DNA Replication in Repair

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1. Introduction

All living organisms face a constant barrage of DNA damage from anthropogenic and naturally occurring external and endogenous sources, yet DNA provides the blueprint for all other cellular structures. Unlike these other structural and functional molecules, DNA is not turned over in a cycle of breakdown and rebuild; DNA, by the nature of its function as an informational macromolecule and its double stranded structure, is faithfully repaired and copied to maintain the encoded information. In the case of multicellular organisms, the focus of this review, fidelity of information prevents disease, both heritable (e.g. genetic disorders), and within an individual (e.g. cancer). However, errors in DNA replication and repair serve as the grist of evolution; in some sense, these errors are essential to life as we know it.

With few exceptions, the repair of DNA damage requires the action of one or more DNA polymerases. In many cases, these are specialized polymerases, recruited to the site of damage for their specific biochemical properties. In this literature review, I will present different types of DNA damage, the biochemical systems utilized in the repair of this damage, the role of various enzymes in this repair – emphasizing the role of specific DNA polymerases – and the outcome of repair – including the resulting mutation spectrum – where relevant.

2. DNA polymerases

DNA polymerases are responsible for the replication of DNA. They perform this function by adding free deoxynucleotides to the 3’ end of a DNA strand or RNA primer and extending the strand in the 5’ direction; they are not able to synthesize DNA de novo without this 3’ hydroxyl (Baker & Bell 1998; Hubscher et al. 2002). They are typically composed of several subunits, but an in depth discussion of polymerase components is beyond the scope of this chapter; please see Kawasaki and Sugino for a more in depth discussion of polymerase subunits (Kawasaki & Sugino 2001). The mechanism of nucleotide insertion by a polymerase is a process consisting of 6 steps binding of the DNA template, binding of the incoming dNTP, undergoing a change in conformation to become more catalytically effective, formation of the phosphodiester bond, release of the pyrophosphate group, and translocation to the next template base or dissociation from DNA (Kuchta et al. 1988; Kuchta et al. 1987; Patel et al. 1991; Washington et al. 2000; Washington et al. 2001; Wong et al. 1991). The structure of a polymerase is similar to that of a right human hand grasping a DNA strand, and is thus described as having finger, palm and thumb subdomains (Steitz 1998). Synthesis is carried out using the opposite strand as a template—the semi-conservative model of DNA synthesis (Meselson & Stahl 1958).
2.1 DNA polymerase families

Polymerases are organized into 7 families by their sequence, structure, and function. These polymerase families are: A, B, C, D, X, Y and RT. Each family has different properties and roles. For example, A Family polymerases are responsible for the bulk of S-phase DNA synthesis, and Y Family polymerases are responsible for Translesion Synthesis (TLS) and the bypass of some DNA lesions. A brief discussion of bacterial polymerase families follows, as does a more detailed discussion of multicellular eukaryotic polymerase families, representative members, and roles (summarized in Table 1).

A Family polymerases are replicative and repair polymerases that include the \textit{E. coli} replicative polymerase polI, \textit{T. aquaticus} polI, and the human mitochondrial polymerase, polY. This family also includes the \textit{E. coli} T7 polymerase, one of the most studied polymerases. Orthologues of this family include members of the B Family of eukaryotic polymerases (Hubscher \textit{et al.} 2002). The tight active site and 3' primer interactions prevent base pair mismatches, and thus these polymerases have a low error rate. pol\theta is a low fidelity A Family member with roles in repair, possibly including Base Excision Repair (BER) and Interstrand Crosslink Repair (ICLR) (Chan \textit{et al.} 2010; Prasad \textit{et al.} 2009). The B Family of polymerases is closely related to the A Family in structure and function. They are DNA directed DNA polymerases and this family includes the eukaryotic replicative and repair polymerases pol\alpha, pol\delta, and pol\epsilon. pol\alpha with its accompanying primase is responsible for initiation of DNA synthesis (Harrington \\& Perrino 1995). pol\delta and pol\epsilon cooperate to accomplish leading and lagging strand DNA synthesis (Chilkova \textit{et al.} 2007). pol\delta and pol\epsilon are also involved in Homologous Recombination Repair (HRR) of DNA (Asturias \textit{et al.} 2006; Kelman 1997; Maloisel \textit{et al.} 2008). Another B Family member, pol\zeta, is involved in TLS (Haracska \textit{et al.} 2003).

