Frontiers of Mutagenesis and DNA Repair: A Workshop

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Abstract

The Chemical Pathology Study Section, Center for Scientific Review, NIH hosted a 1-day workshop in Ventura, California, in the winter of 2003. There were 32 invited participants including invited speakers. The workshop consisted of three sessions and focused on recent developments in our understanding of the molecular mechanisms of mutagenesis and DNA repair, namely, error-prone DNA polymerases, DNA repair models and DNA repair networks.

Introduction

The overall comprehension of how DNA damage, mutagenesis, and DNA repair processes occur and function is central to our understanding of the various steps in the processes leading to altered cellular function. These approaches are relevant to translational efforts for the diagnosis and treatment of cancer, as well as for other diseases and ailments that have been linked to DNA damage. For example, oxidative stress and DNA damage have been proposed to play a critical role in the pathogenesis of several disorders, including cancer, Parkinson’s disease, Alzheimer’s disease, and even aging. At the molecular level, the presence of DNA adducts in the genome may ultimately lead to a permanent loss in the fidelity of information transferred from DNA to proteins. The latter situation could give rise to the inhibition of protein expression or to the expression of defective proteins that may eventually lead to either transformed cells or cell death. Therefore, our understanding of the basic mechanisms of DNA damage and repair is crucial for determining translational applications to these problems.

Error Prone Polymerases

In the first session, the speakers discussed mutagenesis with a particular emphasis on the emerging family of error-prone DNA polymerases. It was appropriate that Dr. Lawrence Loeb (University of Washington), a leader in the mutagenesis field for many years, gave the first talk in this session. Not only has Dr. Loeb contributed numerous important and innovative concepts to this area such as the role of genetic instability in cancer formation, but he has also trained many outstanding investigators, two of whom, Drs. Thomas Kunkel and Kandace Williams, were also speakers in this workshop. In his presentation entitled “Random and sequential mutagenesis of DNA,” Dr. Loeb described in vitro and in vivo mutagenesis approaches to generate enzymes with altered biochemical properties. For example, active site mutants of Thermus aquaticus and Escherichia coli DNA Polymerase I (Pol I) were generated by insertion of random nucleotide sequences into one conserved active site (motif A) and identified by screening for genetic complementation. These studies revealed that this active site of both polymerases is remarkably tolerant of mutations with the resultant amino acid changes generating different enzymes with altered biochemical properties. The remarkably similar mutations in each DNA Pol I may indicate evolutionary mechanisms of survival during altered environmental conditions. For a more detailed examination of in vivo mutagenesis, a DNA Pol I-deficient E. coli strain transformed with an error-prone version of Pol I was used. An attractive feature of this approach is that in vivo mutations in saturated cultures can be targeted to a specific plasmid reporter gene and are expected to result only from activity of the error-prone Pol I. Studies isolating mutants of β-lactamase that confer resistance to the monobactam, aztreonam, were described in which mutation specificity was exceedingly high, in addition to an almost complete lack of silent mutations. This system demonstrates that enzyme alteration can be both targeted and evolutionarily driven. This new system offers enormous potential for studying the effects of enhanced mutagenesis and for selecting enzymes with improved and/or novel properties.

Next, Dr. Myron Goodman (University of Southern California) presented insights into the mechanism of lesion-bypass synthesis by E. coli Pol V, which is encoded by the Umuc and Umud genes. These genes were initially isolated because they are required for UV-induced mutagenesis. Dr. Goodman recalled the contributions of the late Hatch Echols that led to the discovery of his laboratory, in collaboration with Roger Woodgate, that the Umuc/Umud complex (Pol V) has intrinsic DNA polymerase activity. Earlier models of lesion bypass synthesis had suggested that this process involved a functional interaction between the translesion DNA polymerase and the nucleoprotein filament formed by RecA protein. Recent results that challenge these models were presented. It is now proposed that there are functional interactions between Pol V and monomeric RecA that contribute to both DNA synthesis on an undamaged template and synthesis past a DNA lesion. Furthermore, it was suggested that DNA synthesis by Pol V in conjunction with the single-strand binding protein, SSB, may physically displace RecA monomers RecA nucleoprotein filaments assembled on the single-strand DNA template ahead of the DNA polymerase. Finally, Dr. Goodman described coculture experiments with E. coli strains lacking one or more of the error-prone DNA polymerases. These studies revealed an unexpected and as yet poorly defined contribution of the error-prone DNA polymerases in enhancing the fitness of a strain, as measured by the ability to outgrow another strain when cocultured.

