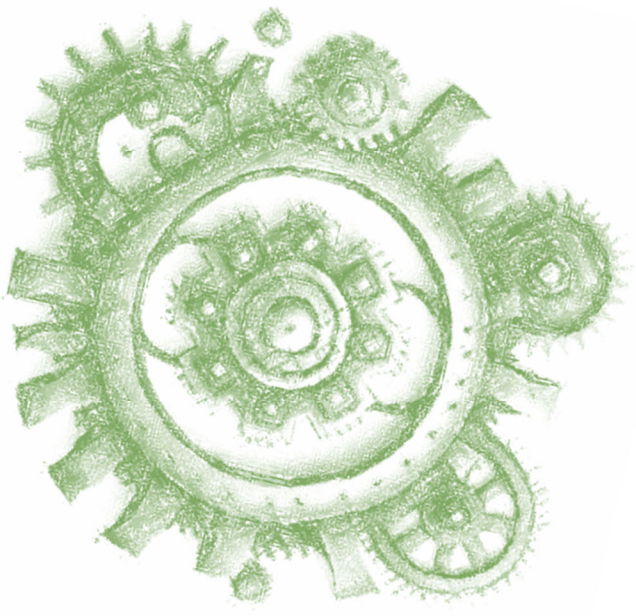
Control of replication associated DNA damage responses by Mismatch Repair

Ijsselsteiijn, R.



Chapter 1:
General introduction

General Introduction:

The integrity of our genomic DNA is essential for a healthy life, but is continuously threatened upon exposure to DNA damaging agents that are omnipresent; the oxygen we breath, the sunlight we often enjoy and the food we eat can result in damage to the DNA of our cells (1). DNA damage alters the structure of the DNA and as such it interferes with the work of DNA and RNA polymerases (1, 2). Persistent damage can ultimately result in genomic instability which, in germ cells, positively contributes to evolution, but can also lead to heritable diseases. In somatic cells mutagenesis is mainly known for its role in cancer. To mitigate the harmful effects of DNA damage cells activate DNA damage responses, including cell cycle arrest, activation of DNA repair pathways, senescence and apoptosis (3).

Understanding the control of DNA damage-induced mutagenesis may contribute to the treatment or prevention of cancer. This chapter describes factors that control the DNA damage response, including DNA damage signaling and mutagenesis, with particular attention to DNA damage induced by ultraviolet light (UV) and by 2-Amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP), two genotoxic agents related to cancer formation in the skin and the gastro-intestinal tract, respectively. The final part of this chapter deals with DNA mismatch repair (MMR), a pathway which main function is thought to be the correction of misincorporations by DNA polymerases. However, MMR may also play an important role in the cellular response to DNA damage.

The risk of being a carnivore on a sunny day: cancer

For diurnal life one of the most common sources of DNA damage is UV radiation from sunlight. Although limited sunlight exposure seems beneficial for human health (4), too much exposure is correlated with skin cancer development (5). Globally, the incidence of skin cancer was over 1.5 million in 2020 (6) and over the years incidence has been rising steadily (5). Skin cancer is a heterogenous disease and can be divided based on the cell type of origin; basal cell carcinomas, squamous cell carcinomas, sebaceous carcinomas and melanocyte originated melanomas (7, 8). The different skin cancer types have different mortality rates, with melanomas being the most lethal and non-melanoma skin cancers being relatively harmless, if diagnosed early on (5). The difference in mortality may be explained by the propensity of melanomas to invade and metastasize, whereas the non-melanoma skin cancers remain more localized (9). Skin cancer incidence is markedly different between different population groups, with skin cancer being the most common cancer in Caucasians (5). White skin contains the least amount of melanin and provides the least protection against sunlight highlighting the importance of UV-radiation as the driving etiological factor in skin cancer (10). UV-light is divided into three groups, UVA (320-400 nm), UVB (280-320 nm) and UVC (200-280 nm). Wavelengths under 315 nanometer are effectively filtered by the ozone layer and thus skin tissue is mostly exposed to UVA and UVB wavelengths (11). UVA is weakly mutagenic and produces both oxidative lesions and low levels of cyclobutane pyrimidine dimers (CPD) (12), while UVB is more mutagenic and produces high levels of CPD and lower levels of 6-4 photoproducts (6-4PP) (11). Following a 30 minutes exposure to sunlight on a clear day, skin cells accumulate nearly two hundred thousand CPD per genome, which lesion frequency is approximately 5-fold higher than the number of endogenous DNA lesions occurring in each human cell during a whole day (13, 14). Photolesions are formed by direct absorption of UV light by DNA resulting in intrastrand crosslinks between two adjacent pyrimidines, but CPD only mildly distort the DNA helix structure, whilst 6-4PP heavily distort the DNA helix (11). This results in 6-4PP being recognized by DNA repair mechanisms more rapidly than CPD (15). As such, sunlight-induced mutagenesis mostly comes from CPD produced by exposure to UVB radiation (16). UVB-induced CPD lesions in human skin are found most often at thymidine-thymidine dimers followed by thymidine-cytosine dimers, whilst cytosine-thymidine and cytosine-cytosine dimers are found least often (17). Curiously, the mutational signature of skin tumors is characterized by mutations on cytosines, specifically cytosine to thymidine transitions and not by mutations on the more commonly induced thymidine CPD (18). This may be explained by deamination of cytosine CPD adducts and the subsequent replication of these deaminated cytosines resulting in aforementioned C>T transitions (18). Moreover, replication of thymidine CPD is a relatively error-free process whereas replication of cytosine CPD more often results in replication errors (19).

Another common source of DNA damage is the diet and this is often associated with the development of colorectal cancer (CRC) (20, 21). CRC is the third most common cancer type world-wide, with nearly 2 million new cases in 2020 and a mortality of

nearly 1 million (6). Moreover, incidence is significantly higher in developed countries, such as those in North America and Western Europe, while the lowest incidence is found in countries found on the African continent (6). Importantly, in developing countries, such as those found in Eastern Europe and Eastern Asian regions, CRC incidence is strongly on the rise (20). The most likely explanation for these geographic difference in CRC incidence are life-style factors such as a diet, consisting of high caloric intake and red meat, and reduced amounts of physical activity, which is more often observed in rapidly developing and wealthy countries (21, 22). Mortality, however, is declining in developed countries as gastro-intestinal screening procedures have been implemented for those at highest risk, due to age or genetic background (23).

It is long known that life-style factors influence CRC development and more than a few DNA damaging compounds have been identified in the diet. Nitrosamines found in, amongst others, processed/cured meats and smoked/salted fish is thought to damage the DNA by forming toxic aldehydes inducing alkylation damage (24). Other dietary mutagens are primary aromatic amines, found in certain food packaging that can subsequently transfer into the food. Moreover, smoking meat can also result in the formation of polycyclic aromatic hydrocarbons, chemical compounds that are associated with CRC as well (25). Another group of common mutagens that are associated with CRC development are the heterocyclic amines (HCA). It has been shown that especially grilling meat at high temperatures results in increased generation of HCA which differs per preparation method and meat type (Table 1). Of all the various HCA, PhIP is the most abundant and seems to be the most mutagenic in mammalian cells (26). PhIP only becomes mutagenic when metabolically activated by the liver after which PhIP is actively transported to the intestines for clearance. Here, PhIP metabolites can react with DNA of intestinal (stem) cells (26, 27). Indeed, measurements of PhIP adducts in human colorectal mucosa show the presence of several PhIP adducts per 10^8 bases (27). In detail, PhIP damages the DNA by binding to the C8-position of guanines resulting in cellular toxicity and increased mutagenesis (28). Not surprisingly, spectra of mutations induced by PhIP consist of mutations specifically on guanines which mostly are guanine to thymidine transversions and guanine to adenine transitions (26). Spectra of PhIP-induced mutations most resemble the COSMIC mutagenic signatures SBS4, SBS18 and SBS29 (29). Interestingly, these signatures do not seem to overlap one-to-one with the CRC mutational signatures. This may be explained by the fact that, unlike skin cancer, where sunlight exposure results in an easy to interpret mutational spectrum, the mutational spectrum of CRC is influenced by many sources and types of DNA damaging agents resulting in scattered mutational signatures. A total of 8 different mutational signatures are found in CRC samples with etiologies ranging from age, defective DNA repair, defective replication proofreading and tobacco chewing (30). A definitive diet-related signature has yet to be uncovered, but epidemiological studies paint a clear picture: there is a significant correlation between intake of DNA damaging agents and colon cancer (21, 31, 32).

Table 1:
Concentrations of PhIP (ng/g) in types of meat at various stages of doneness and preparation methods. Adapted from Gibis *et al.* (133).

Meat:	Temperature (°C)	Time (min)	PhIP (ng/g)
Ground beef patties (fried)	150	4-20	nd-1.8
	230	4-20	1.3-3.2
	277	12	68
Chicken (pan fried)	197-221	14-36	12-70
Chicken (roasted)	175-240	24-40	nd
Pork patties (pan-fried)	177-225	9-21	0.3-10.5
Pork patties (broiled)	177-225	9-21	nd-2.7
Bacon (oven-broiled)	175	7.5	15.9
Bacon (pan-fried)	176	16.1	4.9

Replication fork stalling and the activation of DNA damage signaling

An important line of defense against DNA damage is the activation of DNA damage signaling. When DNA damage stalls the replication machinery it generates single stranded (ss)DNA due to the uncoupling of DNA polymerases and helicases (33). ssDNA is inherently fragile and may break causing toxic double stranded DNA breaks (DSB). To prevent this from happening ssDNA is coated with heterotrimeric Replication Protein A (RPA) to stabilize it (33). This coating also attracts the kinase Ataxia Telangiectasia and Rad3 related (ATR) via its binding partner ATR-Interacting Protein (ATRIP) (34). In parallel, at 3' junctions of ssDNA and dsDNA, the Rad9-Rad1-Hus1 complex is loaded leading to the recruitment of TopBP1 that can also active the ATR kinase (35). The activation of ATR, in turn, is an important event branching out in the activation multiple downstream effectors (36), the most well-known being Checkpoint Kinase 1 (CHK1). ATR phosphorylates CHK1 at serines 317 and 345 resulting in cell cycle arrest, transcription - and replication regulation and promotion of DNA repair.

