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Current Advances in DNA Repair: Regulation of Enzymes and Pathways Involved in Maintaining Genomic Stability

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Abstract

Novel discoveries in the DNA repair field have lead to continuous and rapid advancement of our understanding of not only DNA repair but also DNA replication and recombination. Research in the field transcends numerous areas of biology, biochemistry, physiology, and medicine, making significant connections across these broad areas of study. From early studies conducted in bacterial systems to current analyses in eukaryotic systems and human disease, the innovative research into the mechanisms of repair machines and the consequences of ineffective DNA repair has impacted a wide scientific community. This Forum contains a select mix of primary research articles in addition to a number of timely reviews covering a subset of DNA repair pathways where recent advances and novel discoveries are improving our understanding of DNA repair, its regulation, and implications to human disease. *Antioxid. Redox Signal.* 14, 2461–2464.

Introduction

The numerous mammalian DNA repair pathways serve to protect chromosomal DNA from a variety of insults ranging from DNA mismatches, abasic sites, bulky adduct damage, single-strand and double-strand breaks (DSBs). The different types of damage require different mechanisms for repair. Although there are common mechanisms and components shared between pathways, the different DNA damage and discontinuities bring myriad of possibilities and challenges for accurate repair. Adding to the complexity of DNA repair is that many of the events that induce DNA damage include a component of oxidative stress. DNA DSBs induced by ionizing radiation (IR) are often confounded with reactive oxygen species (ROS) inducing base and sugar damage to the DNA (10, 15). Radiomimetic agents including the chemotheraputic bleomycin also induce oxidative stress to generate single-strand breaks and DSBs (6, 17). Overwhelming the DNA repair systems is a common mechanism employed to treat cancer. The chemotherapeutic agent cisplatin, which forms intra- and interstrand DNA adducts, also induces significant oxidative stress (9, 13, 18). It is therefore not surprising that a subset of DNA repair proteins have been demonstrated to be regulated by redox conditions. Discovery of the mechanism of these regulatory events hold great potential for impacting our understanding of cellular stress responses, regulation of enzymatic activity, and how numerous cancer therapies impart their activity.

DSB Repair *via* Nonhomologous End Joining

One of the more challenging DNA repair pathways is the nonhomologous end joining (NHEJ) DSB repair pathway. This pathway is responsible for repairing IR-induced DNA DSBs, which can often include a wide variety of DNA adducts and discontinuities surrounding the DNA DSB (10). Cellular NHEJ has been studied by measuring repair after IR induced damage, though the limitations involved in these analyses revolve around the complexity of the IR-induced damage response and the inability to target the breaks to specific regions or sequence of DNA. This has been overcome by the use of rare restriction enzyme sites integrated into genomic DNA and induced expression of the restriction enzyme to generate a DSB, the repair of which is monitored by a reporter gene that is only expressed after NHEJ catalyzed rejoining (14, 22). Although this elegant approach solves the targeting issues, the systems employed thus far fail to recapitulate the complexity of an IR-induced break or the oxidative conditions that accompany IR treatment. We have demonstrated that the DNA binding activity and structure of Ku, an essential component of the NHEJ pathway, is regulated by redox conditions (1). These data corroborate other reports that NHEJ is influenced by cellular redox conditions (2, 5, 20) and thus further development of cellular NHEJ assay systems is necessary to accurately recapitulate the cellular repair of IR-induced DNA DSBs. NHEJ has been reconstituted *in vitro* using purified proteins and synthetic DNA substrates mimicking simple

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DSBs (3) and only in a few cases included complex lesions (4, 11, 15). The processing of complex lesions in NHEJ requires DNA polymerases in the Pol X family, and the review by Ramsden (19) highlights a number of DNA polymerases and their important role in NHEJ. Specifically, this review focuses on DNA polymerases in the Pol X family, detailing the structural features, biological roles, polymerase fidelity, DNA diversification, and polymerase structure and function. Briefly, Pol X family polymerases share similar structures, including a 30 kDa domain required for DNA synthesis. LigD, one discussed polymerase found in mycobacterium, contains both a polymerase and a ligase domain. Data discussed demonstrate that deletion of the polymerase domain still renders the ligase domain active. Genomic diversification, aided by NHEJ polymerases, occurs by polymerase TdT, which performs most of the template-independent additions introduced during V(D)J. The final section of the review focuses on the different structures and function of DNA polymerases. Since DSBs contain ends that can be highly damaged the NHEJ polymerases run into a number of challenges, including end bridging and DNA sequences protruding from the 3′ or 5′ overhang, all of which are overcome by the diversity of the NHEJ DNA polymerases.