C Family polymerases represent the main bacterial chromosomal synthetic polymerases (Lamers \textit{et al.} 2006). They are fast moving, have proofreading capability, and are structurally and possibly evolutionarily distinct from the other polymerase families (Bloom \textit{et al.} 1997; Lamers \textit{et al.} 2006). Another distinct family are the D Family polymerases. They are archaeal polymerases that are assumed to function as replicative polymerases (Ishino \textit{et al.} 1998; Yamasaki \textit{et al.} 2010).

X Family polymerases are extensively involved in a variety of DNA repair mechanisms. This family includes pol\beta, pol\omega, pol\lambda, and pol\mu, as well as terminal deoxynucleotidyl transferase (TdT). Some of the X Family members, including pol\beta and pol\lambda, can cleave a 5’ abasic deoxyribose sugar, a critical function in BER and possibly required for Non-Homologous End Joining (NHEJ) (Fan \\& Wu 2004; Garcia-Diaz \textit{et al.} 2001). TdT expression is limited to developing leukocyte lineages where where it plays a critical role in V(D)J Recombination, a specific type of NHEJ (Mahajan \textit{et al.} 1999). pol\lambda and pol\mu are required for NHEJ (Fan \\& Wu 2004; Mahajan \textit{et al.} 2002). pol\omega works in concert with pol\zeta in sister chromatid cohesion and HRR (Edwards \textit{et al.} 2003).

The Y Family of polymerases, including pol\eta, pol\t, pol\kappa, and REV-1 are involved in TLS. Each polymerase in this family has a different bypass preference. For example, pol\eta will bypass cyclohexymine dimers (CTD), inserting two adenosines opposite the lesion in an error free manner. Loss of pol\eta because of its involvement in CTD bypass gives a Xeroderma pigmentosum variant phenotype as with many Nucleotide Excision Repair (NER) enzymes (Johnson \textit{et al.} 1999b; Washington \textit{et al.} 2000). pol\kappa is not able to replicate past a CTD, however, it is involved in the nucleotide incorporation opposite an abasic site and the 3’ thymine of a (6-4) photopoduct (Johnson \textit{et al.} 2000). Although it can insert bases opposite
the lesion, this polymerase cannot extend the nascent DNA strand. While polκ cannot insert bases opposite damage as polŋ and pol can, it does extend opposite the 3’ end of the lesion (Washington et al. 2002). REV-1 is thought to play a supporting role as well, triggering synthesis of the other Y family members at these lesions (Guo et al. 2003; Ohashi et al. 2004; Tissier et al. 2004). The roles of polymerases in TLS are discussed in more detail below.

<table>
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<th>Polymerase Family</th>
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<td>BER?, ICLR?</td>
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<td>N/A</td>
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Table 1. DNA Polymerases Involved in DNA Repair and Their Roles

The RT Family of polymerases includes RNA-directed DNA polymerases that use RNA primers or are involved in viral reverse transcription, like the eukaryotic polymerases responsible for telomere maintenance, telomerase (Gotte et al. 1999; Greider & Blackburn 1989). RT stands for Reverse Transcriptase, the primary function of members of this polymerase family.

### 3. DNA repair

The goal of all DNA repair is to maintain the integrity of the genome with minimal, and ideally no changes to the original DNA sequence. In the case of single strand damage, such as spontaneous depurination, oxidation, alkylation, and ultraviolet (UV) light photoproducts, this is readily achieved by Direct Reversal Repair (DRR), in which an enzyme directly returns the lesion to its former, undamaged state, or utilizing the
antiparallel DNA strand as template, as in TLS, or following excision of the damage as in BER and NER. However, when both strands are damaged, repair mechanisms including Double Strand Break Repair (DSBR) and Interstrand Crosslink Repair (ICLR) are utilized, and repair becomes increasingly complicated and in many cases mutagenic. The type of DNA damage incurred dictates the mechanism(s) of DNA repair. Initial recognition of the lesion directly recruits, or signals for the recruitment of repair factors. The exact repair mechanism implemented for a specific type of lesion may vary, depending upon available factors or cell cycle status.