DNA damage can also result in DSB, which upon processing activates DNA damage signaling. Two apical kinases are activated upon DSB generation, namely DNA-dependent Protein Kinase catalytic subunit (DNA-PKcs), which is recruited by Ku80 and is mainly involved in DSB repair, and Ataxia Telangiectasia Mediated (ATM), which has a wide-array of downstream effectors (36). In vitro, ATM is recruited and activated by members of the Mre11-Rad50-Nbs1 (MRN) complex at the site of damage (37, 38), although there is some MRN-independent activation reported (36). ATM in turn can activate the tumor suppressor p53 responsible for cell cycle arrest, senescence or apoptosis (36). Moreover, ATM is known to phosphorylate H2AX, a modification known as yH2AX, which triggers a chromatin-based signaling cascade that may amplify the DSB signaling and promote repair (39). Chromatin is also modified by KAP-1 which relaxes chromatin formation and promotes DSB repair upon phosphorylation by ATM

(40, 41). Finally, ATM also stimulates DSB repair by promoting DSB end resection, exposing stretches of ssDNA that are required for the DSB repair pathway homologous recombination (HR) (36).

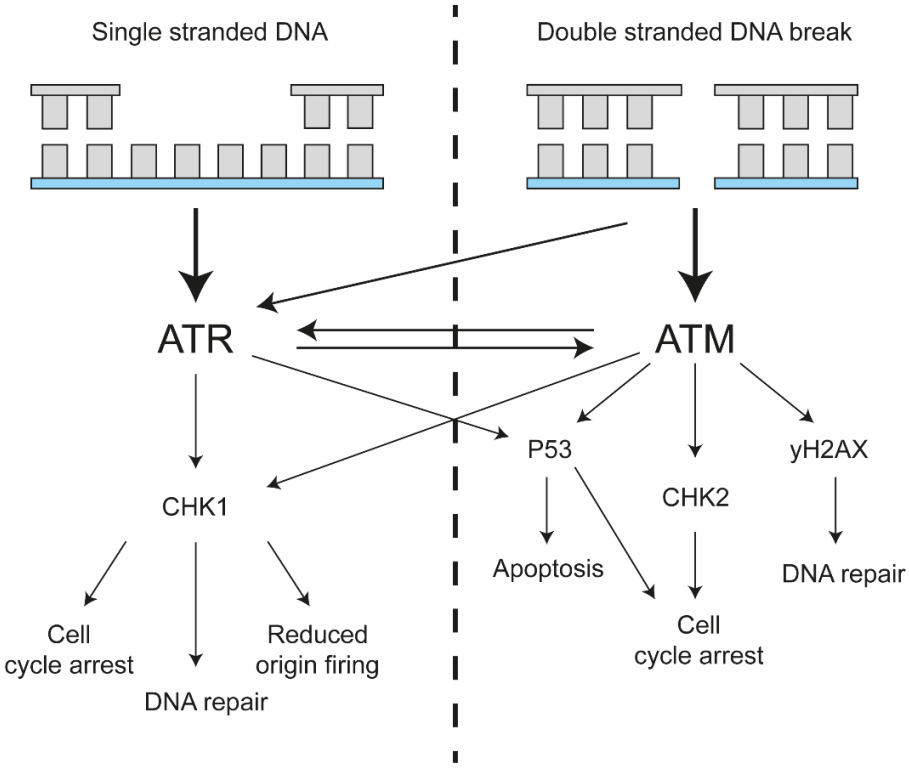


Figure 1: Simplified overview of the ATR/ATM DNA damage signaling axes
Persistent single stranded DNA (ssDNA) formations can activate ATR to induce a range of protective responses. The main effector kinase of ATR is CHK1 and activation of CHK1 can result in reduced origin firing, cell cycle arrest and increased DNA repair. Double stranded DNA breaks (DSB) can activate both ATR and ATM. Both pCHK2 and P53 can induce cell cycle arrest, phosphorylated H2AX can stimulate (DSB) DNA repair and apoptosis can be triggered via P53. ATM and ATR display overlapping substrate specificities, for instance ATR can activate P53 and ATM may activate CHK1.

Obviously, the ATR/ATM signaling axes are intermingled: ATR, activated by ssDNA, can be recruited to a DSB after resection (42, 43) and ATM can be recruited to DSB formed when ssDNA formations break (36, 44). Moreover, there also seems to be significant overlap between the substrates of ATM and ATR: both ATM and ATR can activate p53 as well as CHK1 (Fig. 1) (36, 45, 46).

DNA repair pathways to prevent genome instability

One of the outcomes of DNA damage signaling is the promotion of the various DNA repair pathways (36). DNA damage comes in many forms, such as the aforementioned DSB, intrastrand crosslinks (such as those induced by UV), helix-distorting DNA lesions (such as those induced by PhIP), interstrand crosslinks and adducts resulting from exposure to alkylating and oxidating agents. Moreover, many of these lesions stall the replication machinery leading to formation of ssDNA. Each DNA lesion type is associated with one or more DNA repair pathways that are able to remove the damage

and restore the normal DNA structure (Table 2), thereby preventing mutagenesis. Therefore, loss of any of these pathways often results in cancer formation. For instance, loss of HR is often found in breast cancer (47) and loss of nucleotide excision repair (NER), which repairs bulky and helix-distorting lesions like those induced by UV, results in skin cancer and other skin-associated malignancies (48). When a DNA lesion goes unrepaired it may lead to different outcomes based on lesion type and lesion density. For instance, DNA damage from alkylation and oxidation are sometimes still instructive and mis-instruct DNA polymerases during replication leading to point mutations (49, 50). The more toxic lesion types such as bulky and DNA helix distorting adducts always stall the replication machinery, resulting in stretches of ssDNA that can become DSB (44, 51, 52). DSB can cause large genomic rearrangements, especially when they are repaired by error-prone end joining pathways (53). Loss of one copy of a segment of DNA, also known as loss of heterozygosity (LOH), is a term closely associated with cancer, as LOH is an important genetic event contributing to the loss of tumor suppressor genes (54). LOH is especially dangerous for individuals that have inherited a dysfunctional copy of a tumor suppressor gene, for instance inheritance of a faulty copy of BRCA2, an important gene in the aforementioned HR DNA repair pathway. Indeed, these *BRCA2*-heterozygous cells become HR-deficient more easily, thus resulting in a predisposition for cancer (55).

Table 2: The varying types of DNA damage are caused by different sources, repaired by specialized DNA repair pathways and replicated by different polymerases. Abbreviations: BER (Base Excision Repair), MMR (Mismatch Repair), NER (Nucleotide Excision Repair), FA (Fanconi Anemia), HR (Homologous Recombination), alt/canonical NHEJ (alt/canonical Non-Homologous End Joining), SSBR (single strand break repair).

DNA damage type:	Example sources:	DNA Repair Pathways:	Polymerases:
Alkylating	Diet, Bile, cytostatic drugs (134-136)	Methyltransferases, BER, NER (50)	Replicative/TLS (137)
Oxidating	Mitochondria, inflammation (138, 139)	BER, MMR, NER (140)	Replicative/TLS (141-143)
Helix distorting	Diet, UV (11, 133)	NER (144, 145)	TLS (62)
Interstrand crosslinks	Diet, cytostatic drugs (146)	FA, NER, HR (146)	TLS (147)
Double stranded DNA break	Ionizing radiation, stalled replication forks, repair intermediates (148)	alt/canonical NHEJ, HR (149, 150)	Replicative/TLS (150, 151)
Single stranded DNA break	Repair intermediates, Oxidation (152, 153)	SSBR (152, 153)	Replicative/PolB (152, 153)

When DNA damage persists: DNA damage tolerance

In replicating cells, DNA lesions that escape repair can result in stalling and eventual collapse of the replication fork which results in the generation of DSB (44). To prevent DSB-associated mutagenicity and toxicity the cell activates DNA damage tolerance (DDT) mechanisms that allow replication of a damaged DNA template (56). Two major pathways for DDT have so far been uncovered: an error free pathway called template switching and translesion synthesis (TLS), an inherently error-prone pathway (56). Coordination which pathway performs the bypass seems to be directed post-translational modifications at the ring-forming, homotrimeric Proliferating Cell Nuclear Antigen (PCNA). The PCNA ring encircles DNA, keeps DNA polymerases on the DNA and acts as a sliding clamp during DNA replication. PCNA mono-ubiquitination by Rad18 at lysine 164 is suggested to trigger TLS bypass (57), although TLS may also be activated independently from PCNA ubiquitination (58, 59). Conversely, template switching seems to be activated in vitro by SUMOylation or polyubiquitination at lysine 164 of PCNA (57, 60, 61), however in vivo data for template switching and its biological relevance is scarce. So far two modes of action have been postulated for template switching: recombination-mediated template switching, in which the replicating strand invades the already synthesized sister chromatid and template switching by fork reversal (56). Fork reversal occurs upon annealing of the nascent DNA strands and reannealing of the parental strands of a replication fork resulting in a four-way “chicken foot” structure. Both of these mechanisms are best explained visually and Chang et al. have published a clear picture of how this may work (56).

