Molecular & Cellular Oncology 2:1, e970065; January/February/March 2015; Published with license by Taylor & Francis Group, LLC

Molecular mechanisms involved in initiation of the DNA damage response

Kevin J Barnum and Matthew J O'Connell*

Department of Oncological Sciences and the Graduate School of Biomedical Sciences; Icahn School of Medicine at Mount Sinai; New York, NY USA

Keywords: checkpoint, DNA damage, excision, nuclease, recombination, repair, replication, transcription

Abbreviations: ALT, alternative lengthening of telomeres; DDR, DNA damage response; DSB, double-strand break; FPC, fork protection complex; HR, homologous recombination; ICL, interstrand crosslink; MMR, mismatch repair; NER, nucleotide excision repair; NJEH, non-homologous end joining; TIF, telomere dysfunction-induced focus

DNA is subject to a wide variety of damage. In order to maintain genomic integrity, cells must respond to this damage by activating repair and cell cycle checkpoint pathways. The initiating events in the DNA damage response entail recognition of the lesion and the assembly of DNA damage response complexes at the DNA. Here, we review what is known about these processes for various DNA damage pathways.

Introduction

Cells experience a wide variety of DNA damage lesions, which can occur as often as 10,000 times per day.[1] These lesions interfere with DNA replication, transcription, and genome integrity; therefore, the damage must be repaired to ensure homeostasis and the prevention of disease. The DNA damage response (DDR), which entails both recruitment of DNA repair enzymes and the activation of checkpoints to effect cell cycle arrest, is essential for maintenance of genomic integrity. Defects in the DDR are implicated in several diseases, including cancer, neurodegenerative disease, and immune dysfunction (**Table 1**).[1] There is a wide variety of DNA damage lesions but a limited number of repair pathways and an even smaller number of checkpoint pathways. Therefore, lesion-specific initiating events must be channeled into these repair and checkpoint pathways. Here, we review the initial recognition and processing events that occur in DNA repair pathways. These processes are highly conserved, and unless stated, all processes described occur in mammalian systems. We will not focus on the downstream events, which are described in detail in other excellent reviews.[1,2]

*Correspondence to: Matthew J O'Connell; Email: matthew.oconnell@mssm.edu
Submitted: 08/18/2014; Revised: 09/04/2014; Accepted: 09/06/2014
http://dx.doi.org/10.4161/23723548.2014.970065

The Early Events of a DNA Damage Response

In all contexts, initiation of the DNA damage response requires the same general steps.

Recognition: The first step is recognition of the lesion. Importantly, there is an enormous variety of potential lesions and the cell requires mechanisms to recognize all of these lesions as DNA damage. The cell must therefore contain factors that recognize, bind, or interact with damaged DNA more tightly than with normal DNA, which is in vast excess.

Recruitment of repair and checkpoint proteins: Next, DNA damage repair and checkpoint response proteins must be recruited to the site of the lesion. This occurs either through recruitment by recognition proteins to the lesion or by recognition of structures at the site of the lesion by the repair proteins themselves.

Processing: Often, primary lesions are not able to initiate the repair and checkpoint response themselves. In these cases, primary lesions are processed to structures that are recognized by other DNA damage response proteins. Indeed, because of the wide variety of lesions and the small number of DNA damage responses, it has been proposed that there is a common processing intermediate that is able to initiate the DNA damage response.[3,4]

Checkpoint activation: Checkpoint activation occurs after the recruitment of checkpoint proteins to a particular structure. It is often assumed that checkpoint activation begins upon establishment of the lesion and ceases upon the completion of repair. The repair process, however, most likely destroys the structure required for checkpoint activation, in which case activation of the checkpoint would cease upon the initiation, rather than the completion, of repair. Note that the requirements for the recruitment of repair enzymes and checkpoint proteins may be different, thus processing of lesions may be required for both to occur efficiently.

Repair: There are 2 main modes of repair. The first involves lesions that affect one DNA strand; repair of these lesions involves excision of the lesion and resynthesis of the damaged DNA using the undamaged complementary strand. The other mechanism is double-stranded DNA break (DSB) repair, either by homologous recombination (HR) or non-homologous end joining (NHEJ). In HR, DSB resection occurs and DNA is resynthesized using the sister chromatid as a template. Because of the requirement for a sister chromatid, homologous recombination is restricted to S and G2 phases. In G1 phase, DSBs are

Table 1. Examples of human syndromes associated with early defects in the DNA damage response

Pathway	Genes mutated	Disease	References
MMR	MLH1, MSH2, MSH6, PMS2	Lynch syndrome	7
NER	CSA, CSB, XPB, XPD, XPG	Cockayne syndrome	16
	XPA, XPB, XPC, XPD, XPE, XPF, XPG	Xeroderma pigmentosum	
DSB repair	ATM	Ataxia telangiectasia	34
	MRE11	Ataxia telangiectasia-like disorder	
	NBS1	Nijmegen breakage syndrome	
Replication stress	ATR	Seckel syndrome	33
ICL repair	FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ, FANCL, FANCM, FANCN, FANCO, FANCP, FANCQ	Fanconi anemia	60
Ribonucleotide removal	RNASEH2	Aicardi-Goutiéres syndrome	58

Abbreviations: DSB, double strand break; ICL, interstrand crosslink; MMR, mismatch repair; NER, nucleotide excision repair.

repaired by NEHJ, but this is not a homology-based process and can result in the joining of non-sister chromatids. Importantly, all intermediates in the processing and repair of lesions represent structures other than a DNA duplex and can be recognized as DNA damage, so repair can begin through one pathway and be shunted into another via a common intermediate.

Here, we will first discuss excision-based pathways and then recombination-based pathways.