The final speaker in this session was Dr. Thomas Kunkel (National Institute of Environmental Health Sciences), who focused on the base selectivity of template-dependent human DNA polymerases. Dr. Kunkel has made many important contributions to our understanding of the molecular mechanisms that govern the fidelity of DNA synthesis and has extended these studies to the recently discovered family of error-prone DNA polymerases in human cells that have the ability to copy past DNA lesions but also make a large number of mistakes when copying undamaged DNA. He highlighted structural features of the Pol Y family of DNA polymerases that appear to underlie the ability of these DNA polymerases to accommodate DNA templates
containing lesions into their active site, and he discussed how this altered active geometry may account for the low fidelity of synthesis on undamaged templates. Next, Dr. Kunkel summarized our current understanding of what these error-prone DNA polymerases do. In the case of XPV error-prone DNA polymerase, which is defective in the variant form of the cancer-prone human disease xeroderma pigmentosum, its cellular function, to copy past T-T pyrimidine dimers, has been demonstrated by genetic and biochemical studies. By contrast, many of the other enzymes do not have a defined cellular function. On the basis of the types of errors made by Pol η and Pol λ, he presented a model in which these enzymes may contribute to somatic hypermutation of immunoglobulin genes. Additional work is needed to identify associated factors that may modulate the DNA synthetic activity of these polymerases and to delineate the mechanisms by which they are recruited to their sites of action but are prevented from copying undamaged DNA. At present, several of the genes encoding error-prone DNA polymerases have been inactivated by gene targeting, but this approach has not been particularly informative because gene inactivation has frequently caused embryonic lethality. Finally, Dr. Kunkel described a human disease, progressive external ophthalmoplegia, that is associated with a mutant form of mitochondrial DNA polymerase γ that has reduced catalytic efficiency and fidelity.

**Repair Models**

The second session focused on several experimental models for investigating DNA repair processes. The first speaker was Dr. Rodney Nairn (M. D. Anderson Cancer Center) who described some recent studies performed in a unique model for melanoma, *Xiphophorus* (platyfish-swordtail) interspecies hybrids. This animal model was first studied in the early 20th century, and seminal genetic experiments using *Xiphophorus* hybrids were fundamental to the oncogene-tumor suppressor gene concept. Dr. Nairn and colleagues have exploited a particular *Xiphophorus* hybrid model first described in 1993 by Richard Setlow et al. (1) in which UV irradiation of newly born fish induces melanomas. In this experimental system, a sex-linked oncogene, *Xiphophorus melanoma receptor kinase*, and an autosomal tumor suppressor gene, *Diff*, define a model in which melanoma-susceptible hybrids inherit a copy of the sex-linked *Xiphophorus melanoma receptor kinase* oncogene from the pigmented parent and are also homozygous for the *Diff* tumor suppressor gene from the nonpigmented hybrid parent. They have determined that only the upstream regulatory nucleic acid sequences are very different among identical *CDKN2X* alleles from the different species.

