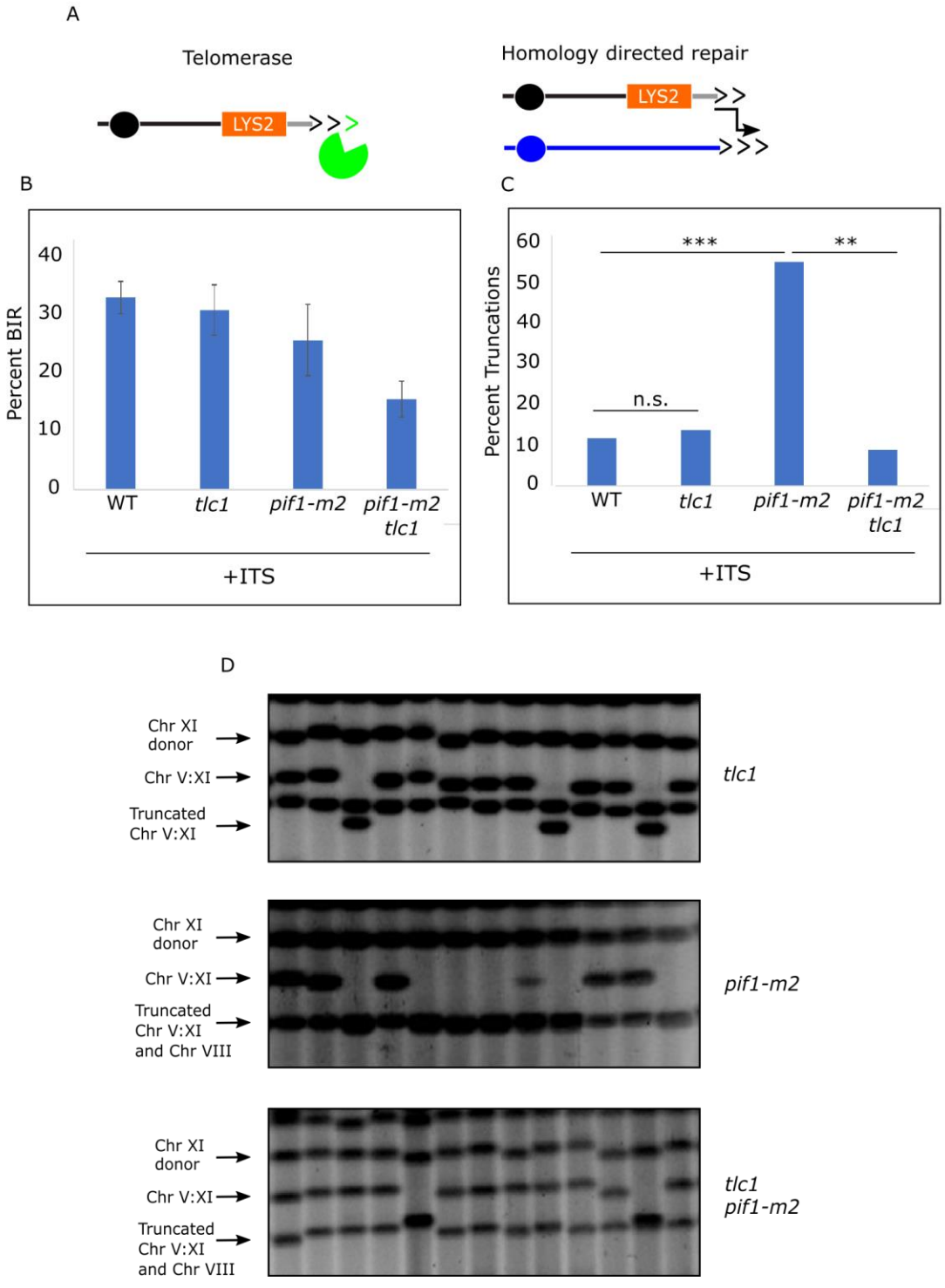

A
Telomerase
LYS2
Homology directed repair
LYS2
B
Percent BIR
40
30
20
10
0
WT
tlc1
pif1-m2
pif1-m2 tlc1
+ITS
C
Percent Truncations
60
50
40
30
20
10
0
***
**
n.s.
WT
tlc1
pif1-m2
pif1-m2 tlc1
+ITS
D
Chr XI donor
Chr V:XI
Truncated Chr V:XI
tlc1
Chr XI donor
Chr V:XI
Truncated Chr V:XI and Chr VIII
pif1-m2
Chr XI donor
Chr V:XI
Truncated Chr V:XI and Chr VIII
tlc1 pif1-m2

Figure 3-4 Telomere Synthesis is Driven by Homology Directed Repair and De Novo Synthesis by Telomerase

A. Schematic of possible methods of telomere creation, showing *de novo* telomere addition by telomerase (left panel) and homology directed repair using natural telomeres (right panel). B. Percent BIR completion (33, 30.8, 25.7, and 15.5 for WT *tlc1*Δ*, pif1-m2,* and *tlc1*Δ *pif-m2* respectively). C. Percent of colonies recovered from BIR assay found to have truncations (12, 14, 56, 9.1 percent respectively). Colonies screened were 115, for the WT strain and 28 for each of the mutants. p-value .003 between *tlc1*Δ *pif1-m2* and *pif1-m2*, p-value $6x10^{-7}$ between WT and *pif1-m2* determined using chi square analysis.

3-5 Disassembly of the D-loop by Mph1 is Critical for Formation of Truncated BIR Products

Since both methods of telomere formation require a free single stranded 3' DNA end, we hypothesized that a critical step in the formation of chromosome truncations may be the disassembly of the D-loop via helicase. To test this hypothesis, we deleted *MPH1*, a helicase that disassembles D-loops and is required for template switching during BIR (Stafa et al. 2014; Prakash et al. 2009). We found that the BIR frequency increased to 54.3 and 57.3 percent for the strains with and without the ITS respectively. These values were not significantly different from each other but were significantly increased from the strains containing *MPH1* (Figure 3-5A). This was consistent with previous studies showing an increased frequency of BIR in the absence of Mph1 (Luke-Glaser and Luke 2012; Stafa et al. 2014; Mehta, Beach, and Haber 2017). We analyzed the sizes of BIR products from 75 independent Lys+ *mph1* +ITS colonies by PFGE and all contained full-length BIR products (Figure 3-5B), indicating that disassembly of the D-loop by Mph1 is critical to the formation of chromosome truncations at the ITS.

We also investigated the opposite situation, *MPH1* overexpression. We reasoned that an increased level of Mph1 protein in the cell would disassemble D-loops more frequently leading to fewer BIR events as reported previously (Luke-Glaser and Luke 2012), and more truncation events. We found this to be the case. *MPH1,* when overexpressed by 2 micron plasmid under the control of a galactose inducible promoter, reduced BIR as expected (0.91 and 2.3 percent for without and with the ITS respectively Figure 3-5A). We also found a significant increase in the formation of chromosome truncations following *MPH1* overexpression, to 74% (Figure 3-5B), indicating that instability of the D-loop during BIR leads to the formation of truncated chromosome products.

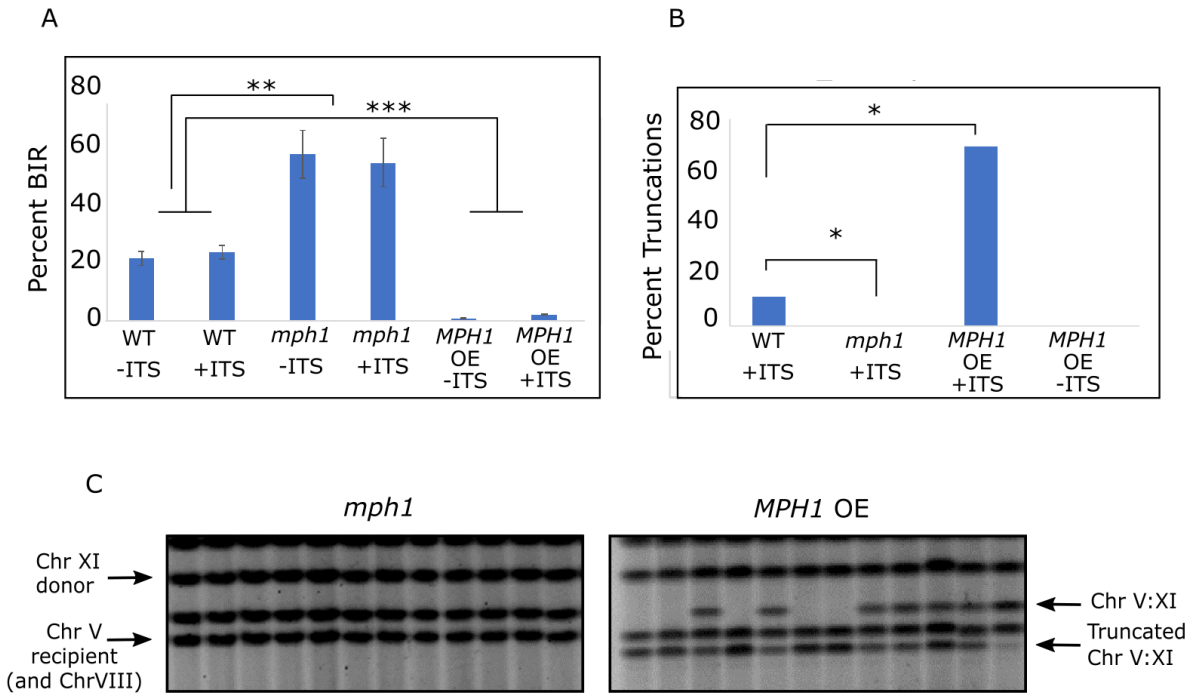


Figure 3-5 Disassembly of the D-loop by Mph1 is Critical for Formation of Truncated BIR Products

A. Percent BIR completion in WT (left two bars 21.4% and 23.5% for – and + ITS respectively), *mph1*Δ (57.3% and 54.3% for – and + ITS respectively), and *MPH1* overexpression (0.91% and 2.3% for – and + ITS respectively) B. Percent of colonies recovered from BIR assay found to have truncations: 14/115 for WT, 0/75 for *mph1,* 17/23 for *MPH1* OE +ITS, 0/27 for *MPH1* OE -ITS. WT vs *mph1*Δ p-value 0.014, WT vs *MPH1* OE p-value 5.5×10^{-5}, p-value for WT +ITS vs *MPH1* OE -ITS is 0.06 due to smaller sample size. C. Representative pulsed field gel of *mph1* colonies showing no truncation events (left gel) and *MPH1* OE showing several truncations. Many lanes show both the truncated product and the full length product, due to the gel containing two colonies per lane in order to increase sample size. Bands were counted separately in the total n.

3-6 Clipping of Heterologous Tail by Rad1/Rad10 is Not Required for New Telomere Formation

It is possible, based on the Mph1 data, that DNA synthesis during BIR can easily pass through the ITS and the telomere is formed due to recognition of the ITS sequence in the ssDNA released behind the polymerase. In this case, the truncated chromosome products should be reliant on the removal of the heterologous DNA between the ITS and the 3' end that needs to be extended during HDR or by telomerase. To determine whether BIR preferentially terminated at the ITS or extends beyond the and thus requires heterologous tail removal we tested the requirement for *RAD10*. The Rad1/Rad10 complex is responsible for endonucleolytic removal of 3' heterologous tails during SSA and the strand invasion step of HDR (Fishman-Lobell and Haber 1992; Ivanov and Haber 1995). We found a decrease in truncated products (from 12 percent in WT to 7 percent in *rad10*). However, this was not statistically significant (p-value 0.2 determined by chi square), indicating that clipping by Rad1/Rad10 is not essential for the formation of truncated products, and that the DNA synthesis is likely to dissociate at the ITS rather than to synthesize past it.

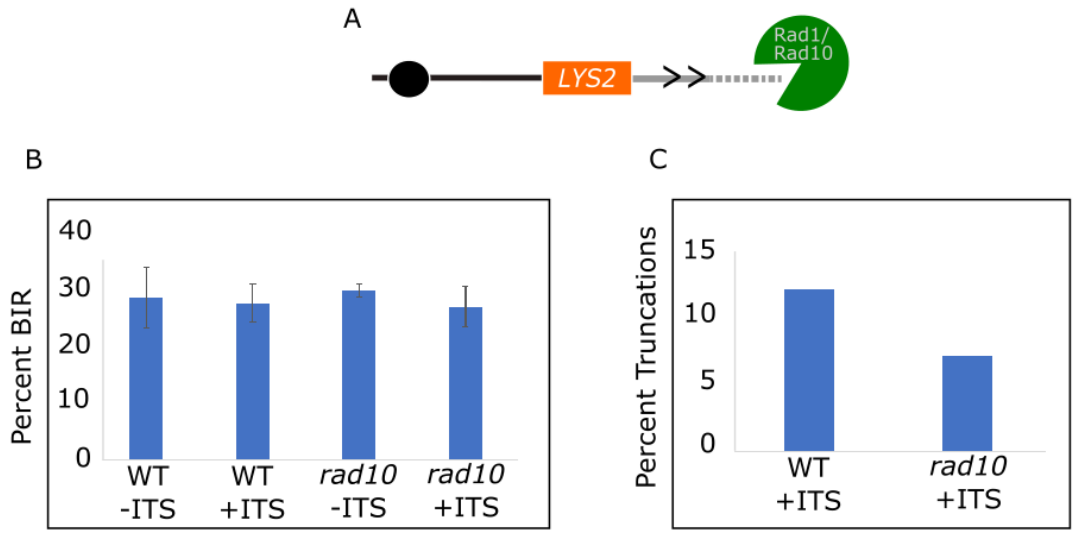


Figure 3-6 Clipping of Heterologous Tail by Rad1/Rad10 is Not Required for New Telomere Formation

A. Shows a model of Rad1/Rad10 clipping activity. B. BIR rates unchanged between all strains (29, 28, 30.4, and 27.4 for WT -ITS, WT +ITS, *rad10* -ITS, and *rad10* +ITS respectively). C. Persistence of truncations in absence of Rad10, with a decrease from 12% to 7% in *rad10*.

3-7 Interstitial Telomere Sequences Added Further from the Site of Initial Strand Invasion Do Not Cause Termination of BIR

To further understand the mechanism for the disruption of BIR by the ITS, we analyzed the BIR outcomes of a strain containing an ITS in a different location. It has been shown that template switching during BIR occurs more frequently closer to the site of strand invasion than further away (Smith, Llorente, and Symington 2007). We therefore hypothesized that creation of truncated products may function similarly, and may then also occur more frequently when the ITS is closer to the site of strand invasion. To test this, we created a BIR strain containing an ITS 48kb downstream from the site of strand invasion ("48kb ITS" Figure 3-7A). As in the 2.5kb ITS strain, no difference was seen in overall BIR completion between the strain with no ITS and the strain containing the 48kb ITS (Figure 3-7B). Unlike the 2.5kb ITS strain however, no truncations were seen in any of the 67 colonies screened (Figure 3-7C). This indicates that similar to template switching during BIR, these truncation events are more likely to occur closer to the start of BIR. However, whether this is due to the 2.5kb ITS capturing BIR products that would otherwise have failed, a change in stability of the D-loop over time, or other factors such as approaching replication forks is not yet clear.

