Published in final edited form as:
Mutat Res. 2013 ; 0: 1–3. doi:10.1016/j.mrfmmm.2013.04.009.

Mutation Research / Fundamental and Molecular Mechanisms of Mutagenesis Special Issue: DNA Repair and Genetic Instability

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DNA repair, DNA damage tolerance and the DNA damage response are complex biochemical events within the cell that are of critical importance with regard to genome stability and the onset of carcinogenesis, as well as chemotherapeutic efficacy during cancer treatment. DNA repair, similar to DNA replication, exhibits high fidelity in normal circumstances. However, in the presence of excessive DNA damage, or dysfunctional DNA repair mechanisms, error-prone DNA repair and DNA damage tolerance can lead to genetic instability and carcinogenesis, as well as increased sensitivity or resistance to chemotherapeutics. A detailed knowledge of the sequential molecular and biochemical steps within these cellular pathways is essential for successful clinical treatment of cancer and inherited DNA replication or repair syndromes. This special issue contains articles that illustrate a wide range of topics to better understand the overarching theme of DNA repair and genetic instability in the context of human disease.

DNA glycosylases are the enzymes that initiate base excision repair (BER), a process in all living organisms that protects against a specific subset of base lesions produced by endogenous events, environmental chemicals and ionizing radiation. DNA glycosylases are exceptionally diverse and numerous, accounting for the extensive list of lesions repaired by BER. Susan Wallace and coauthors describe the most current biochemical and structural data for Neil3, a specific glycosylase recently discovered in vertebrate cells. Neil3 has homology to bacterial Fpg/Nei proteins, but with diverse substrate specificity, different structural features and an uncertain biological role. Another pair of DNA glycosylases, Methyl-CpG Domain Protein 4 (MBD4) and Thymine DNA Glycosylase (TDG), remove uracil or thymine from U:T or G:T mispairs respectively, generated by deamination of cytosine or 5-methylcytosine at CpG sites within the genome. New evidence presented by Joann Sweasy indicates that these two glycosylases, previously believed to be redundant, have diverse functions within the cell. Tumor-associated mutations of both glycosylases that generate altered protein function are also discussed in this comprehensive review.

5-Fluorouracil (5-FU) causes the introduction of uracil into DNA by the inhibition of TTP synthesis, and is itself incorporated into DNA. Both base substitutions are recognized by uracil DNA glycosylase (UDG), and single-strand selective monofunctional uracil DNA glycosylase (SMUG1). In the study reported by Michael Wyatt and colleagues, the individual roles of UDG and SMUG1 were examined during cellular response to 5-FU. Using exposure protocols that mimic clinical treatment, they discovered that loss of SMUG1, but not UDG, prolongs S phase and Chk1 phosphorylation after 5-FU treatment,

implicating a specific role for SMUG1 in DNA damage recognition and repair after cellular exposure to this chemical.

MUTYH is the human ortholog of MutY, a highly conserved glycosylase, and is integral to one of the most complicated BER pathways. This BER enzyme recognizes and removes adenine incorrectly inserted during DNA replication opposite a template containing the oxidative 8-oxodG lesion. Subsequent repair activity by the BER pathway recreates the 8-oxodG:C base pair that is then recognized by the OGG1 DNA glycosylase and removed with additional BER repair activity to reconstitute the correctly paired G:C base pair. Successful, coordinated activity of these two glycosylases, along with a multitude of other BER proteins, prevents G:C to T:A transversions induced by oxidative damage. Importantly, MUTYH-associated polyposis (MAP) is associated with germline mutations in this BER glycosylase. The review of this BER pathway by Margherita Bignami and associates is a timely and thorough characterization of protein structure, molecular mechanism, and functional studies of MUTYH variants, including relevance of inactivation and of variants in colorectal cancer risk.

The repair of interstrand crosslinks (ICLs) produced by bifunctional alkylating agents and other anticancer drugs involves coordinated activity of specific proteins from multiple DNA repair pathways. Key roles have been identified that involve proteins from Nucleotide Excision Repair (NER) and Homologous Recombination (HR). Recent studies have also indicated a role for the BER pathway in mediating the cytotoxicity of ICLs. Steve Patrick and Anbarasi Kothandapani have contributed an in depth review that examines potential mechanisms and consequences of BER-mediated modulation of ICL repair.

