Control of replication associated DNA damage responses by Mismatch Repair

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Chapter &:

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Summary

Mutagenesis, the process that generates alterations in the DNA sequence, underlies many serious illnesses plaguing mankind, most notably cancer. Mutagenesis often occurs during DNA replication and **Chapter 1** discusses in detail the workings of this process. In short: during the replication of double-stranded DNA, the DNA double-helix is unwound allowing for the duplication of both strands by replicative DNA polymerases. When a replicative polymerase incorporates an incorrect nucleotide during DNA replication a 'mismatch' is generated which can lead to a mutation in the next replication cycle. DNA mismatch repair (MMR) can detect and remove these mismatches thereby preventing mutations in the DNA. **Chapter 1** describes how the MMR process on base-base mismatches works: the MMR-proteins MSH2 and MSH6 form a heterodimer that is able to recognize mismatches and subsequently recruit a heterodimer consisting of MLH1 and PMS2 to generate a nick in the DNA-strand containing the misincorporation. Finally, EXO1 is recruited to excise a part of the newly generated strand, including the mismatch. However, the necessity of EXO1 in the MMR pathway is being debated as other nucleases, amongst which FAN1, could possibly substitute for EXO1 if EXO1 is not available. Other proteins may also play a role in the MMR pathway, such as PMS1 and MLH3. These proteins are homologs of MLH1 and PMS2, but their exact role is, as of yet, unclear. Furthermore, loss of PMS1 or MLH3 does not lead to MMR-deficiency. In essence, there are four MMR core-genes, *MSH2*, *MSH6*, *MLH1*, *PMS2*. Loss of any of these genes leads to MMR-deficiency resulting in hyper-mutagenesis and an increased risk of cancer. Furthermore, **Chapter 1** describes the different types of DNA damage and the associated DNA repair pathways. DNA damage alters the DNA structure, and when left unrepaired, it leads to replicative blocks that activate DNA damage signaling, and ultimately results in cell death. However, DNA damage signaling can also activate DNA damage tolerance mechanisms, such as translesion synthesis (TLS). TLS is performed by so-called TLS polymerases that can replicate damaged DNA thereby quenching DNA damage signaling and preventing cell death. However, TLS polymerases are error-prone which leads to increased mutagenesis. Our cellular DNA is constantly threatened by DNA damaging agents, for instance found in the diet, thus it is advantageous to suppress TLS-induced mutagenicity as much as possible. This thesis investigated whether the MMR pathway could be involved in reducing mutagenesis from DNA damaging agents by controlling TLS and DNA damage responses.

Chapter 2 is a graphical review that summarizes the role of MMR in dealing with responses associated with DNA damage. MMR plays an unmistakable role in four different lesion types: alkylation, oxidation, interstrand crosslinks, and helix-distorting. Loss of MMR can result in increased resistance to these various lesion types and often leads to increased mutagenicity. Additionally, **Chapter 2** details the implications of loss of MMR for cancer development and treatment. DNA-damaging agents are commonly used to combat cancer, but loss of MMR may increase resistance to such drugs and may even result in an environment where pre-cancerous MMR-deficient cells can thrive, leading to the formation of new tumors. Furthermore, in **Chapter 2** it is

hypothesized that individuals with Lynch syndrome (LS), a cancer predisposition syndrome caused by the inheritance of one dysfunctional copy of an MMR gene, are at greater risk from the adverse effects of DNA-damaging agents.

Chapter 2 described the important role of MMR in reducing the adverse effects of DNA damaging agents, such as mutagenesis and loss of DNA damage signaling. However, several questions remained: (i) how does MMR reduce mutagenesis from DNA damaging agents, (ii) how does MMR contribute to activation of damage signaling, and (iii) which MMR-related proteins play a role in dealing with DNA damage? The aim of **Chapter 3** was to answer these questions by generating a panel of isogenic mouse embryonic stem cell lines deficient for *Msh6*, *Mlh1*, *Pms2*, *Exo1*, *Fan1*, *Mlh3* or *Pms1* and exposing these cells to mutagenic ultraviolet (UV) radiation. All these cell lines contained an additional *Xpa*-deficiency to prevent the repair of UV lesions. **Chapter 3** describes that *Msh6*, *Mlh1*, and *Pms2* protect against UV-induced mutagenesis, but the other investigated genes do not show such a phenotype. In the case of *Exo1* and *Fan1*, this may be because these genes are redundant with other exonucleases. The same can be said for the MMR homologs *Mlh3* and *Pms1*; these genes may share functional redundancy with *Pms2*. Moreover, **Chapter 3** confirmed previously published data that *Msh6*-deficient cells have greatly reduced UV-induced DNA damage signaling and associated single-stranded DNA formation compared to wild-type cells. These data led to the hypothesis that Msh6 (together with Msh2) may control the DNA damage response by removing TLS replication errors, thereby generating single-stranded DNA tracts that activate DNA damage signaling. However, **Chapter 3** also shows that, in contrast to loss of Msh6, loss of Mlh1 or Pms2 does not result in the reduction of damage signaling and single-stranded DNA formation, which may suggest that some Mlh1/Pms2-independent processes take place that generate these ssDNA gaps. Taken together, the data in **Chapter 3** suggest that all four canonical MMR genes, *Msh2*, *Msh6*, *Mlh1* and *Pms2*, play a role in suppressing UV-induced mutagenesis.

