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Review of Native Fragile Sites in Saccharomyces Cerevisiae: A Comparison to Human Fragile Sites

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ABSTRACT

Saccharomyces cerevisiae (*S. cerevisiae*) was the first eukaryote to have its full genome sequenced, which makes it one of the longest studied genomes. The scientific community has established that *S. cerevisiae* is a useful model in the study of human diseases due to the homology that exists in numerous human and yeast genes. Yeast artificial chromosomes (YACs) have been developed that contain inserts of human DNA. These YACs can be used to study the mechanisms that cause DNA fragility in humans by placing a known human fragile site within a YAC. Though it is useful to study human DNA inserted into yeast to monitor the inherent fragility of the sequence, studying the nature of native yeast fragile sites may have benefits as well. The types of native fragile sites in *Saccharomyces cerevisiae* include *Mec1* mutant, hydroxyurea, palindromic, ty-element, and *Pol1* mutant induced fragile sites. Many of the mechanisms of yeast DNA fragility are similar to those of human DNA. The purpose of this review is to compare and contrast the mechanisms of fragility in *S. cerevisiae* and human DNA. Though human and yeast fragile sites are not always caused by similar means, with further study of the native fragile sites in *S. cerevisiae*, more similarities may be found that can give further insight into the human fragile sites and diseases caused by them.

INTRODUCTION

Saccharomyces cerevisiae

Saccharomyces cerevisiae (*S. cerevisiae*), often referred to as baker's or budding yeast, is a type of fungi found naturally on the outside of grapes and figs (Goffeau et al., 1996). The names "baker's" and "brewer's" yeast reflect the ways that *S. cerevisiae* has been used for centuries. *S. cerevisiae* was used to leaven dough and to produce ethanol through fermentation in brewing. In addition to being used to make beer and bread, *S. cerevisiae* has become common in many research labs due in part to its having been the first eukaryote to have its full genome sequenced (Goffeau et al., 1996). As a result, much has been learned about budding yeast, which coupled with it being an easy organism to grow and work with, makes it a prevalent model organism (Botstein, Chervitz, & Cherry, 1997).

Moreover, *S. cerevisiae* is a unicellular organism, unlike humans, which are multicellular. However, both organisms are eukaryotes and as a result many homologous genes, genes that code for similar traits, have been found, making *S. cerevisiae* important in research pertaining to human genetic disease (Botstein et al., 1997). The existence of homologous genes in a unicellular organism affords researchers the ability to study the genes in a less complex organism. These results can then be applied to better understand the homologous gene in the more complex organism. Due to varying similarities to mammals, yeast has been used as models in the study of the effects of pharmacological molecules (Mattiazzi, Petrovic, & Krizaj, 2012), neurological diseases (Sarto-Jackson & Tomaska, 2016), energy metabolism (Zhang, Vermuri, & Nielsen, 2010), and fragile sites (Lemoine, Degtyareva, Lobachev, & Petes, 2005).

Fragile Sites

A fragile site is an area of a gene that experiences frequent breaks for any number of reasons. While fragile sites occur in many organisms, we will focus on fragility in the *S. cerevisiae* and human genomes. Yeast fragile sites are not separated into specific

categories; they are defined by the mechanism that causes the fragility. Studies have indicated that certain yeast fragile sites behave similarly to human fragile sites. For example, both human and yeast fragile sites are susceptible to replication stress (Raveendranathan et al., 2006). In addition, two kinases in yeast that play direct roles in fragile site stability, *Mec1* and *Tel1*, are homologs of human ATR and ATM proteins, respectively (Cha & Kleckner, 2002). There are instances of breaks due to knock-out of *Mec1*, which mirrors common fragile sites (CFSs; Cha & Kleckner, 2002).

There are two categories of human fragile sites: "common" fragile sites and "rare" fragile sites (Durkin & Glover, 2007). Common fragile sites may occur in the whole population (Zlotorynski et al., 2003); these fragile sites cluster around areas of replication stress and occur more frequently than rare fragile sites (Durkin & Glover, 2007). Many common fragile sites can be induced by a form of replication stress, including DNA polymerase inhibitors such as aphidicolin (Glover, Berger, Coylr, & Echo, 1984). These are of particular interest because they have been linked to tumorigenesis (Smith, Huang, & Wang, 1998). It has also been suggested that common fragile sites may be used to indicate a predisposition to lung cancer (Karadag et al., 2002). Being linked to such serious diseases warrants further study of fragile sites, which may lead to a more effective understanding and prevention of these diseases.

