275 TRANSCRIPTIONAL BYPASS OF MODIFIED GUANINE BASES AND UNPAIRED REGIONS OF DNA: BIOCHEMICAL AND MODELING STUDIES. *Dimitri A[1], Burns JA[1], Broyde S[1], Geacintov NE[1], Farley SA[1], Guengerich F[2], Rizzo C[2], Goodenough AK[2], Scicchitano DA[1].* [1]New York University, New York, NY, United States, [2]Vanderbilt University, Nashville, TN, United States.

This study examines the effect of small DNA lesions and DNA secondary structure on the behavior of human RNA polymerase II (hRNAPII) during transcription elongation *in vitro*. There is significant evidence that so-called bulky DNA adducts are able to impede transcription **(1)**. Here we focus on small DNA lesions: O^6-methylguanine (O^6meG), 5-guanidino-4-nitroimidazole (NIM), and 1-N^2-ethenoguanine (εG). Our results indicate that lesion bypass occurs during transcription by hRNAPII past O^6meG, while the enzyme stalls when facing NIM and εG. Each of these three guanine-derived lesions exhibits different hydrogen-bonding capabilities that affect their ability to pair with incoming nucleotides during transcription elongation and quite probably play a pivotal role in determining polymerase behavior. For example, O^6meG induces the incorporation of uridine opposite the lesion, which is consistent with the ability of O^6meG to hydrogen bond with uracil. Computer modeling using INSIGHT II will shed further light on the base-pairing events that occur within the enzyme's active site. DNA secondary structures have been implicated in mutational processes associated with trinucleotide-repeat diseases such as Huntington's. Here we also report the effect on transcription of a twenty-nucleotide, single-strand loop engineered into the DNA template strand. The results show formation of multiple truncated transcripts with lengths corresponding to the position where the single-strand loop resides in the template DNA, suggesting that the transcription complex stalls due to the presence of the loop. Further studies of various secondary structures will be pursued to establish the relationships among DNA structure, hRNAPII stalling, and DNA repair. The results for small guanine lesions and loops in DNA further support the idea that several factors influence the behavior of RNA polymerase II during transcription elongation past such structures. These include (1) the ability of the enzyme's active site to accommodate the lesion; (2) the identity of the incoming base; (3) the sequence context; and (4) the size, shape and stereochemistry of the lesion. **(1)** Scicchitano, D. A., Olesnicky, E., and Dimitri, A. (2004) *DNA Repair* 3, 1537.

276 BENZO(a)PYRENE (BP)-DNA ADDUCT REDUCTION IN THE PRESENCE OF CHLOROPHYLLIN (CHL) IS INDEPENDENT OF BP-METABOLIZING CYTOCHROME P450 INDUCTION IN HUMAN MCL-5 CELLS. *Divi RL[1], Orozco CC[1], Weston A[2], Poirier MC[1].* [1]National Cancer Institute, NIH, Bethesda, MD, United States, [2]National Institute for Occupational Safety and Health, CDC, Morgantown, WV, United States.