TLS does not require a sister chromatid and bypasses the damage by replicating the damaged nucleotides directly. TLS is comprised of specialized DNA polymerases that, as compared with replicative DNA polymerases, have a more relaxed open catalytic domain that can more easily encompass damaged nucleotides (62). Moreover, TLS polymerases contain no proofreading domains, allowing for the bypass without getting stuck on probable mis-incorporations (62). TLS is split into two main families, the Y-family polymerases (REV1, Eta/η, Iota/ι, Kappa/κ) and the B-family polymerase Polζ (63). Generally speaking, a Y-family polymerases performs the initial nucleotide insertion opposite the damaged nucleotide and the B-family polymerase extends past the damaged nucleotide before replicative polymerases can take over. However, studies have shown that TLS is more complex, for instance, Polκ can also perform the extension step at certain lesions in vitro (64, 65). Moreover, PrimPol, a member of the archaeal eukaryotic primases is important in restarting replication downstream of the lesion (66), but also displays polymerase activity and can bypass oxidative and UV lesions in human cells (67-69). Lastly, Polymerase Theta, a member of the A-family of DNA polymerases, has also been shown to perform TLS in human fibroblasts (70, 71).

Due to a more relaxed catalytic domain and lack of proofreading, TLS is a relatively error-prone process and can sometimes be inefficient, however, this differs per lesion and polymerase (72). For instance, the bypass of T-T CPD lesions by Polη is efficient and relatively error-free, whereas deficiency for Polη results in error-prone bypass by Polκ, Polι or Polζ and increased UV-sensitivity in both mouse embryonic fibroblasts

and human cells (19, 73, 74). Conversely, C-C CPD is bypassed in an error-prone manner by Polη due to the deamination of cytosine to uracil. Uracil originating from cytosine CPD is bypassed "correctly" by Polη and matched with adenine, thus resulting in the well-known C>T mutations that are found in UV-induced skin cancers (75). Furthermore, in MEF, the strongly helix-distorting 6-4PPs depend on TLS polymerases Rev1/Polζ for bypass (76) and not on Polη. Another type of bulky lesions often found in human DNA will be those induced by the diet related chemical PhIP. The dG-C8-PhIP adduct is mostly bypassed by Rev1 in vitro, and the subsequent extension reaction is performed by Polκ (64). The bypass by Rev1 is also relatively error-free, as Rev1 exclusively incorporates cytosines opposite the damage (77).

Importantly, the bypass of DNA lesions by TLS results in quenching of DNA damage signaling, as the replication fork is no longer stalled and patches of ssDNA are converted to dsDNA (78). This prevents replication fork collapse, DSB formation, persistent signaling and apoptosis. As such control of TLS is key in the balance between mutagenesis and apoptosis; too much error-prone bypass can lead to DNA-associated diseases such as cancer, but too little leads to excessive apoptosis and senescence resulting in rapid aging syndromes and developmental issues as is seen in mice with TLS defects (79, 80).

Control of TLS

Control of TLS is important to minimize the negative effects of this pathway. TLS is controlled on multiple levels: the expression levels of TLS polymerases, post-translational modifications, recruitment of TLS polymerases to the lesion and competition with other DNA polymerases. Expression levels of TLS polymerases during different phases of the cell cycle have been measured in mammalian cells and no significant differences were found for most TLS pols tested, except for Polη which is slightly higher expressed in G2 (81). Also, the overall mutagenicity of TLS opposite 6-4PP or (+)-trans benzo(a)pyrene diolepoxide N2 guanine (N(2)-BPDE-dG), an important food and tobacco smoke adduct, is similar between the different cell cycle stages, however there is a cell stage specific mutagenic signature (81). This may highlight two different modes of TLS-mediated bypass. When a mildly distorting lesion such as a CPD is encountered it may be bypassed directly, also known as "on the fly", without repriming of the replication machinery (Fig.2). In contrast, when a lesion is strongly DNA helix distorting, such as a 6-4PP, the replication machinery reprimes downstream of the lesion (82) (Fig. 2) leaving a ssDNA gap. At a later stage these ssDNA gaps are likely filled in, in a more error-prone fashion, dependent on Polζ and Rev1 (76). Moreover, microRNA is shown to regulate TLS activity further by directly inhibiting the expression of Rev1 or by regulating the expression of Rad18, thus affecting TLS recruitment (83). The activity of TLS polymerases is also regulated by post-translational modifications. For instance, using a cell-free model with purified human proteins, it was shown that phosphorylation of Polη results in release of sequestration by PDIP38 and an increase in its binding strength to PCNA (84). Moreover, in yeast, Rev1 is phosphorylated in response to DNA damage and this

phosphorylation is, at least in a NER-deficient background, required for proficient TLS (85, 86).

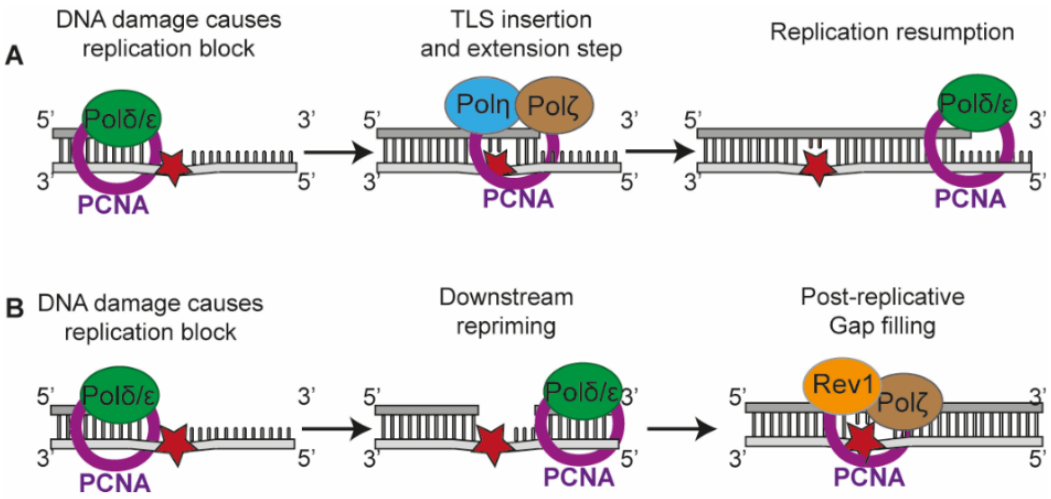


Figure 2: Modes of action for TLS
TLS can be performed in two ways: either "on the fly" during replication or post-replicative gap filling. A: After a replication block at a DNA lesion (asterisk) an inserter TLS polymerase is recruited (like Polη) to replicate the DNA lesion followed by extension by an extender polymerase (for instance Polζ). Lastly, replicative polymerases can take over again and proceed replicating the DNA. B: During persistent replication blockage the replication machinery can also be reprimed downstream of the lesion to continue DNA synthesis. Afterwards post-replicative TLS gap filling takes place dependent on TLS polymerases Rev1 and Polζ.

How polymerases are chosen and compete with each other is still a matter of debate. One theory is the model of a "molecular toolbelt" in which PCNA is a kind of polymerase hub that binds multiple TLS polymerases directly or via the "molecular bridge" Rev1. Most TLS polymerases actually have binding domains specifically for PCNA in support of this idea (87). Thus, when PCNA stalls on a lesion, replicative polymerases are replaced by TLS polymerases until the bypass is completed before switching back. Moreover, Polζ shares the subunits Pol31 and Pol32 with Polδ. These subunits are important for PCNA-mediated TLS. Pol32 interacts with PCNA, thus it seems likely that Polζ and Polδ compete directly for access to the PCNA sliding clamp (88). Mono-ubiquitination of PCNA has been suggested to play a major role here, which stimulates the activity of TLS polymerases and allows them to outcompete replicative polymerases (87). Yet, studies in mammalian cells have shown that PCNA mono-ubiquitination is not always essential for TLS bypass. For instance, binding of Polη to PCNA does not require PCNA mono-ubiquitination in human cells (58). Moreover, in MEF mutated to prevent PCNA mono-ubiquitination, approximately 30% of TLS activity remains (59). This shows that PCNA mono-ubiquitination is important for regulating TLS, but not essential.

Mutagenicity of TLS polymerases might also be controlled more directly. Although TLS polymerases do not exhibit proofreading activity themselves, they may be assisted by

replicative DNA polymerases that can proofread. In yeast it has been shown that Polα errors may be corrected by Polδ (89).

Moreover, simultaneous loss of both proofreading activities of Polδ and Polε resulted in mutagenesis higher than the sum of loss of the single proofreading activities, suggesting in trans proofreading (90). As such Polδ or Polε may also proofread TLS errors to minimize the mutagenicity of lesion bypass. In fact, in human cells it has been shown that when Polη creates a mismatch further extension from this mismatched terminus becomes much slower allowing for proofreading by Polδ or Polε (91). However, further research has to be done to show this for other TLS polymerases, too.

MMR and the control of replication errors

The bulk of the DNA is undamaged and is replicated by the replicative polymerases. In short Polα creates template primers and the replicative polymerases Polδ/Polε start replicating from these primers and do so either in a continuous manner during leading strand synthesis (Polε) or discontinuous during lagging strand synthesis (Polδ) (92). DNA replication on undamaged templates has a very low error-rate with approximately 1 error per billion bases (63). This high fidelity can be attributed to three factors: (I) the tight catalytic domain steering proper base pairing, (II) the proofreading domain that identifies and allows for the removal of mismatches and (III) post-replicative MMR (63). The MMR pathway is able to identify and excise misincorporations that slip through proofreading thereby increasing the fidelity of DNA replication by a factor between hundred to thousand-fold and protecting cells from mutagenesis (63, 93). The MMR pathway was first identified in the 1970s in prokaryotes (94, 95) and was soon after studied in one of the simpler eukaryotic systems, Saccharomyces cerevisiae, before ultimately being translated to humans. A simple description of eukaryotic MMR follows four steps: (I) identification of a mismatch by MSH2/MSH6 (MutSα) or MSH2/MSH3 (MutSβ), (II) nicking of the daughter strand by MLH1/PMS2 (MutLα), (III) removal of the misincorporation by exonucleases and (IV) resynthesis of the excised strand and ligation (96) (Fig. 3). However, the molecular mechanisms of MMR are complex, especially in eukaryotes and the next sections will discuss MMR step-by-step in detail.