Excision-Based Pathways

Excision-based pathways are used to repair lesions in which the bases are damaged or mismatched but the sugar-phosphate backbone is intact. They entail the resection of damaged or mismatched DNA and the resynthesis of new DNA using the complementary strand as a template. Because it uses the complementary strand and not a sister chromatid, excision repair can occur in any phase of the cell cycle. The initiating events in excision repair are relatively well understood.

Mismatch repair

Despite the proofreading functions of DNA polymerases, incorrectly paired bases are incorporated at a low frequency. In addition, chemically modified bases can alter base pairing. Mismatch repair (MMR) is a system that removes these mismatched bases in addition to mono-, di-, and trinucleotide insertion/deletion loops (IDLs), which cause expansion and contraction of repeats.

MMR is very well understood in *E. coli*, in which mismatches are detected by MutS. MutS subsequently recruits MutL and MutH, and the mismatched base is excised. In *E. coli*, strand discrimination is based on adenine methylation, as the template strand is methylated whereas methylation of the nascent strand is delayed.[5]

In eukaryotes, multiple genes encode MutS and MutL homologs. Mismatches are detected by members of the MutS homolog (MSH) protein family, which bind the DNA as heterodimer clamps by recognizing the distortion of the DNA helix resulting from mismatches and IDLs that induce an ~60° bend. After the formation of a complete ring around the DNA the MSH complex actually dissociates from the lesion and diffuses along the DNA as a sliding clamp. The MSH complex recruits a MutL homolog (MLH) complex, which has endonucleolytic activity and generates nicks in the DNA. From these nicks, the exonuclease EXO1 resects the damaged strand, and polymerases and ligases synthesize new DNA and seal the resulting nick respectively.[5]

A major question in the MMR field is how the strand with the mismatch is identified in eukaryotes, as eukaryotes do not have the hemimethylation system present in *E. coli*. It was observed that mismatched bases on the lagging strand were more efficiently repaired than those on the leading strand.[6] Moreover, MLH complexes were shown to make nicks preferentially in strands that already contain nicks, similar to Okazaki fragments of the lagging strand. For the leading strand, it is thought that orientation-specific binding of MLH to PCNA and the asymmetrical endonucleolytic activity of the MLH complex contribute to its ability to nick the nascent strand instead of the template strand.[5]

Mutations in the MMR genes *MLH1*, *MSH2*, *MSH6*, and other genes result in Lynch syndrome, in which patients are highly susceptible to cancers of the colon, endometrium, ovary, and the upper GI tract. These tumors have relatively normal karyotypes and are instead characterized by microsatellite instability, which results from defective repair of IDLs.[7]

Base excision repair

Base excision repair (BER) removes modified bases, including alkylated bases that are produced by several common therapeutics. In addition, it repairs apurinic/apyrimidinic (AP) sites, the most common spontaneous lesion in cells.[1] The initiating event in BER involves the recognition and cleavage of the modified base from the sugar by a DNA glycosylase. There are 11 nuclear DNA glycosylases in humans, and each recognizes a group of modified bases and mismatches. Notably, they flip the base out from the DNA before cleaving it from the deoxyribose. This is followed by excision of the backbone by any one of a variety of pathways, which each feed into one of the 2 general forms of BER, short-patch and long-patch repair. In short-patch repair, only a single nucleotide is removed, and the gap is filled by Polβ and ligated by ligase 1 or 3. In long-patch repair, 2–10

nucleotides are removed, and the process requires flap endonuclease 1 (FEN1), PCNA, DNA polymerases β, δ, or ε, and ligase 1.[8]

Nucleotide excision repair

Bases in DNA are constantly subject to modification by products of metabolism, UV light, and environmental mutagens. These modified bases are removed by nucleotide excision repair (NER). Because of the importance of this pathway for the removal of UV-damaged bases, many of the genes fall into a group of genes that are mutated in xeroderma pigmentosum (XP), a genetic disorder that results in hypersensitivity to UV light and skin tumorigenesis in UV-exposed regions.[1]

The most common UV-induced lesions are cyclobutane dimers and 4–6 photoproducts. Both distort the double helix and are a physical impediment to DNA and RNA polymerases. Recognition of UV-induced lesions occurs via the XPC–RAD23 or, for cyclobutane dimers, DDB1–DDB2 complexes.[9] These complexes recognize the destabilization of the DNA helix induced by the lesion and bind to the modified bases. They then recruit several other XP proteins that promote unwinding of the DNA, excision of the damaged strand, and resynthesis.[10]

RNA polymerase as a detector of DNA damage

During transcription, RNA polymerase frequently undergoes stalling as a result of barriers to transcription, which can occur at difficult-to-replicate regions, protein–DNA complexes, and DNA damage lesions that evoke NER.[11]

It has been known for some time that damage in transcribed regions is more rapidly repaired than damage in other regions and that this occurs specifically on the template strand.[12] Indeed, RNA polymerase itself is able to detect lesions, and there are also specialized mechanisms that allow for their repair. This is essential, because an impassable lesion in the transcribed region of an essential gene would be disastrous. In addition, upon stalling at a site of damage RNA polymerase could be evicted from a damaged gene, and in a large gene, where the likelihood of damage is high and transcription of a single copy can take several hours, the abortion of transcription is costly.[13] Furthermore, it has been proposed that RNA polymerase is an ideal detector of DNA damage, because in its stalled state it is bound extremely tightly to the DNA at the lesion.[14]