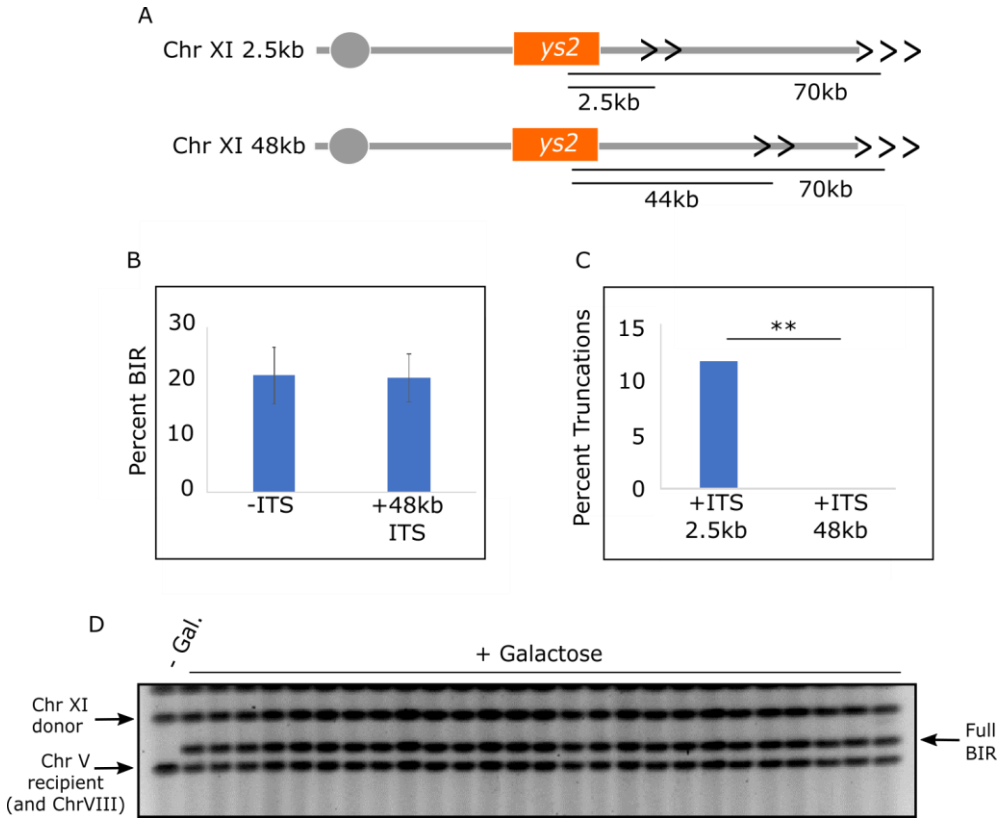


Figure 3-7 Interstitial Telomere Sequences Added Further from the Site of Initial Strand Invasion Do Not Cause Termination of BIR

A. Showing a schematic of the donor chromosome (Chr XI) from the two strains used showing the different location of the ITS. B. Showing that as with the 2.5kb ITS, there is no change in BIR rates when the ITS is placed 48kb away (BIR rates 21.1 and 20.6 for -ITS and +48kb ITS). C. Showing the formation of no truncated BIR products (14/115 for 2.5kb and 0/67 for 48kb ITS. p-value .003 determined by chi square. D. Representative PFGE image from colonies containing 48kb ITS showing no truncated chromosome products.

3-8 Unique Features of the ITS Contribute to Formation of Truncated Chromosomes

Since Mph1 plays a central role in template switching during BIR, and our data point to a role of HDR in formation of chromosome truncations at the ITS, we wanted to address whether other sequences could promote chromosome truncations by template switching. If this were the case we would predict that if the ITS were replaced with another telomere seeding sequence, or any sequence near a telomere, we would recover truncated BIR products.

We created two new strains (Figure 3-8A), one where the ITS was replaced with a 64 bp tract consisting of $(GT)_{32}$, that retains the ability to seed telomeres but is predicted to not form G quadruplexes and lacks a Rap1 binding site (Lustig 1992), and one where the ITS was replaced with an 80 bp sequence from the conserved region of the yeast Y' element, a naturally occurring sub-telomeric repeat found at of many yeast chromosomes described in section 1-6.2. The BIR frequency was not changed by insertion of the $(GT)_{32}$ or Y' element sequence and we failed to observe any truncated chromosomes in any of the colonies analyzed by PFGE (Figure 3-8B and C).

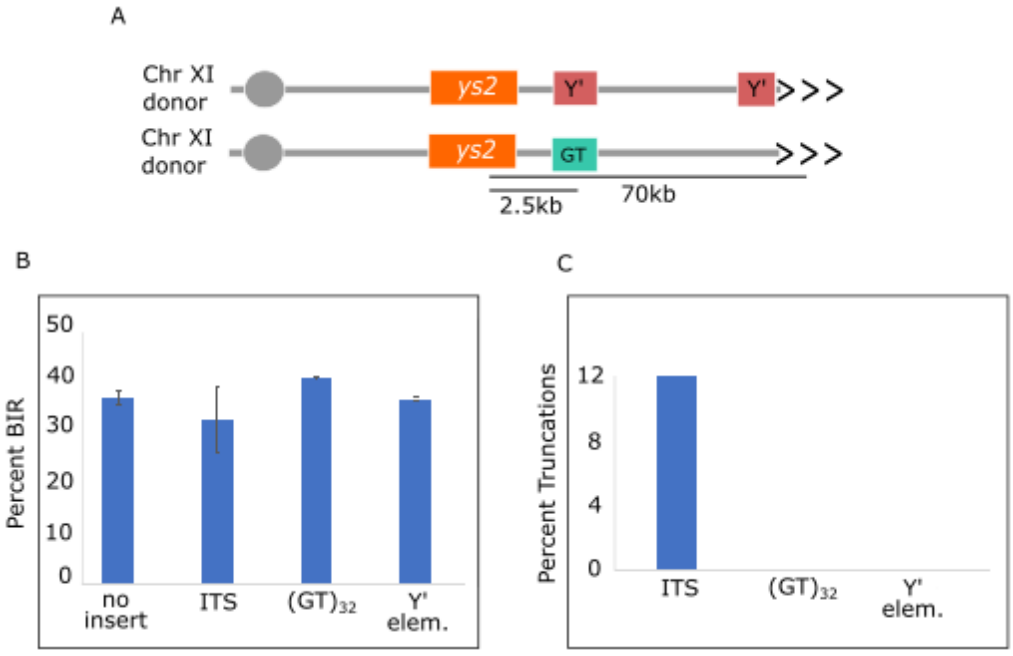


Figure 3-8 Unique Features of the ITS Contribute to Formation of Truncated Chromosomes

A. Showing a schematic of the alternate sequences used in place of the ITS. Top chromosome shows inserted Y' element and the natural Y' element. Bottom shows the inserted tract of GT repeats. B. No different between BIR percents containing the three different inserts (35.9, 31.7, 39.7, and 35.7 for strains containing no insert, ITS, $(GT)_{32,}$ and Y' element respectively. Error bars are standard deviation). C. Truncation percents showing 12% for the strain containing the ITS and 0 for all other inserts. n= 115, 28, and 54 for strains containing no insert, ITS, $(GT)_{32,}$ and Y' element respectively. p-value .05 between ITS and $(GT)_{32}$ insert, p-value .006 between ITS and Y' element insert determined by chi square analysis.

3-9 $(GT)_{32}$ Tracts and Y' Element Insertions Can Form Truncations When BIR is Less Processive

The data thus far indicate that unique features of the ITS, such as Rap1 binding or G quadruplex formation contribute to the formation of truncations, potentially by disrupting synthesis during BIR. If this is true, then the alternate sequences, $(GT)_{32}$ tracts and Y' element insertions, should be able to form truncations if BIR is artificially disrupted. To test this, we created strains with *pol32*Δ and *pif1-m2* mutations, which are known to decrease the processivity of DNA synthesis during BIR (Lydeard et al. 2007; Buzovetsky et al. 2017; Wilson et al. 2013; Vasianovich, Harrington, and Makovets 2014; Saini et al. 2013; Deem et al. 2008; Smith, Lam, and Symington 2009). The BIR frequency is reduced to around 1% and 15% in the *pol32*Δ and *pif1-m2* mutants, respectively (Figure 3-9A).

In the *pol32*Δ derivative with the ITS sequence, 27% of the rare BIR products recovered contained chromosome truncations, while 11% of colonies analyzed from the $(GT)_{32}$ strain and 1% of the clones analyzed from the Y' element strain exhibited the shorter BIR product. (Figure 3-9B). In strains lacking nuclear Pif1, we found that 56% of the BIR products terminated at the ITS, 37% at the $(GT)_{32}$ insert, and 29% of the BIR products derived from the Y' insert strain had terminated in the vicinity of the Y' element (Figure 3-9B).

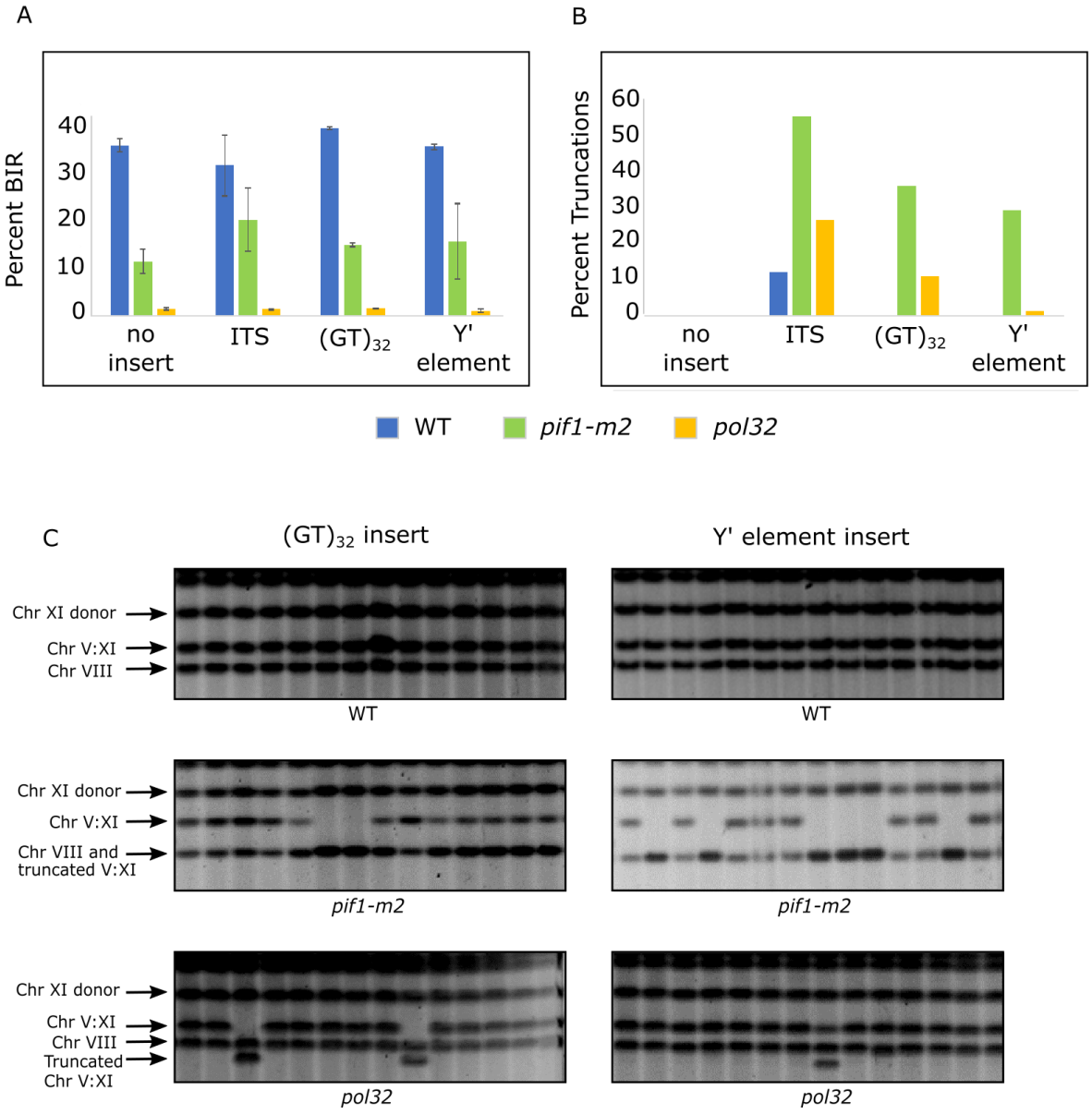

A
Percent BIR
40
30
20
10
0
no insert
ITS
(GT)32
Y' element
B
Percent Truncations
60
50
40
30
20
10
0
no insert
ITS
(GT)32
Y' element
WT
pif1-m2
pol32
C
(GT)32 insert
Y' element insert
Chr XI donor
Chr V:XI
Chr VIII
WT
WT
Chr XI donor
Chr V:XI
Chr VIII and truncated V:XI
pif1-m2
pif1-m2
Chr XI donor
Chr V:XI
Chr VIII
Truncated Chr V:XI
pol32
pol32

Figure 3-9 $(GT)_{32}$ Tracts and Y' Element Insertions Can Form Truncations When BIR is Less Processive

A. Percent BIR completion in all strains (For no insert, ITS, $(GT)_{32}$, and Y' element respectively, BIR completion percents are as follows. WT: 35.9, 31.8, 39.7, 35.6. *pif1-m2*: 11.5, 20.3, 15, 15.7. *pol32*: 1.5, 1.4, 1.5, 1.1.). Error bars represent standard deviation. B. Percent of colonies recovered from BIR assay found to have truncations. (For no insert, ITS, $(GT)_{32}$, and Y' element respectively, truncation percents are as follows. WT: 0, 12, 0, 0. *pif1-m2*: 0, 56, 36.5, 29.6. *pol32*: 0, 27, 11.1, 0). C. Representative sections of PFGE of strains containing GT repeats (left three gels) and Y' element inserts (right three gels) showing no truncated chromosomes in the WT background, but truncations appearing in the *pif1-m2* and *pol32* background. The *pif1-m2* strains were created by crossing and contain a slightly smaller chr VIII that runs at the same size as the truncated chr V:XI product. To increase number of colonies screened, some gels were run with two colonies per lane, this can be seen in the $(GT)_{32}$ *pif1-m2* gel.

Statistical note:

-Different inserts (ITS, $(GT)_{32}$ and Y' element) did not significantly change the BIR completion in the same backgrounds.

-All WT, *pif1-m2*, and *pol32* strains were significantly different from each other except for WT vs *pif1-m2* in the +ITS strain.