Recent studies were described in which different promoters of *CDKN2X* alleles, one from each of the parental fish species, were used to drive reporter genes after transfection into two different *Xiphophorus* cell lines. One cell line was derived from a platyfish-swordtail melanoma and the other from an embryonic cell line. Expression from *CDKN2X* promoters was significantly greater (>3-fold) in the melanoma-derived cells (which also overexpress the *Xiphophorus melanoma receptor kinase* oncogene). Analysis of promoters using deletions and site-specific mutagenesis (*i.e.*, targeted to the CR1 sequence found adjacent to *Mitf* binding sequences and a SP transcription factor consensus sequence) indicated that these were important regulatory sites. Interestingly, the SP consensus sequence was perfect in the nonpigmented species but differed by a one-nucleotide change in the pigmented species. When altered in the pigmented species promoter by site-directed mutagenesis to a nonpigmented species consensus sequence, the reporter was down-regulated in the fish cell lines. This result suggests that the pigmented species allele may not be subject to negative regulatory signals, consistent with other findings showing significant (>10-fold) overexpression of the pigmented species *CDKN2X* allele compared with the nonpigmented species allele in melanomas. A model for the putative role of *CDKN2X* in melanomagenesis was presented to explain how the G1-S checkpoint could be deranged in melanocytes by insufficient expression of *CDKN2X* in fish that are melanoma susceptible. This model was developed by Dr. Nairn and colleagues to address the hypothesis that *Xiphophorus melanoma receptor kinase 2* provides strong growth signals upstream from *CDKN2X* (and other cell cycle regulators) and that these signals provoke early malignant changes in melanocytes when the regulation of specific cell cycle parameters is defective.

Dr. Winfried Edelmann (Albert Einstein College of Medicine) then presented an interesting and unique mouse model for the study of DNA mismatch repair (MMR) proteins and cancer. Dr. Edelmann has generated two new mouse lines that carry precise knock-in mutations in murine *MutS* homologues (*Msh*). The first mouse line he discussed carries a *G674A* missense mutation located in the conserved P-loop domain of *Msh2*. This site is located in the COOH-terminal portion of this gene and contains the ATPase domain, characterized by the Walker ‘type A’ motif and therefore responsible for ATP binding and hydrolysis. The second mouse line carries a *Msh6*T1217D missense mutation located in the interacting domain for Msh2-Msh6 heterodimeric interaction.

Both of these knock-in mouse models demonstrate significant differences in phenotype from wild-type mice and from the MMR knockout mouse models previously generated by Dr. Edelmann’s research group and by others. For example, although the *Msh2G674A* knock-in embryonic stem cell extracts exhibit wild-type binding affinity to an oligomer containing a G:T mismatch, the extracts do not exhibit the wild-type reduction of G:T binding affinity in the presence of ATP. Furthermore, although MMR activity within these mutant cell extracts is deficient, the cisplatin-induced apoptotic DNA damage response is similar to that which occurs in wild-type cells. Overall survival of the *Msh2G674A* mice is prolonged beyond the homozygous *Msh2* null mouse model by a few months; however, all knock-in mutant mice are dead by 12 months of age. These results indicate that the DNA damage-induced apoptosis function of Msh2 can delay but not prevent tumorigenesis.

Recent studies have also been directed toward characterization of the *Msh6*T1217D mouse model. Dr. Edelmann and colleagues have established that tumors derived from these mutant mice have high microsatellite instability, unlike the homozygous *Msh6* null mouse model. The mutation frequency of this mutant knock-in mouse model is also high, with heterozygous *Msh6*T1217D/+ mice displaying intermediate mutation frequency between wild-type and homozygous *Msh6*T1217D/T1217D mice. These ongoing studies demonstrate different functions of individual MMR proteins and the ability to separate these functions for further study by precise mutational targeting.