Pawelczak *et al.* (16) continue the focus on the NHEJ pathway but highlight the importance of DNA chemistry and sequence in end-joining enzymology. Detailed in this article is the end processing activity of the NHEJ nuclease Artemis and the importance of DNA-PK, the trimeric complex consisting of Ku70, Ku80, and DNA-PKcs. Briefly, DSBs induced by exogenous agents such as IR do not result in easily ligatable blunt ends. IR exposure results in the significant generation of ROS, which result, in base and sugar damage to DNA. The resulting damage is highly complex and requires processing before joining of the DNA DSBs. Detailed are the four main steps to NHEJ, which include damage recognition, synaptic complex formation, DNA end processing, and ligation. The authors propose a series of innovative models that provide the structural framework for further defining the roles of proteins in the NHEJ pathway. *cis*-Activation of DNA-PK and *trans*-activation of DNA-PK are two initial models proposed detailing the end processing step of NHEJ by Artemis. The *cis*-activation of DNA-PK results in Artemis-mediated *trans* cleavage; that is, DNA-PK is activated by a DNA termini on which it sits, whereas the Artemis protein cleaves the DNA termini associated with a second DNA-PK molecule. The *trans*-activation of DNA-PK, activation of the trimeric complex from a DNA termini associated with a second DNA-PK complex, results in *cis* cleavage by Artemis. This *cis* cleavage implies that Artemis cleaves the DNA termini of the DNA-PK complex opposite of the one it is on. Another proposed model focuses on the association of DNA termini through the interaction of two Ku80 molecules, specifically the C-terminal region of the Ku80 molecules on two different pieces of DNA. This model demonstrates the importance of the C-terminal region flexibility in bridging two DNA termini. Although the details of NHEJ remain elusive, this review highlights major findings of the pathway and proposes a number of models consistent with the existing data.

Excision Repair

The excision repair pathways share common features in basic repair mechanisms that involve recognition of the DNA damage site, excision of the damaged bases followed by resynthesis of new DNA, and ligation to complete the process. The base excision repair (BER) and nucleotide excision repair (NER) pathways respond to specific DNA damage that result from chemical modification of DNA, whereas the mismatch repair pathway responds to normal bases that are part of noncanonical DNA structures, including DNA mismatches and insertion/deletion loops. BER is the pathway known to repair DNA base damage caused by ROS. ROS can be generated from IR exposure, as mentioned above, in addition to ultraviolet (UV) light and endogenous metabolic products. The review from Sobol's group (21) delineates the subpathways associated with the BER pathway that become initiated by a number of different glycosylases. Although these glycosylases are necessary for BER initiation, the chemical intermediates yielded vary. The review details the macromolecules that can be damaged by ROS and states that the resulting DNA damage includes nucleotide base modifications, AP sites, and single-/double-strand DNA breaks and crosslinks. The different types of lesion induced by ROS are all repaired *via* BER, which is composed of three basic steps: lesion recognition/strand scission, gap tailoring, and DNA synthesis/ligation. The authors discuss three variations to this general model and states that the most common subpathway is initiated *via* bi-functional DNA glycolyases. The second BER subpathway is initiated by monofunctional glycolyases and initiates a short-patch BER pathway. Finally, bifunctional DNA glycosylases associated with β-elimination are detailed. Authors also briefly discuss mitochondrial BER and the role of BER in cancer, demonstrating the importance BER plays in medicine.

The NER pathway is known to repair bulky DNA adducts caused by a number of agents, including damage induced by UV exposure and the common chemotherapeutic cisplatin. Damaged DNA is recognized in the NER pathway *via* a trimeric protein complex consisting of Xeroderma Pigmentosum Group C (XPC), Rad23B, and Centrin 2. Together, this complex recognizes and binds to bulky DNA adducts initiating the repair of the damaged DNA. The trimeric complex recognizes a wide range of DNA lesions and the ability of this complex to recognize such a variety of damage remains elusive. Detailed in the primary research article by the Naegeli group (8) is XPC's ability to bind DNA, recognize damage, relocate to damage, and recruit additional NER proteins. The authors identified and mutated four conserved amino acid residues, previously demonstrated to be in contact with undamaged and damaged nucleotides surrounding a UV-lesion based on a cocrystal structure. All four conserved amino acids demonstrate a decrease in DNA binding activity and damage recognition, and most mutants had reduced repair capacity. Overall, the data provided in the article support the cocrystal structure previously proposed by demonstrating that the amino acids predicted to interact with DNA surrounding a UV-induced lesion do so and are necessary for efficient NER. Additionally, the amino acids that contact the DNA are responsible for the formation of the stable platform necessary for NER.