3-10 Summary

Here we showed that if BIR encounters an interstitial telomere sequence (ITS) placed in its path, BIR terminates at the ITS 12% of the time, with the formation of a new telomere at this location. We find that the ITS can be converted to a functional telomere by either direct addition of telomeric repeats by telomerase, or by homology-directed repair using natural telomeres. This termination and creation of a new telomere is promoted by Mph1 helicase, which is known to disassemble D-loops, and does not require the clipping activity of Rad1/Rad10 indicating that dissociation is likely to happen at the ITS, or quite close it, since Polymerase delta itself can remove several mismatched bases (Jin et al. 2005; Kunkel 1988), rather than much after it.

Interestingly, truncated chromosome products were not formed by an ITS inserted further from the site of strand invasion, indicating that an ITS may be a way to "catch" BIR products that would otherwise have failed to complete BIR, or other possibilities such as a change in stability of the D-loop later in BIR, or collision with a replication fork which prevents BIR from reaching the end of the chromosome.

We also showed that other sequences that have the potential to form new telomeres, but lack the unique features of a perfect telomere sequence, do not terminate BIR at a significant frequency in wild-type cells. However, these sequences can cause chromosome truncations if BIR is made less processive by loss of Pol32 or Pif1. These findings together indicate that features of the ITS itself such as secondary structure and telomeric protein binding pose a challenge to BIR and increase the vulnerability of the D-loop to dissociation by Mph1, promoting telomere formation at the site instead of completion of BIR.

Chapter 4: Competition Between Break Induced Replication and Gene Conversion

4-1 Context

While carrying out the experiments described in Chapter 3, we came upon an unusual result. Initially, we constructed the +ITS strains using the *HphMX* (hygromycin B resistance) gene inserted 44kb downstream, from the *ys2* donor, rather than the *URA3* gene described in Chapter 3. When colonies of this strain that survived HO cleavage were scored on SC-lys and YPD + G418 media, we found a large proportion were Lys^+ $G418^r$, instead of the expected Lys^+ $G418^s$ phenotype, indicating retention of recipient sequence at the other end of the DSB. Since this occurred in the control strains lacking the ITS and containing only the marker, we knew it wasn't a disruptive effect of the ITS, and had to be related to our marker choice.

Upon closer investigation, we found that the *KanMX* marker used in our BIR system and the *HphMX* inserted onto the donor, both contain the MX promoter and terminator regions providing two regions of homology of 344Bp and 198bp in the promoters and terminators respectively. We hypothesized that this homology was allowing some cells to carry out extremely long tract gene conversion (LTGC) of 44kb, instead of BIR (Figure 4-1). We then decided to investigate and understand these Lys^+ Kan^+ colonies.

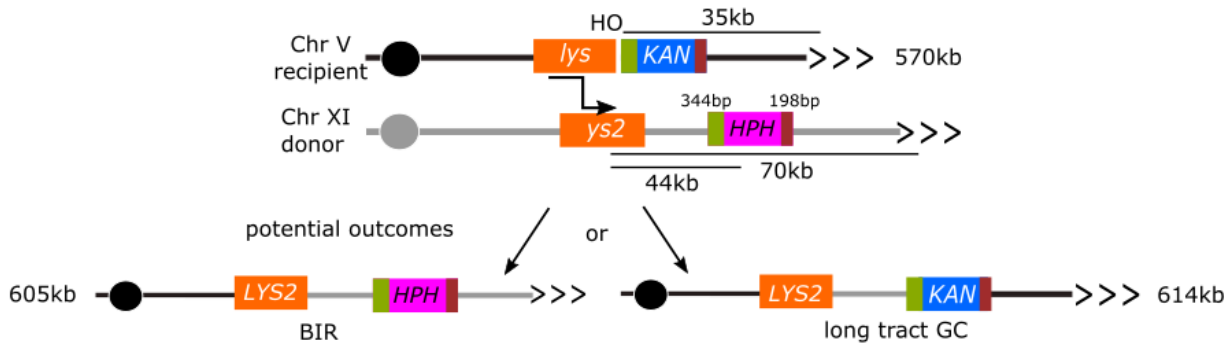


Figure 4-1 Experimental System

Schematic (not to scale) of strain and possible repair outcomes. The *HphMX* cassette in purple and the *KanMX* cassette in blue share homology in their promoter and terminator regions marked here by green and red segments respectively. The repair could lead to BIR (left side) or long tract gene conversion (right side) using the homology from the MX region.

4-2 Gene Conversion Events and BIR Events Compete

We first measured the frequency of BIR in strains with and without the *HphMX* insert. Galactose survival rates were not significantly different between the strains with and without the *HphMX* insert (61.7 and 54.6 respectively). However, half of the colonies recovered from the strain containing *HphMX* retained resistance to G418 (Figure 4-2A) indicating that they had might have undergone GC instead of BIR, retaining the end of chromosome V.

To determine whether or not this was the case, we separated intact chromosomes from Lys^+ colonies that either retained resistance to G418 or had lost resistance to G418, by PFGE. We then performed a southern blot hybridization analysis using a sequence telomere-proximal to the *HphMX* insert on the donor chromosome as a hybridization probe to test for the completion of BIR (Figure 4-2B bottom). Full BIR should contain this sequence on the recipient and the donor, while GC would only contain it on the donor.

This probe hybridizes to the donor chromosome XI in every lane, as expected, and also hybridizes to the band corresponding with the repair product in the BIR colony not containing any insert on the donor (Figure 4-2B second lane). As predicted, the band corresponding to the repair product from the cells that retained G418 resistance does not hybridize with this probe, confirming that GC has occurred in place of BIR and the end of chromosome XI was not copied onto the recipient. Most of the G418 sensitive Lys^+ colonies from the strain containing the *HphMX* insert exhibit the expected hybridization products (Figure 4-2B lanes 3-5). However, one of the G418 sensitive Lys^+ colonies only showed the donor chromosome signal, indicating that GC using the terminator had occurred (Figure 4-2B lane 6).

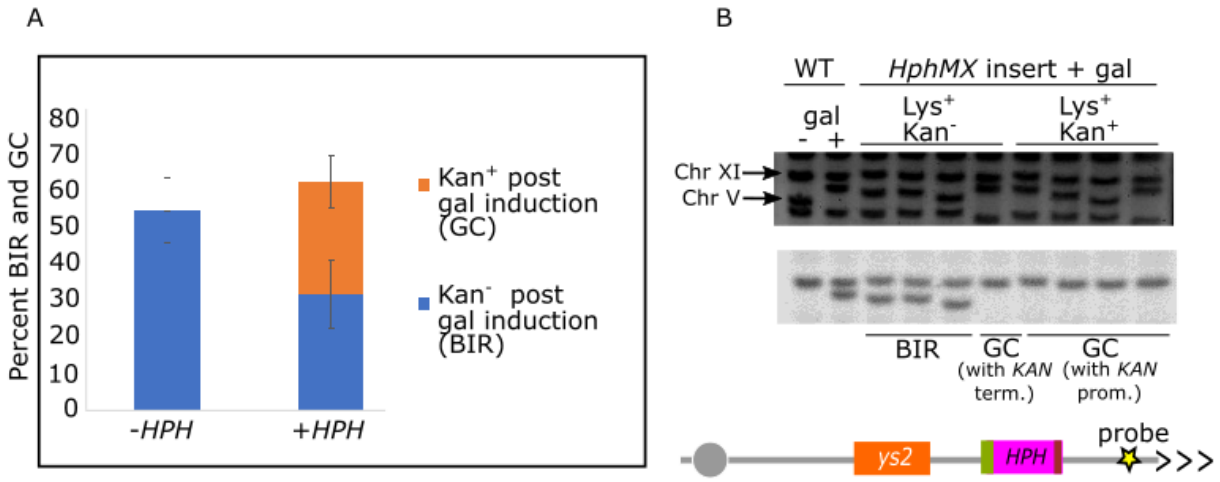


Figure 4-2 Gene Conversion Events and BIR Events Compete

A. Percent of cells surviving DSB induction (61.7 and 54.6 percent for cells without and with the *HphMX* insert respectively) on galactose. Cells surviving DSB were replica plated from galactose to G418 and the percent resistant was counted. The percent that retain resistance to G418 is shown in orange (Kan^+). No cells lacking the *HphMX* insert retained G418 resistance, while 50.5 percent of the cells containing the insert retained G418 resistance. B. Cropped image of the PFGE and southern blot. Southern blot shows the probe hybridization to the donor chromosome XI in all lanes (upper band) and absence of the downstream donor sequence in Kan^+ DSB repair products (right four lanes), as well as the absence of the downstream donor sequence in one Kan^- product (fifth lane from right), and the presence of the donor sequences on the BIR repair product (lanes 2-5). BIR products are slightly different sizes due to the starting size of chromosome V being slightly different in different clones. This does not affect the cells' ability to carry out BIR. C. Schematic showing location of southern blot probe on the donor chromosome.

4-3 Distance from Site of Strand Invasion Influences Use of Homology for GC

Considering that there appeared to be a balance between LTGC and BIR, we asked if one of the factors influencing pathway choice was the placement of the homology, as had been suggested by Jain et al. (Jain et al. 2009). We hypothesized that homology placed closer to the site of invasion would be more likely to be used for a GC event than homology further away. To test this we created a second strain where the *HphMX* insert was moved closer to the telomere. In this strain the cell must carry out 55kb of DNA synthesis before encountering the homology of the MX region, as opposed to 44kb. As expected, the percent of repair products that retained the end of chromosome V decreased with the increased distance.

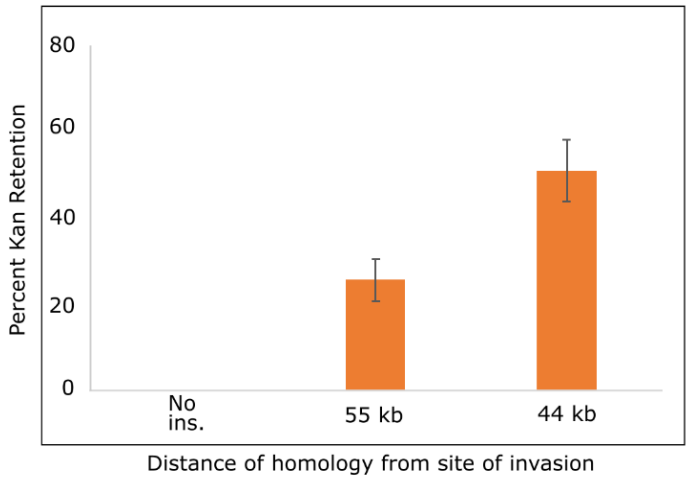


Figure 4-3 Distance from Site of Strand Invasion Influences Use of Homology for GC

Percent cells retaining G418 resistance after DSB repair for strains containing no insert (0% retention), or the insert 44kb, or 55kb away which retained the Kan marker 50.5 and 24.3 percent of the time respectively.

4-4 There is Little Competition for GC from Homology on Other Chromosomes

To gain further insight into how this distant homology is exerting an effect on the repair outcome, we asked whether similar homology on a chromosome uninvolved with repair could just as efficiently compete with BIR. To test this we created a strain where a third marker containing the MX homology region, *NatMX* was inserted in unrelated chromosome XII (Figure 4-4A).

We then compared BIR and GC outcomes reasoning that if this third piece of available homology was competing with the others for a GC event, we would see a decrease in the number of *Kan*$^+$ repair products. We saw no decrease in the number of *Kan*$^+$ repair products in the strain containing the *NatMX* marker (25.3 and 24.6 percent Kan$^+$ in the HphMX strain or the HphMX NatMX strain, respectively) indicating that it does not commonly interact with the chromosome undergoing repair (Figure 4-4B).

To further analyze the repair products, we developed a PCR assay (Figure 4-4C) to detect any use of the *NatMX* homology that was below detection in the plating assay. 24 colonies were analyzed by PCR using primers designed to detect the three possible outcomes: full length BIR, long tract gene conversion using *KanMX*, or a template switch-like event using *NatMX* homology. Out of the 24 colonies examined, 5 (21%) showed the LTGC, and 1 (4%) show the possible use of *NatMX,* although the size of this band is larger than the expected PCR product and may be an artifact.

The lack of *NatMX* involvement supports the idea that the two ends of the DSB may remain engaged in some way, that promotes GC, and the event is not a template switch-like event where the new DNA is released and re-invades at a site of homology elsewhere. If the template switch model were the case here we would expect *NatMX* to engage with *HphMX* just as often as *KanMX* does.

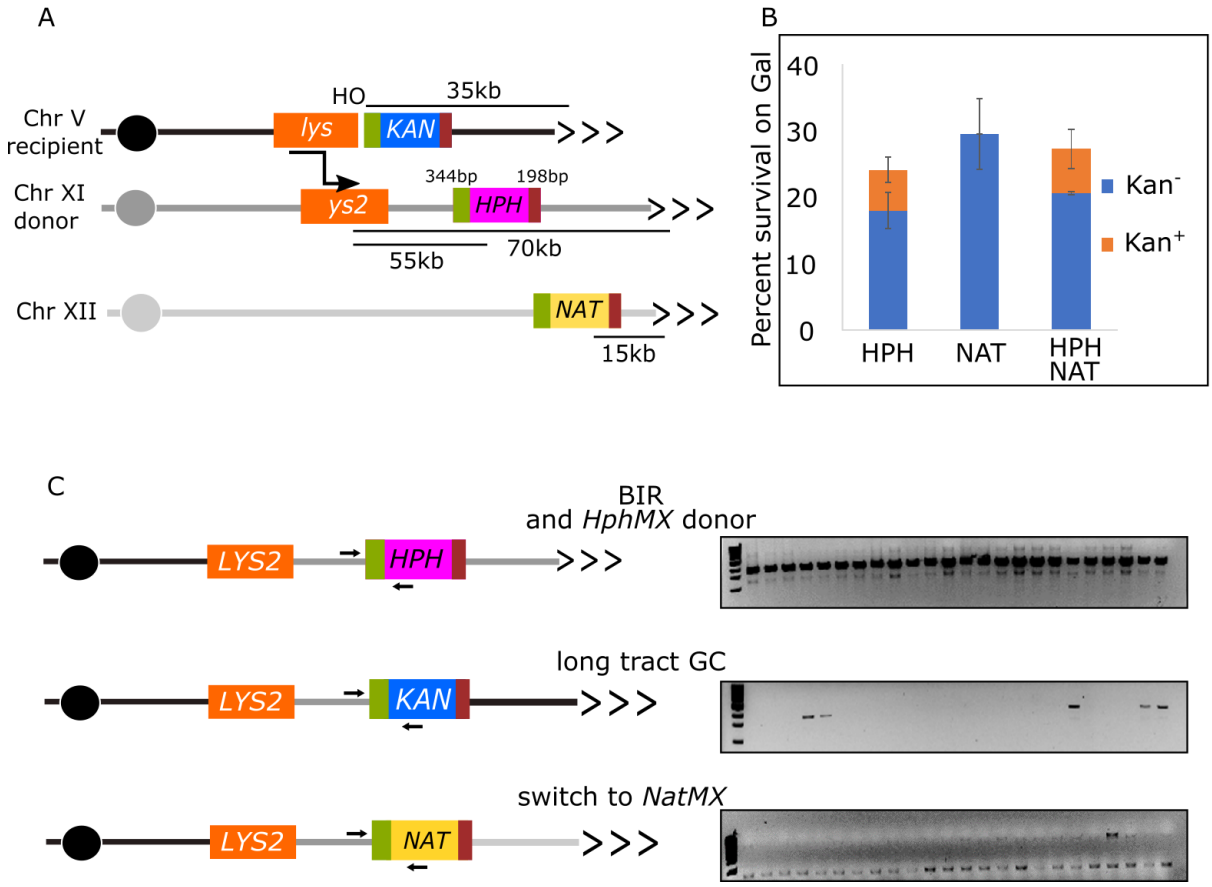


Figure 4-4 There is Little Competition for GC from Homology on Other Chromosomes

A. Schematic of chromosomes containing inserts. chromosome V and XI are the same as in Figure 5-1, and chromosome XII has a *NatMX* marker 15kb from the telomere. B. BIR percents for each strain (24.3, 29.8, and 27.6 for strains containing *HphMX*, *NatMX*, or both, respectively. Total bars) and percent Kan retention (25.3, 0, and 24.6 for strains containing *HphMX*, *NatMX*, or both, respectively. Orange segments). C. Left panel showing schematic of PCR primers used. Right panel shows PCR products. Top row shows band produced when *HphMX* is present in original location and/or in a full-length BIR product. All lanes show this band. Middle row shows bands produced when LTGC occurs. Five lanes show this band. Bottom band shows potential band produced in the case of template switching to the *NatMX*. One lane may show this band.