Yeast as a Model for the Study of Fragile Sites

Two methods have been established to study fragile sites in *S. cerevisiae*. First, a yeast artificial chromosome (YAC) containing a fragile human DNA sequence can be placed in the yeast genome (Wilke et al., 1995). Placing human DNA into yeast cells is advantageous because it allows the DNA sequence to be studied out of context. It also allows the human DNA to be studied in a less complex organism, which can be efficient economically and practically. To determine if a sequence of human DNA is inherently fragile, it is vital to remove it from the context of the human chromosome to reduce variables. Outside the context of

human cells, we can examine whether the sequence itself is the cause of fragility, or if it is fragile due to factors that take place within human cells. Studying YACs is economical and practical because yeast are easier to reproduce and sustain than human cells.

Moreover, native yeast fragile sites can also be studied. Due to the similarities, they can provide insights into the causes of human fragile sites. This review will be discussing yeast native fragile sites induced by low DNA polymerase α, mec1Δ, Ty elements, DNA replication defects, and palindromic cruciform structures.

Yeast Native Fragile Sites

Low DNA Polymerase Alpha Induced Fragile Sites

DNA polymerase alpha must be present for yeast to undergo DNA replication (Lemoine et al., 2005). Adding a *GAL1* promoter to the *POL1* gene can allow for the control of DNA polymerase alpha production. The *GAL1* promoter causes the *POL1* gene to be replicated only when galactose is present (Lemoine et al., 2005). Therefore, when no galactose is present, the *POL1* gene is not replicated and DNA polymerase alpha is not made (Lemoine et al., 2005). **Figure 1.** illustrates how media containing different levels of galactose affect yeast containing the *GALPOL1* gene. The results of this experiment showed that DNA replication stress induced by low levels of DNA polymerase alpha caused increased fragility in *S. cerevisiae*. This included duplications, deletions, and translocations between Ty elements (Lemoine et al., 2005).

Similarly, human common fragile sites can be induced by DNA polymerase alpha inhibitors such as aphidicolin (Glover et al., 1984). Comparably, induced DNA fragility at common fragile sites also includes duplications, deletions, and translocation (Zlotorynski et al., 2003; Kurahashi & Emanuel, 2001). However, these translocations occur at AT-rich palindromic repeats (Kurahashi & Emanuel, 2001). Additionally, human CFSs and native yeast fragile sites are late replicating (Le Beau et al., 1998; Lemoine et al., 2005). When DNA polymerase alpha is inhibited in both yeast and human cells it causes sensitivity to DNA damaging molecules, including hydroxyurea (HU; Lemoine et al., 2005; Zeeland et al., 1982).