### 3.1 Direct reversal repair

DRR represents a set of enzymes that catalyze direct repair of the damaged base/s, returning the DNA to its previous, undamaged configuration and sequence. Examples of DNA damage and their repair proteins would include $\textit{O}^6$-alkylguanine being repaired by $\textit{O}^6$-alkylguanine DNA alkyltransferase (AGT), 1-alkyladenine being repaired by AlkB dioxygenase human homologues AHB2 and AHB3, cyclopyrimidine dimers (CPD) being repaired by DNA photolyase and 6-4 photoproducts being repaired by 6-4 photolyase (Duncan et al. 2002; Kim et al. 1993; Wibley et al. 2000; Zhao, X. et al. 1997). As these specific enzyme mechanisms execute a direct catalytic repair of DNA damage, they do not require DNA synthesis in repair. However, some of these same forms of DNA damage, for example CPD, can be repaired through alternate, DNA polymerase requiring mechanisms, such as NER.

### 3.2 Base excision repair

BER is utilized in the repair of DNA damage incurred on a single strand, where there is little structural alteration of the DNA backbone. This damage includes apurinic sites resulting from spontaneous depurination, oxidized or alkylated bases, or base mismatches resulting from 5-methylcytosine deamination (T/G mismatch) or polymerase errors. Because there is little backbone distortion, these lesions tend not to block replicative polymerases, and as a result, this damage can be highly mutagenic if not detected and repaired. For example, an unrepaired 8-oxoguanine at DNA synthesis will lead to G:C to T:A transversions common to many solid tumors (Bruner et al. 2000).

#### 3.2.1 Steps of BER

In BER, specific glycosylases recognize and bind specific lesions (Banerjee et al. 2006; Bruner et al. 2000; Engelward et al. 1997; Klungland et al. 1999; Parsons 2003) with the assistance of accessory proteins such as MutM (Banerjee et al. 2006) (Figure 1). Examples include 8-oxoguanine DNA glycosylase recognizing and binding 8-oxoguanine (Bruner et al. 2000) or alkyladenine DNA glycosylase recognizing and binding alkyladenine (Engelward et al. 1997). The damaged base is flipped out and cleaved by the glycosylase (Banerjee et al. 2006) generating an abasic site. Apurinic-apyrimidinic endonuclease (APE1) will then nick or break the DNA backbone (Mol et al. 2000; Srivastava et al. 1998) (Figure 1).

At this point, two possible pathways, Short Patch Repair (SPR) and Long Patch Repair (LPR), diverge (Kubota et al. 1996). In SPR, pol$\beta$ will cleave the 5’ abasic sugar and replace the missing nucleotide (Garcia-Diaz et al. 2001). X-ray Repair Cross Complementing Protein 1 (XRCC1), along with its binding partner, DNA Ligase III will seal the backbone nick. In LPR, Replication Factor C (RFC) loads Proliferating Cell Nuclear Antigen (PCNA) at the
incision (Kelman 1997). PCNA will facilitate polβ binding (a common step in the recruitment of many polymerases to damaged sites) and polβ will synthesize a stretch of DNA, creating a 5' flap (Srivastava et al. 1998). This flap is trimmed by Flap Endonuclease 1 (FEN1), and DNA Ligase I seals the remaining nick or break in the DNA backbone (Prasad et al. 2000; Srivastava et al. 1998) (Figure 1). In both cases, the DNA is repaired in an error-free manner, if the damage is recognized before S-phase and DNA synthesis.

![Fig. 1. The generalized steps of BER](image)

### 3.3 Nucleotide excision repair
Whereas the damage to which BER responds does not significantly distort the DNA backbone, NER explicitly recognizes this backbone distortion utilizing two different systems—Transcription Coupled Repair (TCR, (Bohr et al. 1985; Mellon et al. 1987)) and Global Genome Repair (GGR, (Aboussekhra et al. 1995; Araujo et al. 2000)). The types of damage recognized by these systems primarily consist of bulky adducts, including UV photoproducts (cyclopyrimidine dimmers and 6-4 photoproducts (Mellon et al. 1987; Ng et al. 2003)) or N-acetoxy-2acetyl-aminofluorene induced adducts (Ng et al. 2003). These bulky adducts will stall DNA polymerases as well as transcription machinery, and are therefore less mutagenic than the types of damage repaired by BER, but they can be cytotoxic (Mitchell et al. 2003).