For the recognition step eukaryotes express two heterodimers with slightly overlapping functionality, MSH2/MSH6 and MSH2/MSH3, also known as MutSα and MutSβ, respectively. MutSα is mainly involved in the recognition of base:base mismatches and small insertion deletion (indel) loops (Fig. 2), whereas MutSβ is involved in MMR for larger indel loops (97). MutSα can recognize mispairs by slightly bending the DNA duplex to monitor its rigidity. In the case of a mismatch, the DNA duplex bends further compared to correctly paired DNA, allowing for better interaction with MutSα catalytic sites (98). Interestingly, not every mismatch is recognized with similar efficiency. Many fundamental studies use the G-T mismatch to investigate which proteins might play a role in MMR and with good reason as the G-T mismatch is recognized 8-fold more often than, for instance, a C-A mismatch in vitro (99). Moreover, not only mismatches on undamaged DNA templates are recognized, but DNA lesions are recognized as well. Several studies using human (cancer) cells have shown that MutSα is able to

recognize a wide variety of DNA lesions, including O^6-methylguanine, the cisplatin d(GpG) intrastrand crosslink (100), UV-induced photolesions (101), aminofluorene - and acetylaminofluorene adducts (102) and benzo[c]phenanthrene adenine adducts (103). The recognition of a misincorporation opposite a DNA lesion is also important in suppressing mutagenesis stemming from DNA damaging agents in mammalian cells, as loss of MutSα results in DNA damage-induced hypermutagenesis (1, 104, 105). Mismatch recognition by MutSα of mismatches opposite damaged nucleotides seems to be similar to misincorporations opposite undamaged nucleotides: in the case of a O6-methylguanine-thymidine mismatch the structure is remarkably similar to an undamaged G-T mismatch (98). However, how MutSα might recognize a misincorporation opposite a bulky DNA lesion remains to be determined. After successful recognition MutSα exchanges ADP for ATP to trigger a confirmational change in MutSα, that allows for the recruitment of proteins involved in the next step: the nicking of the newly synthesized daughter strand.

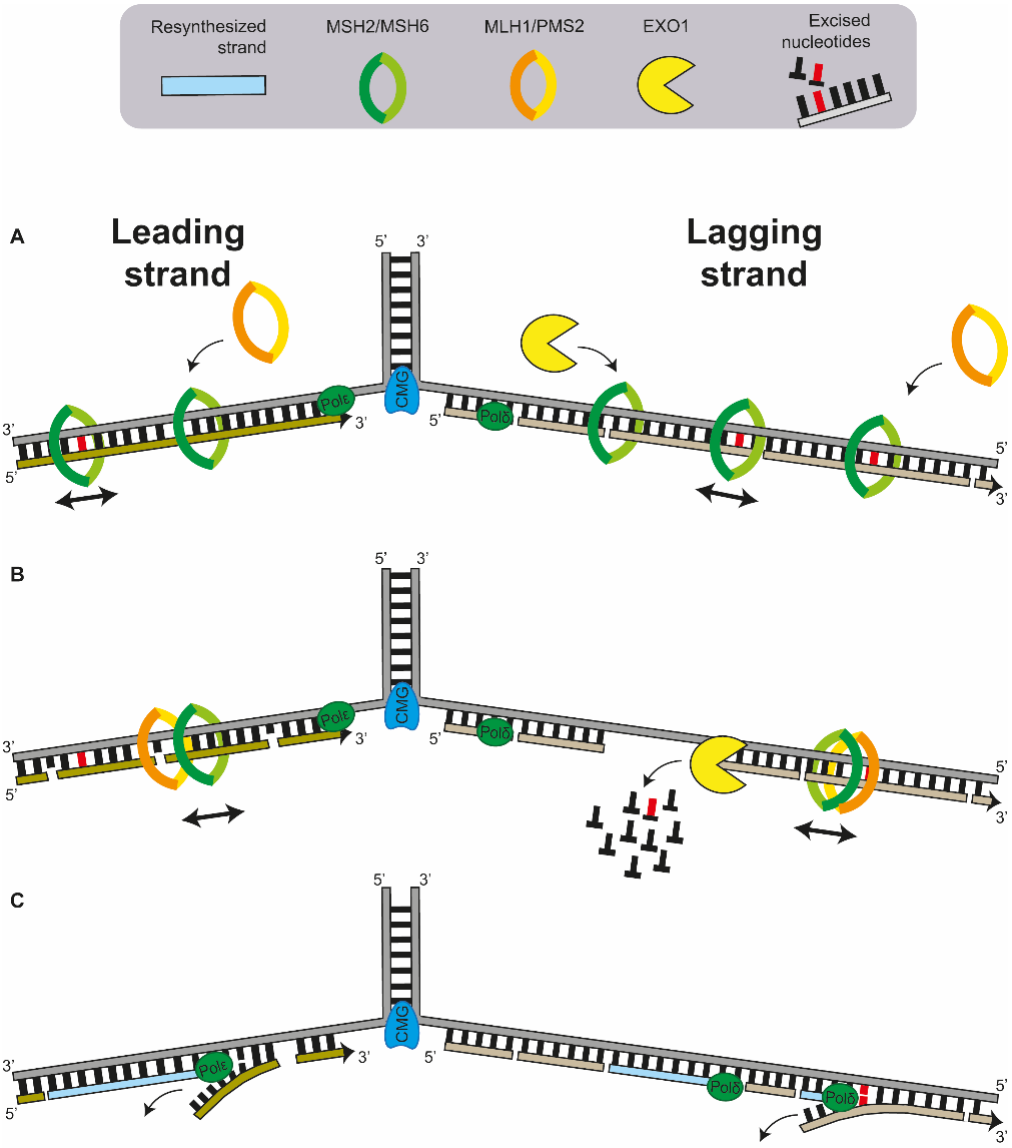


Figure 3: Eukaryotic Mismatch Repair on lagging and leading strands
A: Mismatch repair starts by recognition of a mismatched base (■) by MSH2/MSH6 which forms a sliding clamp. This allows for the activation of multiple MSH2/MSH6 complexes and triggers the recruitment of MLH1/PMS2 or EXO1 depending on strand and directionality. B: MLH1/PMS2 also slide over the DNA and can be activated by PCNA (not shown) to make multiple incisions. EXO1 degrades the daughter strand nucleotide by nucleotide, including the mismatch, which may occur on both strands. C. Replicative polymerases can resynthesize the strand thereby either displacing the still present daughter strand, including the mismatch, or simple gap filling if there was exonuclease activity.

The recognition of the daughter strand by MMR is a topic that is markedly different between eukaryotes and prokaryotes. For instance, during E. Coli MMR the newly synthesized daughter strand is recognized by the methylation status of the strand (106). In eukaryotes strand discrimination may be directed by how PCNA is loaded at nicks by clamp loaders. Its orientation allows for a way to discriminate the template strand from the daughter strand (107). As such PCNA is able to steer the endonucleolytic heterodimer MLH1/PMS2, also known as MutLα, to the daughter strand to create a nick which provides an entrance for the subsequent excision step (108). However, nicking may not always be essential due to the presence of nicks due to unligated Okazaki fragments in the lagging strand that may also serve as an entry for excision (96) (Fig. 3).

Excision of the daughter strand is often described to be performed by the exonuclease EXO1 in eukaryotic MMR. EXO1 acts in 5'-3' direction and in mismatch repair excises a stretch of the newly synthesized strand including the mismatch (Fig. 3) (109). Curiously, although EXO1 is important for MMR in vitro, Exo1 deficiency only displays a weak mutator phenotype in mice (110), which may hint at other exonucleases that play a role in MMR as well. Indeed, some overlap between exonuclease functionality has been proposed, as mutant Exo1 MEF upregulate the exonucleases Artemis, Mre11 and Fan1, which contributes to restoring MMR activity (111). Interestingly, in human cells, FAN1 and MRE11 are able to bind to MLH1 providing additional evidence for their role in MMR (112, 113). Studies using mammalian cells have shown that cells deficient for both FAN1 and EXO1 have significantly decreased MMR efficiency compared to either EXO1 or FAN1 single mutants, indeed suggesting a genetic redundancy between the two exonucleases (114). Conversely, FAN1 is also reported to frustrate MMR rather than act as a backup for EXO1 by sequestering MLH1 and thereby preventing the binding of MLH1 to MutSβ and possibly MutSα (115). Moreover, exonuclease-independent pathways exist (Fig. 3) that rely on continued endonucleolytic degradation performed by MutLα followed by unwinding by the replicative polymerases (116). After excision the ssDNA is coated by RPA for stability until the daughter strand is resynthesized and the remaining nicks are ligated (96).

Apart from the MMR genes described, two MMR homologs may also play a minor role during MMR, namely MLH3 and PMS1, which can both bind to MLH1. MLH3 shares some sequence homology with PMS2 and contains an endonuclease domain (117). MLH3 has a clear role in meiosis, where its endonuclease activity is important for cleaving recombination intermediates (118). During MMR in somatic cells, however,

MLH3 is suggested to be a very limited backup of PMS2 as demonstrated by both in vitro and in vivo experiments with single and double knock-out mice (119, 120). In contrast to MLH3, PMS1 does not have an endonuclease domain and the MLH1/PMS1 complex is considered an accessory factor (121). In yeast, scMLH2 (the ortholog of human PMS1) prevents excessive genomic rearrangements during meiotic recombination. Moreover, the scMLH1/scMLH2 complex is also recruited to mismatches, but its role in MMR remains unclear (122).