The best-understood RNA polymerase-coupled repair process is a modified form of NER called transcription-coupled NER (TC-NER), which differs from NER in non-transcribed regions (global genomic or GG-NER) in the initial events and the proteins required. The unique proteins in TC-NER are CSA and CSB, both of which are mutated in Cockayne syndrome. Patients with Cockayne syndrome exhibit the photosensitivity of XP in addition to progeroid symptoms such as hearing loss, cachexia, cataracts, retinopathy, and neurodegeneration.[15,16] CSB has been shown to copurify with RNA polymerase II and to bind more tightly to the stalled polymerase.[17,18] CSB recruits CSA to the stalled complex, which is followed by recruitment of NER factors that allow unwinding, excision, and resynthesis. XPC and DDB1/DDB2, which in GG-NER recognize the lesion and recruit the rest of the NER enzymes, are not required for TC-NER; instead, RNA polymerase II is thought to serve the role of these proteins.[13]

Checkpoint activation during excision repair

It is unclear whether the cell cycle checkpoint response is activated by the lesions themselves or by excision-based repair processes, particularly at the G2/M transition. If a cell enters mitosis with a single unrepaired DSB, all DNA from the breakpoint to the telomere is lost. However, the physiological consequence of entering mitosis with lesions that are removed by excision is not obvious. Both the doses and wavelengths of UV light used commonly for cell-based experiments are supraphysiological, and can induce other lesions including strand breaks and protein–DNA crosslinks. In considering NER repair of UV-damaged DNA, there is evidence from XP patient cell lines that the repair machinery is required for checkpoint activation during G1 and G2.[19] However, conflicting evidence from another paper shows that, of the XP factors, only XPA is required for checkpoint activation, suggesting that processing does not contribute.[20] Additionally, it was shown that checkpoint activation does not occur from UV-induced lesions until they cause replication stress in S phase.[21] Indeed, at low doses of UV-C irradiation, *Schizosaccharomyces pombe* cells progress through the entire cell cycle and arrest only after the subsequent S phase in a Chk1-dependent manner, implying that checkpoint activation does not occur directly from the lesion, but from indirect effects during DNA replication.[22]

Recombination-Based Pathways

Double-strand breaks

Double-strand breaks (DSBs) are the most catastrophic of DNA lesions. Inefficient repair of DSBs can lead to loss of large amounts of genetic material and may result in cell death or cellular transformation. DSBs can be induced directly by intrinsic reactive oxygen species, or by radio- or chemotherapy. Alternatively, they can arise indirectly by replication across single-strand breaks, such as those that result from type I topoisomerase dysfunction or inhibition. As mentioned above, prior to DNA replication, DSBs are repaired by the NHEJ pathway, in which the free ends are ligated. After replication, however, the sister chromatid can be used for homologous recombination (HR) repair.

DSBs prior to DNA replication

Recognition of DSBs occurs via proteins that bind free DNA ends. In the NHEJ pathway, the Ku70/80 complex binds DSB ends.[23] This leads to recruitment of the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs), which with Ku70/Ku80 composes the DNA-PK complex. DNA-PK is a phosphoinositde-3-kinase–related kinase (PIKK), and is related to the ataxia telangiectasia mutated (ATM) and AT and Rad3-related (ATR) kinases. DNA-PKcs phosphorylates several proteins involved in NHEJ, including itself. These autophosphorylations are thought to modulate access to the DNA ends so that ligatable ends are created but extensive end resection does not occur.[24]

XRCC4/LigIV is then recruited to promote ligation of the DNA ends.

DSBs after DNA replication

In the homologous recombination (HR) pathway, the MRE11/RAD50/NBS1 (MRN) complex binds the DNA ends; this process is promoted by poly(ADP-ribose) polymerase 1 (PARP1), which synthesizes poly(ADP-ribose) chains on nearby chromatin proteins.[25] The MRN complex contains 3′5′ exonucleolytic activity that promotes limited resection of the DNA ends. In *Saccharomyces cerevisiae*, it has been shown that Mre11 and Sae2, the yeast homolog of the human protein CtIP, promote initial resection of DSBs. This is followed by more extensive resection carried out by the 5′3′ exonuclease EXO1.[26] EXO1 belongs to the XPG-related family of 5′3′ endo/exonucleases, which also contains FEN1 and ASTE1. EXO1 and FEN1 have both been shown to be involved in DSB resection, as has the *S. pombe* homolog of ASTE1, Ast1.[27] Note that *S. cerevisiae*, historically the primary model for DSB repair, does not have an ASTE1 homolog. The extensive resection effected by these nucleases creates ssDNA with a 3′-overhang that allows the assembly of checkpoint signaling proteins and the initiation of homology-based repair.

The ssDNA generated by resection is bound by replication protein A (RPA). This RPA-ssDNA acts as a platform for the recruitment of 2 checkpoint complexes. In the first, ATR is recruited to RPA-coated ssDNA by its binding partner, ATR-interacting protein (ATRIP). The other complex is the Rad9–Rad1–Hus1 (9–1–1) complex, which is a PCNA-like ring that is loaded by a complex containing Rad17 and RFC2–5. ATR-dependent phosphorylation of ATRIP, the 9–1–1 complex, and RPA leads to the recruitment of mediators, which in turn recruit the checkpoint kinase Chk1. Chk1 undergoes ATR-dependent phosphorylation and activation and phosphorylates Wee1 and Cdc25, which regulate Cdc2.[28] In addition, an alternative mode of ATR activation was recently reported to involve the Nbs1-dependent recruitment of ATR to RPA–ssDNA followed by RPA phosphorylation; this mode was found to be required for the repair of replication-associated DSBs.[29]

Although the pathway leading to Chk1 activation is relatively well defined, how the pathway is initiated from the original lesion is not as clear. It has been established that RPA-coated ssDNA is essential for ATR/ATRIP recruitment and Chk1 activation[30], but studies have shown that this structure by itself is unable to activate Chk1. These papers showed that both a 5′ primer-template junction and the initiation of DNA replication are required for Chk1 activation.[4,31] How this structure is generated in certain circumstances, such as DSB repair, is relatively well understood as 5′3′ resection produces both ssDNA and the free 5′ end at the primer-template junction, but it is not as clear in other circumstances, such as during replication stress (see below). In these cases, it is likely that processing of the lesion is needed to produce the required structures.