4-5 Summary

In some situations, one-ended breaks are clearly one-ended, such as eroded telomeres and collapsed replication forks. But in other situations it is less clear, and a cell must “decide” how to deal with a break and “decide” whether there are two available ends, as well as whether there is homology for both ends. We find that in agreement with previous studies (Jain et al. 2009; 2016; Mehta, Beach, and Haber 2017) there exists in cells a balance between GC and BIR.

We show that even over distances of tens of kb, cells can still use relatively small tracts of homology (a few hundred base pairs) to capture the extended invading strand and complete repair by GC. Distance does influence the cell’s choice of pathway, where the homology 55kb away about half as likely to be used by the cell to heal a two-ended break than the closer homology 44kb away.

This GC event happens frequently between the *HphMX* and *KanMX* markers but does not appear to involve homology on unrelated chromosomes, as demonstrated by the lack of interaction between the *HphMX* and *NatMX*. Whether this is because both ends of the break remain tethered for some time, or because the *KanMX* broken end also invades at the *HphMX* and begins synthesizing as well, is not clear and requires further investigation.

Chapter 5: Discussion

5-1 The Particular Sequence Encountered by BIR Can Lead to Mutagenesis

Typically, homologous recombination has been considered “error free”, however all DNA repair events can be a source of errors. Traditional two-ended DSB repair leads to an increase in mutations in the area repaired (Hicks, Kim, and Haber 2010), especially if the area is complex or repetitive such as a minisatellite (Jeffreys et al. 1994). The healing of one-ended double strand breaks by break induced replication is particularly prone to mutagenesis, due to the extensive DNA synthesis that occurs without the formation of a full replisome, and the long tracts of ssDNA created (Deem et al. 2011). It is important to understand the characteristics of mutagenicity in BIR, since BIR has been shown to play a critical role in the survival of cells experiencing replication stress (Minocherhomji et al. 2015; Costantino et al. 2014).

In this study we show that during BIR the particular sequence encountered by the migrating D-loop has an effect on the outcome of repair. We found that an ITS located 2.5 kb centromere distal to the site of strand invasion can cause termination of BIR synthesis and addition of a telomere to the ITS, resulting in a BIR product 67.5 kb shorter than would occur had DNA synthesis continued to the end of the donor chromosome. We also show that the effects other similar sequences have on BIR can be influenced by the processivity of DNA synthesis, where the less processive BIR is, the more mutagenic various sequences can be.

5-1.1 Telomere Formation

We investigated the mechanism of formation of the telomere, which we considered to be either homology directed repair using a natural telomere as a template, or the direct action of telomerase at the ITS site. Our data show that truncated chromosomes can be formed even in the absence of *TLC1*, which encodes the RNA template for telomerase. This indicates that HDR can be used by the cell to generate a telomere at the ITS. On the other hand, cells lacking

nuclear Pif1 -which normally prevents telomerase activity at a DSB- show an increase in the frequency of formation of chromosome truncations. This increase was dependent on *TLC1*, which indicates that telomerase can also act at the ITS to generate a new telomere.

While these data do indeed show that both methods *can* be used by the cell to form telomeres at the ITS, there are several caveats to this conclusion when it is applied to cells, since none of the experiments done to determine the origin of the new telomeres could be performed in biologically undisturbed cells.

The first caveat is that the type II survivors derived from the *tlc1*Δ strain used to investigate the requirement for telomerase, have very long and heterogeneous telomeres, up to several kb (Teng and Zakian 1999), as well as extrachromosomal telomeric circles (Cesare and Griffith 2004). This increase in telomere sequences can provide additional templates for HDR, tipping the balance toward HDR; thus, telomerase might play a more important role in terminating BIR at the ITS in WT cells than is apparent in the *tlc1*Δ mutant. Because of the need for homologous recombination proteins to initiate BIR, we cannot evaluate the role of HR proteins in the generation of truncated BIR products.

The second caveat of the telomere formation data is the many roles of Pif1 that are relevant to BIR. In addition to its role in repressing telomerase activity at DSB ends, Pif1 also plays a role unwinding G quadruplexes (Dahan et al. 2018), as well as a role in the processivity of Polymerase delta during BIR. To address this second caveat, we investigated whether the increase in truncations seen in the *pif1-m2* mutant was dependent on telomerase. The data from the *tlc1 pif1-m2* double mutant did in fact show that the increase in truncations in the absence of Pif1 is dependent on *TLC1*, which helps to clarify that the telomerase inhibitor role of Pif1 is in fact why we see the increase in truncation formation at the ITS in the absence of Pif1. This supports the suggestion that telomerase can act here. However, as we show in later experiments, the *pif1-m2* mutation also increases the truncation formation in the strain with the

Y' element insertion, which cannot use telomerase to form telomeres. Therefore, Pif1 must be playing at least two roles in the formation of truncations: increasing polymerase processivity and decreasing the action of telomerase at the DNA end, and the disruption of either role can increase the formation of truncated chromosome products during BIR.

Even with these caveats, it is still clear that both methods of telomere synthesis *can* be used by the cell. Further experiments would be required to determine which method, if either, is dominant in WT undisturbed cells. One approach to address this is the use of a galactose inducible mutant *TLC1* template. In this experiment, the *TLC1* with a different template than WT *TLC1* would be turned on only during BIR. Colonies with truncations could then be sequenced to determine whether the mutant *TLC1* sequence or the WT *TLC1* sequence was added to the newly formed telomere, indicating telomerase activity or HDR, respectively.

5-1.2 BIR Processivity in Truncation Formation

In WT cells, truncated BIR products are only recovered from the strain with the perfect telomere sequence (ITS) inserted on the donor chromosome. When we tested other sequences that share homology to the yeast Y' elements just internal to yeast telomeres, we found these sequences did not cause truncated chromosomes to be formed at a detectable level. We also found that $(GT)_{32}$ sequences, which can act as telomere seeding sequences that lack the hallmarks of telomeres, such as the ability to form G quadruplexes and bind telomeric proteins like Rap1, do not cause chromosome truncations. This indicates that the unique features of the telomeric sequence promote truncation events. It is possible however that at a high enough sample size a truncation event caused by one of the two inserts would eventually be found in a WT background.

We also find that Mph1 is required to form chromosome truncations. This requirement is consistent with previous studies showing that Mph1 dissociates Rad51-generated D-loops in vitro, has a negative effect on BIR, and promotes template switching (Prakash et al. 2009; Luke-Glaser and Luke 2012; Stafa et al. 2014). We propose that Mph1 dissociates the D-loop when it traverses the ITS and the resulting 3' ssDNA end containing the telomere repeats can be used to form a telomere, either by the action of telomerase or by invasion at a natural telomere and a second round of BIR.

Taken together, the sequence specificity of the ITS, and the dependence on Mph1 indicates that the ITS increases the vulnerability of the D-loop to the activity of Mph1. Further research will be necessary to determine if the increased vulnerability to Mph1 disassembly is caused by stalling or slowing of the polymerase at the ITS site, or by disruption or deformation of the D-loop by the ITS making it easier for Mph1 to disassemble it. Both of these scenarios may be caused by tight binding of proteins like Rap1, which has been shown to be disruptive to replication at telomeres, or by G quadruplex formation ahead or behind the polymerase in the G rich strand opposite the template, or in the newly synthesized G-rich strand.

Further experiments could be done, such as 2D gels to identify signs of stalled DNA synthesis, or ChIP to determine if the polymerase slows or spends more time at the ITS. These experiments would be challenging, however, since BIR is asynchronous, but they might yield valuable insight into why and how ITSs can cause these telomeres to be formed. Further experiments to clarify the role of Rap1 and G quadruplexes would be insightful as well. Rap1 is an essential protein and cannot be deleted, but downregulation of Rap1 using a degron system or overexpression of Rap1 might reveal a role in truncation formation that could be assessed by comparing the number of BIR products with chromosome truncations. Similarly, modulating the strength of G quadruplex formation either through changing the sequence of the inserted ITS to a stronger G quadruplex forming sequence (Piazza et al. 2015), or by the addition of G

quadruplex stabilizing drugs might shed light of the role this DNA secondary structure may be playing in the formation of telomeres at the ITS.

To investigate the role of decreased processivity that may be occurring as BIR DNA synthesis traverses the ITS, we impaired the processivity of BIR in two other ways and evaluated BIR outcomes. We found that when disruption of BIR is caused by loss nuclear Pif1 or deletion of *POL32*, the formation of truncated chromosome products is greatly increased in. In addition, the Y' elements and GT repeats which do not generate chromosome truncations at a detectable frequency in WT cells, now gain the ability to terminate BIR. The increase in chromosome truncations was greatest in strains containing tracts of GT repeats, that can most likely use both telomerase and homology directed repair. Truncations were still seen, though to a lesser degree, in strains containing homology to Y' elements, that can only use HDR and have a longer amount of DNA to synthesize before reaching the end of the telomere.

These experiments are in agreement with earlier studies investigating the outcomes of BIR in the absence of Pol32 (Deem et al. 2008; Smith, Lam, and Symington 2009). Both the study by Deem et al, and the study by Smith et al. showed more mutagenic outcomes of BIR caused by lack of processivity. Smith et al. showed using a CFV assay that 51% of the fragments transformed into cells lacking Pol32 underwent a half crossover instead of carrying out BIR. Deem et at found using a BIR assay at the *MAT* locus that half crossovers represented >23% of repaired colonies in the *pol32* strains.

It is also important to note that in our system, neither the lack of Pif1 or Pol32 nor over expression of MPH1 caused truncations to form at non telomere-seeding locations. This shows there is not simply uncontrolled telomere formation at random locations caused by BIR disruption or increased telomerase activity, as seed sequence or homology near a telomere was still required. In most cases in *MPH1* OX*, pif1-m2* or *pol32*, the lack of processivity of BIR simply

caused cell death because the cells could not complete the BIR process. These mutations only lead to truncated BIR products when there was a telomere seeding sequence.

The experiments with the Y' element and GT repeats also provide another piece of data that indicates that both methods of telomere synthesis can be used by the cell: The difference in number of truncations formed in the strains with the GT repeats vs the y' element. GT repeats can presumably use either method of telomere creation, since telomerase can recognize GT repeats, and GT repeats should exist in the natural telomere to provide a homologous site for strand invasion and HR. The Y' element strain however, is not recognized by telomerase as part of a telomere, and is forced to only use HDR, and in turn always has fewer truncations. This of course also has caveats, most notably that Y' element homology is less abundant than telomere homology, and the DNA to be synthesized is somewhat longer. But the use of 1 vs 2 possible methods to create a telomere can be one explanation of the difference in truncation number. To clarify this further, it would be interesting to test these two inserts in a *tlc1*Δ strain, as well as the *pif1-m2 tlc1*Δ strain.

5-1.3 Similarity and Relationship to Template Switching

It is difficult to directly compare the frequencies of various other template switch events, with the events triggered by the three sequences described in this thesis because each sequence has a different amount of homology available in the cell. For example, Smith et al. showed that in their chromosome fragment system, template switching happened at least once in 15-25 percent of the clones analyzed (Smith, Llorente, and Symington 2007). This template switch frequency however is coming from a cell with two copies of the donor chromosome. In this case, regardless of where D-loop dissociation occurs, there is a homologous template available to switch to, unlike in the ITS study where successful switching can occur almost

exclusively in the ITS. This limitation should decrease the frequency of events. However, in our ITS study, if dissociation happens at the ITS there are at least 32 other templates for it to switch to each of ~250bp, for a total of ~8kb telomere sequences in the cell. This abundance of templates might act to increase the frequency again. It is difficult to assess whether the truncations seen in the current study occur more or less frequently than random template switch by the numbers of ITS-caused truncated chromosomes alone.

Interestingly, Smith et al. also found template switch events between other sequences. In 11 out of 94 transformants analyzed, the CFV was a different size, and in five out of the five clones further analyzed, another template switch event had occurred between delta elements, which are part of the family of Ty elements that make up 3% of the yeast genome, for a total of 377kb Ty elements. Ty elements are particularly rich on chromosome III, making up 4.7% of the chromosome where this experiment was carried out (Richard, Kerrest, and Dujon 2008; J. M. Kim et al. 1998). Switch events between delta elements seem in some ways more like the events triggered by the ITS inserts in the current study. The frequency of the switching between delta element also seems similar, around 12%, but again the amount of homology available is quite different: hundreds of kb in the case of Ty elements, vs 8kb in the case of telomeres.

When this is taken together, it seems that the ITS is the “perfect storm” for these template switch events, while the $GT_{(32)}$ and Y’ elements are each missing at least one of the elements: tight protein binding or secondary structure formation, abundance of perfect homology in the cell, and the ability to recruit telomerase. These other sequences therefore need a “push” in the form of a disruption of the processivity of BIR in order to see the truncation events at an observable frequency.

5-1.4 Model

We propose that the ITS, while not able to drive the formation of truncated BIR products in the absence of D-loop disassembly by Mph1, is still disruptive enough to cause the D-loop to be particularly vulnerable to disassembly. This disruption may be caused by secondary DNA structures due to the high GC content of the ITS, or to bound telomeric proteins like Rap1. Any of these factors might lead to stalling of the polymerase allowing more time for Mph1 to act, or malformation of the D-loop making it easier for Mph1 to act. The 3' end newly released by Mph1 is then free to form a telomere either through the recruitment and action of telomerase or through a second round of invasion and HDR at a telomere (Figure 5-1.3).

The combination of the ITS being disruptive *enough*, and having abundant homology throughout the cell in the form of 32 telomeres as well as telomerase, allows the ITS to end BIR synthesis early by falling prey to the vulnerabilities of DNA synthesis during BIR. This also supports why ITSs are not so unstable that they cause regular spontaneous loss of chromosome arms: typical S phase DNA synthesis is much less vulnerable and much more processive.