#### 3.3.1 Steps of NER
TCR is activated by the stalling of RNA polymerase II upon encountering a bulky adduct. This recruits Cockayne Syndrome Proteins A and B (CSA and CSB, (Henning et al. 1995)), Xeroderma Pigmentosum Protein A (XPA), Binding Protein 2 (XAB2, (Nakatsu et al. 2000)), and High Mobility Group Nucleosome Binding Protein 1 (HMGN1, (Birger et al. 2003)) to the site of damage. This system for sensing DNA damage can only function on actively transcribed genes. With GGR, the bulky adducts are recognized by the Xeroderma Pigmentosum Protein C (XPC)-Homologue of RAD23 B (HR23B) complex (Figure 2), with help from Xeroderma Pigmentosum Protein E (XPE, also known as Damaged DNA Binding Protein 2 (DDB2)) in the case of photodimers (Kulaksiz et al. 2005; Yokoi et al. 2000).
Following damage recognition, these two repair systems utilize the same enzymes to catalyze repair. These include the basal transcription factor (TFIIH), with the helicases Xeroderma Pigmentosum Protein B (XPB, 3’ to 5’ helicase) and Xeroderma Pigmentosum Protein D (XPD, 5’ to 3’ helicase), as well as Xeroderma Pigmentosum Protein A (XPA) and Xeroderma Pigmentosum Group G (XPG) (Tantin 1998; Tantin et al. 1997). Once bound, the TFIIH complex opens the lesion. XPA recruits Replication Protein A (RPA), which stabilizes the single stranded DNA (ssDNA) (Li et al. 1995a; Li et al. 1995b). The Excision Repair Cross Complementation Group 1–Xeroderma Pigmentosum Group F (ERCC1-XPF) heterodimer then incises the DNA backbone 5’ of the damage, while XPG incises the backbone on the 3’ side of the adduct (O’Donovan et al. 1994; Sijbers et al. 1996).

With 25-30nt surrounding the lesion removed, RFC loads PCNA, as in BER (Kelman 1997). At this stage, variations in the final steps may be observed with dividing and non-dividing cells (Fousteri & Mullenders 2008). In the case of dividing cells, DNA polDž and poldž will synthesize DNA across the gap, and DNA Ligase I will seal the resulting nick (Aboussekhra et al. 1995; Araujo et al. 2000; Araujo & Wood 1999). In the DNA of non-dividing cells (out of S-phase), polk might instead be utilized to bridge the gap, with XRCC1-DNA Ligase III sealing the nick (Moser et al. 2007; Ogi & Lehmann 2006). Again, as with BER, the utilization of the undamaged strand results in error-free repair.

3.4 Translesion synthesis

The DNA lesions produced in cells by a variety of chemical or physical agents can sometimes escape the repair mechanisms of the cells, including NER. Because these lesions distort the structure of the DNA, the high-fidelity polymerases are no longer able to bind and replicate past the lesions. Other polymerases, the low-fidelity, TLS polymerases, are recruited at the replication fork and are involved in replication past the DNA damage (Burgers et al. 2001; Ohmori et al. 2001). As noted above, the most significant classes of TLS enzymes encountered in eukaryotes are the members of the Y-family of DNA polymerases (polŋ, τ, κ and Rev1) also known as UmuC/DinB/Rev1/Rad30 superfamily, in addition to a member of the B-family of polymerases, polζ (Ohmori et al. 2001; Zhao, B. et al. 2004).
3.4.1 Steps of TLS
The first step in DNA repair is the recruitment of TLS polymerases at the stalled replication fork by a monoubiquitnated, sumoulated, or otherwise modified PCNA (Haracska et al. 2001a; Haracska et al. 2001b; Waters et al. 2009). Rev-1 is suspected to act as a trigger in mobilizing the other polymerases, polŋ, polît, and polk, to insert a base or bases opposite the damaged nucleotide/s (Guo et al. 2003; Ohashi et al. 2004; Tissier et al. 2004). After bringing the polymerases to the lesion site during the repair process, the same Rev-1 will bind polŋ, polît is a member of the B-family of polymerases that is not very efficient in inserting the nucleotides opposite lesions, but can extend the primer termini (Haracska et al. 2003). Once this polymerase binds, it will begin to synthesize a stretch of DNA opposite the damage site, completing the bypass of the lesion. Two generalized models for TLS include polymerase switching, in which a replicative polymerase stalls, PCNA is modified, and a TLS polymerase extends from the lesion, and once bypassed, replication resumes. The other model is gap filling, in which a gap is left following replication fork stalling at a damaged site, and TLS fills this gap. These models are reviewed in Waters et al. (Waters et al. 2009).

3.5 Double strand break repair
Unlike the damage repaired by DRR, BER, and NER, DSB involve both strands of DNA. This eliminates the ability of DSBR to utilize DNA’s built in backup, the opposing DNA strand, as it too is damaged. This damage is typically caused by ionizing radiation, neighboring single strand breaks, natural processes such as V(D)J recombination, meiotic and mitotic crossing over, yeast mating type switching, and the collapse or stalling of replication forks (Khanna & Jackson 2001; Sugawara et al. 2000).