Chapter 4 investigates the hypothesis that the control of UV light-induced mutagenesis by MMR correlates with the extent of error-prone TLS replication. To this end, *Polymerase H* (*Polh*)-deficient cells were generated and analyzed. *Polh* encodes for TLS Polymerase eta, which can bypass UV damage in a relatively error-free manner. When *Polh* is lost, more error-prone TLS polymerases bypass UV lesions, resulting in enhanced UV-induced mutagenesis. Thus, if MMR reduces mutagenicity resulting from TLS errors, then loss of MMR must be even more detrimental in *Polh*-deficient cells than in *Polh*-proficient cells. To evaluate this hypothesis, *PolhMsh6* and *PolhMlh1* double knock-out cell lines were generated. In support, loss of Msh6 or Mlh1 in *Polh*-deficient cells further increased UV-induced mutagenesis, even higher than the sum of UV-induced mutagenesis in the single knock-out cell lines. Additionally, the removal of TLS errors by MMR would result in single-stranded DNA tracts and concomitant induction of DNA damage signaling. Loss of Msh6 in *Polh*-deficient cells led to reduced UV-induced single-stranded DNA formation and damage signaling. Consistent with the results described in **Chapter 3**, a deficiency for *Mlh1* in a *Polh*-deficient background

did not result in the loss of single-stranded DNA formation and damage signaling. Interestingly, previously published works have shown that MMR proteins Msh2/Msh6 can recruit TLS polymerases to the site of damage and, in addition, might be involved in promoting error-free template switching to bypass a DNA lesion. These findings together with the work presented in **Chapter 4** may provide a model to explain the control of the DNA damage response by MMR in a two-step process: (i) MMR removes TLS errors, thereby preventing mutagenesis and promoting DNA damage signaling and checkpoint activation, and (ii) Msh2/Msh6 subsequently promotes error-free bypass either by TLS or by template switching which will quench DNA damage signaling and checkpoint activation.

Patients with LS display a remarkable restricted cancer tropism as most LS patients develop colon cancer. **Chapter 5** aimed to investigate the hypothesis that cells containing one dysfunctional copy of an MMR gene, such as intestinal stem cells of LS patients, are at greater risk from the adverse effects of food-derived genotoxic compounds. To test this hypothesis, mouse embryonic stem cell models were generated that resemble LS cells by containing only one wild type allele of either the MMR gene *Msh2* or *Mlh1*. These cells were exposed to 2-Amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP), a mutagen found in meat grilled at high temperatures that induces helix-distorting DNA lesions. **Chapter 5** shows that *Msh2* or *Mlh1* heterozygous cells lost MMR at a significantly higher frequency than WT cells when exposed to PhIP. Moreover, previous literature has shown that loss of MMR may result in aggravated hypermutagenesis from various DNA-damaging agents. In **Chapter 5**, these findings were corroborated by showing that defects in either one of the four core MMR genes (*Msh2*, *Mlh1*, *Msh6* and *Pms2*) enhanced PhIP-induced mutagenesis in mouse embryonic stem cell lines. Finally, loss of Msh2 resulted in the loss of protective DNA damage signaling, which may give these MMR-deficient cells a growth advantage. Taken together, the data in **Chapter 5** suggest that PhIP may drive oncogenesis in LS patients on three levels: (i) by increasing the frequency at which MMR-heterozygous cells become MMR-deficient; (ii) the resulting MMR-deficient cells have lost the capability to suppress PhIP-induced mutagenesis; and (iii) they have also lost protective DNA damage signaling. As such, this data may be important in advising LS individuals to adopt a lifestyle that reduces exposure to DNA-damaging agents, such as those found in unhealthy diets. Moreover, the findings in **Chapter 5** may explain why LS patients predominantly develop colon cancer, as this tissue type is constantly exposed to dietary DNA-damaging agents.

In conclusion, this thesis shows the importance of MMR in the regulation of DNA-damage induced mutagenesis and the implications of these findings for both healthy individuals and LS-patients. This thesis describes a pathway in which MMR is first required to remove the misincorporation opposite a DNA damage and is subsequently required to facilitate the (error-free) bypass of the DNA lesion by recruiting either TLS polymerases or template switch components. **Chapter 6** discusses the results and models described in this thesis in more detail and in light of previously published works. Moreover, **Chapter 6**, describes possible next steps for the research presented in this

thesis, such as the continued investigation of which proteins, for example exonucleases other than Exo1 and Fan1, are involved in MMR-activity on damaged DNA. Another interesting next step would be to investigate how MMR recognizes and removes misincorporations opposite damaged DNA. For instance, this could involve measuring MMR activity on substrates with a misincorporation opposite a DNA damage. Additionally, studying the binding of Msh2/Msh6 to a PhIP-adduct containing mismatch would provide valuable insights. Finally, this thesis shows that DNA damage is particularly hazardous for individuals that have lost MMR partially or completely and **Chapter 6** discusses the clinical implications of this finding in detail. Altering the diet to minimize DNA damaging agents in the colon would be good advice for patients with an MMR-defect.