The DNA mismatch repair (MMR) pathway in yeast and human cells has expanded significantly from the post-replication mutation avoidance system first characterized in bacteria. Dysfunctional human MMR is now firmly established as a causative mechanism for Hereditary Nonpolyposis Colon Cancer (HNPCC), and also for acquired resistance to monofunctional alkylating agents. The comprehensive review by Kandace Williams and colleagues focuses primarily on the DNA binding heterodimer MutSα (MSH2 + MSH6). This review explores current knowledge regarding heterodimer structure and function, molecular mechanisms of mismatch detection and binding, and cellular regulation. These authors also include a discussion of several non-canonical roles of this MMR heterodimer, such as potential functions of the N-terminal disordered domain of MSH6, and MMR-dependent DNA damage response induced by O^6me-Guanine. Interactions with other DNA repair pathways such as BER, double-strand break (DSB) repair and ICL repair are reviewed, as well as roles for MMR during antibody diversity and trinucleotide repeat expansion.

Microsatellite instability (MSI) is observed as varying lengths of repeating microsatellite sequences throughout the genome. This phenomenon commonly develops because DNA replication occurs without coordinated proofreading by the MMR pathway, a fundamental requirement for high fidelity DNA replication. The degree of MSI at specific locations is used clinically for diagnosis of HNPCC and MMR deficient tumors. It has been discovered however, that mutation rates of individual microsatellite regions vary greatly, depending on motif size, sequence, and length, as well as the involvement of cellular pathways or events other than dysfunctional MMR. Kristin Eckert and colleagues present compelling evidence that specific di- and tetra-nucleotide forms of MSI found in sporadic cancers arise by distinct genetic mechanisms. The origins and pathological significance for these unique MSI events are explored in depth in this review.

Fanconi anemia (FA) is a rare recessive genetic disorder that derives from mutations in one of fifteen genes coding for proteins that are within the FA pathway. FA cells are highly sensitive to DNA cross-linking agents and reactive oxygen species. The most common clinical events associated with this disease are acute myeloid leukemia and bone marrow failure with squamous cell carcinoma (SCC) as an additional, extreme risk. Susanne Wells and colleagues review the mechanisms of action of the FA pathway as it contributes to cellular stress response, DNA repair and SCC sensitivity.

Bloom syndrome (BS) is another rare recessive disorder but results from loss of function of the recQ-like BLM helicase. This genetic disorder is characterized by short stature (amongst several other physical features), and predisposition to the development of cancer. Strikingly, cells from patients with BS exhibit genomic instability because of increased chromosomal recombination, including hyper-recombination of rDNA repeats. Samir Acharya and colleagues within Joanna Grodon's laboratory have identified a direct interaction of DNA topoisomerase I with the C-terminus of BLM in the nucleolus. The results of this study suggest that BLM and DNA topoisomerase I coordinately modulate RNA:DNA hybrid formation.

The integrity of chromosomal DNA is maintained not only by high fidelity DNA replication and repair, but also by a complex cellular network that surveys for base lesions, broken strands, or blocked replication forks, known collectively as the DNA damage response (DDR). The exact biochemical nature of the DDR is dependent on several variables and may involve activation of cell cycle checkpoints, the initiation of DNA repair, as well as the onset of cellular senescence, cell death, and/or DNA damage tolerance (DDT). DDT involves recruitment of translesion DNA synthesis (TLS) polymerases, or lesion bypass polymerases, after DNA replication. The putative reason for this response is to increase genetic stability in the presence of DNA damage in replicating cells, but may instead result in increased mutagenesis. Christine Canman and co-authors review specialized functions of several lesion bypass polymerases with regard to specific DNA repair pathways, mutagenesis, and genetic stability. Complementary to the Canman review, Motoshi Suzuki and colleagues describe the potential involvement of core DNA replication proteins and replication steps that have been implicated in the process of carcinogenesis, including the RAD6-RAD18 TLS pathway. These authors also discuss specific mutations in several associated genes that have been identified in human cancer.