In the case of the $GT_{(32)}$ and Y' element inserts, they do not appear to be as disruptive as the ITS is, and require BIR disruption is other ways such as loss of Pif1 or Pol32 in order to be used in the formation of truncated chromosomes. We did not test directly the requirement of Mph1 in these non-ITS events, but it seems likely that Mph1 is required here too, particularly since loss of Pif1 is known to only prevent the progression of D-loop extension, and not to disassemble D-loops entirely (Wilson et al. 2013).

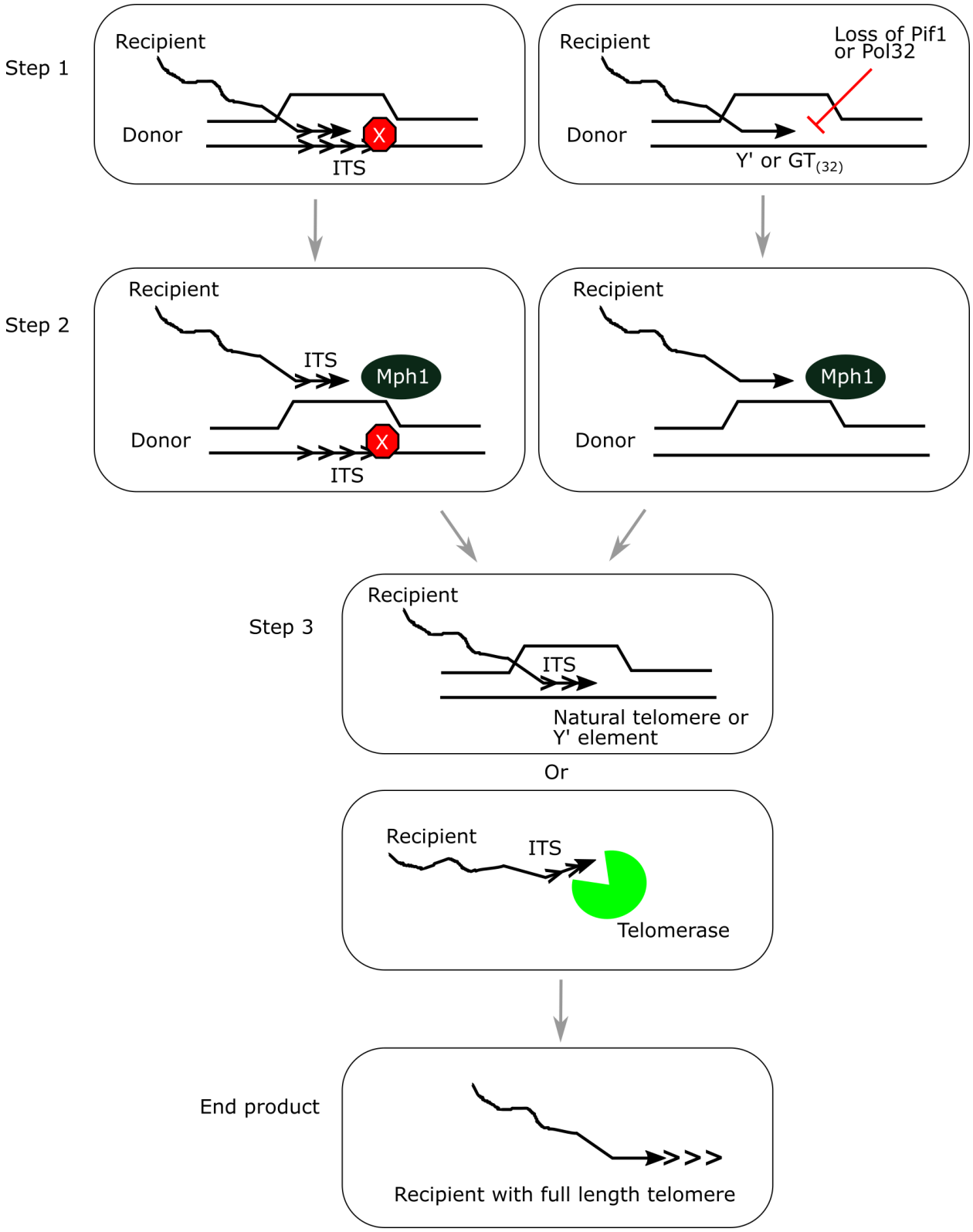

Step 1
Recipient
Donor
X
ITS
Recipient
Loss of Pif1
or Pol32
Donor
Y' or GT(32)
Step 2
Recipient
ITS
Mph1
Donor
X
ITS
Recipient
Mph1
Donor
Step 3
Recipient
ITS
Natural telomere or
Y' element
Or
Recipient
ITS
Telomerase
End product
Recipient with full length telomere

Figure 5-1.4 Model

Showing proposed steps in the formation of truncated chromosome products of BIR. From top to bottom the steps shown are first, stalling or other disruption in the processivity of BIR. At the ITS this is shown as the red stop sign, at the other $GT_{(32)}$ or Y' element inserts this is shown as the effects of the loss of Pif1 or Pol32. Second, Mph1 disassembly of the D-loop. Third, formation of a new telomere by either direct action of telomerase or homology dependent repair (depending on sequence). Lastly, the end product is shown which is similar for all inserts: a recipient chromosome with a full-length telomere added at the site of the insertion.

5-1.5 Broader Applications and Unanswered Questions

It is possible that other forms of replication stress such as hydroxyurea exposure, which depletes nucleotide pools, or polymerase inhibitors such as aphidicolin sometimes used to model the replication stress of cancer cells, would also decrease the processivity of BIR and result in the formation of truncated chromosome products. It has already been shown that BIR may be used in mammalian cells to complete replication of fragile sites during early mitosis, and may be responsible for certain rearrangements in breast cancer cells (Minocherhomji et al. 2015; Costantino et al. 2014). Thus, the tendency of BIR to form these truncations may be relevant to mutagenesis in these replication-stressed and vulnerable cells.

One unanswered question is the effect of the location of the ITS on BIR and truncation formation. We originally placed the ITS close to the site of invasion for two reasons: first, because we were hoping to "catch" early BIR attempts that might start and "give up" before completing BIR, and second, because there is some evidence that template switching occurs more frequently early in BIR (Smith, Llorente, and Symington 2007). When we placed to ITS 55 kb away from the site of invasion, no truncated chromosomes were found. Whether this is because there is some increase in stability of the D-loop later in BIR as has been proposed by some or because many more BIR events start BIR than finish BIR, and we did in fact "catch" BIR events with the ITS that would be otherwise lost, is unclear. It would be interesting to see if there is an incremental decrease in truncation formation if the ITS were placed at various sites further from the site of invasion.

We also considered the effect of a reverse orientation ITS. However we would not be able to recover events with telomere addition at this site. To investigate if the reversed sequence is in fact disruptive to BIR in any way (as the forward facing ITS appears to be) we would have to look for a decrease in BIR survival caused by the disruptive ITS sequence. Given

the variability of BIR completion and the small expected decrease in survival, it is unlikely that we would see a reproducible decrease, so this was not tested.

It also appears that cells lacking processivity, such as the *pol32*, *pif1-m2*, and *MPH1* OX strains, have an increase in survival when the strains contain an ITS. This was significant in *MPH1* OE, (p-value 0.002) *pif1-m2* (p-value 0.008) but did not reach significance in the *pol32*Δ strains potentially because the BIR rates were so low. It is possible that the ITS functions as a way to end BIR early in these strains, without which the cells simply fail to complete BIR leading to overactivation of the DNA damage checkpoint and cell death. Further testing to analyze the strength and implications of these data is required.

5-2 Connections Between BIR and GC

We also addressed the interplay between BIR and GC. This issue has been addressed previously in several studies by the Haber and Malkova labs where they showed competition between BIR and GC. Mehta et al. in particular studied the extent of homology required, showing that less homology leads to more BIR while more homology leads to more GC (Mehta, Beach, and Haber 2017). Jain et al.(2009) studied and the distance between the two donors required for a two ended break to be repaired as two ended showing that the further the two homology donors are, the less likely the cell is to use GC over BIR (Jain et al. 2009). Jain et al.(2016) looks into the mechanisms of a proposed checkpoint that the cell uses to “decide” whether to carry out GC or BIR (Jain et al. 2016). Deem et al. also showed the GC can be used over BIR even in cases of very small tracts of homology (46bp) (Deem et al. 2008).

The present study did not address the proposed checkpoint, but the data we found showing that distance between the two donor homologies influences the repair pathway support what was found by these previous studies. Our data also go on to show that GC can be used

over an even longer distance than previously showed, and with less homology. Jain et al. showed that GC decreases to just under 50% when the two homologies were placed 26kb apart. While we showed a similar rate of GC with homologies further away, at 44kb, we showed that even at a distance of 55kb, cells will still perform gene conversion close to 25% of the time, even when the homology is small, ~300 bp in this case, compared with ~700bp in the Jain et al. paper.

This data combined with our data showing that BIR forms telomeres at the ITS at a relatively high frequency of 12%, support the idea that cells “hate” BIR. It seems that all steps possible are taken by the cell to avoid BIR and the extensive mutagenicity associated with BIR. This seems true even if those steps taken by the cell are *also* mutagenic, such as very long tract gene conversion that creates hybrid chromosomes, and inappropriate telomere synthesis. The constant “checking” in the form of D-loop disassembly by Mph1, drives template switching, and drives the truncated chromosome products in this study. It is possible that it also drives the extremely long tract gene conversion events found, and supports the idea that cells would “rather” do anything than BIR.

References

Aksenova, Anna Y., Patricia W. Greenwell, Margaret Dominska, Alexander A. Shishkin, Jane C. Kim, Thomas D. Petes, and Sergei M. Mirkin. 2013. “Genome Rearrangements Caused by Interstitial Telomeric Sequences in Yeast.” *Proceedings of the National Academy of Sciences of the United States of America* 110 (49): 19866–71. https://doi.org/10.1073/pnas.1319313110.

Aksenova, Anna Y., and Sergei M. Mirkin. 2019. “At the Beginning of the End and in the Middle of the Beginning: Structure and Maintenance of Telomeric DNA Repeats and Interstitial Telomeric Sequences.” *Genes* 10 (2). https://doi.org/10.3390/genes10020118.

Ashley, T., and D. C. Ward. 1993. “A ‘Hot Spot’ of Recombination Coincides with an Interstitial Telomeric Sequence in the Armenian Hamster.” *Cytogenetics and Cell Genetics* 62 (2–3): 169–71. https://doi.org/10.1159/000133464.

Balakumaran, B. S., C. H. Freudenreich, and V. A. Zakian. 2000. “CGG/CCG Repeats Exhibit Orientation-Dependent Instability and Orientation-Independent Fragility in Saccharomyces Cerevisiae.” *Human Molecular Genetics* 9 (1): 93–100. https://doi.org/10.1093/hmg/9.1.93.

Bechter, Oliver E., Ying Zou, William Walker, Woodring E. Wright, and Jerry W. Shay. 2004. “Telomeric Recombination in Mismatch Repair Deficient Human Colon Cancer Cells after Telomerase Inhibition.” *Cancer Research* 64 (10): 3444–51. https://doi.org/10.1158/0008-5472.CAN-04-0323.

Bertoni, L., C. Attolini, L. Tessera, E. Mucciolo, and E. Giulotto. 1994. “Telomeric and Nontelomeric (TTAGGG)n Sequences in Gene Amplification and Chromosome Stability.” *Genomics* 24 (1): 53–62. https://doi.org/10.1006/geno.1994.1581.

Bhandari, Jayaram, Travis Karg, and Kent Golic. 2019. “Homolog Dependent Repair Following Dicentric Chromosome Breakage in Drosophila Melanogaster.” *Genetics*, May. https://doi.org/10.1534/genetics.119.302247.

Blackburn, Elizabeth H., and Kathleen Collins. 2011. “Telomerase: An RNP Enzyme Synthesizes DNA.” *Cold Spring Harbor Perspectives in Biology* 3 (5). https://doi.org/10.1101/cshperspect.a003558.

Bosco, G., and J. E. Haber. 1998. “Chromosome Break-Induced DNA Replication Leads to Nonreciprocal Translocations and Telomere Capture.” *Genetics* 150 (3): 1037–47.

Boulé, Jean-Baptiste, and Virginia A. Zakian. 2006. “Roles of Pif1-like Helicases in the Maintenance of Genomic Stability.” *Nucleic Acids Research* 34 (15): 4147–53. https://doi.org/10.1093/nar/gkl561.

Bryan, T. M., A. Englezou, J. Gupta, S. Bacchetti, and R. R. Reddel. 1995. “Telomere Elongation in Immortal Human Cells without Detectable Telomerase Activity.” *The EMBO Journal* 14 (17): 4240–48.

Burgers, P. M., and K. J. Gerik. 1998. “Structure and Processivity of Two Forms of Saccharomyces Cerevisiae DNA Polymerase Delta.” *The Journal of Biological Chemistry* 273 (31): 19756–62. https://doi.org/10.1074/jbc.273.31.19756.

Buzovetsky, Olga, Youngho Kwon, Nhung Tuyet Pham, Claire Kim, Grzegorz Ira, Patrick Sung, and Yong Xiong. 2017. “Role of the Pif1-PCNA Complex in Pol δ-Dependent Strand Displacement DNA Synthesis and Break-Induced Replication.” *Cell Reports* 21 (7): 1707–14. https://doi.org/10.1016/j.celrep.2017.10.079.

Cannavo, Elda, and Petr Cejka. 2014. “Sae2 Promotes DsDNA Endonuclease Activity within Mre11–Rad50–Xrs2 to Resect DNA Breaks.” *Nature* 514 (7520): 122–25. https://doi.org/10.1038/nature13771.

Cesare, Anthony J., and Jack D. Griffith. 2004. “Telomeric DNA in ALT Cells Is Characterized by Free Telomeric Circles and Heterogeneous T-Loops.” *Molecular and Cellular Biology* 24 (22): 9948–57. https://doi.org/10.1128/MCB.24.22.9948-9957.2004.

Cesare, Anthony J., and Roger R. Reddel. 2010. “Alternative Lengthening of Telomeres: Models, Mechanisms and Implications.” *Nature Reviews. Genetics* 11 (5): 319–30. https://doi.org/10.1038/nrg2763.

Chen, C., and R. D. Kolodner. 1999. “Gross Chromosomal Rearrangements in Saccharomyces Cerevisiae Replication and Recombination Defective Mutants.” *Nature Genetics* 23 (1): 81–85. https://doi.org/10.1038/12687.

Chen, Jian-Min, Peter D. Stenson, David N. Cooper, and Claude Férec. 2005. “A Systematic Analysis of LINE-1 Endonuclease-Dependent Retrotranspositional Events Causing Human Genetic Disease.” *Human Genetics* 117 (5): 411–27. https://doi.org/10.1007/s00439-005-1321-0.