MMR and cancer

Loss of the MMR pathway results in increased mutagenesis and is therefore associated with cancer (123). This can occur either spontaneously during life, resulting in MMR-deficient tumors, or by inheritance of the MMR-defects. MMR is mainly associated with colorectal cancer (CRC) as most MMR-deficient tumors are found in that tissue. In fact, of all CRC, approximately 15% have a defect in MMR (124). Moreover, approximately 3% of CRC is caused by a heterozygous defect in one of four core genes involved in MMR: MSH2, MSH6, MLH1 and PMS2, known as Lynch syndrome (LS) (125).

LS mainly gives rise to tumors in the colorectum and endometrium and displays a high mutational burden, partly caused by microsatellite instability (MSI) (126, 127). DNA polymerases often “slip” over these microsatellites (small repeated DNA motifs) creating small indel loops that are normally repaired by MMR, preventing mutagenesis (128). This high mutation frequency accelerates carcinogenesis with a tumor developing from a colonic adenoma in less than 3 years in LS, down from the 6-10 years seen in sporadic CRC (126). Interestingly, the high mutational burden also attracts immune cells and thus the prognosis of a patient with an MMR-deficient tumor is distinctly better than the MMR-proficient counterpart (23, 129). LS is also associated with a younger age of onset, as the first tumor develops typically in patients younger than 50 years old (126), which is significantly earlier than the age of onset of sporadic CRC, which is approximately 70 years (23). This younger age of onset may be explained by the fact that LS patients, heterozygous for one of the four core MMR genes, only need to lose the remaining wild-type MMR allele, whereas a healthy individual needs to lose both alleles of a core MMR gene (126).

The age of onset also depends on which MMR gene is mutated. Patients carrying germline mutations in MLH1 and MSH2 show a lower age of onset than carriers with mutations in MSH6 or PMS2 (126). This may possibly be explained by the functional redundancies in the MMR pathway. As discussed above MSH2/MSH3 and MLH1/MLH3 may also contribute to repair of various mismatches and indel loops and these heterodimers are still formed in individuals that have lost MSH6 or PMS2 (97, 119). However, MSH3 or MLH3 are not considered as “LS genes” and are hardly associated with increased risk of developing cancer. The type of cancer caused by MMR defects partly depends on which MMR gene is mutated. For instance, MSH6+/- carriers more often develop endometrial cancer, sometimes without MSI, possibly due to the redundancy with MSH2/MSH3. MSH2+/- and MLH1+/- carriers typically develop

CRC, but MSH2+/- is also associated with extracolonic tumors. PMS2 mutations are also associated with CRC, but sometimes without family history (126). Interestingly, deletions in the EPCAM gene may also lead to LS, specifically the development of CRC, due to the close proximity of this gene to the MSH2 locus (130). Mutations in MSH2 are also most often associated with Muir-Torre, a variant of LS that causes cancer predominantly in the sebaceous glands of the skin (131).

In rare cases it can occur that not one, but two faulty copies of an MMR gene are inherited, giving rise to constitutional MMR deficiency (cMMRd) syndrome. These individuals develop tumors early in life, often before the age of 10, and they no longer predominantly develop CRC, but also tumors in tissues that are associated with rapidly dividing cells during early development, like the brain and the hematopoietic system (132). This tragic syndrome raises an important question: why is cMMRd associated with tumors in all rapidly dividing tissues, but does LS predominantly cause CRC? Moreover, why is sporadic loss of MMR often found in the gastro-intestinal (GI) tract? The answers to these questions will be addressed in this thesis.

In conclusion, both misincorporations generated by replicative DNA polymerases on undamaged DNA and error-prone TLS at bulky or helix-distorting nucleotide lesions contribute to mutagenesis related to cancer formation. Mammalian cells minimize mutagenesis (i) by activating post-replicative MMR, which removes misincorporations that have escaped the proofreading activity of replicative DNA polymerases, and (ii) by activating DNA damage signaling and checkpoint pathways following induction of bulky or helix-distorting nucleotide lesions. The latter will result in inhibition of DNA replication, induction of cell cycle arrests and activation of DNA repair to remove nucleotide lesions prior to DNA replication. If DNA lesions are persistent, they will be replicated by TLS, an inherently error-prone process. How error-prone TLS is controlled remains to be determined. However, scant experimental evidence indicates that some MMR proteins may contribute not only in the activation of DNA damage responses, but also in suppressing DNA damage-induced mutagenesis.

Aim and outline of the thesis

Aim:
This thesis aims to investigate the role of core MMR factors Msh6, Mlh1 and Pms2, MMR homologs Mlh3 and Pms1, and exonucleases Exo1 and Fan1 in protecting mammalian cells from the genotoxic effects of UVC light and the dietary carcinogen PhIP, which induce bulky and helix-distorting DNA lesions. This analysis will contribute to better comprehend the cellular response to bulky and helix-distorting DNA lesions and may help in understanding the cancer tropism found in LS patients who carry a heterozygous defect in one of the four core MMR genes.

Outline:
Chapter 2 of this thesis provides an in-depth overview of the various ways MMR and DNA damage intertwines. In this chapter the role of MMR in suppressing mutagenesis resulting from the four main types of damage, namely methylating, oxidating, helix-distorting and interstrand crosslinks, is reviewed. Moreover, the clinical implications of MMR loss are discussed, how this affects the use of generic cancer treatments for MMR-deficient tumors and what role DNA damage plays in generating a specific gastro-intestinal cancer tropism associated with loss of the MMR genes. **Chapter 3** studies in detail how MMR can suppress mutagenesis in mammalian cells following exposure to UVC light as a prototypic DNA damaging agent. Using mouse embryonic stem cells, multiple MMR and MMR-associated genes are investigated with respect to their role in UV-induced damage signaling and control of mutagenesis, including analyses of spectra of mutations in Mlh1-deficient and Msh6-deficient cells. **Chapter 4** describes studies on the intertwinement between TLS and MMR by making use of MMR and Polη knock-out and double-knockout cells. This study tests the hypothesis that MMR proteins suppress mutagenesis by removing TLS errors post-replicatively, a pathway dubbed post-TLS repair. **Chapter 5** describes a study on the CRC-associated tropism of Lynch syndrome, a syndrome characterized by the mono-allelic inheritance of a faulty MMR gene. Here, PhIP, a common dietary mutagen and carcinogen relevant for the GI tract, is used to investigate (I) whether food-related agents can inactivate MMR in a Lynch-like cell-based model, (II) whether MMR deficiency affects DNA damage signaling induced by PhIP and (III) whether PhIP-induced mutagenesis is further enhanced when MMR is completely lost. Finally, **chapter 6** summarizes the findings of this thesis, discusses new questions and insights provided by the studies presented in this thesis and describes the possibilities for future research.

References

1. Ijsselsteijn R, Jansen JG, De Wind N. DNA mismatch repair-dependent DNA damage responses and cancer. DNA Repair. 2020;93:102923.
2. D'Alessandro G, d'Adda di Fagagna F. Transcription and DNA Damage: Holding Hands or Crossing Swords? Journal of molecular biology. 2017;429(21):3215-29.
3. Huang R, Zhou PK. DNA damage repair: historical perspectives, mechanistic pathways and clinical translation for targeted cancer therapy. Signal Transduct Target Ther. 2021;6(1):254.
4. Vieth R. Critique of Public Health Guidance for Vitamin D and Sun Exposure in the Context of Cancer and COVID-19. Anticancer research. 2022;42(10):5027-34.
5. Apalla Z, Nashan D, Weller RB, Castellsague X. Skin Cancer: Epidemiology, Disease Burden, Pathophysiology, Diagnosis, and Therapeutic Approaches. Dermatology and therapy. 2017;7(Suppl 1):5-19.
6. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: a cancer journal for clinicians. 2021;71(3):209-49.
7. Kyllo RL, Brady KL, Hurst EA. Sebaceous carcinoma: review of the literature. Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al]. 2015;41(1):1-15.
8. Leiter U, Garbe C. Epidemiology of melanoma and nonmelanoma skin cancer--the role of sunlight. Advances in experimental medicine and biology. 2008;624:89-103.
9. Braeuer RR, Watson IR, Wu CJ, Mobley AK, Kamiya T, Shoshan E, et al. Why is melanoma so metastatic? Pigment cell & melanoma research. 2014;27(1):19-36.
10. Alaluf S, Atkins D, Barrett K, Blount M, Carter N, Heath A. Ethnic variation in melanin content and composition in photoexposed and photoprotected human skin. Pigment cell research. 2002;15(2):112-8.
11. Sinha RP, Hader DP. UV-induced DNA damage and repair: a review. Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology. 2002;1(4):225-36.
12. Douki T, Reynaud-Angelin A, Cadet J, Sage E. Bipyrimidine photoproducts rather than oxidative lesions are the main type of DNA damage involved in the genotoxic effect of solar UVA radiation. Biochemistry. 2003;42(30):9221-6.
13. Nakamura J, Mutlu E, Sharma V, Collins L, Bodnar W, Yu R, et al. The endogenous exposome. DNA Repair (Amst). 2014;19:3-13.
14. Kuluncsics Z, Perdiz D, Brulay E, Muel B, Sage E. Wavelength dependence of ultraviolet-induced DNA damage distribution: involvement of direct or indirect mechanisms and possible artefacts. Journal of photochemistry and photobiology B, Biology. 1999;49(1):71-80.
15. Young AR, Chadwick CA, Harrison GI, Hawk JL, Nikaido O, Potten CS. The in situ repair kinetics of epidermal thymine dimers and 6-4 photoproducts in human skin types I and II. The Journal of investigative dermatology. 1996;106(6):1307-13.
16. Freeman SE, Hacham H, Gange RW, Maytum DJ, Sutherland JC, Sutherland BM. Wavelength dependence of pyrimidine dimer formation in DNA of human skin irradiated in situ with ultraviolet light. Proc Natl Acad Sci U S A. 1989;86(14):5605-9.
17. Mouret S, Baudouin C, Charveron M, Favier A, Cadet J, Douki T. Cyclobutane pyrimidine dimers are predominant DNA lesions in whole human skin exposed to UVA radiation. Proc Natl Acad Sci U S A. 2006;103(37):13765-70.
18. Brash DE. UV signature mutations. Photochemistry and photobiology. 2015;91(1):15-26.
19. Yoon JH, Prakash L, Prakash S. Highly error-free role of DNA polymerase eta in the replicative bypass of UV-induced pyrimidine dimers in mouse and human cells. Proc Natl Acad Sci U S A. 2009;106(43):18219-24.
20. Bishehsari F, Mahdavinia M, Vacca M, Malekzadeh R, Mariani-Costantini R. Epidemiological transition of colorectal cancer in developing countries: environmental factors, molecular pathways, and opportunities for prevention. World J Gastroenterol. 2014;20(20):6055-72.
21. Chiavarini M, Bertarelli G, Minelli L, Fabiani R. Dietary Intake of Meat Cooking-Related Mutagens (HCAs) and Risk of Colorectal Adenoma and Cancer: A Systematic Review and Meta-Analysis. Nutrients. 2017;9(5):514.
22. Derry MM, Raina K, Agarwal C, Agarwal R. Identifying Molecular Targets of Lifestyle Modifications in Colon Cancer Prevention. Frontiers in Oncology. 2013;3.
23. Brenner H, Kloor M, Pox CP. Colorectal cancer. Lancet. 2014;383(9927):1490-502.
24. Zhu Y, Wang PP, Zhao J, Green R, Sun Z, Roebothan B, et al. Dietary N-nitroso compounds and risk of colorectal cancer: a case-control study in Newfoundland and Labrador and Ontario, Canada. The British journal of nutrition. 2014;111(6):1109-17.
25. Sinha R, Kulldorff M, Gunter MJ, Strickland P, Rothman N. Dietary benzo[a]pyrene intake and risk of colorectal adenoma. Cancer Epidemiol Biomarkers Prev. 2005;14(8):2030-4.
26. Bellamri M, Walmsley SJ, Turesky RJ. Metabolism and biomarkers of heterocyclic aromatic amines in humans. Genes and environment : the official journal of the Japanese Environmental Mutagen Society. 2021;43(1):29.
27. Friesen MD, Kaderlik K, Lin D, Garren L, Bartsch H, Lang NP, et al. Analysis of DNA adducts of 2-amino-1-methyl-6-phenylimidazo[4,5- b]pyridine in rat and human tissues by alkaline hydrolysis and gas chromatography/electron capture mass spectrometry: validation by comparison with 32P-postlabeling. Chem Res Toxicol. 1994;7(6):733-9.