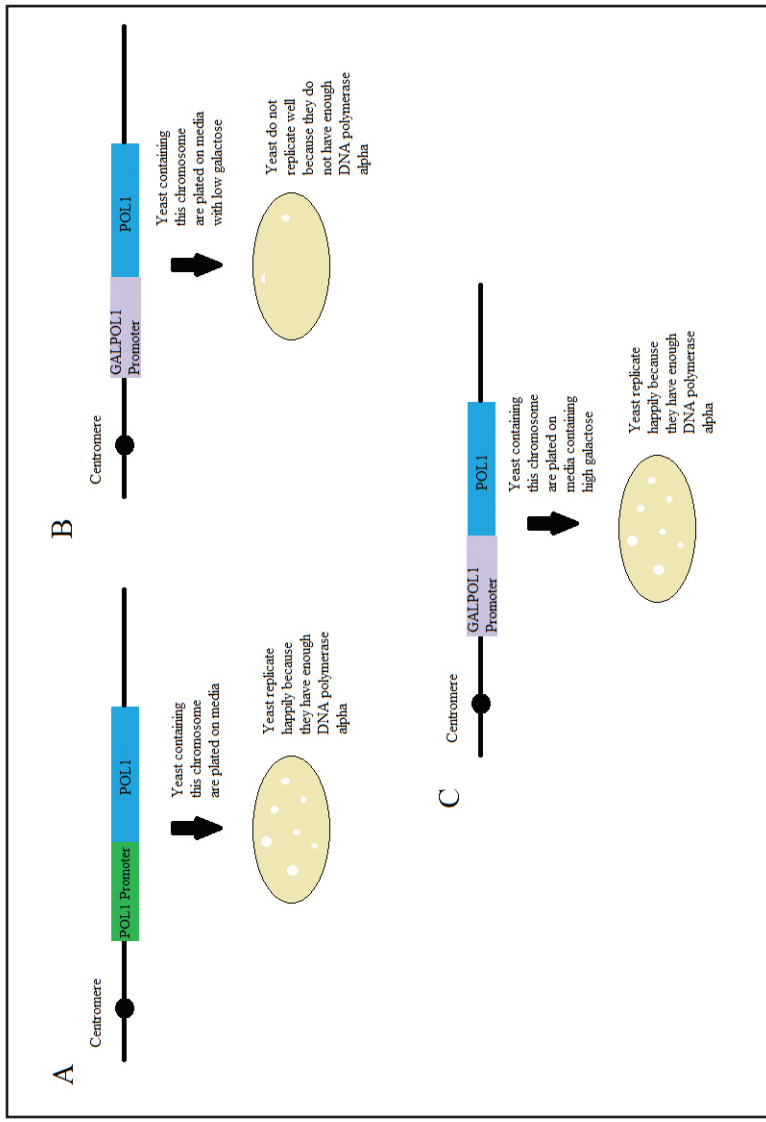
A
Centromere
POL1 Promoter
POL1
Yeast containing this chromosome are plated on media
Yeast replicate happily because they have enough DNA polymerase alpha
B
Centromere
GALPOL1 Promoter
POL1
Yeast containing this chromosome are plated on media with low galactose
Yeast do not replicate well because they do not have enough DNA polymerase alpha
C
Centromere
GALPOL1 Promoter
POL1
Yeast containing this chromosome are plated on media containing high galactose
Yeast replicate happily because they have enough DNA polymerase alpha

Figure 1.

In contrast, fragile sites that occur when DNA polymerase alpha is inhibited cause illegitimate mating in yeast and not humans, because humans are multicellular complex organisms (Lemoine et al., 2005). Moreover, in yeast DNA, polymerase alpha was inhibited using the *GAL1* promoter (Lemoine et al., 2005), while it was inhibited with aphidicolin in humans (Zeeland et al., 1982).

Palindromic Sequence Breaks

DNA fragility can occur at palindromic sequence in the yeast genome (Nag & Kurst, 1997). A palindromic sequence is one that when read five-prime (5') to three-prime (3') on one strand is the same as when reading 5' to 3' on the complimenting strand. Because the sequence is a palindrome, when DNA replication begins and the DNA is temporarily single-stranded, it can hydrogen bond to itself, causing secondary structures such as hairpin loops or cruciform (Nag & Kurst, 1997). **Figure 2.** illustrates a possible cruciform structure. When DNA is formed into these structures the cell can resolve it in a number of ways (Nag & Kurst, 1997). Many resolutions cause double-stranded breaks during meiosis (Nag & Kurst, 1997). Though this particular fragile site is not truly native to yeast, it was inserted via a plasmid vector by which palindromic sequences are thought to occur (Nag & Kurst, 1997). Deletions occur at palindromic areas of the human genome (Kurahashi et al., 2009). Unlike these palindromic sequences in yeast, the palindromic sequences in humans cause problems during mitosis, not meiosis (Kurahashi et al., 2009). The fragility of these human palindromic sequences is induced by replication stress; the sites are not inherently fragile, like the yeast palindromic sequences (Kurahashi et al., 2009).