Chen, Q., A. Ijpma, and C. W. Greider. 2001. “Two Survivor Pathways That Allow Growth in the Absence of Telomerase Are Generated by Distinct Telomere Recombination Events.” *Molecular and Cellular Biology* 21 (5): 1819–27. https://doi.org/10.1128/MCB.21.5.1819-1827.2001.

Chimpanzee Sequencing and Analysis Consortium. 2005. “Initial Sequence of the Chimpanzee Genome and Comparison with the Human Genome.” *Nature* 437 (7055): 69–87. https://doi.org/10.1038/nature04072.

Chiruvella, Kishore K., Zhuobin Liang, and Thomas E. Wilson. 2013. “Repair of Double-Strand Breaks by End Joining.” *Cold Spring Harbor Perspectives in Biology* 5 (5): a012757. https://doi.org/10.1101/cshperspect.a012757.

Ciccia, Alberto, and Stephen J. Elledge. 2010. “The DNA Damage Response: Making It Safe to Play with Knives.” *Molecular Cell* 40 (2): 179–204. https://doi.org/10.1016/j.molcel.2010.09.019.

Colnaghi, Rita, Gillian Carpenter, Marcel Volker, and Mark O’Driscoll. 2011. “The Consequences of Structural Genomic Alterations in Humans: Genomic Disorders, Genomic Instability and Cancer.” *Seminars in Cell & Developmental Biology*, Polarized

growth and movement: How to generate new shapes and structures, 22 (8): 875–85. https://doi.org/10.1016/j.semcdb.2011.07.010.

Conrad, M. N., J. H. Wright, A. J. Wolf, and V. A. Zakian. 1990. “RAP1 Protein Interacts with Yeast Telomeres in Vivo: Overproduction Alters Telomere Structure and Decreases Chromosome Stability.” *Cell* 63 (4): 739–50.

Costantino, Lorenzo, Sotirios K. Sotiriou, Juha K. Rantala, Simon Magin, Emil Mladenov, Thomas Helleday, James E. Haber, George Iliakis, Olli P. Kallioniemi, and Thanos D. Halazonetis. 2014. “Break-Induced Replication Repair of Damaged Forks Induces Genomic Duplications in Human Cells.” *Science (New York, N.Y.)* 343 (6166): 88–91. https://doi.org/10.1126/science.1243211.

Crider, David G., Luis J. García-Rodríguez, Pallavi Srivastava, Leonardo Peraza-Reyes, Krishna Upadhyaya, Istvan R. Boldogh, and Liza A. Pon. 2012. “Rad53 Is Essential for a Mitochondrial DNA Inheritance Checkpoint Regulating G1 to S Progression.” *The Journal of Cell Biology* 198 (5): 793–98. https://doi.org/10.1083/jcb.201205193.

Dahan, Danielle, Ioannis Tsirkas, Daniel Dovrat, Melanie A. Sparks, Saurabh P. Singh, Roberto Galletto, and Amir Aharoni. 2018. “Pif1 Is Essential for Efficient Replisome Progression through Lagging Strand G-Quadruplex DNA Secondary Structures.” *Nucleic Acids Research* 46 (22): 11847–57. https://doi.org/10.1093/nar/gky1065.

Daley, James M., Phillip L. Palmbos, Dongliang Wu, and Thomas E. Wilson. 2005. “Nonhomologous End Joining in Yeast.” *Annual Review of Genetics* 39: 431–51. https://doi.org/10.1146/annurev.genet.39.073003.113340.

Davis, Allison P., and Lorraine S. Symington. 2004. “RAD51-Dependent Break-Induced Replication in Yeast.” *Molecular and Cellular Biology* 24 (6): 2344–51. https://doi.org/10.1128/mcb.24.6.2344-2351.2004.

Day, Henry A., Pavlos Pavlou, and Zoë A. E. Waller. 2014. “I-Motif DNA: Structure, Stability and Targeting with Ligands.” *Bioorganic & Medicinal Chemistry* 22 (16): 4407–18. https://doi.org/10.1016/j.bmc.2014.05.047.

Deem, Angela, Krista Barker, Kelly Vanhulle, Brandon Downing, Alexandra Vayl, and Anna Malkova. 2008. “Defective Break-Induced Replication Leads to Half-Crossovers in Saccharomyces Cerevisiae.” *Genetics* 179 (4): 1845–60. https://doi.org/10.1534/genetics.108.087940.

Deem, Angela, Andrea Keszthelyi, Tiffany Blackgrove, Alexandra Vayl, Barbara Coffey, Ruchi Mathur, Andrei Chabes, and Anna Malkova. 2011. “Break-Induced Replication Is Highly Inaccurate.” *PLoS Biology* 9 (2): e1000594. https://doi.org/10.1371/journal.pbio.1000594.

Diede, S. J., and D. E. Gottschling. 1999. “Telomerase-Mediated Telomere Addition in Vivo Requires DNA Primase and DNA Polymerases Alpha and Delta.” *Cell* 99 (7): 723–33.

Donnianni, Roberto A., and Lorraine S. Symington. 2013. “Break-Induced Replication Occurs by Conservative DNA Synthesis.” *Proceedings of the National Academy of Sciences of the United States of America* 110 (33): 13475–80. https://doi.org/10.1073/pnas.1309800110.

Dunn, B., P. Szauter, M. L. Pardue, and J. W. Szostak. 1984. “Transfer of Yeast Telomeres to Linear Plasmids by Recombination.” *Cell* 39 (1): 191–201. https://doi.org/10.1016/0092-8674(84)90205-8.

Elango, Rajula, Ziwei Sheng, Jessica Jackson, Jenna DeCata, Younis Ibrahim, Nhung T. Pham, Diana H. Liang, et al. 2017. “Break-Induced Replication Promotes Formation of Lethal Joint Molecules Dissolved by Srs2.” *Nature Communications* 8 (1): 1790. https://doi.org/10.1038/s41467-017-01987-2.

Finn, Karen, Noel Francis Lowndes, and Muriel Grenon. 2012. “Eukaryotic DNA Damage Checkpoint Activation in Response to Double-Strand Breaks.” *Cellular and Molecular Life Sciences* 69 (9): 1447–73. https://doi.org/10.1007/s00018-011-0875-3.

Fischer, G., S. A. James, I. N. Roberts, S. G. Oliver, and E. J. Louis. 2000. “Chromosomal Evolution in Saccharomyces.” *Nature* 405 (6785): 451–54. https://doi.org/10.1038/35013058.

Fishman-Lobell, J., and J. E. Haber. 1992. “Removal of Nonhomologous DNA Ends in Double-Strand Break Recombination: The Role of the Yeast Ultraviolet Repair Gene RAD1.” *Science (New York, N.Y.)* 258 (5081): 480–84. https://doi.org/10.1126/science.1411547.

Förstemann, K., and J. Lingner. 2001. “Molecular Basis for Telomere Repeat Divergence in Budding Yeast.” *Molecular and Cellular Biology* 21 (21): 7277–86. https://doi.org/10.1128/MCB.21.21.7277-7286.2001.

Garcia, Valerie, Sarah E. L. Phelps, Stephen Gray, and Matthew J. Neale. 2011. “Bidirectional Resection of DNA Double-Strand Breaks by Mre11 and Exo1.” *Nature* 479 (7372): 241–44. https://doi.org/10.1038/nature10515.

Gehring, K., J. L. Leroy, and M. Guéron. 1993. “A Tetrameric DNA Structure with Protonated Cytosine.Cytosine Base Pairs.” *Nature* 363 (6429): 561–65. https://doi.org/10.1038/363561a0.

Gerald, Jonathan N. Fitz, Jacqueline M. Benjamin, and Stephen J. Kron. 2002. “Robust G1 Checkpoint Arrest in Budding Yeast: Dependence on DNA Damage Signaling and Repair.” *Journal of Cell Science* 115 (Pt 8): 1749–57.

Geronimo, Carly L., and Virginia A. Zakian. 2016. “Getting It Done at the Ends: Pif1 Family DNA Helicases and Telomeres.” *DNA Repair* 44 (August): 151–58. https://doi.org/10.1016/j.dnarep.2016.05.021.

Gilson, E., M. Roberge, R. Giraldo, D. Rhodes, and S. M. Gasser. 1993. “Distortion of the DNA Double Helix by RAP1 at Silencers and Multiple Telomeric Binding Sites.” *Journal of Molecular Biology* 231 (2): 293–310. https://doi.org/10.1006/jmbi.1993.1283.

Gobbini, Elisa, Corinne Cassani, Jacopo Vertemara, Weibin Wang, Fabiana Mambretti, Erika Casari, Patrick Sung, Renata Tisi, Giuseppe Zampella, and Maria Pia Longhese. 2018. “The MRX Complex Regulates Exo1 Resection Activity by Altering DNA End Structure.” *The EMBO Journal* 37 (16). https://doi.org/10.15252/embj.201798588.

Goldstein, A. L., and J. H. McCusker. 1999. “Three New Dominant Drug Resistance Cassettes for Gene Disruption in Saccharomyces Cerevisiae.” *Yeast (Chichester, England)* 15 (14): 1541–53. https://doi.org/10.1002/(SICI)1097-0061(199910)15:14<1541::AID-YEA476>3.0.CO;2-K.

Goto, Greicy H., Sevil Zencir, Yukinori Hirano, Hiroo Ogi, Andreas Ivessa, and Katsunori Sugimoto. 2015. “Binding of Multiple Rap1 Proteins Stimulates Chromosome Breakage Induction during DNA Replication.” *PLOS Genetics* 11 (8): e1005283. https://doi.org/10.1371/journal.pgen.1005283.

Gravel, Serge, J. Ross Chapman, Christine Magill, and Stephen P. Jackson. 2008. “DNA Helicases Sgs1 and BLM Promote DNA Double-Strand Break Resection.” *Genes & Development* 22 (20): 2767–72. https://doi.org/10.1101/gad.503108.

Haber, James E. 2018. “DNA Repair: The Search for Homology.” *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology* 40 (5): e1700229. https://doi.org/10.1002/bies.201700229.

Hicks, Wade M., Minlee Kim, and James E. Haber. 2010. “Increased Mutagenesis and Unique Mutation Signature Associated with Mitotic Gene Conversion.” *Science (New York, N.Y.)* 329 (5987): 82–85. https://doi.org/10.1126/science.1191125.

Ho, Chu Kwen, Gerard Mazón, Alicia F. Lam, and Lorraine S. Symington. 2010. “Mus81 and Yen1 Promote Reciprocal Exchange during Mitotic Recombination to Maintain Genome Integrity in Budding Yeast.” *Molecular Cell* 40 (6): 988–1000. https://doi.org/10.1016/j.molcel.2010.11.016.

Hoeijmakers, Jan H. J. 2009. “DNA Damage, Aging, and Cancer.” *The New England Journal of Medicine* 361 (15): 1475–85. https://doi.org/10.1056/NEJMra0804615.

Holliday, Robin. 2007. “A Mechanism for Gene Conversion in Fungi.” *Genetical Research* 89 (5–6): 285–307. https://doi.org/10.1017/S0016672308009476.

Hu, Yan, Hong-Bo Tang, Ning-Ning Liu, Xia-Jing Tong, Wei Dang, Yi-Min Duan, Xiao-Hong Fu, et al. 2013. “Telomerase-Null Survivor Screening Identifies Novel Telomere Recombination Regulators.” *PLOS Genetics* 9 (1): e1003208. https://doi.org/10.1371/journal.pgen.1003208.

Ira, Grzegorz, and James E. Haber. 2002. “Characterization of RAD51-Independent Break-Induced Replication That Acts Preferentially with Short Homologous Sequences.” *Molecular and Cellular Biology* 22 (18): 6384–92. https://doi.org/10.1128/MCB.22.18.6384-6392.2002.

Ira, Grzegorz, Anna Malkova, Giordano Liberi, Marco Foiani, and James E. Haber. 2003. “Srs2 and Sgs1–Top3 Suppress Crossovers during Double-Strand Break Repair in Yeast.” *Cell* 115 (4): 401–11. https://doi.org/10.1016/S0092-8674(03)00886-9.

Ivanov, E L, and J E Haber. 1995. “RAD1 and RAD10, but Not Other Excision Repair Genes, Are Required for Double-Strand Break-Induced Recombination in Saccharomyces Cerevisiae.” *Molecular and Cellular Biology* 15 (4): 2245–51.

Jain, Suvi, Neal Sugawara, John Lydeard, Moreshwar Vaze, Nicolas Tanguy Le Gac, and James E. Haber. 2009. “A Recombination Execution Checkpoint Regulates the Choice of Homologous Recombination Pathway during DNA Double-Strand Break Repair.” *Genes & Development* 23 (3): 291–303. https://doi.org/10.1101/gad.1751209.

Jain, Suvi, Neal Sugawara, Anuja Mehta, Taehyun Ryu, and James E. Haber. 2016. “Sgs1 and Mph1 Helicases Enforce the Recombination Execution Checkpoint During DNA Double-Strand Break Repair in Saccharomyces Cerevisiae.” *Genetics* 203 (2): 667–75. https://doi.org/10.1534/genetics.115.184317.

Jeffreys, A. J., K. Tamaki, A. MacLeod, D. G. Monckton, D. L. Neil, and J. A. Armour. 1994. “Complex Gene Conversion Events in Germline Mutation at Human Minisatellites.” *Nature Genetics* 6 (2): 136–45. https://doi.org/10.1038/ng0294-136.

Jin, Yong Hwan, Parie Garg, Carrie M. W. Stith, Hanan Al-Refai, Joan F. Sterling, Laura J. W. Murray, Thomas A. Kunkel, Michael A. Resnick, Peter M. Burgers, and Dmitry A. Gordenin. 2005. “The Multiple Biological Roles of the 3’-->5’ Exonuclease of Saccharomyces Cerevisiae DNA Polymerase Delta Require Switching between the Polymerase and Exonuclease Domains.” *Molecular and Cellular Biology* 25 (1): 461–71. https://doi.org/10.1128/MCB.25.1.461-471.2005.

Johnson, F. B., R. A. Marciniak, M. McVey, S. A. Stewart, W. C. Hahn, and L. Guarente. 2001. “The Saccharomyces Cerevisiae WRN Homolog Sgs1p Participates in Telomere Maintenance in Cells Lacking Telomerase.” *The EMBO Journal* 20 (4): 905–13. https://doi.org/10.1093/emboj/20.4.905.

Kang, L. E., and L. S. Symington. 2000. “Aberrant Double-Strand Break Repair in Rad51 Mutants of Saccharomyces Cerevisiae.” *Molecular and Cellular Biology* 20 (24): 9162–72. https://doi.org/10.1128/mcb.20.24.9162-9172.2000.

Kilburn, A. E., M. J. Shea, R. G. Sargent, and J. H. Wilson. 2001. “Insertion of a Telomere Repeat Sequence into a Mammalian Gene Causes Chromosome Instability.” *Molecular and Cellular Biology* 21 (1): 126–35. https://doi.org/10.1128/MCB.21.1.126-135.2001.