28. Brown K, Guenther EA, Dingley KH, Cosman M, Harvey CA, Shields SJ, et al. Synthesis and spectroscopic characterization of site-specific 2-amino-1-methyl-6-phenylimidazo. Nucleic Acids Res. 2001;29(9):1951-9.
29. Holzl-Armstrong L, Moody S, Kucab JE, Zwart EP, Bellamri M, Luijten M, et al. Mutagenicity of 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-b]pyridine (N-OH-PhIP) in human TP53 knock-in (Hupki) mouse embryo fibroblasts. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association. 2021;147:111855.
30. Liu Z, Zhang Y, Dang Q, Wu K, Jiao D, Li Z, et al. Genomic Alteration Characterization in Colorectal Cancer Identifies a Prognostic and Metastasis Biomarker: FAM83A|IDO1. Front Oncol. 2021;11:632430.
31. Bouvard V, Loomis D, Guyton KZ, Grosse Y, Ghissassi FE, Benbrahim-Tallaa L, et al. Carcinogenicity of consumption of red and processed meat. The Lancet Oncology. 2015;16(16):1599-600.
32. Sinha R, Rothman N. Role of well-done, grilled red meat, heterocyclic amines (HCAs) in the etiology of human cancer. Cancer letters. 1999;143(2):189-94.
33. Berti M, Cortez D, Lopes M. The plasticity of DNA replication forks in response to clinically relevant genotoxic stress. Nature reviews Molecular cell biology. 2020;21(10):633-51.
34. Zou L, Elledge SJ. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. Science. 2003;300(5625):1542-8.
35. Delacroix S, Wagner JM, Kobayashi M, Yamamoto K, Karnitz LM. The Rad9-Hus1-Rad1 (9-1-1) clamp activates checkpoint signaling via TopBP1. Genes & development. 2007;21(12):1472-7.
36. Blackford AN, Jackson SP. ATM, ATR, and DNA-PK: The Trinity at the Heart of the DNA Damage Response. Mol Cell. 2017;66(6):801-17.
37. Falck J, Coates J, Jackson SP. Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. Nature. 2005;434(7033):605-11.
38. Lee JH, Paull TT. ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. Science. 2005;308(5721):551-4.
39. Scully R, Xie A. Double strand break repair functions of histone H2AX. Mutat Res. 2013;750(1-2):5-14.
40. Ziv Y, Bielopolski D, Galanty Y, Lukas C, Taya Y, Schultz DC, et al. Chromatin relaxation in response to DNA double-strand breaks is modulated by a novel ATM- and KAP-1 dependent pathway. Nature cell biology. 2006;8(8):870-6.
41. White D, Rafalska-Metcalf IU, Ivanov AV, Corsinotti A, Peng H, Lee SC, et al. The ATM substrate KAP1 controls DNA repair in heterochromatin: regulation by HP1 proteins and serine 473/824 phosphorylation. Molecular cancer research : MCR. 2012;10(3):401-14.
42. Jazayeri A, Falck J, Lukas C, Bartek J, Smith GC, Lukas J, et al. ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks. Nature cell biology. 2006;8(1):37-45.
43. Adams KE, Medhurst AL, Dart DA, Lakin ND. Recruitment of ATR to sites of ionising radiation-induced DNA damage requires ATM and components of the MRN protein complex. Oncogene. 2006;25(28):3894-904.
44. Liao H, Ji F, Helleday T, Ying S. Mechanisms for stalled replication fork stabilization: new targets for synthetic lethality strategies in cancer treatments. EMBO reports. 2018;19(9).
45. Jones GG, Reaper PM, Pettitt AR, Sherrington PD. The ATR-p53 pathway is suppressed in noncycling normal and malignant lymphocytes. Oncogene. 2004;23(10):1911-21.
46. Sahu RP, Batra S, Srivastava SK. Activation of ATM/Chk1 by curcumin causes cell cycle arrest and apoptosis in human pancreatic cancer cells. British journal of cancer. 2009;100(9):1425-33.
47. Nik-Zainal S, Davies H, Staaf J, Ramakrishna M, Glodzik D, Zou X, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. Nature. 2016;534(7605):47-54.
48. Brambullo T, Colonna MR, Vindigni V, Piaserico S, Masciopinto G, Galeano M, et al. Xeroderma Pigmentosum: A Genetic Condition Skin Cancer Correlated-A Systematic Review. BioMed research international. 2022;2022:8549532.
49. Shibutani S, Takeshita M, Grollman AP. Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. Nature. 1991;349(6308):431-4.
50. Fu D, Calvo JA, Samson LD. Balancing repair and tolerance of DNA damage caused by alkylating agents. Nature Reviews Cancer. 2012;12(2):104-20.
51. Mimmler M, Peter S, Kraus A, Stroh S, Nikolova T, Seiwert N, et al. DNA damage response curtails detrimental replication stress and chromosomal instability induced by the dietary carcinogen PhIP. Nucleic Acids Research. 2016;44(21):10259-76.
52. Elvers I, Johansson F, Groth P, Erixon K, Helleday T. UV stalled replication forks restart by re-priming in human fibroblasts. Nucleic Acids Res. 2011;39(16):7049-57.
53. Bunting SF, Nussenzweig A. End-joining, translocations and cancer. Nature reviews Cancer. 2013;13(7):443-54.
54. Nichols CA, Gibson WJ, Brown MS, Kosmicki JA, Busanovich JP, Wei H, et al. Loss of heterozygosity of essential genes represents a widespread class of potential cancer vulnerabilities. Nat Commun. 2020;11(1):2517.
55. Tilanus-Linthorst MM, Lingsma HF, Evans DG, Thompson D, Kaas R, Manders P, et al. Optimal age to start preventive measures in women with BRCA1/2 mutations or high familial breast cancer risk. Int J Cancer. 2013;133(1):156-63.
56. Chang DJ, Cimprich KA. DNA damage tolerance: when it's OK to make mistakes. Nature chemical biology. 2009;5(2):82-90.
57. Moldovan GL, Pfander B, Jentsch S. PCNA, the maestro of the replication fork. Cell. 2007;129(4):665-79.