In other studies, plasmids containing palindromic sequences were introduced into *Saccharomyces cerevisiae* and were shown to be resolved using mechanisms characteristic of Holliday junction resolution (Cote & Lewis, 2008). **Figure 2.** illustrates a possible cruciform resolution via a Holliday junction resolution. It is proposed that after resolution leading to one or more hairpin loops, nuclease Mre11 can remove the hairpin caps (Cote & Lewis, 2008). Removal of the hairpin caps can allow

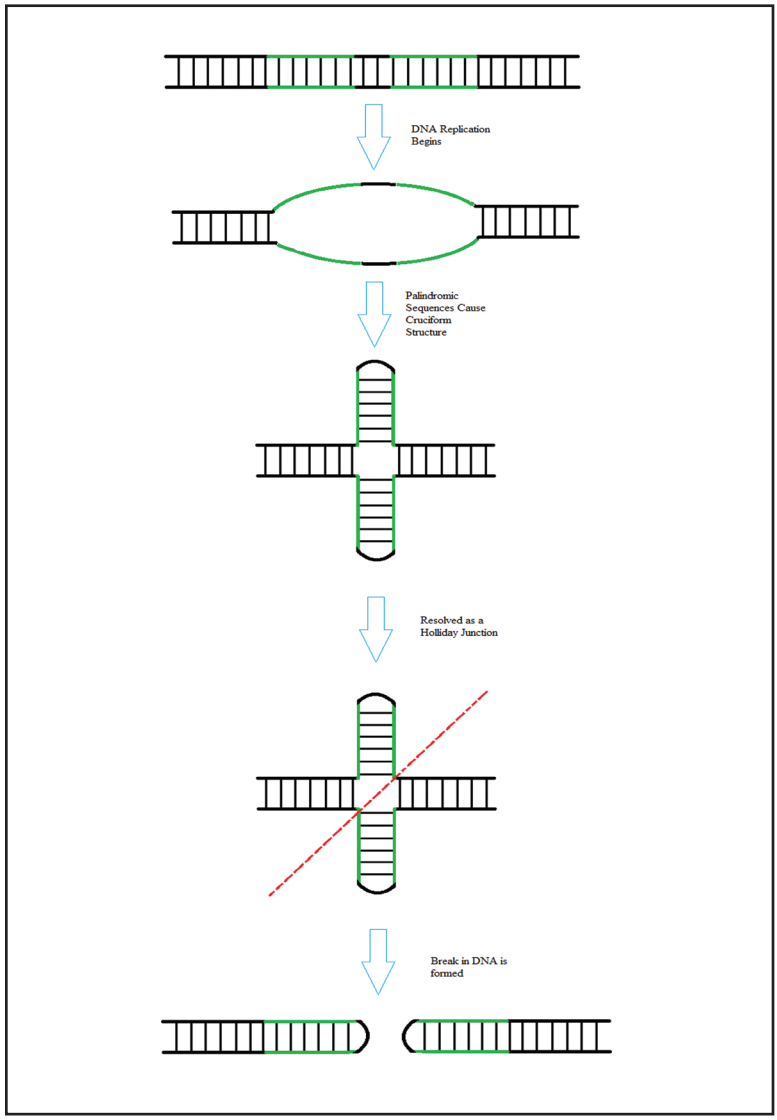

Figure 2. Resolution of Palindromic Sequence as a Holliday Junction. The green sections are used to represent sections of DNA, which are inverted repeats. These result in hairpin loops or cruciform structures when single stranded, as they are during replication. After resolution of the cruciform structure, a break can be formed due to the manner in which it has been resolved. If it is resolved as a Holliday junction, the palindromic sequences remain hydrogen bonded. This illustration is only meant to be a visual representation, and is not to scale.

for repair of the double-strand break (Lobacheb, Gordenin, & Resnick, 2002). Mre11 is a nuclease also found in mammalian cells, including humans (Lee & Paull, 2005).

As stated previously, human DNA also contains palindromic regions (Zlotorynski et al., 2003). However, these palindromes are usually AT-rich, and the sequence inserted into the yeast cell is not (Zlotorynski et al. 2003; Nag & Kurst, 1997). Furthermore, the AT-rich sequences in humans form hairpin loops, opposed to cruciform structures (Zlotorynski et al. 2003; Nag & Kurst, 1997).

