NER of Bulky and Non-Bulky DNA Lesions in Nucleosome Environments

Polycyclic aromatic hydrocarbons (PAH) are byproducts of fossil fuel combustion and are present in our air, food and water; the presence of these genotoxic environmental carcinogens in our environment continues to be a hazard to human health. The PAH are metabolically activated to potent transient epoxide intermediates that react chemically with purine nucleobases that give rise to pre-mutagenic bulky DNA lesions. Another source of formation of genotoxic DNA lesions is the inflammatory response that produces reactive oxygen species (ROS) that also cause DNA damage. Such lesions, if not repaired by human DNA repair mechanisms, interfere with normal DNA synthesis and transcription by generating mutations and miscoded proteins that can initiate and promote the development of human cancers.

The human nucleotide excision repair (NER) apparatus in humans is an important cellular defense system that excises an astounding variety of structurally diverse DNA lesions. However, within the last decade, evidence has emerged that some DNA lesions are repaired very slowly, while others are entirely resistant to NER. However, much of this information has been obtained from NER experiments conducted with free DNA substrates, while in human cells the DNA exists in the form of chromatin where it is tightly wrapped around disk-shaped histone octamers called nucleosome particles (NCP). The NCPs are strung like beads on a DNA string that are densely packed in the nucleus. DNA lesions are known to be less accessible to NER (and other proteins) in the absence of chromatin remodeling activity. However, the DNA damaging reactive intermediates are molecules that are small molecules like the reactive oxygen species or polycyclic aromatic hydrocarbon metabolites, that can diffuse through chromatin more easily because of their small sizes and thus cause DNA damage.

The mechanisms of DNA repair in the context of chromatin and its nucleosome subunits is poorly understood and is currently a hot and expanding area of research. How DNA repair proteins gain access to DNA lesions positioned at different target sites in nucleosomes, and how the histone environment affects NER of different DNA lesions, is the focus of this project.

The nucleosomes consist on an octamer core particle that consists of four different histones H2A, H2B, H3, and H4 dimers. The DNA molecules are wrapped around the periphery of the disc-like octamer with each DNA strand coiled around the octamer, with the DNA backbone alternately facing the solvent environment, or the octamer protein surface with a periodicity of ten base pairs. In nature, the histones are post-translationally modified by modifying some of the amino acids by acetylation, phosphorylation, ubiquitination, methylation, etc., to alter the dynamic unwrapping and rewrapping of nucleosomes to allow for DNA replication, transcription, or DNA repair, among the many processes governed by post-translational modification (PTM) of histones.

NER of bulky and small-sized DNA lesions in nucleosomes. The first part deals with the recognition and repair of small-sized and bulky DNA lesions embedded in solvent-exposed (Out), or inward facing DNA lesions that are in contact with the nucleosomal octamer core. In this part we used opposite forms of nucleosomes: (1) nucleosomes prepared with recombinant histones without any modifications of the amino acids (Rec-NCPs), and native PTM NCPs with random post-translationally modified histones extracted from human HeLa cells. The objectives were to determine the impact of nucleosomes on the repair of two structurally very different but representative types of bulky and non-bulky DNA lesions depicted in Figure 1. (2) The second
part was focused on the kind of PTMs that facilitates the repair of DNA lesions in nucleosomes, the building blocks of chromatin.

I. Nucleotide Excision Repair and Impact of Site-Specific 5',8-Cyclopurine and Bulky DNA Lesions on Physical Properties of Nucleosomes.