58. Acharya N, Yoon JH, Gali H, Unk I, Haracska L, Johnson RE, et al. Roles of PCNA-binding and ubiquitin-binding domains in human DNA polymerase eta in translesion DNA synthesis. Proc Natl Acad Sci U S A. 2008;105(46):17724-9.
59. Hendel A, Krijger PH, Diamant N, Goren Z, Langerak P, Kim J, et al. PCNA ubiquitination is important, but not essential for translesion DNA synthesis in mammalian cells. PLoS Genet. 2011;7(9):e1002262.
60. Mohiuddin M, Evans TJ, Rahman MM, Keka IS, Tsuda M, Sasanuma H, et al. SUMOylation of PCNA by PIAS1 and PIAS4 promotes template switch in the chicken and human B cell lines. Proc Natl Acad Sci U S A. 2018;115(50):12793-8.
61. Branzei D, Vanoli F, Foiani M. SUMOylation regulates Rad18-mediated template switch. Nature. 2008;456(7224):915-20.
62. Vaisman A, Woodgate R. Translesion DNA polymerases in eukaryotes: what makes them tick? Critical reviews in biochemistry and molecular biology. 2017;52(3):274-303.
63. McCulloch SD, Kunkel TA. The fidelity of DNA synthesis by eukaryotic replicative and translesion synthesis polymerases. Cell Res. 2008;18(1):148-61.
64. Fukuda H, Takamura-Enya T, Masuda Y, Nohmi T, Seki C, Kamiya K, et al. Translesional DNA synthesis through a C8-guanyl adduct of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in Vitro: REV1 inserts dC opposite the lesion, and DNA polymerase kappa potentially catalyzes extension reaction from the 3'-dC terminus. J Biol Chem. 2009;284(38):25585-92.
65. Washington MT, Johnson RE, Prakash L, Prakash S. Human DINB1-encoded DNA polymerase kappa is a promiscuous extender of mispaired primer termini. Proc Natl Acad Sci U S A. 2002;99(4):1910-4.
66. Mouron S, Rodriguez-Acebes S, Martinez-Jimenez MI, Garcia-Gomez S, Chocron S, Blanco L, et al. Repriming of DNA synthesis at stalled replication forks by human PrimPol. Nature structural & molecular biology. 2013;20(12):1383-9.
67. Wan L, Lou J, Xia Y, Su B, Liu T, Cui J, et al. hPrimpol1/CCDC111 is a human DNA primase-polymerase required for the maintenance of genome integrity. EMBO reports. 2013;14(12):1104-12.
68. Garcia-Gomez S, Reyes A, Martinez-Jimenez MI, Chocron ES, Mouron S, Terrados G, et al. PrimPol, an archaic primase/polymerase operating in human cells. Mol Cell. 2013;52(4):541-53.
69. Bianchi J, Rudd SG, Jozwiakowski SK, Bailey LJ, Soura V, Taylor E, et al. PrimPol bypasses UV photoproducts during eukaryotic chromosomal DNA replication. Mol Cell. 2013;52(4):566-73.
70. Yoon JH, Johnson RE, Prakash L, Prakash S. DNA polymerase theta accomplishes translesion synthesis opposite 1,N(6)-ethenodeoxyadenosine with a remarkably high fidelity in human cells. Genes & development. 2019;33(5-6):282-7.
71. Yoon JH, McArthur MJ, Park J, Basu D, Wakamiya M, Prakash L, et al. Error-Prone Replication through UV Lesions by DNA Polymerase theta Protects against Skin Cancers. Cell. 2019;176(6):1295-309 e15.
72. Quinet A, Lerner LK, Martins DJ, Menck CFM. Filling gaps in translesion DNA synthesis in human cells. Mutation research Genetic toxicology and environmental mutagenesis. 2018;836(Pt B):127-42.
73. Jansen JG, Temviriyanukul P, Wit N, Delbos F, Reynaud CA, Jacobs H, et al. Redundancy of mammalian Y family DNA polymerases in cellular responses to genomic DNA lesions induced by ultraviolet light. Nucleic Acids Res. 2014;42(17):11071-82.
74. Ziv O, Geacintov N, Nakajima S, Yasui A, Livneh Z. DNA polymerase zeta cooperates with polymerases kappa and iota in translesion DNA synthesis across pyrimidine photodimers in cells from XPV patients. Proc Natl Acad Sci U S A. 2009;106(28):11552-7.
75. Sugiyama T, Keinard B, Best G, Sanyal MR. Biochemical and photochemical mechanisms that produce different UV-induced mutation spectra. Mutat Res. 2021;823:111762.
76. Temviriyanukul P, van Hees-Stuivenberg S, Delbos F, Jacobs H, de Wind N, Jansen JG. Temporally distinct translesion synthesis pathways for ultraviolet light-induced photoproducts in the mammalian genome. DNA Repair (Amst). 2012;11(6):550-8.
77. Yang W, Gao Y. Translesion and Repair DNA Polymerases: Diverse Structure and Mechanism. Annual Review of Biochemistry. 2018;87(1):239-61.
78. Friedberg EC. Suffering in silence: the tolerance of DNA damage. Nature Reviews Molecular Cell Biology. 2005;6(12):943-53.
79. Jansen JG, Tsaalbi-Shtylik A, de Wind N. Roles of mutagenic translesion synthesis in mammalian genome stability, health and disease. DNA Repair (Amst). 2015;29:56-64.
80. Martin-Pardillos A, Tsaalbi-Shtylik A, Chen S, Lazare S, van Os RP, Dethmers-Ausema A, et al. Genomic and functional integrity of the hematopoietic system requires tolerance of oxidative DNA lesions. Blood. 2017;130(13):1523-34.
81. Diamant N, Hendel A, Vered I, Carell T, Reissner T, de Wind N, et al. DNA damage bypass operates in the S and G2 phases of the cell cycle and exhibits differential mutagenicity. Nucleic Acids Res. 2012;40(1):170-80.
82. Quinet A, Tirman S, Cybulla E, Meroni A, Vindigni A. To skip or not to skip: choosing repriming to tolerate DNA damage. Mol Cell. 2021;81(4):649-58.
83. Natarajan V. Regulation of DNA repair by non-coding miRNAs. Non-coding RNA research. 2016;1(1):64-8.
84. Peddu C, Zhang S, Zhao H, Wong A, Lee EYC, Lee M, et al. Phosphorylation Alters the Properties of Pol eta: Implications for Translesion Synthesis. iScience. 2018;6:52-67.
85. Sabbioneda S, Bortolomai I, Giannattasio M, Plevani P, Muzi-Falconi M. Yeast Rev1 is cell cycle regulated, phosphorylated in response to DNA damage and its binding to chromosomes is dependent upon MEC1. DNA Repair (Amst). 2007;6(1):121-7.

86. Pages V, Santa Maria SR, Prakash L, Prakash S. Role of DNA damage-induced replication checkpoint in promoting lesion bypass by translesion synthesis in yeast. Genes & development. 2009;23(12):1438-49.
87. Leung W, Baxley RM, Moldovan GL, Bielinsky AK. Mechanisms of DNA Damage Tolerance: Post-Translational Regulation of PCNA. Genes. 2018;10(1).
88. Baranovskiy AG, Lada AG, Siebler HM, Zhang Y, Pavlov YI, Tahirov TH. DNA polymerase delta and zeta switch by sharing accessory subunits of DNA polymerase delta. J Biol Chem. 2012;287(21):17281-7.
89. Pavlov YI, Frahm C, Nick McElhinny SA, Niimi A, Suzuki M, Kunkel TA. Evidence that errors made by DNA polymerase alpha are corrected by DNA polymerase delta. Current biology : CB. 2006;16(2):202-7.
90. Tran HT, Gordenin DA, Resnick MA. The 3'-->5' exonucleases of DNA polymerases delta and epsilon and the 5'-->3' exonuclease Exo1 have major roles in postreplication mutation avoidance in Saccharomyces cerevisiae. Mol Cell Biol. 1999;19(3):2000-7.
91. Bebenek K, Matsuda T, Masutani C, Hanaoka F, Kunkel TA. Proofreading of DNA polymerase eta-dependent replication errors. J Biol Chem. 2001;276(4):2317-20.
92. O'Donnell M, Langston L, Stillman B. Principles and concepts of DNA replication in bacteria, archaea, and eukarya. Cold Spring Harb Perspect Biol. 2013;5(7).
93. Kunkel TA. Evolving views of DNA replication (in)fidelity. Cold Spring Harbor symposia on quantitative biology. 2009;74:91-101.
94. Wagner R, Jr., Meselson M. Repair tracts in mismatched DNA heteroduplexes. Proc Natl Acad Sci U S A. 1976;73(11):4135-9.
95. Wildenberg J, Meselson M. Mismatch repair in heteroduplex DNA. Proc Natl Acad Sci U S A. 1975;72(6):2202-6.
96. Jiricny J. The multifaceted mismatch-repair system. Nature reviews Molecular cell biology. 2006;7(5):335-46.
97. Genschel J, Littman SJ, Drummond JT, Modrich P. Isolation of MutSβ from Human Cells and Comparison of the Mismatch Repair Specificities of MutSβ and MutSα. Journal of Biological Chemistry. 1998;273(31):19895-901.
98. Warren JJ, Pohlhaus TJ, Changela A, Iyer RR, Modrich PL, Beese LS. Structure of the human MutSalpha DNA lesion recognition complex. Mol Cell. 2007;26(4):579-92.
99. Gradia S, Acharya S, Fishel R. The role of mismatched nucleotides in activating the hMSH2-hMSH6 molecular switch. J Biol Chem. 2000;275(6):3922-30.
100. Duckett DR, Drummond JT, Murchie AI, Reardon JT, Sancar A, Lilley DM, et al. Human MutSalpha recognizes damaged DNA base pairs containing O6-methylguanine, O4-methylthymine, or the cisplatin-d(GpG) adduct. Proc Natl Acad Sci U S A. 1996;93(13):6443-7.
101. Wang H, Lawrence CW, Li GM, Hays JB. Specific binding of human MSH2.MSH6 mismatch-repair protein heterodimers to DNA incorporating thymine- or uracil-containing UV light photoproducts opposite mismatched bases. J Biol Chem. 1999;274(24):16894-900.
102. Li GM, Wang H, Romano LJ. Human MutSalpha specifically binds to DNA containing aminofluorene and acetylaminofluorene adducts. J Biol Chem. 1996;271(39):24084-8.
103. Wu J, Gu L, Wang H, Geacintov NE, Li GM. Mismatch repair processing of carcinogen-DNA adducts triggers apoptosis. Mol Cell Biol. 1999;19(12):8292-301.
104. Borgdorff V, Pauw B, van Hees-Stuivenberg S, de Wind N. DNA mismatch repair mediates protection from mutagenesis induced by short-wave ultraviolet light. DNA Repair (Amst). 2006;5(11):1364-72.
105. Ijsselsteijn R, van Hees S, Drost M, Jansen JG, de Wind N. Induction of mismatch repair deficiency, compromised DNA damage signaling and compound hypermutagenesis by a dietary mutagen in a cell-based model for Lynch Syndrome. Carcinogenesis. 2021.
106. Li GM. Mechanisms and functions of DNA mismatch repair. Cell Res. 2008;18(1):85-98.
107. Kunkel TA, Erie DA. Eukaryotic Mismatch Repair in Relation to DNA Replication. Annual review of genetics. 2015;49:291-313.
108. Kadyrov FA, Dzantiev L, Constantin N, Modrich P. Endonucleolytic function of MutLalpha in human mismatch repair. Cell. 2006;126(2):297-308.
109. Tran PT, Erdeniz N, Symington LS, Liskay RM. EXO1-A multi-tasking eukaryotic nuclease. DNA Repair (Amst). 2004;3(12):1549-59.
110. Wei K, Clark AB, Wong E, Kane MF, Mazur DJ, Parris T, et al. Inactivation of Exonuclease 1 in mice results in DNA mismatch repair defects, increased cancer susceptibility, and male and female sterility. Genes & development. 2003;17(5):603-14.
111. Desai A, Gerson S. Exo1 independent DNA mismatch repair involves multiple compensatory nucleases. DNA Repair (Amst). 2014;21:55-64.
112. Rikitake M, Fujikane R, Obayashi Y, Oka K, Ozaki M, Hidaka M. MLH1-mediated recruitment of FAN1 to chromatin for the induction of apoptosis triggered by O(6) -methylguanine. Genes to cells : devoted to molecular & cellular mechanisms. 2020;25(3):175-86.
113. Her C, Vo AT, Wu X. Evidence for a direct association of hMRE11 with the human mismatch repair protein hMLH1. DNA Repair (Amst). 2002;1(9):719-29.
114. Kratz K, Artola-Boran M, Kobayashi-Era S, Koh G, Oliveira G, Kobayashi S, et al. FANCD2-Associated Nuclease 1 Partially Compensates for the Lack of Exonuclease 1 in Mismatch Repair. Mol Cell Biol. 2021;41(9):e0030321.