There is competition between the systems that effect NHEJ and HR. It has been suggested that each is controlled by a pair of proteins; p53-binding protein 1 (53BP1) and its associated proteins rap1-interacting factor 1 (RIF1) and pax transactivation domain-interacting protein (PTIP) have been shown to promote NHEJ, whereas BRCA1 and CtIP have been shown to promote HR. Furthermore, these complexes are thought to antagonize each other, and the balance is regulated in a cell cycle-dependent manner, such that NHEJ predominates during G1, and HR predominates during S/G2.[32]

Defects in the initiation of the DSB response in tissues that have a high proliferative burden and are highly metabolically active can lead to disease. Mutation in ATR leads to Seckel syndrome, which is characterized by microcephaly and developmental delay.[33] Mutation in ATM results in ataxia telangiectasia, which manifests as premature aging, neurological dysfunction, abnormal blood vessel formation, immunodeficiency, and increased cancer risk. Ataxia telangiectasia-like disorder is caused by MRE11 mutations and manifests with ataxia, but only mild immune abnormalities and no telangiectasia. Nijmegen breakage syndrome (NBS) is caused by mutation in the MRN complex member NBS1, which is involved in the early stages of DSB repair. It is characterized by microcephaly, immunodeficiency, abnormal sexual development, and increased risk of cancer.[34]

Replication stress

Faithful replication and division of the genetic material requires that the DNA be replicated once and only once. Failure to do so results in changes in copy number and can lead to cellular death or transformation.

Throughout S phase several endogenous and exogenous factors can interfere with the coordinated completion of DNA replication, resulting in replication stress. The definition of replication stress and what constitutes it, however, is rapidly evolving. Causes may include DNA lesions, nicks, and gaps that result from repair and other processes of DNA metabolism, ribonucleotides that are erroneously incorporated into the DNA, particular DNA sequences that are intrinsically difficult to replicate, and collisions with the transcriptional machinery.[35] In addition, replication stress can occur as a result of dysregulated cell cycle signaling or as a result of chemotherapy, which can result in dysregulated supply of deoxyribonucleotides, origins, histones, and other requirements for DNA replication. Indeed, it is thought that replication stress is an early event in cellular transformation.[36-38]

Barriers to replication can be generally categorized as unprogrammed and programmed. Unprogrammed barriers occur randomly and include DNA damage lesions and breaks in the sugar-phosphate backbone. Programmed barriers, on the other hand, are barriers that are meant to impede the progression of DNA polymerase in every cell cycle and are typically thought to restrict the direction of replication through a region, such as in the rDNA where transcriptional activity is high and DNA polymerase is allowed to progress only in the same direction as the transcriptional machinery.

Upon stalling at a barrier the fork may collapse, whereby the components of the replisome dissociate from the DNA. This collapsed fork structure cannot be used directly to restart replication, and replication must be restarted using the recombination

machinery.[39,40] Cells respond to this replication stress by initiating a checkpoint cascade that regulates replication dynamics and arrests the cell cycle.[41]

The replisome includes a large complex called the fork protection complex (FPC), which in humans includes the subunits CLASPIN, TIMELESS, and TIPIN. One of the functions of the FPC is to mediate stalling and stabilization of the stalled replisome. Mrc1, the potential fission yeast homolog of human CLASPIN, mediates stalling in response to unprogrammed barriers, such as bulky DNA lesions and nucleotide depletion.[42] The human proteins TIMELESS and TIPIN mediate stalling at both unprogrammed sites and programmed sites.[43]

Upon fork stalling at sites of DNA damage, the helicase uncouples from the polymerase, creating a significant amount of ssDNA that becomes coated by RPA. This RPA–ssDNA is thought to lead to the recruitment of ATR/ATRIP and contribute to activation of the Chk1 cascade, thereby leading to cell cycle arrest and the stabilization of replication forks.[42] As discussed above, activation of the ATR–Chk1 pathway also requires a 5′ primer-template junction; however, the source of this structure during replication stress is unclear. It is possible that it is supplied by the nearest Okazaki fragment or that processing is required to produce a primer-template junction elsewhere. As discussed above, an alternative NBS1-dependent mode of ATR activation that is reported not to require the 5′ primer-template junction and that results in the phosphorylation of RPA rather than CHK1 was recently shown to be involved in the response to replication fork collapse.[28]

In addition to the ATR response, at unprogrammed sites of stalling *S. pombe* Mrc1 transmits a signal to Cds1, the *S. pombe* homolog of the checkpoint protein CHK2.[44] Cds1 contributes locally to replication fork stabilization and globally to cell cycle arrest.

Telomere stress

During replication of linear DNA, the ends of the DNA shorten with each round of replication. The genetic material is protected from degradation by repetitive DNA sequences located at the ends of the DNA called telomeres. When the telomeres reach a critically short length the cells enter senescence and stop dividing, which is thought to underlie physiological aging. They can escape this senescence, however, by reactivating telomerase, the enzyme that lengthens telomeres, a process that is associated with cellular transformation. Because the ends of linear chromosomes represent DSBs, telomeres are susceptible to resection and recombination. To prevent this, they are bound by an elaborate structure of proteins called the shelterin complex that protects the ends and suppresses recombination and checkpoint signaling.[45]

DNA damage lesions within the telomeres have recently been shown to be refractory to repair and to elicit a persistent DNA damage response.[46] In addition, during cellular senescence from a variety of stimuli, telomeres are a center for the assembly of DNA damage response complexes that arrest the cell cycle.[47]