The non-bulky 5',8-cyclopurine lesions (cdG and cdA) and bulky, stereoisomeric polycyclic aromatic benzo[a]pyrene diole epoxide-derived cis and trans-\(N^2\)-guanine adducts (BPDE-G) embedded in free double-stranded DNA are good substrates of nucleotide excision repair (NER) in human cell extracts. When embedded at the In or Out rotational settings near the dyad axis in nucleosome particles (NCPs) reconstituted either with recombinant histones (Rec-NCP) or with native histones extracted from HeLa cells (HeLa-NCP), the cyclopurine lesions are completely resistant to NER. The BPDE-dG adducts, located at the same NCP settings, are strongly resistant to NER in Rec-NCPs, but are good substrates of NER in HeLa-NCPs. The relative NER efficiencies in HeLa cell extracts of all four BPDE-G adduct samples in HeLa cell NCPs are reduced by a common factor of 2.2 ± 0.3 relative to the NER efficiencies in free DNA. The relative NER response of the bulky BPDE-dG adducts in HeLa-NCPs is approximately proportional to the NER efficiencies in free DNA, and are not directly correlated with the observed differences in the thermodynamic destabilization of HeLa NCPs, nor rotational settings, or hydroxyl radical footprint patterns. However, FRET fluorescence depolarization measurements reflect differences in NER efficiencies and transient unwrapping phenomena between Rec- and HeLa-NCPs. The multi-step NER mechanism is initiated by the binding of the DNA damage-sensing NER factor XPC-RAD23B to a transiently opened BPDE-modified DNA sequence. The latter must be at least 30 base pairs in length, the footprint of XPC-RAD23B. The results are consistent with the hypothesis that the overall NER efficiency depends on a competition between the NCP rewrapping and XPC-RAD23B binding rates.

The strong differences in NER activities between post-translationally modified and recombinant histone nucleosomes are consistent with the notion that the NER activities depend on the transient unwrapping and partial exposure of nucleosomal DNA sequences to the NER factor XPC-RAD23B. Electrophoretic mobility shift assays indicate that the octamer histone core particles are not evicted in the process. The complete NER resistance of the cyclopurine lesions is attributed to the relatively small physical size of the cdG and cdA lesions that is not significantly different from natural nucleotides. In contrast to the larger-sized and bulky BPDE-dG adducts, the 5',8-cyclopurine lesions do not sufficiently perturb the local DNA-
II. New insights into histones and post-translational modifications (PTM) that favor DNA repair by the NER mechanism.

Overview. We developed suitable approaches for extracting post-translationally modified histones from human HeLa cells, followed by extensive purification of native H2A, H2B, H3 and H4 histones. At the same time we obtained recombinant, non-modified histones from commercial sources that we selectively modified by chemical means to mimic specific post-translational modifications. The availability of different modified and unmodified recombinant histones, allowed us to synthesize nucleosomes with different combinations in order to pinpoint which of the four histones and which specific post-translational modifications favors the repair of DNA lesions by the nucleotide excision repair mechanism in human cell extracts.

In one set of experiments we prepared nucleosomes with the four native, randomly post-translationally modified native HeLa nucleosomes by removing, one type of native HeLa histone at a time, and replacing it by a recombinant histone. In this way, we were able to identify native H3 nucleosomes as the critical ingredient of the successful nucleotide excision repair activity in NER-active HeLa cell extracts. In these experiments, we prepared 147 base pair long DNA duplexes that contained a single cis-BPDE-dG adduct in the middle, and then incubated these constructs in NER-active HeLa cell extracts for up to 60 min. The cis-BPDE-dG adducts are excellent substrates of NER which is evident from the ladder of dual incision oligonucleotide products 25 – 30 base pairs in lengths. These short dual incision products are readily separated from the un-incised 147-mer DNA strands using electrophoretic mobility shift assays after loading the incubated samples onto denaturing polyacrylamide gels. Replacing the native H2A, H2B, and H4 HeLa histones by their recombinant counterparts in nucleosomes bearing cis-BPDE-dG adducts, does not significantly affect the yield of NER dual incision products. However, if the nucleosomal HeLa H3 histones are replaced by recombinant H3 histones, the NER activity is completely abolished; by contrast, swapping H4 histones in an analogous manner does not have any significant impact on the NER product yields.

It is well known that native nucleosomes in chromatin are post-translationally modified by methylation, ubiquitination, acetylation, phosphorylation, etc. We have discovered that acetylation of H3, but not any of the other types of PTMs present in human HeLa cells is critically important for successful NER of the bulky cis-BPDE-dG lesions. This is an important finding because it demonstrates that only acetylation is important in facilitating nucleotide excision repair of DNA lesions in nucleosomes. The next key step was to determine whether the same mechanism is operative at the chromatin level in human cells. However, this was beyond the scope of this project. Here we summarize the experimental evidence on which these important conclusions are based.