Mutant Mec1 Linked Fragile Sites

Mec1 has been shown to play an important role in the prevention of DNA breaks at replication slow zones (RSZ) in yeast (Cha & Kleckner, 2002). During DNA replication in S-phase, effective replication of DNA requires dNTPs to be present. These dNTPs are synthesized in part by Rnr1 a ribonucleotide reductase. Rnr1 is inhibited by Sml1, which causes lower levels of dNTPs to be present. Lower levels of dNTPs can cause replication problems in RSZs (Cha & Kleckner, 2002). Through its downstream target Rad53, Mec1 inhibits Sml1, which allows Rnr1 to produce dNTPs. Having enough dNTPs lowers the formation of double strand breaks (DSBs) at RSZs. However, when Mec1 is replaced with mec1-ts, a thermosensitive gene, double strand breaks occur at RSZs under restrictive temperatures (Cha & Kleckner. 2002). Under these temperature sensitive conditions, it has been found that knocking out Rrm3 decreases DSBs at RSZs (Hashash, Johnson, & Cha, 2011). This occurs because rr3Δ leads to Sml1 degradation (Hashash et al., 2011). To support the hypothesis that RSZs are fragile because of low dNTP concentration, mec1-ts cells were treated with hydroxyurea (Hashash et al., 2011). Hydroxyurea (HU) inhibits Rnr1, which causes dNTPs to not be up regulated. In mec1-ts cells that were treated with HU, more breaks occurred at RSZs (Hashash et al., 2011). However, when exposed to excessively high concentrations of HU, fewer breaks occurred at RSZs because the replication forks collapsed before the RSZs could be reached (Hashash et al., 2011).

To further understand S-phase checkpoint kinases, Mec1 and Rad53 mutants *mec1-1* and *rad53-1* were introduced in yeast (Raveendranathan et al., 2006). These mutant genes prevent the Mec1 and Rad53 kinases from being produced (Raveendranathan et al., 2006). Yeast cells that do not produce Mec1 and Rad53 exhibited hindered DNA replication due to replication fork collapse (Raveendranathan et al., 2006). A cascade of events linked to Mec1 causes the production of dNTPs, which are required for DNA replication, and when these are in limited supply due to the *mec1-1* or *rad53-1* mutation, replication slow zones were the result (Raveendranathan et al., 2006).

In addition, it has been shown that Mec1 and Tel1 activity in response to DNA damage causes phosphorylation of histone H2A, resulting in γ-H2AX (Szilard et al., 2011). Searching for and mapping γ-H2AX allows for the identification of many fragile sites on the yeast genome (Szilard et al., 2011). A homologous pathway occurs in human cells. Mec1 homolog ATR and Tel1 homolog ATM respond to DNA damage, and this leads to the phosphorylation of histone H2A, which results in γ-H2A, as shown in **Figure 3.** (Szilard et al., 2011).

In addition to the similarities between yeast and human phosphorylation of histones, the human homolog of MEC1, ATR, is also involved in a replication checkpoint (Wan, Kulkami, &Wang, 2010). ATR and MEC1 are both needed in response to DNA polymerase alpha inhibition (Wan et al., 2010; Song, Dominska, Greenwell, & Petes, 2014). ATR and MEC1 are also both involved in a pathway with ribonucleotide reductases (Zhang, Jones, Martin, Caplen, & Pommier, 2009; Cha & Kleckner, 2002).

Hydroxyurea Induced Fragile Sites

To study the effects of hydroxyurea (HU) induced replication stress, Feng, Di Rienzi, Raghuraman, and Brewer (2011) subjected yeast DNA to HU and used ssDNA detection to monitor replication fork movement. After HU exposure they were able to map breaks in the chromosome, which clustered around areas containing replication forks (Feng et al., 2011). Additionally,