Kim, J. M., S. Vanguri, J. D. Boeke, A. Gabriel, and D. F. Voytas. 1998. “Transposable Elements and Genome Organization: A Comprehensive Survey of Retrotransposons Revealed by the Complete Saccharomyces Cerevisiae Genome Sequence.” *Genome Research* 8 (5): 464–78. https://doi.org/10.1101/gr.8.5.464.

Kim, Jane C., Samantha T. Harris, Teresa Dinter, Kartik A. Shah, and Sergei M. Mirkin. 2017. “The Role of Break-Induced Replication in Large-Scale Expansions of (CAG)N•(CTG)n Repeats.” *Nature Structural & Molecular Biology* 24 (1): 55–60. https://doi.org/10.1038/nsmb.3334.

Kok, Marleen, Myriam Chalabi, and John Haanen. 2019. “How I Treat MSI Cancers with Advanced Disease.” *ESMO Open* 4 (Suppl 2): e000511. https://doi.org/10.1136/esmoopen-2019-000511.

Koszul, Romain, Sandrine Caburet, Bernard Dujon, and Gilles Fischer. 2004. “Eucaryotic Genome Evolution through the Spontaneous Duplication of Large Chromosomal

Segments." *The EMBO Journal* 23 (1): 234–43. https://doi.org/10.1038/sj.emboj.7600024.

Koszul, Romain, Bernard Dujon, and Gilles Fischer. 2006. "Stability of Large Segmental Duplications in the Yeast Genome." *Genetics* 172 (4): 2211–22. https://doi.org/10.1534/genetics.105.048058.

Kraus, Eliyahu, Wai-Ying Leung, and James E. Haber. 2001. "Break-Induced Replication: A Review and an Example in Budding Yeast." *Proceedings of the National Academy of Sciences* 98 (15): 8255–62. https://doi.org/10.1073/pnas.151008198.

Kreuzer, Kenneth N. 2000. "Recombination-Dependent DNA Replication in Phage T4." *Trends in Biochemical Sciences* 25 (4): 165–73. https://doi.org/10.1016/S0968-0004(00)01559-0.

Krogh, Berit Olsen, and Lorraine S. Symington. 2004. "Recombination Proteins in Yeast." *Annual Review of Genetics* 38: 233–71. https://doi.org/10.1146/annurev.genet.38.072902.091500.

Kulkarni, Avanti, and David M. Wilson. 2008. "The Involvement of DNA-Damage and -Repair Defects in Neurological Dysfunction." *American Journal of Human Genetics* 82 (3): 539–66. https://doi.org/10.1016/j.ajhg.2008.01.009.

Kunkel, T. A. 1988. "Exonucleolytic Proofreading." *Cell* 53 (6): 837–40. https://doi.org/10.1016/s0092-8674(88)90189-4.

Kyrion, G, K A Boakye, and A J Lustig. 1992. "C-Terminal Truncation of RAP1 Results in the Deregulation of Telomere Size, Stability, and Function in Saccharomyces Cerevisiae." *Molecular and Cellular Biology* 12 (11): 5159–73.

Lam, Enid Yi Ni, Dario Beraldi, David Tannahill, and Shankar Balasubramanian. 2013. "G-Quadruplex Structures Are Stable and Detectable in Human Genomic DNA." *Nature Communications* 4: 1796. https://doi.org/10.1038/ncomms2792.

Larrivée, Michel, and Raymund J. Wellinger. 2006. "Telomerase- and Capping-Independent Yeast Survivors with Alternate Telomere States." *Nature Cell Biology* 8 (7): 741–47. https://doi.org/10.1038/ncb1429.

Le, S., J. K. Moore, J. E. Haber, and C. W. Greider. 1999. "RAD50 and RAD51 Define Two Pathways That Collaborate to Maintain Telomeres in the Absence of Telomerase." *Genetics* 152 (1): 143–52.

Lee, Ja Yil, Tsuyoshi Terakawa, Zhi Qi, Justin B. Steinfeld, Sy Redding, YoungHo Kwon, William A. Gaines, Weixing Zhao, Patrick Sung, and Eric C. Greene. 2015. "DNA RECOMBINATION. Base Triplet Stepping by the Rad51/RecA Family of Recombinases." *Science (New York, N.Y.)* 349 (6251): 977–81. https://doi.org/10.1126/science.aab2666.

Leffak, Michael. 2017. "Break-Induced Replication Links Microsatellite Expansion to Complex Genome Rearrangements." *BioEssays* 39 (8): 1700025. https://doi.org/10.1002/bies.201700025.

Lesage, P., and A. L. Todeschini. 2005. “Happy Together: The Life and Times of Ty Retrotransposons and Their Hosts.” *Cytogenetic and Genome Research* 110 (1–4): 70–90. https://doi.org/10.1159/000084940.

Lin, Chi-Ying, Hsih-Hsuan Chang, Kou-Juey Wu, Shun-Fu Tseng, Chuan-Chuan Lin, Chao-Po Lin, and Shu-Chun Teng. 2005. “Extrachromosomal Telomeric Circles Contribute to Rad52-, Rad50-, and Polymerase Delta-Mediated Telomere-Telomere Recombination in Saccharomyces Cerevisiae.” *Eukaryotic Cell* 4 (2): 327–36. https://doi.org/10.1128/EC.4.2.327-336.2005.

Lisby, Michael, Jacqueline H. Barlow, Rebecca C. Burgess, and Rodney Rothstein. 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.

Liu, Yuan, and Samuel H. Wilson. 2012. “DNA Base Excision Repair: A Mechanism of Trinucleotide Repeat Expansion.” *Trends in Biochemical Sciences* 37 (4): 162–72. https://doi.org/10.1016/j.tibs.2011.12.002.

Llorente, Bertrand, Catherine E. Smith, and Lorraine S. Symington. 2008. “Break-Induced Replication: What Is It and What Is It For?” *Cell Cycle (Georgetown, Tex.)* 7 (7): 859–64. https://doi.org/10.4161/cc.7.7.5613.

Long, Ashlee, Jill S. Napierala, Urszula Polak, Lauren Hauser, Arnulf H. Koeppen, David R. Lynch, and Marek Napierala. 2017. “Somatic Instability of the Expanded GAA Repeats in Friedreich’s Ataxia.” *PloS One* 12 (12): e0189990. https://doi.org/10.1371/journal.pone.0189990.

Luke-Glaser, Sarah, and Brian Luke. 2012. “The Mph1 Helicase Can Promote Telomere Uncapping and Premature Senescence in Budding Yeast.” *PLOS ONE* 7 (7): e42028. https://doi.org/10.1371/journal.pone.0042028.

Lundblad, V., and E. H. Blackburn. 1993. “An Alternative Pathway for Yeast Telomere Maintenance Rescues Est1- Senescence.” *Cell* 73 (2): 347–60.

Lustig, A. J. 1992. “Hoogsteen G-G Base Pairing Is Dispensable for Telomere Healing in Yeast.” *Nucleic Acids Research* 20 (12): 3021–28. https://doi.org/10.1093/nar/20.12.3021.

Lydeard, John R., Suvi Jain, Miyuki Yamaguchi, and James E. Haber. 2007. “Break-Induced Replication and Telomerase-Independent Telomere Maintenance Require Pol32.” *Nature* 448 (7155): 820–23. https://doi.org/10.1038/nature06047.

Lydeard, John R., Zachary Lipkin-Moore, Suvi Jain, Vinay V. Eapen, and James E. Haber. 2010. “Sgs1 and Exo1 Redundantly Inhibit Break-Induced Replication and de Novo Telomere Addition at Broken Chromosome Ends.” *PLoS Genetics* 6 (5): e1000973. https://doi.org/10.1371/journal.pgen.1000973.

Lydeard, John R., Zachary Lipkin-Moore, Yi-Jun Sheu, Bruce Stillman, Peter M. Burgers, and James E. Haber. 2010. “Break-Induced Replication Requires All Essential DNA Replication Factors except Those Specific for Pre-RC Assembly.” *Genes & Development* 24 (11): 1133–44. https://doi.org/10.1101/gad.1922610.

MacNeill, S A, S Moreno, N Reynolds, P Nurse, and P A Fantes. 1996. “The Fission Yeast Cdc1 Protein, a Homologue of the Small Subunit of DNA Polymerase Delta, Binds to Pol3 and Cdc27.” *The EMBO Journal* 15 (17): 4613–28.

Malkova, A., E. L. Ivanov, and J. E. Haber. 1996. “Double-Strand Break Repair in the Absence of RAD51 in Yeast: A Possible Role for Break-Induced DNA Replication.” *Proceedings of the National Academy of Sciences of the United States of America* 93 (14): 7131–36. https://doi.org/10.1073/pnas.93.14.7131.

Maloisel, Laurent, Francis Fabre, and Serge Gangloff. 2008. “DNA Polymerase Delta Is Preferentially Recruited during Homologous Recombination to Promote Heteroduplex DNA Extension.” *Molecular and Cellular Biology* 28 (4): 1373–82. https://doi.org/10.1128/MCB.01651-07.

McVey, Mitch, Varandt Y. Khodaverdian, Damon Meyer, Paula Gonçalves Cerqueira, and Wolf-Dietrich Heyer. 2016. “Eukaryotic DNA Polymerases in Homologous Recombination.” *Annual Review of Genetics* 50 (November): 393–421. https://doi.org/10.1146/annurev-genet-120215-035243.

Mehta, Anuja, Annette Beach, and James E. Haber. 2017. “Homology Requirements and Competition between Gene Conversion and Break-Induced Replication during Double-Strand Break Repair.” *Molecular Cell* 65 (3): 515-526.e3. https://doi.org/10.1016/j.molcel.2016.12.003.

Meselson, M., and J. J. Weigle. 1961. “Chromosome Breakage Accompanying Genetic Recombination in Bacteriophage.” *Proceedings of the National Academy of Sciences* 47 (6): 857–68. https://doi.org/10.1073/pnas.47.6.857.

Mimitou, Eleni P., and Lorraine S. Symington. 2008. “Sae2, Exo1 and Sgs1 Collaborate in DNA Double-Strand Break Processing.” *Nature* 455 (7214): 770–74. https://doi.org/10.1038/nature07312.

———. 2009. “DNA End Resection: Many Nucleases Make Light Work.” *DNA Repair* 8 (9): 983–95. https://doi.org/10.1016/j.dnarep.2009.04.017.

Minocherhomji, Sheroy, Songmin Ying, Victoria A. Bjerregaard, Sara Bursomanno, Aiste Aleliunaite, Wei Wu, Hocine W. Mankouri, Huahao Shen, Ying Liu, and Ian D. Hickson. 2015. “Replication Stress Activates DNA Repair Synthesis in Mitosis.” *Nature* 528 (7581): 286–90. https://doi.org/10.1038/nature16139.

Mondello, C., L. Pirzio, C. M. Azzalin, and E. Giulotto. 2000. “Instability of Interstitial Telomeric Sequences in the Human Genome.” *Genomics* 68 (2): 111–17. https://doi.org/10.1006/geno.2000.6280.

Moore, Anthony, Margaret Dominska, Patricia Greenwell, Anna Y. Aksenova, Sergei Mirkin, and Thomas Petes. 2018. “Genetic Control of Genomic Alterations Induced in Yeast by Interstitial Telomeric Sequences.” *Genetics* 209 (2): 425–38. https://doi.org/10.1534/genetics.118.300950.

Moore, I. K., M. P. Martin, and C. E. Paquin. 2000. “Telomere Sequences at the Novel Joints of Four Independent Amplifications in Saccharomyces Cerevisiae.” *Environmental and Molecular Mutagenesis* 36 (2): 105–12.

Moreau, S., E. A. Morgan, and L. S. Symington. 2001. “Overlapping Functions of the Saccharomyces Cerevisiae Mre11, Exo1 and Rad27 Nucleases in DNA Metabolism.” *Genetics* 159 (4): 1423–33.

Morrical, Scott W. 2015. “DNA-Pairing and Annealing Processes in Homologous Recombination and Homology-Directed Repair.” *Cold Spring Harbor Perspectives in Biology* 7 (2): a016444. https://doi.org/10.1101/cshperspect.a016444.

Morrow, D. M., C. Connelly, and P. Hieter. 1997. “‘Break Copy’ Duplication: A Model for Chromosome Fragment Formation in Saccharomyces Cerevisiae.” *Genetics* 147 (2): 371–82.

Mosig, Gisela. 1987. “The Essential Role of Recombination in Phage T4 Growth.” *Annual Review of Genetics* 21 (1): 347–71. https://doi.org/10.1146/annurev.ge.21.120187.002023.

Murga, Matilde, Emilio Lecona, Irene Kamileri, Marcos Díaz, Natalia Lugli, Sotirios K. Sotiriou, Marta E. Anton, Juan Méndez, Thanos D. Halazonetis, and Oscar Fernandez-Capetillo. 2016. “POLD3 Is Haploinsufficient for DNA Replication in Mice.” *Molecular Cell* 63 (5): 877–83. https://doi.org/10.1016/j.molcel.2016.07.007.

Obodo, Udochukwu C., Esther A. Epum, Margaret H. Platts, Jacob Seloff, Nicole A. Dahlson, Stoycho M. Velkovsky, Shira R. Paul, and Katherine L. Friedman. 2016. “Endogenous Hot Spots of De Novo Telomere Addition in the Yeast Genome Contain Proximal Enhancers That Bind Cdc13.” *Molecular and Cellular Biology* 36 (12): 1750–63. https://doi.org/10.1128/MCB.00095-16.

Oh, Julyun, and Lorraine S. Symington. 2018. “Role of the Mre11 Complex in Preserving Genome Integrity.” *Genes* 9 (12). https://doi.org/10.3390/genes9120589.

Paeschke, Katrin, Karin R. McDonald, and Virginia A. Zakian. 2010. “Telomeres: Structures in Need of Unwinding.” *FEBS Letters* 584 (17): 3760–72. https://doi.org/10.1016/j.febslet.2010.07.007.

Pâques, F., and J. E. Haber. 1997. “Two Pathways for Removal of Nonhomologous DNA Ends during Double-Strand Break Repair in Saccharomyces Cerevisiae.” *Molecular and Cellular Biology* 17 (11): 6765–71. https://doi.org/10.1128/mcb.17.11.6765.

Piazza, Aurèle, Michael Adrian, Frédéric Samazan, Brahim Heddi, Florian Hamon, Alexandre Serero, Judith Lopes, Marie-Paule Teulade-Fichou, Anh Tuân Phan, and Alain Nicolas. 2015. “Short Loop Length and High Thermal Stability Determine Genomic Instability Induced by G-Quadruplex-Forming Minisatellites.” *The EMBO Journal* 34 (12): 1718–34. https://doi.org/10.15252/embj.201490702.

Piazza, Aurèle, and Wolf-Dietrich Heyer. 2018. “Multi-Invasion-Induced Rearrangements as a Pathway for Physiological and Pathological Recombination.” *BioEssays: News and*

Reviews in Molecular, Cellular and Developmental Biology 40 (5): e1700249. https://doi.org/10.1002/bies.201700249.

———. 2019. “Homologous Recombination and the Formation of Complex Genomic Rearrangements.” *Trends in Cell Biology* 29 (2): 135–49. https://doi.org/10.1016/j.tcb.2018.10.006.