There are three main pathways for the repair of DSB: — Single Strand Annealing (SSA), NHEJ, and HRR. Local differences in DNA sequence, the availability of repair factors, the availability of a homologous sequence, and cell cycle status affect which mechanism of DSBR is utilized. NHEJ, of which there are two alternate biochemical pathways, the primary, Ligase IV dependent NHEJ (D-NHEJ) and the backup, Ligase III dependent NHEJ (B-NHEJ), is utilized for most DSB in mammalian cells (Mladenov & Iliakis 2011). SSA can occur where there are direct repeats in DNA sequence, and there are lower levels of NHEJ components (Fishman-Lobell et al. 1992). HRR functions predominantly in S and G2 phases of the cell cycle, when homologous substrates are readily available (Aylon et al. 2004).

3.5.1 Steps of NHEJ
DSBR by NHEJ can follow a main D-NHEJ (named for the requirement of DNA-PKcs) pathway responsible for the bulk of DSBR in mammalian cells, or a Backup B-NHEJ. Both pathways repair DSB in similar manners, but utilize different proteins at each step (Mladenov & Iliakis 2011). For simplicity, Figure 3 will only list D-NHEJ, but B-NHEJ follows similar catalytic steps, as discussed below.

In mammalian D-NHEJ, the DSB is recognized and bound by Ku70/Ku80 heterodimer. This leads to binding of the DNA Dependent Protein Kinase catalytic subunit (DNA-PKcs) yielding a functional DNA-PK holoenzyme (Cary et al. 1997). The ends are modified by polynucleotide kinase (PNK) and terminal deoxynucleotide transferase (TDT), also, an as yet unidentified endonuclease (possibly Artemis, a substrate of DNA-PK) will then resect the 5’ ends leaving 3’ ssDNA. These ends will be filled by DNA polλ and μ, and joined by the DNA Ligase IV-XRCC4 complex, enhanced by XLF Like Factor (XLF) (Bryans et al. 1999; Fan & Wu 2004; Mahajan et al. 2002; Yano et al. 2008) (Figure 3).
Less is known about B-NHEJ than D-NHEJ, but B-NHEJ seems to act more slowly than D-NHEJ, though it can ultimately achieve the same ends, the repair of DSB (Iliakis 2009; Wang, H. et al. 2003; Wang, M. et al. 2006; Wu et al. 2008). This alternate pathway was identified in cell lines deficient in many of the D-NHEJ proteins (Nevaldine et al. 1997; Wang, H. et al. 2001a; Wang, H. et al. 2001b). In B-NHEJ, Poly [ADP-ribose] polymerase 1 (PARP-1), usually associated with single strand break repair, is responsible for end recognition and binding (McKinnon & Caldecott 2007; Wang, M. et al. 2006). A role for the MRN complex in processing of the break has been suggested based upon reduced end joining in D-NHEJ deficient cells when Mre11 is inhibited (Rass et al. 2009). DNA Ligase III and XRCC1 are regulated by PARP-1, and a role for Ligase III has been demonstrated in NHEJ (McKinnon & Caldecott 2007; Windhofer et al. 2007). Enhancement of Ligase III activity by Histone H1, suggests a role in B-NHEJ as well (Rosidi et al. 2008). No direct evidence for the role of a specific polymerase has been identified in B-NHEJ, however, it in known that PARP-1 interacts with DNA polα, suggesting possible involvement in this pathway (Dantzer et al. 1998).

![Fig. 3. Generalized steps of NHEJ](image)

If the two ends that are joined by NHEJ are from a single double strand break, the outcome can be conservative or lead to deletions of varying sizes, depending upon the extent of processing of the ends. If, however, the two ends that are rejoined were from separate DSBs, the result will be a translocation. While NHEJ is quite proficient at rejoining DSB ends and eliminating this highly cytotoxic DNA damage, it does not involve a mechanism to choose which ends to rejoin, and is, thus, considered to be a non-conservative mechanism for DNA repair.