Methods. The single cis-BPDE-dG adduct were positioned in 147-mer DNA near the nucleosome dyad axis at the IN rotational setting (Figure 2). The nucleosome particles were reconstituted from this DNA and hybrid nucleosomes and recombinant or native histones H2A, H2B, H3 and H4 extracted from human HeLa cells. We used standard biochemical methods to extract and purify all four histones ('HeLa' histones) derived from normal HeLa S3 cells. The
unmodified histones, called recombinant (‘Rec’) histones without any PTMs, were purchased from commercial sources. Standard dialysis methods were used to assemble the NCP particles with different (hybrid) combinations of PTM HeLa and recombinant histones (Figures 3 and 4).

The DNA repair assay. The different nucleosome core particles containing single BPDE-dG adducts were incubated for 60 minutes in extracts from human HeLa cells (Figure 3). The ‘0 min’ lane shows the negative control samples before incubation. The NER dual incision repair products produced appear as the ladder of bands that correspond to the 24-30 nucleotide-long oligonucleotide excision products that are resolved by Electrophoretic Mobility Shift Assay (EMSA) methods. The un-incised 147-mer bands are represented by the intense bands at the top of the gel. The ‘All four’ lane represents experiments with NCPs assembled with the four native histones extracted from HeLa cells. The Rec-H4 lane represents an experiment with NCPs assembled from a mixture of H2A, H2B, and H3 HeLa histones with Rec-H4 as the fourth histone. There is little difference in NER yields between the Rec-H3 and ‘All Four’ – HeLa NCP samples. However, when the HeLa-H3 histones are replaced by the recombinant Rec-H3 histone, the NER activity is totally abolished. This results clearly indicate that the post-translational modification of H3 histones plays the critical role in the repair of DNA lesion by the NER mechanism.

Additional results confirming this conclusion are shown in Figure 4. With NCPs prepared with recombinant histones only, the NER activity is negligibly small. Replacing the Rec-H2A and H2B histone pair by HeLa-H2A and H2B histones, yields the same negative result. Replacing Rec-H4 by HeLa-H4, also does not lead to the recovery of NER activity. However, substituting Rec-H3 with post-translationally modified HeLa-H3 restores the full NER activity, even though H2A, H2B, and H4 are all recombinant histones.

The results (Figure 4) not only confirm that the PTM of H3 is important for NER, but that the modification of only H3 is needed for observing full NER activity in HeLa cell extracts.
What remained to be shown is, which post-translational modification is the most important one in determining the NER response of the NCPs assembled from recombinant H2A, H2B, H4 and HeLa-H3 (Figure4).

Our initial guess was that either acetylation, or phosphorylation of H3 was responsible for the positive NER response of NCPs containing single BPDE-dG adducts. We therefore chemically acetylated or phosphorylated recombinant H3 (H3(Ac) and H3(P, respectively) using standard published protocols to generate the following NCPs.


The results are shown in Figure 5. The red bar shows the NER response of NCPs assembled with all four native HeLa-histones containing the single cis-BPDE-dG adduct. The bar in magenta depicts the NER response of NCPs with all four recombinant histones, but with H3 phosphorylated. The gray bar is the same kind of NCP, but with Rec-H3 acetylated. It is evident that, within experimental error, the NER response of the fully recombinant NCP with acetylated H3 exhibits the same NER response in removing the BPDE-dG adduct as the HeLa-NCP derived from native, post-translationally modified HeLa histones. **The important implications from these series of experiments is that the acetylation of H3 plays the key role in nucleotide excision repair of bulky adducts.**

**Conclusions.** We have pinpointed which of the many molecular parameters affect the repair of cancer-causing bulky DNA lesions by one of the primary cellular defense mechanisms, the nucleotide excision repair pathway. These studies were focused on a systematic approach to elucidate the nature of the parameters that influence the NER of two different classes of DNA lesions (bulky polycyclic aromatic hydrocarbon adduct and small-sized oxidatively derived guanine and adenine lesions) in nucleosomes, the building blocks of chromatin. Both classes of DNA lesions have been identified in human cells and mammalian tissues. The lack of repair of the non-bulky 5’,8-cyclopurine lesions in nucleosomes is a serious risk factor and threat to human health.

![Fig. 5. Effects of post-translational modification of Rec-H3 on NER activities of NCPs prepared with all four recombinant histones, but with H3 either phosphorylated (Rec-H3(P) or acetylated (Rec-H3(Ac)).](image)