The final presentation in this section was by Dr. Kendace Williams (Medical College of Ohio), who also studies MMR using a human cell culture system. The focus of her investigations is to understand how MMR and other DNA repair systems may contribute to mutagenic hot spots. Dr. Williams demonstrated that although the hMutS complex (hMsh2 and hMsh6) within both HCT 116 (hMLH1−/−) and HCT 116 + Ch. 3 (hMLH1+) nuclear extracts bind equally to an oligomer containing a G:T mismatch at the H-ras codon 12 hot spot of mutation, there is no discernable binding affinity for a G:A mismatch at this same location by either the MMR-proficient or the MMR-deficient nuclear extract in all experimental conditions tested. Studies by her research group demonstrated that hMsh2 and hMsh6 can be equally coimmunoprecipitated from both the MMR-proficient and MMR-deficient human nuclear extracts with or without the presence of a DNA mismatch. However, addition of 1.5 mM ATP results in decreased coimmunoprecipitation of these heterodimeric proteins.
only from the MMR-deficient human cell extract, indicating a potential role for the hMutLα complex as a stabilization factor for the hMutSα complex. Results of a MMR assay from the MMR-proficient nuclear extracts demonstrate increased efficiency of correct MMR of G:T→G:C located at H-ras codon 12, with significantly less efficient correct repair of G:A→G:C, corresponding well with a lack of binding affinity for this mismatch within the nuclear extracts. Rather, MMR-proficient nuclear extracts are 1.5 times more likely to incorrectly repair G:A→T:A than to correctly repair this mismatch, which may be because of the activity of a different DNA repair system for this particular mismatch at this oncogenic location. MMR-deficient nuclear extracts demonstrate the expected reduced ability to repair either mismatch, correctly or incorrectly. These investigations are ongoing at a non hotspot of mutation, as well as at different phases of the cell cycle, to more clearly define the various roles of MMR proteins during different stages of the cell cycle and during initiation of human cancer.

Repair Networks

The final session had two talks that analyzed the interactions between the various DNA repair pathways and subpathways. Dr. Phil Hanawalt (Stanford University), who codiscovered excision repair four decades ago, presented data on the “Regulation of the subpathways of nucleotide excision repair.” He discussed our current biochemical understanding of the two subpathways of nucleotide excision repair: global genomic repair (GGR), and transcription-coupled repair (TCR), which is selective for the transcribed DNA strand in expressed genes. Some of the proteins involved in the recognition of DNA damage (including RNA polymerase) are also responsive to natural variations in the secondary structural features of DNA. Gratuitous repair events in undamaged DNA might then contribute to genomic instability. However, the damage recognition enzymes for GGR are normally maintained at very low levels unless the cells are genomically stressed. Hanawalt et al. have shown that GGR is controlled through the SOS stress response in E. coli and through the activated p53 tumor suppressor in human cells. The up-regulation of the uvrA and uvrB genes in bacteria and the p53-dependent expression of p48 (DDB2) in human cells are required for the efficient recognition of UV-induced cyclobutane pyrimidine dimers. Dr. Hanawalt presented microarray results from UV-irradiated cells and pointed out some of the significant up-regulated genes in DNA repair and recombination. The p53-dependent inducible responses in human cells were shown to be important because they operate upon chemical carcinogen DNA damage (e.g., benzo(a)pyrene and benzo(g)chrysene DNA adducts) at levels to which humans are environmentally exposed. Interestingly, most rodent tissues are deficient in the p53-dependent GGR pathway. Because rodents are used as surrogates for environmental cancer risk assessment, it is essential that we understand how they differ from humans with respect to DNA repair and oncogenic responses to environmental genotoxins. In the case of terminally differentiated mammalian cells, Hanawalt described a new paradigm in which GGR is attenuated, but both strands of expressed genes are repaired efficiently. These findings may have important implications for the fate of neurons in Alzheimer’s disease as they attempt to reenter the cell cycle after accumulating DNA damage for many years in dormant regions of the genome.