### 3.5.2 Steps of SSA

SSA requires a more specific set of conditions than NHEJ, specifically neighboring repeats either side of the DSB, and will predominantly occur in S-phase (Frankenberg-Schwager et al. 2009; Sugawara et al. 2000). At the site of the DSB, there is a 5' to 3' resection, likely by the MRN complex, that leaves 3' tails. RAD52 binds the 3' ends, and these tails are stabilized by the ssDNA binding protein, RPA (Stasiak et al. 2000; Van Dyck et al. 1999; Wold 1997). These factors are sufficient for annealing of the repeat sequences. FEN1 or ERCC1/XPF then trim the 3' overhangs (Al-Minawi et al. 2008; Gottlich et al. 1998). The remaining gaps are filled and ligated by DNA polε and DNA Ligase III (Gottlich et al. 1998) (Figure 4).

As neighboring repeats are annealed, sometimes at distances of 40bp to 1-2kb apart, SSA results in deletions of varying sizes (Gottlich et al. 1998; Richardson & Jasin 2000). With multiple genomic DSB, SSA has also been demonstrated to yield translocations (Richardson & Jasin 2000). Presumably, these result from homologous sequences on non-homologous chromosomes annealing and being joined by the SSA machinery.
3.5.3 Steps of HRR
Like SSA, HRR requires a homologous sequence, typically a sister chromatid; thus most HRR occurs in late S or G2 phases of the cell cycle. Following DSB formation, there is a 5’ resection leaving 3’ tails. Rad51 recombinase (made up of Rad51B and Rad51C) binds these single stranded regions, a homologous region is identified, and a D-loop is formed (Sung & Robberson 1995). This complicated process utilizes a number of other proteins, including the MRN complex which, along with BRCA1 and CtIP, again serves a role in resection; BRCA2, which facilitates Rad51 loading and facilitates recombination; RPA, which acts to stabilize the ssDNA and promote strand exchange with Rad51; Rad54, which aids in chromatin remodeling; and Hop2-Mnd1, which help promote D-loop capture and processing (Chen et al. 2008; Pellegrini et al. 2002; Solinger et al. 2001; Stauffer & Chazin 2004; Sung & Robberson 1995; Vignard et al. 2007). (Other protein systems are responsible for meiotic recombination and are not discussed in detail here, but are reviewed in (Smith & Nicolas 1998)).
DNA pol will then extend from the 3’ end of the invading strand, elongating the D-loop (McIlwraith et al. 2005). The invading strand is then displaced and anneals with the second DSB end, being ligated by DNA Ligase I (Goetz et al. 2005) (Figure 5). This mechanism of repair avoids the formation of a Holiday junction and the risk of crossover (referred to as Synthesis Dependent Strand Annealing or SDSA) (Ferguson & Holloman 1996). However, where the goal is crossover, as in meiotic recombination, the extended D-loop will bind the second DSB end creating a Holiday junction, leading to crossover and non-crossover products (referred to as the DSBR pathway) (Szostak et al. 1983).

A specialized version of HRR, called Break Induced Replication (BIR), acts to preserve single DSB ends such as those at chromosomal termini, or, as we will discuss in ICLR, at stalled replication forks. In BIR similar factors are utilized to process the ends, invade a homologous or repeat sequence, extend, and resolve the lesion. Of note, the pol primase is required for initiation of replication by polD (Maloisel et al. 2008). polD is required for long-range synthesis, and, unique to BIR, Pol32 is required but does not function in other HRR pathways (Asturias et al. 2006; Lydeard et al. 2007).

HRR is the only truly conservative repair mechanism for double strand breaks, with both SDSA and DSBR, when a homologous chromosome provides the template for repair. Unlike NHEJ and SSA, it does not typically lead to translocations or deletions, but DSBR can lead to crossing over of chromatids. In BIR, if the substrate for recombination is a homologous chromatid, repair is conservative; however, if a repeat from a non-homologous sequence is utilized, a translocation may result.

### 3.6 Interstrand crosslink repair

There are certain chemicals, endogenous and exogenous, that covalently link both strands of a DNA molecule together; these agents include malondialdehyde, mitomycin C, or psorlen (Scharer 2005). With an ICL, much like a DSB, the anti-parallel strands can no longer be used as template for one another. ICLs covalently link both strands of DNA together preventing strand separation required for transcription and replication, making ICL inducing agents particularly potent killers of cycling cells (Dronkert & Kanaar 2001). For this reason, many ICL inducing agents are used as chemotherapeutic agents in the treatment of cancer (Lawley & Phillips 1996; Lord et al. 2002; Ryu et al. 2004). Stalled replication forks will also utilize ICLR under certain circumstances (McCabe et al. 2008).