Additionally, telomere stress resulting from shortening of the telomeres leads to loss of the shelterin complex and activation of the damage response. Shortening of the telomeres is associated with aging, and is simulated experimentally by inhibition of telomerase. Uncapping is also studied directly by disruption of the shelterin complex. Indeed, loss of shelterin complex members results in the activation of ATM and the formation of telomere dysfunction-induced foci (TIFs), which contain γ-H2AX, the MRN complex, 53BP1–RIF1, and phosphorylated ATM and ATR.[48] The recruitment of 53BP1–RIF1 after uncapping by loss of the shelterin member TRF2 inhibits resection and promotes NHEJ.[49] In *S. cerevisiae*, checkpoint activation upon telomere uncapping also contributes to telomere stability by inhibiting Exo1-mediated resection.[50]

Although the response to uncapping of the telomeres stimulates ATM, it is not the same as the genomic damage response. Loss of telomere protection results in ATM activation, but does not lead to Chk2 activation. Therefore, cells with uncapped telomeres proceed through G2 and M and into G1, where p53, which is upregulated by ATM, halts the cell cycle.[51]

In *S. cerevisiae*, checkpoint activation when telomere stress is induced by inhibition of telomerase activity requires Mrc1, a protein involved in the response to replication block, suggesting that this response is dependent on replication of the DNA through telomeres.[52] In contrast, when telomeres are uncapped Mrc1 does not contribute to checkpoint activation, but protects telomeres from resection by the exonuclease Exo1.[53]

However, not all proliferating cells are telomerase-positive. Indeed, 15% of cancers maintain their telomeres through a process called alternative lengthening of telomeres (ALT). This is thought to involve homologous recombination between telomeres, although the mechanism is currently unclear.[54]

Incorporation of RNA into DNA

The integrity of DNA depends on incorporation of the correct DNA bases opposite the template. Ribonucleotides, however, are incorporated by replicative polymerases with low frequency. These are removed by RNase H2, the RNase that removes primers during Okazaki fragment processing.[55] Loss of RNase H2 results in deletions that result from nucleotide slippage as a result of nicking of the DNA by topoisomerase 1 followed by aberrant religation. In the absence of RNase H2, the helicase Srs2 and the nuclease Exo1 act to remove the Top1-nicked strand.[56]

RNase HII deficiency is one cause of Aicardi-Goutiéres syndrome, a condition similar to systemic lupus erythematosus in which there is inflammation throughout the body and particularly in the nervous system.[57] Immune activation may be due to the production of novel antigens that result from mutation.[58]

Interstrand crosslinks

Interstrand crosslinks (ICLs) are covalent linkages of the 2 DNA strands and are produced by common chemotherapeutics such as platinum compounds. The DNA at these locations cannot be melted and therefore cannot be passed by polymerases. Because of this, ICLs threaten both DNA replication and transcription.

ICLs are repaired by proteins from several of the pathways described above. Proteins implicated in the recognition of ICLs

include XPC, the TC-NER machinery, and MutS homologues, but the molecular mechanism by which this occurs is unclear as the structural defect recognized by these proteins in the case of ICLs is not apparent.[59] Subsequent steps of repair involve the excision and flipping out of a segment of DNA surrounding the lesion on one strand followed by the synthesis of new DNA, which in turn is followed by the excision and resynthesis of the other strand. In G1 and early S phase, this synthesis is carried out by translesion bypass polymerases, whereas in late-S and G2 phase it can occur by homologous recombination.

During S phase, an alternative mode of ICL repair can be carried out by a group of genes that are mutated in Fanconi anemia, a disease characterized by sensitivity to crosslinking agents, hematological failure, and malignancy.[60] A large complex of FANC proteins (A, B, C, E, F, G, L) is recruited by FANCM,[61] a protein that is known to bind branched DNA structures such as stalled replication forks.[62] The complex then monoubiquitinates the FANCD2/FANCI complex, which recruits several other factors, including other FANC proteins, nucleases, translesion polymerases, and homologous recombination proteins that in turn contribute to excision of the lesion and the synthesis of new DNA.[63]

The ATR response is activated by ICLs via the FA machinery, although this process does not require certain mediators of the "canonical" ATR activation pathway.[64] In addition, ATR-mediated phosphorylation of FANC proteins is involved in ICL repair itself.[63] ICLs do not initiate the replication checkpoint via the FPC, however, because both the helicase and the polymerase are halted, therefore uncoupling does not occur.[42]

Summary

Given the wide variety of DNA damage lesions, it is not surprising that many mechanisms are involved in their detection and in the initiation of the DNA damage response. We have attempted to review those mechanisms here. The level of complexity and overlap between these pathways, however, is greater than we have been able to address, and is also a major reason why certain details remain unclear.

Future Work

There are still many gaps in our knowledge of the initiation of the DNA damage response, particularly for recombination-based processes. It is likely that lesions are processed in complex and parallel pathways that give rise to structures that are used for both repair and checkpoint activation.

One outstanding question concerns the spatiotemporal regulation of repair processes. There must be interaction between repair initiation and the cell cycle, as the impact of a lesion depends on the phase of the cell cycle, and the phase of the cell cycle, in turn, impacts on whether and how a lesion will be repaired. In addition to this temporal restriction, repair may be restricted to certain areas of the nucleus. Studies applying conformation capture techniques to DNA damage response factors will be challenging, but may be useful in elucidating details of the processes described here.

Furthermore, the interactions between the repair and checkpoint pathways are unclear, particularly with regard to their individual requirements for activation and cessation. Understanding these processes will enable us to better understand genome stability and to design better therapeutics for cancer and other aging-related diseases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank our colleagues and collaborators for stimulating discussion.

Funding

This work was supported by NIH grants RO1-GM087326 (MJO) and T32-CA078207 (KJB).