The primary research article by Earley and Turchi (12) also analyzes the NER pathway, and discusses human diseases associated with a number of deficiencies in different DNA repair pathways. Data presented in this article focuses on the detection of XPA protein and Replication Protein A (RPA) *via*

an enzyme-linked immunosorbent assay (ELISA)-based assay. Data demonstrate that binding of purified XPA and RPA to single or duplex DNA can be detected *via* the ELISA. Additionally, binding of the RPA-XPA complex can be detected on double-strand duplex platinated DNA. In addition to RPA and XPA, authors tested the ability to detect additional NER proteins, XPF, XPG, ERCC1, and TFIIH, from whole cell extracts. Interestingly, RPA was able to be detected in normal lung tissue samples and tumor tissue samples, suggesting the importance of this detection method for the presence of NER activity in carcinoma patients. Authors conclude that the ELISA is a technique capable of identifying the levels of proteins in tumor samples and using these identified levels to presume the DNA repair capacity of individual patients.

Coming full circle, Dr. Chou (7) reviews DNA polymerases that are essential for the replication of DNA. This review article highlights the importance of Pol eta, a polymerase with the ability to perform translesion synthesis. Pol eta is a unique polymerase that varies from typical high fidelity polymerases due to the lack of intrinsic 3′–5′ exonuclease proofreading activity. This lack of fidelity renders the polymerase capable of replicating DNA opposite of damaged DNA. Pol eta replicates not only DNA opposite of a cyclobutane pyrimidine dimer lesion, but also cisplatin and O_6-methylguanines adducts. Regulation of Pol eta has been identified to occur *via* alternative splicing and protein–protein interactions, in addition to monoubiquitination and phosphorylation. Blocking the unique replicative ability of Pol eta to perform this replication process leads to the possibility of targeting Pol eta as an anticancer target.

Conclusions

This Forum highlights novel research and discoveries in the DNA repair field, focusing on three main repair pathways: NHEJ, BER, and NER. Highlighted in the NHEJ section is the necessity of end processing and the complexity associated with DSB damage and repair. NHEJ polymerases and nucleases possess the ability to repair the most severely damaged DNA bases in the genome. Polymerase diversity plays an important role in overcoming the complexity of DSBs, especially the damage concentrated nearest the break. The nuclease Artemis and the trimeric complex DNA-PK are also highlighted for their essential role in NHEJ. A number of models relating the activation of DNA-PK and cleavage by Artemis are proposed by Pawelczak *et al.* that provide a potential framework for the processing of DNA DSBs. The excision repair section highlights the importance of both the BER and NER pathways. The BER pathway, essential for the repair of ROS, possesses three main subpathways, all of which are initiated by different glycosylases. Detailed in the review is the importance of these different DNA glycosylases all of which render variable chemical intermediates, while still following the three basic BER intermediate steps. The importance of damage recognition in the NER pathway is then discussed in the excision repair section. Although it is known that the trimeric damage recognition complex, XPC/Rad23B/Cen2, recognizes and binds to bulky DNA adducts initiating the NER pathway, the ability of this complex to recognize such variable DNA damage remains unclear. Naegeli's group delineates a two-step damage recognition process, involving an unstable intermediate followed by a stable platform XPC-DNA intermediate necessary for the initiation of the NER pathway. The excision repair section concludes with the novelty of the ELISA designed to detect essential NER proteins. This assay may be employed to characterize the levels of NER proteins, which may translate into the capacity of NER. Determining the efficiency of the NER machinery may affect the therapeutic regimen for a cancer patient. Finally, discussed in the Forum is the importance of DNA polymerases in DNA replication. Specifically, Pol eta, a translesion polymerase that lacks fidelity, is necessary for DNA replication opposite of a damaged base. Blockage of this polymerase has potential to be the target of anticancer agents that would decrease the number of errors incorporated into genomic DNA. Overall, this Forum details a number of important advancements made in the DNA repair field, ranging from the diversity of polymerases, nucleases, and delineation of damage recognition. The Forum also highlights the importance of end processing, repair pathway activity, and discusses the potential for new targets in the chemotherapeutic field, building yet another bridge between science and medicine.

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Abbreviations Used

BER = base excision repair
DSBs = double-strand breaks
ELISA = enzyme-linked immunosorbent assay
IR = ionizing radiation
NER = nucleotide excision repair
NHEJ = nonhomologous end joining
ROS = reactive oxygen species
RPA = replication protein A
UV = ultraviolet
XPC = Xeroderma Pigmentosum Group C

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