The genetic disorder Fanconi Anemia (FA) is intimately associated with ICLR, much the way Xeroderma Pigmentosum is associated with NER. There are currently 15 FA groups, representing defects in 15 proteins involved in ICL repair. These genes are, FANCA, B (Fanconi Anemia Associated Protein of 95kDa, FAAP95), C, D1 (BRCA2), D2, E, F, G, I, J (BRCA1 Interacting Protein C-terminal Helicase 1, BRIP1), L, M (Fanconi Anemia Associated Protein of 250KD, FAAP250), N (Partner and Localizer of BRCA2, PALB2), O (Rad51C), and P (SLX4) (Kitao & Takata 2011). The numerous proteins involved in ICLR reflect the complexity of repairing this type of damage. The model presented here (Figure 6) is speculative; it is based upon published biochemical functions of the proteins involved and the formation of recombination intermediates in FA cells between non-homologous chromosomes (Newell et al. 2004). The ICLR pathway likely represents a last ditch mechanism of repair for this extremely cytotoxic damage where there are no homologous substrates for HRR of these lesions (McCabe et al. 2009). TLS is one other option for damage bypass, but does not constitute repair.
3.6.1 TLS in response to ICL
If there are incisions on both sides of an ICL on one strand, TLS has been proposed to replicate past the lesion, reducing its cytotoxicity and permitting continuation of the cell cycle. TLS utilizing error prone polζ or polη might replicate past the ICL following DSB formation (reviewed in (Dronkert & Kanaar 2001)). However, polη mutants show normal sensitivity to ICL, suggesting no role for this polymerase in repair (Grossmann et al. 2001). Little is known about this pathway compared to NER and HR repair; however, it is thought that this pathway helps the cells to bypass an ICL to reduce cytotoxicity as opposed to actually repairing the lesion (Dronkert & Kanaar 2001). Further, the severity of the FA phenotype with respect to ICL damage suggests TLS is, at most, a minor mechanism of ICL repair.

3.6.2 Steps of ICLR
It has been suggested that the distortion created by the ICL or the ensuing chromatin change could be one of the initial signals for repair (Dronkert & Kanaar 2001). DSB signaling, including ATM kinase activity, also plays a clear role in ICLR, as DSB are an important intermediate in repair, though data suggests that DSB do not activate the FA pathway (Rogakou et al. 1999; Sobeck et al. 2007). The collapsed replication fork at the site of ICL damage, or in response to hydroxyurea treatment, collapses and regresses with the help of RECA (Robu et al. 2001). This leads to loading of the FA core complex via the DNA translocase activity of FANCM/MHF complex (Singh et al. 2010; Yan et al. 2010). The FA core complex is comprised of FANCA, B, C, E, F, G, L and M, and is required for monoubiquitination of FANCD2 by the E3 ligase FANCL, in concert with the E2 subunit UBE2T (Machida et al. 2006) (Figure 6).

Central to the FA pathway are FANCD2 and its paralog FANCI (Smogorzewska et al. 2007). FANCD2 monoubiquitination is traditionally looked upon as the marker of activation of the FA pathway. Monoubiquitination is required for FANCD2 and FANCI localization to chromatin (Garcia-Higuera et al. 2001; Smogorzewska et al. 2007). Monoubiquitinated FANCD2/FANCI colocalizes with BRCA1 in response to DNA damage and at synaptonemal complexes (Garcia-Higuera et al. 2001). Additionally, FANCD2 has been shown to interact in a constitutive manner with FANCD1/BRCA2 and co-localizes with RAD51 in nuclear foci (Hussain et al. 2004). FANCD2 also interacts with the MRN complex, which may serve a role in processing a recombination intermediate (Nakanishi et al. 2002). FANCA, a core complex component, has been shown to interact with several other proteins. Independently of the other FA proteins, FANCA interacts directly with BRCA1 without the requirement for DNA damage, suggesting a constitutive interaction (Folias et al. 2002). Additionally, FANCA has been suggested to aid in the recruitment of the SWI/SNF complex subunit, brahma-related gene 1 (BRG1), and may be involved in chromatin remodeling at the site of action of the FA pathway (Otsuki et al. 2001).