References

1. Ciccia A, Elledge SJ. The DNA damage response: making it safe to play with knives. Mol Cell 2010; 40:179-204; PMID:20965415; http://dx.doi.org/10.1016/j.molcel.2010.09.019
2. O'Connell MJ, Walworth NC, Carr AM. The G2-phase DNA-damage checkpoint. Trends Cell Biol 2000; 10:296-303; PMID:10856933; http://dx.doi.org/10.1016/S0962-8924(00)01773-6
3. Zou L, Elledge SJ. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. Science 2003; 300:1542-8; PMID:12791985; http://dx.doi.org/10.1126/science.1083430
4. MacDougall CA, Byun TS, Van C, Yee MC, Cimprich KA. The structural determinants of checkpoint activation. Genes Dev 2007; 21:898-903; PMID:17437996; http://dx.doi.org/10.1101/gad.1522607
5. Jiricny J. Postreplicative mismatch repair. Cold Spring Harb Perspect Biol 2013; 5:a012633; PMID:23545421; http://dx.doi.org/10.1101/cshperspect.a012633
6. Pavlov YI, Mian IM, Kunkel TA. Evidence for preferential mismatch repair of lagging strand DNA replication errors in yeast. Curr Biol 2003; 13:744-8; PMID:12725731; http://dx.doi.org/10.1016/S0960-9822(03)00284-7
7. Lynch HT, Lynch PM, Lanspa SJ, Snyder CL, Lynch JF, Boland CR. Review of the Lynch syndrome: history, molecular genetics, screening, differential diagnosis, and medicolegal ramifications. Clin Genet 2009; 76:1-18; PMID:19659756; http://dx.doi.org/10.1111/j.1399-0004.2009.01230.x
8. Krokan HE, Bjoras M. Base excision repair. Cold Spring Harb Perspect Biol 2013; 5:a012583; PMID:23545420; http://dx.doi.org/10.1101/cshperspect.a012583
9. Fitch ME, Nakajima S, Yasui A, Ford JM. In vivo recruitment of XPC to UV-induced cyclobutane pyrimidine dimers by the DDB2 gene product. J Biol Chem 2003; 278:46906-10; PMID:12944386; http://dx.doi.org/10.1074/jbc.M307254200
10. Scharer OD. Nucleotide excision repair in eukaryotes. Cold Spring Harb Perspect Biol 2013; 5:a012609; PMID:24086042; http://dx.doi.org/10.1101/cshperspect.a012609
11. Svejstrup JQ. Contending with transcriptional arrest during RNAPII transcript elongation. Trends Biochem Sci 2007; 32:165-71; PMID:17349792; http://dx.doi.org/10.1016/j.tibs.2007.02.005
12. Mellon I, Hanawalt PC. Induction of the Escherichia coli lactose operon selectively increases repair of its transcribed DNA strand. Nature 1989; 342:95-8; PMID:2554145; http://dx.doi.org/10.1038/342095a0
13. Hanawalt PC, Spivak G. Transcription-coupled DNA repair: two decades of progress and surprises. Nat Rev Mol Cell Biol 2008; 9:958-70; PMID:19023283; http://dx.doi.org/10.1038/nrm2549
14. Lindsey-Boltz LA, Sancar A. RNA polymerase: the most specific damage recognition protein in cellular responses to DNA damage? Proc Natl Acad Sci U S A 2007; 104:13213-4; PMID:17684092; http://dx.doi.org/10.1073/pnas.0706316104
15. Vermeulen W, Fousteri M. Mammalian transcription-coupled excision repair. Cold Spring Harb Perspect Biol 2013; 5:a012625; PMID:23906714; http://dx.doi.org/10.1101/cshperspect.a012625
16. Rapin I. Disorders of nucleotide excision repair. Handbook of clinical neurology 2013; 113:1637-50;