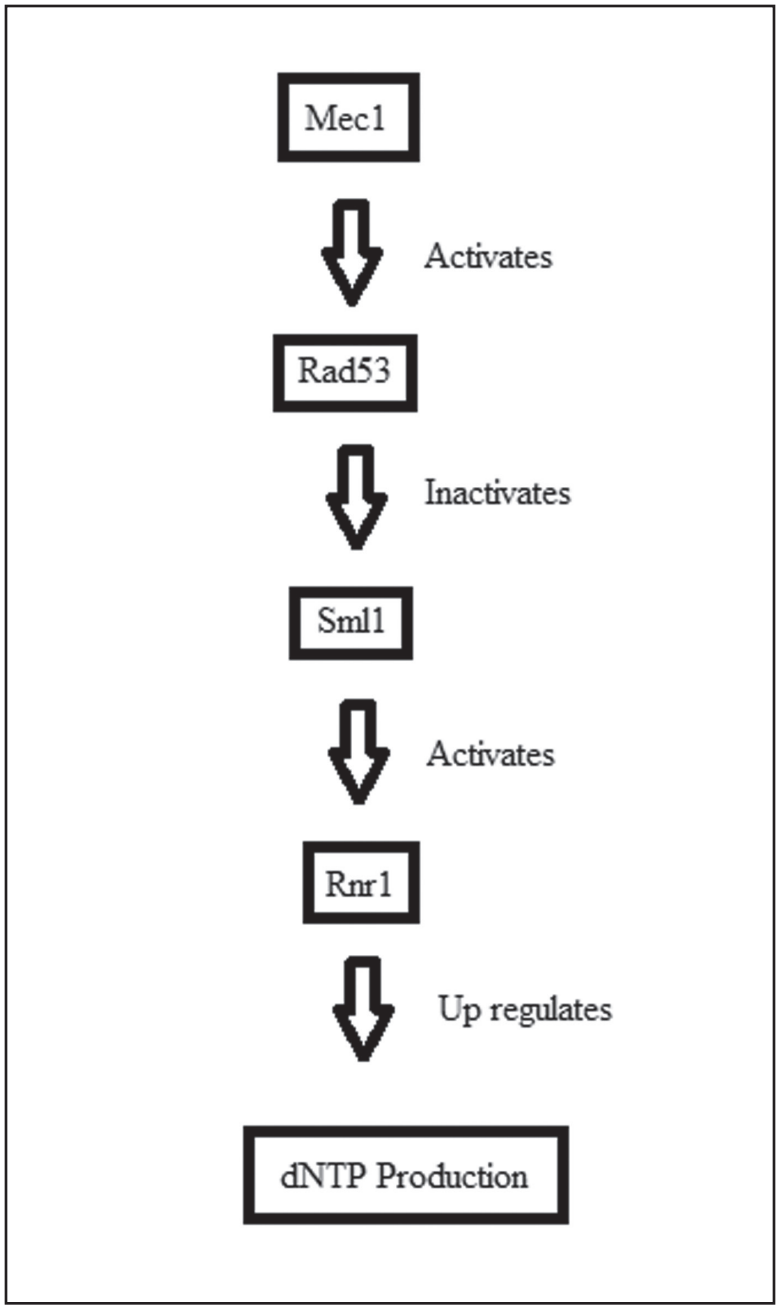

Figure 3. Flow Chart Highlighting the Pathway from Mec1 to dNTP Production.

single-stranded DNA (ssDNA) was found before chromosome breaks were observed, indicating that ssDNA may be involved in the development of double-strand breaks (Feng et al., 2011). A specialized yeast strain containing *mec1-4*, which is temperature sensitive and which was not subjected to HU, was also shown to have ssDNA preceding chromosome breaks (Feng et al., 2011). This further supports the hypothesis that ssDNA is involved in chromosome breakage. The proposed cause of ssDNA in this study is uncoupled DNA synthesis or cutting of already synthesized DNA (Feng et al., 2011).

Similarly, HU can cause replication stress in human cells, which leads to ssDNA (Mortusewicz, Evers, & Helleday, 2016). Double-strand DNA breaks in human cells also occur at ssDNA (Mortusewicz et al., 2016). Humans have an ssDNA-binding protein that stabilizes sites of replication that can be stalled due to hydroxyurea (Mortusewicz et al., 2016).

CONCLUSIONS

The similarities between the genome of *Saccharomyces cerevisiae* and the human genome allows us to study fragile sites in yeast and apply this knowledge to human fragile sites. Many of the mechanisms that cause native yeast fragile sites also cause human fragile sites. In many cases, homologous genes are involved in similar pathways. These homologous genes are the similarities left over from the common ancestor of yeast and humans. Because the yeast genome is much smaller and yeast are easier to work with than human cells, yeast will continue to be an important model organism for studying fragile sites. Even after we have exhausted the similarities between them, yeast can still be used to study human fragile sites out of context by placing human DNA in the yeast genome. The discoveries to be made by studying fragile sites in yeast are many, and they will likely help to establish treatments for diseases linked to fragile sites, as well as to establish mechanisms for mapping fragile sites on chromosomes.

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