When considering the mechanisms of DNA repair, DNA replication and lesion bypass, the predominant DNA substrate for all of these processes is Watson-Crick B-form DNA. However, there are more than ten different types of 'non-B' DNA conformations and many play important roles in regulating gene expression, gene rearrangements and in dictating chromatin status. In their, review, Karen Vasquez and Guliang Wang focus on the interactions of DNA repair proteins with non-B DNA and their roles in genetic instability and advance the possibility that proteins and DNA involved in such interactions may represent plausible targets for selective therapeutic intervention.

Fidelity of DNA repair is critical, as DNA sequence alterations can contribute to an increase in gene mutations, gene dysfunction and cellular abnormalities. Homologous recombination (HR) is a highly error-free pathway involved in the repair of DNA double-strand breaks (DSBs). Unfortunately, familial breast cancers, among others, present with defects in HR-mediated repair and therefore require the cancer cell to use more error-prone pathways for DSB repair such as microhomology-mediated non-homologous end joining (mmNHEJ). Error-prone DSB repair mediated by the mmNHEJ pathway can promote the formation of somatic copy number variations (CNVs) that in turn can promote cancer formation and progression. In their review, Lisa Wiesmueller and colleagues define CNVs, describe

mechanisms of CNV formation and detail the latest technologies for CNV detection and analysis. Further, they summarize the latest data on CNVs with regard to breast cancer susceptibility genes and breast cancer biology.

Another critical aspect of genome stability involves the maintenance of telomere length. Telomeres are DNA-protein structures composed of (TTAGGG)n sequence repeats in vertebrates that protect chromosome ends and prevent the eventual loss of coding DNA due to the end replication problem. Maintenance of telomere length occurs via expression of the enzyme telomerase or in a telomerase-independent manner termed alternative lengthening of telomeres (ALT). This review, by Joanna Groden and colleagues, summarizes recent clinical data and findings in mammalian cells that identify the genetic mutations permissive to ALT, the DNA repair proteins involved in ALT mechanisms and the importance of telomere maintenance mechanisms for tumor progression.

Model systems such as bacteria (*Escherichia coli*), yeast (*Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*), the fruit fly (*Drosophila melanogaster*) and the nematode (*Caenorhabditis elegans*) have provided significant advances in our understanding of DNA repair, DNA lesion bypass and DDR mechanisms. Recently, zebrafish (*Danio rerio*) have emerged as a versatile model organism for the study of DNA repair and the DDR for both gene identification and discovery as well as a model for investigating the role of individual DNA repair genes in development, cancer formation and chemotherapy response. In this timely and thorough review, Phyllis Strauss discusses this model system, emphasizing that the zebrafish genome contains nearly all of the genes involved in the different DNA repair pathways in eukaryotes, including direct reversal (DR), MMR, nucleotide excision repair (NER), BER, HR, nonhomologous end joining (NHEJ) and TLS. Further, they introduce recent work on DNA damage and DNA repair studies in zebrafish, with special emphasis on the role of BER in zebrafish during early embryological development.

Pluripotent cells, organ-specific stem cells, somatic proliferating cells and post-mitotic cells are all subject to genotoxic insult. The resulting genomic DNA damage triggers a set of signaling events known collectively as the DDR, initiating DNA repair processes, facilitating cell cycle arrest and depending on the extent of damage, triggering the onset of cell death or senescence. However, emerging evidence indicates that DNA repair and the DDR function differently in different cellular contexts, with the expectation that stem cells are likely to address DNA damage differently from their somatic counterparts. In this extensive review, Eugenia Dogliotti and colleagues detail information on the common and distinct mechanisms controling genome integrity that are utilized by different cell types along the self-renewal/differentiation program, with special emphasis on their roles in the prevention of aging and disease.

The goal of this themed issue has been to provide a broad view of the many ways that genomic stability is controlled within the cell. Human DNA repair disorders are just beginning to be defined in depth, and already many patients with inherited and sporadic cancers involving DNA repair defects are now in clinical trials using targeted agents that can exploit these defects for selective or preferential response. What is very clear, however, is that an enormous amount of basic research remains to be accomplished to better understand the biochemistry and molecular biology of our DNA replication, DNA repair, and genome maintenance functions before fully realizing the many clinical opportunities on the horizon.