PMID:23622385; http://dx.doi.org/10.1016/B978-0-444-59565-2.00032-0
17. van Gool AJ, Citterio E, Rademakers S, van Os R, Vermeulen W, Constantinou A, Egly JM, Bootsma D, Hoeijmakers JH. The Cockayne syndrome B protein, involved in transcription-coupled DNA repair, resides in an RNA polymerase II-containing complex. EMBO J 1997; 16:5955-65; PMID:9312053; http://dx.doi.org/10.1093/emboj/16.19.5955
18. Selby CP, Sancar A. Human transcription-repair coupling factor CSB/ERCC6 is a DNA-stimulated ATPase but is not a helicase and does not disrupt the ternary transcription complex of stalled RNA polymerase II. J Biol Chem 1997; 272:1885-90; PMID:8999876; http://dx.doi.org/10.1074/jbc.272.3.1885
19. Marini F, Nardo T, Giannattasio M, Minuzzo M, Stefanini M, Plevani P, Muzi Falconi M. DNA nucleotide excision repair-dependent signaling to checkpoint activation. Proc Natl Acad Sci U S A 2006; 103:17325-30; PMID:17088560; http://dx.doi.org/10.1073/pnas.0605446103
20. Bomgarden RD, Lupardus PJ, Soni DV, Yee MC, Ford JM, Cimprich KA. Opposing effects of the UV lesion repair protein XPA and UV bypass polymerase eta on ATR checkpoint signaling. EMBO J 2006; 25:2605-14; PMID:16675950; http://dx.doi.org/10.1038/sj.emboj.7601123
21. Ward IM, Minn K, Chen J. UV-induced ataxia-telangiectasia-mutated and Rad3-related (ATR) activation requires replication stress. J Biol Chem 2004; 279:9677-80; PMID:14742437; http://dx.doi.org/10.1074/jbc.C300554200
22. Callegari AJ, Kelly TJ. UV irradiation induces a postreplication DNA damage checkpoint. Proc Natl Acad Sci U S A 2006; 103:15877-82; PMID:17043220; http://dx.doi.org/10.1073/pnas.0607343103
23. Downs JA, Jackson SP. A means to a DNA end: the many roles of Ku. Nat Rev Mol Cell Biol 2004; 5:367-78; PMID:15122350; http://dx.doi.org/10.1038/nrm1367
24. Neal JA, Meek K. Choosing the right path: does DNA-PK help make the decision? Mutat Res 2011; 711:73-86; PMID:21376743; http://dx.doi.org/10.1016/j.mrfmmm.2011.02.010
25. Haince JF, McDonald D, Rodrigue A, Dery U, Masson JY, Hendzel MJ, Poirier GG. PARP1-dependent kinetics of recruitment of MRE11 and NBS1 proteins to multiple DNA damage sites. J Biol Chem 2008; 283:1197-208; PMID:18025084; http://dx.doi.org/10.1074/jbc.M706734200
26. Mimitou EP, Symington LS. Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. Nature 2008; 455:770-4; PMID:18806779; http://dx.doi.org/10.1038/nature07312
27. Kuntz K, O'Connell MJ. Initiation of DNA damage responses through XPG-related nucleases. EMBO J 2013; 32:290-302; PMID:23211746; http://dx.doi.org/10.1038/emboj.2012.322
28. Kuntz K, O'Connell MJ. The G(2) DNA damage checkpoint: could this ancient regulator be the Achilles heel of cancer? Cancer Biol Ther 2009; 8:1433-9; PMID:19574738; http://dx.doi.org/10.4161/cbt.8.15.9081
29. Shiotani B, Nguyen HD, Hakansson P, Marechal A, Tse A, Tahara H, Zou L. Two distinct modes of ATR activation orchestrated by Rad17 and Nbs1. Cell Rep 2013; 3:1651-62; PMID:23684611; http://dx.doi.org/10.1016/j.celrep.2013.04.018
30. Zou L, Elledge SJ. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. Science 2003; 300:1542-8; PMID:12791985; http://dx.doi.org/10.1126/science.1083430
31. Stokes MP, Van Hatten R, Lindsay HD, Michael WM. DNA replication is required for the checkpoint response to damaged DNA in Xenopus egg extracts. J Cell Biol 2002; 158:863-72; PMID:12213834; http://dx.doi.org/10.1083/jcb.200204127
32. Escribano-Diaz C, Orthwein A, Fradet-Turcotte A, Xing M, Young JT, Tkac J, Cook MA, Rosebrock AP, Munro M, Canny MD, et al. A cell cycle-dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice. Mol Cell 2013; 49:872-83; PMID:23333306; http://dx.doi.org/10.1016/j.molcel.2013.01.001
33. O'Driscoll M, Gennery AR, Seidel J, Concannon P, Jeggo PA. An overview of three new disorders associated with genetic instability: LIG4 syndrome, RS-SCID and ATR-Seckel syndrome. DNA Repair (Amst) 2004; 3:1227-35; PMID:15279811;
34. Taylor AM, Groom A, Byrd PJ. Ataxia-telangiectasia-like disorder (ATLD)-its clinical presentation and molecular basis. DNA Repair (Amst) 2004; 3:1219-25; PMID:15279810; http://dx.doi.org/10.1016/j.dnarep.2004.04.009
35. Zeman MK, Cimprich KA. Causes and consequences of replication stress. Nat Cell Biol 2014; 16:2-9; PMID:24366029; http://dx.doi.org/10.1038/ncb2897
36. Bester AC, Roniger M, Oren YS, Im MM, Sarni D, Chaoat M, Bensimon A, Zamir G, Shewach DS, Kerem B. Nucleotide deficiency promotes genomic instability in early stages of cancer development. Cell 2011; 145:435-46; PMID:21529715; http://dx.doi.org/10.1016/j.cell.2011.03.044
37. Saldivar JC, Miuma S, Bene J, Hosseini SA, Shibata H, Sun J, Wheeler LJ, Mathews CK, Huebner K. Initiation of genome instability and preneoplastic processes through loss of Fhit expression. PLoS Genet 2012; 8:e1003077; PMID:23209436;
38. Burrell RA, McClelland SE, Endesfelder D, Groth P, Weller MC, Shaikh N, Domingo E, Kanu N, Dewhurst SM, Gronroos E, et al. Replication stress links structural and numerical cancer chromosomal instability. Nature 2013; 494:492-6; PMID:23446422; http://dx.doi.org/10.1038/nature11935
39. Labib K, Hodgson B. Replication fork barriers: pausing for a break or stalling for time? EMBO Rep 2007; 8:346-53; PMID:17401409; http://dx.doi.org/10.1038/sj.embor.7400940