Exposure to polycyclic aromatic hydrocarbons (PAHs), environmental pollutants, is associated with DNA damage and cancer induction in exposed human populations. Studies have shown that xenobiotic DNA adduct formation is inhibited by CHL, and because this compound is a potential chemopreventive agent the underlying mechanisms are of great interest. Here, we evaluated DNA adduct formation induced by BP, and BP-activating enzyme expression and protein levels in the presence or absence of CHL. For this purpose, we used human lymphoblast (MCL-5) cells that express *CYP1A1, CYP1B1* and other cytochrome P450s. In cells treated for 24 hr with 4 :M BP there were 167 10β-(deoxyguanosin-N^2-yl)-7β,8α,9α-trihydroxy-7,8,9,10-tetrahydro-BP (BPdG) adducts/10^8 nucleotides, measured by immunoassay using antiserum elicited against 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-benzo[*a*]pyrene (BPDE)-DNA. Co-exposure with 4 :M BP and 4 :M CHL reduced the BPdG adduct level to 120/10^8 nucleotides, while 24 h of CHL pre-exposure reduced BPdG levels to 111/10^8 nucleotides, and in cells pre- and co-exposed with CHL had 83 BPdG adducts/10^8 nucleotides. Expression of *CYP1A1, CYP1B1 and CYP3A4,* determined by real time-polymerase chain reaction, was increased by 3.0-, 2.3- and 2.1-fold, respectively, in cells treated with BP alone. In cells treated with CHL alone, *CYP3A4* was down-regulated and the other genes were not altered. The combination of CHL and BP in the co- and pre-exposed groups resulted in 3- to 42-fold induction of *CYP1A1* and *CYP1B1*, while *CYP3A4* expression was unchanged. Western blot analysis revealed ~2-fold increase in CYP1A1 levels in cells exposed to BP alone, BP with CHL pre- and co-exposure, and CHL alone. CYP1B1 and CYP3A4 levels were also increased in cells exposed to BP with CHL pre- and co-treatments. Thus, the lowest BPdG adducts were formed in cells having 2-fold higher levels of CYP1A1, 1B1 and 3A4 proteins, compared to the unexposed controls. The CHL-induced reduction in BP-DNA adduct formation appears to be independent of the BP-activating enzyme levels examined here, and may involve additional mechanisms. The capacity of CHL to reduce BP-DNA adducts in human lymphoblasts suggests that CHL may be a useful chemopreventive agent in PAH-exposed humans.

277 DNA LESION-SPECIFIC INTERACTION AND CO-LOCALIZATION OF REPLICATION PROTEIN A (RPA) AND THE MRE11/RAD50/NBS1 (MRN) COMPLEX AT STALLED REPLICATION FORKS AND IN REPAIR FOCI. *Robison JG[1], Bissler JJ[2], Dixon K[3].* [1]University of Cincinnati, Cincinnati, OH, United States, [2]Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, [3]University of Arizona, Tucson, AZ, United States.

The DNA damage response, triggered by DNA replication stress or DNA damage, involves sensors that recognize DNA damage and transducers that activate downstream effectors of DNA repair and cell cycle checkpoints. Replication protein A (RPA) and the Mre11/Rad50/Nbs1 (MRN) complex appear to participate as both sensors and effectors in response to most types of DNA damage. After DNA damage, subunits of both protein complexes became phosphorylated (presumably by ATM and/or ATR) and these phosphorylated proteins could be detected together in nuclear foci by immunohistochemistry using phosphoprotein-specific antibodies. Interestingly, the extent of co-localization and phosphorylation of these proteins appeared to differ depending on the type of DNA damage. When DNA replication was stalled as a result of hydroxyurea-induced depletion of nucleotide precursors or the introduction of photo-dimers in DNA by UV radiation, RPA and MRN complexes were found together in nuclear foci. In contrast, when DNA replication was stalled due to the introduction of DNA crosslinks by mitomycin C, only the MRN complex and not RPA could be visualized in foci. These results are consistent with a model in which the participation of the two complexes depends on the structure of the DNA at the stalled forks. The response of the two proteins also differed when DNA strand breaks were induced with either camptothecin (CAMPT) or etoposide (ETOP). RPA and MRN co-localized at sites of CAMPT induced damage but not ETOP. With ETOP, either RPA or MRN foci were observed, but never in the same cell. However, RPA appeared to be necessary for formation of MRN foci. When the RPA-p70 subunit was depleted with siRNA, formation of MRN foci was reduced. Furthermore, when Mre11 was depleted with siRNA, RPA foci were observed, but the RPA-p34 subunit in the foci was not phosphorylated. These observations have lead to a model in which RPA is required for recruitment of MRN, which in turn activates ATM and/or ATR at the damage site. The amount of RPA that remains at the damage site is determined by the structure of the DNA lesion and/or the specific DNA repair pathway activated. References: J Biol Chem 279 (2004) 34802; J Biol Chem 280 (2005) 12927. [Supported by NIH grants R01-NS34782 to KD, and R01-DK061458 to JJB.]

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