Biochemical studies have identified several proteins forming large complexes with the FA proteins. Included in the BRAFT complex are five FA proteins (FANCA, C, E, F, and G), the Bloom syndrome helicase (BLM), replication protein A (RPA) and topoisomerase IIIa (Topo3a). This complex has a DNA duplex unwinding capability that requires BLM, but not FANCA. However, BLM is not required for FANCD2 monoubiquitination, suggesting BLM functions in this pathway downstream of core complex signaling for activation of the FA pathway (Meetei et al. 2003). FANCJ is a BRCA1 interacting protein that functions as an ATP-dependent 5′-3′ helicase (Cantor et al. 2001; Cantor et al. 2004).
interaction of FA proteins with BLM, a 3’-5’ helicase, these data suggest the ability of FA complexes to open stretches of DNA in both directions (Ellis et al. 1995). The discovery of FANCD1 as BRCA2 directly linked the FA pathway and HRR pathway (Hirsch et al. 2004). BRCA2 is known to regulate RAD51 controlling the formation of the RAD51/ssDNA nucleoprotein filament required for strand pairing during HRR in DSBR (Davies et al. 2001; Sharan et al. 1997). In addition, BRCA2 binds FANCD2 and G placing the core complex and FANCD2 at sites of homologous recombination repair (Hussain et al. 2004). Another recombination and FA was uncovered with the identification of FANCN as the partner and localizer of BRCA2 (PALB2) (Reid et al. 2007; Tischkowitz et al. 2007; Xia et al. 2006). As its name suggests, PALB2 interacts with BRCA2 and is responsible for its localization to chromatin; thus, PALB2 is required for BRCA2’s function in homologous recombination repair and cell cycle checkpoints (Xia et al. 2007; Xia et al. 2006). FANCM, in addition to its early binding role, also serves a catalytic function in the processing or resolution of the recombination intermediate, as cells from a FANCM patient form radials in response to ICL inducing agents, but the FANCM defect does not impact FANCD2 monoubiquitination (Singh et al. 2009).

Fig. 6. Hypothetical Steps of ICLR

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All of this suggests the next step in repair involves a HRR or HRR like repair mechanism, likely similar to BIR with a non-homologous substrate, as failure to complete repair yields a radial formation (Figure 7), and these radials are between non-homologous chromosomes (Newell et al. 2004). It would seem the variety of functions pulled together for ICLR serve to stabilize the stalled replication fork, initiate recombination in the absence of an available direct homologue by identifying repeats or microhomology suitable for recombination on non-homologous chromosomes, synthesis along this sequence past the region affected by the ICL (possibly involving polθ/Ligase IV, (Chan et al. 2010)), and subsequent resolution of the recombination intermediate (Figure 6).

Fig. 7. A. Reorganization of non-homologous chromosome recombination intermediates from the model in Figure 6 into a chromosomal context (large circles represent centromeres) demonstrates the possibility for intermediates from this mechanism to yield radials. B. Portion of a metaphase spread of ICL treated FA mutant cells and a radial formation.

4. Discussion

With the exception of DRR and possibly a small subset of NHEJ, DNA damage repair requires DNA replication. BrdU incorporation, a general marker of DNA synthesis, has been successfully used as a surrogate marker for DNA damage repair (Kalle et al. 1993; Kao et al. 2001). The requirement of replication for repair relates to the double stranded structure of DNA, and the idea of having a built in backup copy of information on the opposing strand. To a certain extent, this holds true even in the case of double stranded damage, though the backup may be a repeat sequence on the same stretch of DNA or on a non-homologous chromosome, in the case of SSA and ICLR respectively.

Different types of DNA damage will utilize any of a variety of DNA polymerases, based upon the structure/function of this polymerase. The evolution of various DNA polymerase families with specific roles supports the importance of replication as an indispensable tool in the repair of DNA damage. TLS utilizes polymerases with open active sites to permit synthesis past an adduct (Trincao et al. 2001). Long-range synthesis in HRR requires polθ, because of its processivity (Asturias et al. 2006). BIR requires the normally dispensable polθ subunit, pol32, to facilitate replication restart in response to this specific recombination based repair mechanism (Lydeard et al. 2007). The importance of polθ in NER, is demonstrated by the similarity of disease spectrum present in XP variant and the XP group genes constituting catalytic functions in the repair of bulky adducts (Johnson et al. 1999a).
The evolutionary persistence of many of these polymerases demonstrates that this intimate involvement of DNA replication in repair is an indispensible facet of life, as we know it.

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6. References


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Since the discovery of the DNA structure researchers have been highly interested in the molecular basis of genome inheritance. This book covers a wide range of aspects and issues related to the field of DNA replication. The association between genome replication, repair and recombination is also addressed, as well as summaries of recent work of the replication cycles of prokaryotic and eukaryotic viruses. The reader will gain an overview of our current understanding of DNA replication and related cellular processes, and useful resources for further reading.

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