40. McGlynn P, Lloyd RG. Recombinational repair and restart of damaged replication forks. Nat Rev Mol Cell Biol 2002; 3:859-70; PMID:12415303; http://dx.doi.org/10.1038/nrm951
41. Jossen R, Bermejo R. The DNA damage checkpoint response to replication stress: A Game of Forks. Front Genet 2013; 4:26; PMID:23493417; http://dx.doi.org/10.3389/fgene.2013.00026
42. Tourriere H, Pasero P. Maintenance of fork integrity at damaged DNA and natural pause sites. DNA Repair (Amst) 2007; 6:900-13; PMID:17379579;
43. Leman AR, Noguchi E. Local and global functions of Timeless and Tipin in replication fork protection. Cell Cycle 2012; 11:3945-55; PMID:22987152; http://dx.doi.org/10.4161/cc.21989
44. Tanaka K, Russell P. Mrc1 channels the DNA replication arrest signal to checkpoint kinase Cds1. Nat Cell Biol 2001; 3:966-72; PMID:11715017; http://dx.doi.org/10.1038/ncb1101-966
45. Webb CJ, Wu Y, Zakian VA. DNA repair at telomeres: keeping the ends intact. Cold Spring Harb Perspect Biol 2013; 5; PMID:23732473;
46. Fumagalli M, Rossiello F, Clerici M, Barozzi S, Cittaro D, Kaplunov JM, Bucci G, Dobreva M, Matti V, Beausejour CM, et al. Telomeric DNA damage is irreparable and causes persistent DNA-damage-response activation. Nat Cell Biol 2012; 14:355-65; PMID:22426077; http://dx.doi.org/10.1038/ncb2466
47. Hewitt G, Jurk D, Marques FD, Correia-Melo C, Hardy T, Gackowska A, Anderson R, Taschuk M, Mann J, Passos JF. Telomeres are favoured targets of a persistent DNA damage response in ageing and stress-induced senescence. Nat Commun 2012; 3:708; PMID:22426229; http://dx.doi.org/10.1038/ncomms1708
48. Takai H, Smogorzewska A, de Lange T. DNA damage foci at dysfunctional telomeres. Curr Biol 2003; 13:1549-56; PMID:12956959; http://dx.doi.org/10.1016/S0960-9822(03)00542-6
49. Zimmermann M, Lottersberger F, Buonomo SB, Sfeir A, de Lange T. 53BP1 regulates DSB repair using Rif1 to control 5' end resection. Science 2013; 339:700-4; PMID:23306437; http://dx.doi.org/10.1126/science.1231573
50. Jia X, Weinert T, Lydall D. Mec1 and Rad53 inhibit formation of single-stranded DNA at telomeres of Saccharomyces cerevisiae cdc13-1 mutants. Genetics 2004; 166:753-64; PMID:15020465; http://dx.doi.org/10.1534/genetics.166.2.753
51. Cesare AJ, Hayashi MT, Crabbe L, Karlseder J. The telomere deprotection response is functionally distinct from the genomic DNA damage response. Mol Cell 2013; 51:141-55; PMID:23850488; http://dx.doi.org/10.1016/j.molcel.2013.06.006
52. Grandin N, Bailly A, Charbonneau M. Activation of Mrc1, a mediator of the replication checkpoint, by telomere erosion. Biol Cell 2005; 97:799-814; PMID:15760303; http://dx.doi.org/10.1042/BC20040526
53. Tsolou A, Lydall D. Mrc1 protects uncapped budding yeast telomeres from exonuclease EXO1. DNA Repair (Amst) 2007; 6:1607-17; PMID:17618841;
54. Cesare AJ, Reddel RR. Alternative lengthening of telomeres: models, mechanisms and implications. Nat Rev Genet 2010; 11:319-30; PMID:20351727; http://dx.doi.org/10.1038/nrg2763
55. Nick McElhinny SA, Kumar D, Clark AB, Watt DL, Watts BE, Lundstrom EB, Johansson E, Chabes A, Kunkel TA. Genome instability due to ribonucleotide incorporation into DNA. Nat Chem Biol 2010; 6:774-81; PMID:20729855; http://dx.doi.org/10.1038/nchembio.424
56. Potenski CJ, Niu H, Sung P, Klein HL. Avoidance of ribonucleotide-induced mutations by RNase H2 and Srs2-Exo1 mechanisms. Nature 2014; PMID:24896181;
57. Crow YJ, Leitch A, Hayward BE, Garner A, Parmar R, Griffith E, Ali M, Semple C, Aicardi J, Babul-Hirji R, et al. Mutations in genes encoding ribonuclease H2 subunits cause Aicardi-Goutieres syndrome and mimic congenital viral brain infection. Nat Genet 2006; 38:910-6; PMID:16845400; http://dx.doi.org/10.1038/ng1842
58. Crow YJ. Aicardi-Goutieres syndrome. Handb Clin Neurol 2013; 113:1629-35; PMID:23622384; http://dx.doi.org/10.1016/B978-0-444-59565-2.00031-9
59. Muniandy PA, Liu J, Majumdar A, Liu ST, Seidman MM. DNA interstrand crosslink repair in mammalian cells: step by step. Crit Rev Biochem Mol Biol 2010; 45:23-49; PMID:20039786; http://dx.doi.org/10.3109/10409230903501819
60. Khincha PP, Savage SA. Genomic characterization of the inherited bone marrow failure syndromes. Semin Hematol 2013; 50:333-47; PMID:24246701; http://dx.doi.org/10.1053/j.seminhematol.2013.09.002
61. Kim JM, Kee Y, Gurtan A, D'Andrea AD. Cell cycle-dependent chromatin loading of the Fanconi anemia core complex by FANCM/FAAP24. Blood 2008; 111:5215-22; PMID:18174376; http://dx.doi.org/10.1182/blood-2007-09-113092
62. Gari K, Decaillet C, Stasiak AZ, Stasiak A, Constantinou A. The Fanconi anemia protein FANCM can promote branch migration of Holliday junctions and replication forks. Mol Cell 2008; 29:141-8; PMID:18206976; http://dx.doi.org/10.1016/j.molcel.2007.11.032
63. Deans AJ, West SC. DNA interstrand crosslink repair and cancer. Nat Rev Cancer 2011; 11:467-80; PMID:21701511; http://dx.doi.org/10.1038/nrc3088
64. Tomida J, Itaya A, Shigechi T, Unno J, Uchida E, Ikura M, Masuda Y, Matsuda S, Adachi J, Kobayashi M, et al. A novel interplay between the Fanconi anemia core complex and ATR-ATRIP kinase during DNA cross-link repair. Nucleic Acids Res 2013; 41:6930-41; PMID:23723247; http://dx.doi.org/10.1093/nar/gkt467