Pinto, Ricardo Mouro, Ella Dragileva, Andrew Kirby, Alejandro Lloret, Edith Lopez, Jason St Claire, Gagan B. Panigrahi, et al. 2013. “Mismatch Repair Genes Mlh1 and Mlh3 Modify CAG Instability in Huntington’s Disease Mice: Genome-Wide and Candidate Approaches.” *PLoS Genetics* 9 (10): e1003930. https://doi.org/10.1371/journal.pgen.1003930.

Platt, Jesse M., Paul Ryvkin, Jennifer J. Wanat, Greg Donahue, M. Dan Ricketts, Steven P. Barrett, Hannah J. Waters, et al. 2013. “Rap1 Relocalization Contributes to the Chromatin-Mediated Gene Expression Profile and Pace of Cell Senescence.” *Genes & Development* 27 (12): 1406–20. https://doi.org/10.1101/gad.218776.113.

Podvin, Sonia, Holly T. Reardon, Katrina Yin, Charles Mosier, and Vivian Hook. 2019. “Multiple Clinical Features of Huntington’s Disease Correlate with Mutant HTT Gene CAG Repeat Lengths and Neurodegeneration.” *Journal of Neurology* 266 (3): 551–64. https://doi.org/10.1007/s00415-018-8940-6.

Prakash, Rohit, Dominik Satory, Eloïse Dray, Almas Papusha, Jürgen Scheller, Wilfried Kramer, Lumir Krejci, et al. 2009. “Yeast Mph1 Helicase Dissociates Rad51-Made D-Loops: Implications for Crossover Control in Mitotic Recombination.” *Genes & Development* 23 (1): 67–79. https://doi.org/10.1101/gad.1737809.

Putnam, Christopher D., Vincent Pennaneach, and Richard D. Kolodner. 2004. “Chromosome Healing through Terminal Deletions Generated by de Novo Telomere Additions in Saccharomyces Cerevisiae.” *Proceedings of the National Academy of Sciences* 101 (36): 13262–67. https://doi.org/10.1073/pnas.0405443101.

Reddel, Roger R. 2014. “Telomere Maintenance Mechanisms in Cancer: Clinical Implications.” *Current Pharmaceutical Design* 20 (41): 6361–74.

Rehman, Adeeb H., Robert P. Jones, and Graeme Poston. 2019. “Prognostic and Predictive Markers in Liver Limited Stage IV Colorectal Cancer.” *European Journal of Surgical Oncology: The Journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology*, June. https://doi.org/10.1016/j.ejso.2019.06.038.

Reynolds, I. S., S. J. Furney, E. W. Kay, D. A. McNamara, J. H. M. Prehn, and J. P. Burke. 2019. “Meta-Analysis of the Molecular Associations of Mucinous Colorectal Cancer.” *The British Journal of Surgery* 106 (6): 682–91. https://doi.org/10.1002/bjs.11142.

Richard, Guy-Franck, Alix Kerrest, and Bernard Dujon. 2008. “Comparative Genomics and Molecular Dynamics of DNA Repeats in Eukaryotes.” *Microbiology and Molecular Biology Reviews : MMBR* 72 (4): 686–727. https://doi.org/10.1128/MMBR.00011-08.

Roumelioti, Fani-Marlen, Sotirios K. Sotiriou, Vasiliki Katsini, Maria Chiourea, Thanos D. Halazonetis, and Sarantis Gagos. 2016. “Alternative Lengthening of Human Telomeres

Is a Conservative DNA Replication Process with Features of Break-Induced Replication." *EMBO Reports* 17 (12): 1731–37. https://doi.org/10.15252/embr.201643169.

Ruff, Patrick, Roberto A. Donnianni, Eleanor Glancy, Julyun Oh, and Lorraine S. Symington. 2016. "RPA Stabilization of Single-Stranded DNA Is Critical for Break-Induced Replication." *Cell Reports* 17 (12): 3359–68. https://doi.org/10.1016/j.celrep.2016.12.003.

Saini, Natalie, Sreejith Ramakrishnan, Rajula Elango, Sandeep Ayyar, Yu Zhang, Angela Deem, Grzegorz Ira, James E. Haber, Kirill S. Lobachev, and Anna Malkova. 2013. "Migrating Bubble during Break-Induced Replication Drives Conservative DNA Synthesis." *Nature* 502 (7471): 389–92. https://doi.org/10.1038/nature12584.

Sakofsky, Cynthia J., Steven A. Roberts, Ewa Malc, Piotr A. Mieczkowski, Michael A. Resnick, Dmitry A. Gordenin, and Anna Malkova. 2014. "Break-Induced Replication Is a Source of Mutation Clusters Underlying Kataegis." *Cell Reports* 7 (5): 1640–48. https://doi.org/10.1016/j.celrep.2014.04.053.

Samassekou, O., and J. Yan. 2011. "Polymorphism in a Human Chromosome-Specific Interstitial Telomere-Like Sequence at 22q11.2." *Cytogenetic and Genome Research* 134 (3): 174–81. https://doi.org/10.1159/000328862.

Schaffitzel, C., I. Berger, J. Postberg, J. Hanes, H. J. Lipps, and A. Plückthun. 2001. "In Vitro Generated Antibodies Specific for Telomeric Guanine-Quadruplex DNA React with Stylonychia Lemnae Macronuclei." *Proceedings of the National Academy of Sciences of the United States of America* 98 (15): 8572–77. https://doi.org/10.1073/pnas.141229498.

Schulz, V. P., and V. A. Zakian. 1994. "The Saccharomyces PIF1 DNA Helicase Inhibits Telomere Elongation and de Novo Telomere Formation." *Cell* 76 (1): 145–55.

Shim, Eun Yong, Woo-Hyun Chung, Matthew L. Nicolette, Yu Zhang, Melody Davis, Zhu Zhu, Tanya T. Paull, Grzegorz Ira, and Sang Eun Lee. 2010. "Saccharomyces Cerevisiae Mre11/Rad50/Xrs2 and Ku Proteins Regulate Association of Exo1 and Dna2 with DNA Breaks." *The EMBO Journal* 29 (19): 3370–80. https://doi.org/10.1038/emboj.2010.219.

Shinohara, Akira, Miki Shinohara, Tsutomu Ohta, Shimako Matsuda, and Tomoko Ogawa. 1998. "Rad52 Forms Ring Structures and Co-Operates with RPA in Single-Strand DNA Annealing." *Genes to Cells* 3 (3): 145–56. https://doi.org/10.1046/j.1365-2443.1998.00176.x.

Shore, D., and K. Nasmyth. 1987. "Purification and Cloning of a DNA Binding Protein from Yeast That Binds to Both Silencer and Activator Elements." *Cell* 51 (5): 721–32. https://doi.org/10.1016/0092-8674(87)90095-x.

Simonet, Thomas, Laure-Emmanuelle Zaragosi, Claude Philippe, Kevin Lebrigand, Clémentine Schouteden, Adeline Augereau, Serge Bauwens, et al. 2011. "The Human TTAGGG Repeat Factors 1 and 2 Bind to a Subset of Interstitial Telomeric Sequences and Satellite Repeats." *Cell Research* 21 (7): 1028–38. https://doi.org/10.1038/cr.2011.40.

Smith, Catherine E., Alicia F. Lam, and Lorraine S. Symington. 2009. "Aberrant Double-Strand Break Repair Resulting in Half Crossovers in Mutants Defective for Rad51 or the DNA

Polymerase Delta Complex." *Molecular and Cellular Biology* 29 (6): 1432–41. https://doi.org/10.1128/MCB.01469-08.

Smith, Catherine E., Bertrand Llorente, and Lorraine S. Symington. 2007. "Template Switching during Break-Induced Replication." *Nature* 447 (7140): 102–5. https://doi.org/10.1038/nature05723.

Sohn, Sea H., Asha S. Multani, Pankaj K. Gugnani, and Sen Pathak. 2002. "Telomere Erosion-Induced Mitotic Catastrophe in Continuously Grown Chinese Hamster Don Cells." *Experimental Cell Research* 279 (2): 271–76. https://doi.org/10.1006/excr.2002.5614.

Stafa, Anamarija, Roberto A. Donnianni, Leonid A. Timashev, Alicia F. Lam, and Lorraine S. Symington. 2014. "Template Switching during Break-Induced Replication Is Promoted by the Mph1 Helicase in Saccharomyces Cerevisiae." *Genetics* 196 (4): 1017–28. https://doi.org/10.1534/genetics.114.162297.

Strathern, J. N., B. K. Shafer, and C. B. McGill. 1995. "DNA Synthesis Errors Associated with Double-Strand-Break Repair." *Genetics* 140 (3): 965–72.

Sugiyama, T., J. H. New, and S. C. Kowalczykowski. 1998. "DNA Annealing by RAD52 Protein Is Stimulated by Specific Interaction with the Complex of Replication Protein A and Single-Stranded DNA." *Proceedings of the National Academy of Sciences of the United States of America* 95 (11): 6049–54. https://doi.org/10.1073/pnas.95.11.6049.

Sun, H., D. Treco, and J. W. Szostak. 1991. "Extensive 3'-Overhanging, Single-Stranded DNA Associated with the Meiosis-Specific Double-Strand Breaks at the ARG4 Recombination Initiation Site." *Cell* 64 (6): 1155–61.

Symington, Lorraine S. 2016. "Mechanism and Regulation of DNA End Resection in Eukaryotes." *Critical Reviews in Biochemistry and Molecular Biology* 51 (3): 195–212. https://doi.org/10.3109/10409238.2016.1172552.

Symington, Lorraine S., and Jean Gautier. 2011. "Double-Strand Break End Resection and Repair Pathway Choice." *Annual Review of Genetics* 45: 247–71. https://doi.org/10.1146/annurev-genet-110410-132435.

Szostak, J. W., T. L. Orr-Weaver, R. J. Rothstein, and F. W. Stahl. 1983. "The Double-Strand-Break Repair Model for Recombination." *Cell* 33 (1): 25–35. https://doi.org/10.1016/0092-8674(83)90331-8.

Teng, S. C., and V. A. Zakian. 1999. "Telomere-Telomere Recombination Is an Efficient Bypass Pathway for Telomere Maintenance in Saccharomyces Cerevisiae." *Molecular and Cellular Biology* 19 (12): 8083–93. https://doi.org/10.1128/mcb.19.12.8083.

Toh, Geraldine W.-L., Neal Sugawara, Junchao Dong, Rachel Toth, Sang Eun Lee, James E. Haber, and John Rouse. 2010. "Mec1/Tel1-Dependent Phosphorylation of Slx4 Stimulates Rad1-Rad10-Dependent Cleavage of Non-Homologous DNA Tails." *DNA Repair* 9 (6): 718–26. https://doi.org/10.1016/j.dnarep.2010.02.013.

Usdin, Karen, Nealia C. M. House, and Catherine H. Freudenreich. 2015. “Repeat Instability during DNA Repair: Insights from Model Systems.” *Critical Reviews in Biochemistry and Molecular Biology* 50 (2): 142–67. https://doi.org/10.3109/10409238.2014.999192.

VanHulle, Kelly, Francene J. Lemoine, Vidhya Narayanan, Brandon Downing, Krista Hull, Christy McCullough, Melissa Bellinger, Kirill Lobachev, Thomas D. Petes, and Anna Malkova. 2007. “Inverted DNA Repeats Channel Repair of Distant Double-Strand Breaks into Chromatid Fusions and Chromosomal Rearrangements.” *Molecular and Cellular Biology* 27 (7): 2601–14. https://doi.org/10.1128/MCB.01740-06.

Vasianovich, Yulia, Lea A. Harrington, and Svetlana Makovets. 2014. “Break-Induced Replication Requires DNA Damage-Induced Phosphorylation of Pif1 and Leads to Telomere Lengthening.” *PLoS Genetics* 10 (10): e1004679. https://doi.org/10.1371/journal.pgen.1004679.

Wang, S S, and V A Zakian. 1990. “Sequencing of Saccharomyces Telomeres Cloned Using T4 DNA Polymerase Reveals Two Domains.” *Molecular and Cellular Biology* 10 (8): 4415–19.

Welcsh, Piri L., and Mary-Claire King. 2001. “BRCA1 and BRCA2 and the Genetics of Breast and Ovarian Cancer.” *Human Molecular Genetics* 10 (7): 705–13. https://doi.org/10.1093/hmg/10.7.705.

Wellinger, Raymund J., and Virginia A. Zakian. 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.

White, C. I., and J. E. Haber. 1990. “Intermediates of Recombination during Mating Type Switching in Saccharomyces Cerevisiae.” *The EMBO Journal* 9 (3): 663–73.

Wilson, Marenda A., YoungHo Kwon, Yuanyuan Xu, Woo-Hyun Chung, Peter Chi, Hengyao Niu, Ryan Mayle, et al. 2013. “Pif1 Helicase and Polδ Promote Recombination-Coupled DNA Synthesis via Bubble Migration.” *Nature* 502 (7471): 393–96. https://doi.org/10.1038/nature12585.

Wood, Ashley M., Jannie M. Rendtlew Danielsen, Catherine A. Lucas, Ellen L. Rice, David Scalzo, Takeshi Shimi, Robert D. Goldman, Erica D. Smith, Michelle M. Le Beau, and Steven T. Kosak. 2014. “TRF2 and Lamin A/C Interact to Facilitate the Functional Organization of Chromosome Ends.” *Nature Communications* 5 (November): 5467. https://doi.org/10.1038/ncomms6467.

Wyatt, Haley D. M., and Stephen C. West. 2014. “Holliday Junction Resolvases.” *Cold Spring Harbor Perspectives in Biology* 6 (9): a023192. https://doi.org/10.1101/cshperspect.a023192.

Zakian, V. A., H. M. Blanton, and L. Wetzel. 1986. “Distribution of Telomere-Associated Sequences in Yeast.” *Basic Life Sciences* 40: 493–98.

Zhang, Jia-Min, Tribhuwan Yadav, Jian Ouyang, Li Lan, and Lee Zou. 2019. “Alternative Lengthening of Telomeres through Two Distinct Break-Induced Replication Pathways.” *Cell Reports* 26 (4): 955-968.e3. https://doi.org/10.1016/j.celrep.2018.12.102.

Zhu, Zhu, Woo-Hyun Chung, Eun Yong Shim, Sang Eun Lee, and Grzegorz Ira. 2008. “Sgs1 Helicase and Two Nucleases Dna2 and Exo1 Resect DNA Double-Strand Break Ends.” *Cell* 134 (6): 981–94. https://doi.org/10.1016/j.cell.2008.08.037.

Zierhut, Christian, and John F. X. Diffley. 2008. “Break Dosage, Cell Cycle Stage and DNA Replication Influence DNA Double Strand Break Response.” *The EMBO Journal* 27 (13): 1875–85. https://doi.org/10.1038/emboj.2008.111.

Zou, Lee. 2013. “Four Pillars of the S-Phase Checkpoint.” *Genes & Development* 27 (3): 227–33. https://doi.org/10.1101/gad.213306.113.