115. Goold R, Hamilton J, Menneteau T, Flower M, Bunting EL, Aldous SG, et al. FAN1 controls mismatch repair complex assembly via MLH1 retention to stabilize CAG repeat expansion in Huntington's disease. Cell Rep. 2021;36(9):109649.
116. Goellner EM, Putnam CD, Kolodner RD. Exonuclease 1-dependent and independent mismatch repair. DNA Repair (Amst). 2015;32:24-32.
117. Kadyrova LY, Gujar V, Burdett V, Modrich PL, Kadyrov FA. Human MutLgamma, the MLH1-MLH3 heterodimer, is an endonuclease that promotes DNA expansion. Proc Natl Acad Sci U S A. 2020;117(7):3535-42.
118. Cannavo E, Sanchez A, Anand R, Ranjha L, Hugener J, Adam C, et al. Regulation of the MLH1-MLH3 endonuclease in meiosis. Nature. 2020;586(7830):618-22.
119. Chen PC, Dudley S, Hagen W, Dizon D, Paxton L, Reichow D, et al. Contributions by MutL homologues Mlh3 and Pms2 to DNA mismatch repair and tumor suppression in the mouse. Cancer Res. 2005;65(19):8662-70.
120. Cannavo E, Marra G, Sabates-Bellver J, Menigatti M, Lipkin SM, Fischer F, et al. Expression of the MutL homologue hMLH3 in human cells and its role in DNA mismatch repair. Cancer Res. 2005;65(23):10759-66.
121. Raschle M, Marra G, Nystrom-Lahti M, Schar P, Jiricny J. Identification of hMutLbeta, a heterodimer of hMLH1 and hPMS1. J Biol Chem. 1999;274(45):32368-75.
122. Pannafino G, Alani E. Coordinated and Independent Roles for MLH Subunits in DNA Repair. Cells. 2021;10(4).
123. Liu D, Keijzers G, Rasmussen LJ. DNA mismatch repair and its many roles in eukaryotic cells. Mutation Research/Reviews in Mutation Research. 2017;773:174-87.
124. Poulogiannis G, Frayling IM, Arends MJ. DNA mismatch repair deficiency in sporadic colorectal cancer and Lynch syndrome. Histopathology. 2010;56(2):167-79.
125. 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. Clinical genetics. 2009;76(1):1-18.
126. Lynch HT, Snyder CL, Shaw TG, Heinen CD, Hitchins MP. Milestones of Lynch syndrome: 1895–2015. Nature Reviews Cancer. 2015;15(3):181-94.
127. Baretti M, Le DT. DNA mismatch repair in cancer. Pharmacology & therapeutics. 2018;189:45-62.
128. Kunkel TA, Erie DA. DNA mismatch repair. Annu Rev Biochem. 2005;74:681-710.
129. Schwitalle Y, Kloor M, Eiermann S, Linnebacher M, Kienle P, Knaebel HP, et al. Immune response against frameshift-induced neopeptides in HNPCC patients and healthy HNPCC mutation carriers. Gastroenterology. 2008;134(4):988-97.
130. Ligtenberg MJ, Kuiper RP, Chan TL, Goossens M, Hebeda KM, Voorendt M, et al. Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3' exons of TACSTD1. Nat Genet. 2009;41(1):112-7.
131. Mangold E, Pagenstecher C, Leister M, Mathiak M, Rutten A, Friedl W, et al. A genotype-phenotype correlation in HNPCC: strong predominance of msh2 mutations in 41 patients with Muir-Torre syndrome. J Med Genet. 2004;41(7):567-72.
132. Lavoine N, Colas C, Muleris M, Bodo S, Duval A, Entz-Werle N, et al. Constitutional mismatch repair deficiency syndrome: clinical description in a French cohort. Journal of Medical Genetics. 2015;52(11):770-8.
133. Gibis M. Heterocyclic Aromatic Amines in Cooked Meat Products: Causes, Formation, Occurrence, and Risk Assessment. Comprehensive reviews in food science and food safety. 2016;15(2):269-302.
134. Jia W, Xie G, Jia W. Bile acid-microbiota crosstalk in gastrointestinal inflammation and carcinogenesis. Nat Rev Gastroenterol Hepatol. 2018;15(2):111-28.
135. Cantwell M, Elliott C. Nitrates, Nitrites and Nitrosamines from Processed Meat Intake and Colorectal Cancer Risk. Journal of Clinical Nutrition & Dietetics. 2017;3:4-27.
136. Marchesi F, Turriziani M, Tortorelli G, Avvisati G, Torino F, De Vecchis L. Triazene compounds: mechanism of action and related DNA repair systems. Pharmacological research. 2007;56(4):275-87.
137. Du H, Wang P, Li L, Wang Y. Repair and translesion synthesis of O (6)-alkylguanine DNA lesions in human cells. J Biol Chem. 2019;294(29):11144-53.
138. Winterbourn CC, Kettle AJ, Hampton MB. Reactive Oxygen Species and Neutrophil Function. Annual Review of Biochemistry. 2016;85(1):765-92.
139. Khansari N, Shakiba Y, Mahmoudi M. Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. Recent patents on inflammation & allergy drug discovery. 2009;3(1):73-80.
140. Bridge G, Rashid S, Martin SA. DNA mismatch repair and oxidative DNA damage: implications for cancer biology and treatment. Cancers. 2014;6(3):1597-614.
141. Dizdaroglu M. Oxidatively induced DNA damage: mechanisms, repair and disease. Cancer letters. 2012;327(1-2):26-47.
142. Petta TB, Nakajima S, Zlatanou A, Despras E, Couve-Privat S, Ishchenko A, et al. Human DNA polymerase iota protects cells against oxidative stress. EMBO J. 2008;27(21):2883-95.
143. Guan J, Yu S, Zheng X. NEDDylation antagonizes ubiquitination of proliferating cell nuclear antigen and regulates the recruitment of polymerase eta in response to oxidative DNA damage. Protein & cell. 2018;9(4):365-79.
144. Fahrer J, Kaina B. Impact of DNA repair on the dose-response of colorectal cancer formation induced by dietary carcinogens. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association. 2017;106(Pt B):583-94.
145. Marteijn JA, Lans H, Vermeulen W, Hoeijmakers JH. Understanding nucleotide excision repair and its roles in cancer and ageing. Nature reviews Molecular cell biology. 2014;15(7):465-81.

146. Semlow DR, Walter JC. Mechanisms of Vertebrate DNA Interstrand Cross-Link Repair. Annu Rev Biochem. 2021;90:107-35.
147. Roy U, Scharer OD. Involvement of translesion synthesis DNA polymerases in DNA interstrand crosslink repair. DNA Repair (Amst). 2016;44:33-41.
148. Mehta A, Haber JE. Sources of DNA double-strand breaks and models of recombinational DNA repair. Cold Spring Harb Perspect Biol. 2014;6(9):a016428.
149. Cannan WJ, Pederson DS. Mechanisms and Consequences of Double-Strand DNA Break Formation in Chromatin. Journal of cellular physiology. 2016;231(1):3-14.
150. Sallmyr A, Tomkinson AE. Repair of DNA double-strand breaks by mammalian alternative end-joining pathways. J Biol Chem. 2018;293(27):10536-46.
151. Donnianni RA, Zhou ZX, Lujan SA, Al-Zain A, Garcia V, Glancy E, et al. DNA Polymerase Delta Synthesizes Both Strands during Break-Induced Replication. Mol Cell. 2019;76(3):371-81 e4.
152. Abbotts R, Wilson DM, 3rd. Coordination of DNA single strand break repair. Free Radic Biol Med. 2017;107:228-44.
153. Caldecott KW. DNA single-strand break repair. Experimental cell research. 2014;329(1):2-8.