The final presentation in this session, presented by Dr. Mark R. Kelley (Indiana University School of Medicine), focused on translational applications of DNA repair, particularly the study of DNA base excision repair and ovarian cancer. Dr. Kelley’s research is directed toward inhibiting the repair and/or redox function of the human apurinic/apyrimidinic (AP) endonuclease (APE1) to increase the sensitivity of ovarian tumor cells to chemotherapy (alkylators and cisplatin) and ionizing radiation. APE1 contributes to the repair of DNA damage formed by alkylators, agents that oxidize DNA and by ionizing radiation. APE1 hydrolyzes the phosphodiester backbone immediately 5’ to an abasic (AP) site. However, APE1 is a multifunctional protein that functions also as a redox factor maintaining transcription factors in an active reduced state. APE1 has been shown to stimulate the DNA binding activity of numerous transcription factors that are involved in cancer promotion and progression such as Fos, Jun, NFKB, PAX, HIF-1α, p53, and others and has been shown to interact with Ku70/80, which is involved in double-strand break repair. APE1 has also been shown to interact, in vitro, with Ogg1, MYH1, Nth1, XPG, and others. Dr. Kelley hypothesizes that the inhibition of APE1’s repair and/or redox function will increase the efficacy of currently used chemotherapeutic agents in ovarian (and other) cancer treatments. He has demonstrated that treatment of a variety of ovarian cancer cell lines with APE1 siRNA sensitizes the cells to killing by cancer chemotherapeutic agents. Data were also presented relating to the targeting of APE1 siRNA to ovarian tumor using folic acid-derivatized liposomes as well as tumor-specific promoters to enhance tumor specificity and safety of this approach. Finally, a small molecule inhibitor of the redox function of APE1, but not its repair activities, was shown to enhance cell killing. In conclusion, the overall significance of this works relates to the ability to imbalance the base excision repair pathway in tumor cells increasing their sensitivity to chemotherapeutic agents using inhibitors of both repair and redox functions of the APE1 enzyme.

Conclusions

This workshop focused on the frontiers of knowledge within several important areas of investigation in regard to DNA mutagenesis and repair. The first session focused on error-prone polymerases and yielded some surprising insights. Although the role of each recently discovered error-prone DNA polymerase in human cells has not yet been elucidated, E. coli model systems demonstrate that not only do these type of polymerases confer growth advantages over strains that do not express them but they can also be used to rapidly drive targeted mutations evolutionarily required for survival. The second session, focusing on repair models, contained enlightened examples of how classic genetic models can be reexamined for important mechanistic insights. The long-standing platyfish-swordtail interspecies hybrid (Xiphophorus) melanoma model of oncogene-tumor suppressor interaction has recently yielded exciting evidence of altered cell cycle regulatory sequences as the likely primary initiation event. Murine knockout models of MMR (and other DNA repair pathways) have been constructed by several investigators within the last several years but only recently has attention focused on knock-in models of missense mutations within specific functional domains. These models are now providing powerful tools to understand structure-function relationships of these important DNA repair complexes. Alternatively, although the fidelity of DNA replication and repair has been studied by a variety of different biochemical or genetic assays, there is still much left to understand in regard to differential interactions of DNA damage and repair at oncogenic hot spots of mutation. Recent advances demonstrate specific types of damage at sensitive sites, introduced at certain portions of the cell cycle, can present significant difficulties for the human cell to recognize and,
hence, to repair correctly. The final session of repair networks presented several innovative approaches to understanding these complex systems. The nucleotide excision repair pathway, composed of the GGR and TCR pathways, has only recently been discovered to have a finely tuned p53-dependent up-regulation of the GGR pathway in humans that can be triggered by bulky adduct-producing chemicals at low environmental levels. Intriguingly, the up-regulation of this DNA repair pathway, reminiscent of the *E. coli* SOS response, does not occur in rodents and is attenuated in terminally differentiated cells, despite adequate repair within both strands of actively transcribed genes. Finally, both the repair and redox functions of the base excision repair pathway enzyme, APE1, are being intensely scrutinized by state-of-the-art techniques to determine whether the manipulation of either function of this enzyme in ovarian cancer cells can help to sensitize these cells to chemotherapy. These studies represent an exciting model system for the translational application of basic DNA repair investigations in the clinic.

**References**


**Appendix**

List of key speakers:

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