113 **ANALYSIS OF THE ATR-MEDIATED DNA DAMAGE AND REPLICATION CHECKPOINTS IN XENOPUS EGG EXTRACTS.**
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To maintain genomic stability, cells depend on the DNA damage and replication checkpoints. ATR (ATM and Rad3-related) and the proteins in the Rad1 complex, Rad1, Hus1 and Rad9, are believed to be upstream components of these checkpoints. It is not understood how these proteins respond to a diverse range of DNA damaging agents, including ultraviolet radiation, ionizing radiation and alkylating agents. We have investigated the involvement of DNA replication in activation of the DNA damage checkpoint in Xenopus egg extracts. We find that DNA damage caused by ultraviolet radiation or methyl methanesulfonate slows replication in a checkpoint-independent manner and is accompanied by the accumulation on chromatin of ATR and components of the Rad1 complex. We also show that the replication proteins RPA and polymerase alpha accumulate on chromatin following DNA damage, suggesting that single-stranded DNA may accumulate. Importantly, we find that the accumulation of ATR, Rad1, RPA and polymerase alpha is blocked by geminin, suggesting that their binding is dependent on the initiation of DNA replication. In addition, several components of the Rad1 complex become phosphorylated after activation of this checkpoint. These phosphorylations are dependent on the kinase activity of ATR and on initiation of DNA replication, but they are not required for the association of the Rad1 complex with chromatin. Finally, we show that the damage-induced phosphorylation of Chk1 and checkpoint arrest are abrogated when replication is inhibited. Taken together, these data suggest that replication is required for activation of the DNA damage checkpoint and may provide a unifying model for ATR activation by diverse lesions during S phase.

114 **AN FDA PERSPECTIVE ON MODE OF ACTION IN MUTAGENICITY AND CARCINOGENICITY RISK ASSESSMENT.**
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Risk assessment plays a central role in FDA's responsibility to assure safety and efficacy of regulated products. Risk assessment is of two principal types: 1) qualitative or semi-quantitative assessments, involving weight of evidence decisions, risk-benefit judgments, and approval/disapproval actions, and 2) quantitative assessments, involving formal hazard identification, exposure assessment, and dose-response assessment to determine quantitative risk of adverse outcomes. Mode of action information can improve risk assessment at all stages. For example, differences in receptor-mediated characteristics or formation of active metabolites between laboratory models and humans may determine whether hazard exists. Knowledge of proximate toxic species aids appropriate exposure assessments, and differentiation between linear, non-linear, and threshold responses plays an important role in dose-response assessment. Current regulatory guidances that address mode of action information in carcinogenic and mutagenic risk assessment include: 1) upper dose level selection in carcinogenicity studies (25x clinical dose *vs.* MTD for agents with evidence of mutagenic activity) (ICH S1C), 2) selection of species and model system based on mechanistic knowledge (ICH S1B), and 3) mutagenicity testing requirements for initiating human testing of drugs based on initial screening (ICH M3). Current unresolved issues related to mode of action include characterization of "non-genotoxic carcinogens" and the importance of "indirect" mechanisms of mutagenicity related to high levels of cellular cytotoxicity. In each case, mechanistic knowledge needs to be related to the key question of whether a particular mode of toxicity observed in laboratory models can be reliably extrapolated to the human.

115 **ALTERED GENE EXPRESSION PATTERNS IN MCF-7 CELLS INDUCED BY THE URBAN DUST COMPLEX MIXTURE SRM 1649 MONITORED USING DNA MICROARRAYS.**
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Human exposures to polycyclic aromatic hydrocarbons (PAHs) occur in complex mixtures. In this study, gene expression patterns were investigated in MCF-7 cells exposed for 24h to Standard Reference Material (SRM) SRM 1649 alone or SRM 1649 plus either benzo[a]pyrene (BP) or dibenzo[a,l]pyrene (DBP). Gene expression was monitored using high density oligonucleotide arrays (Affymetrix U133A) representing more than 22,000 human genes and expressed sequence tags. Duplicate treatments displayed a high degree of reproducibility. Global analyses of the gene expression data revealed alterations of at least 2 fold change [signal log ratio (SLR) $\leq$ -1 or $\geq$ 1] in 120 RNA transcripts in response to SRM 1649 exposure. Increase in expression of CYP1A1 and CYP1B1 was observed in response to BP exposure (SLR of 6.5 and 2.8, respectively). An additive induction of CYP1A1 and CYP1B1 was observed with co-treatment of SRM 1649 and BP (SLR of 7.5 and 3.3, respectively). On the contrary, DBP alone did not show any change in expression of CYP1A1 and CYP1B1, however, co-treatment of SRM 1649 and DBP showed an increase in expression of CYP1A1 and CYP1B1 (SLR of 2.4 and 2, respectively). The effect of complex PAH mixtures on the metabolic activation of carcinogenic PAH by CYP enzymes were correlated with the results from gene expression studies. CYP enzyme activity was very similar to SRM 1649 alone in comparison with the co-treatment of SRM 1649 and BP. Thus, we conclude that the data not only provides a transcriptional signature for chemical carcinogen exposure but also suggests that a major factor in carcinogenic activity of PAH within complex mixtures is the ability of the complex mixture to promote or inhibit the activation of carcinogenic PAH by the induction of CYP metabolic enzymes.

116 **CLINICAL CONSEQUENCES OF INTRAUTERINE MUTAGEN EXPOSURES.**
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The susceptibility of developing humans to irreversible maldevelopment emphasizes the need for continued environmental surveillance and reduction of mutagen exposures. At levels producing maternal toxicity, ionizing radiation is embryolethal. Birth defects have been reported following single exposures 1 Gy or greater, but growth of the developing central nervous system continues to be compromised at doses 10-fold lower. Chemotherapy during pregnancy has similar effects. Chronic low level mutagen exposures such as to industrially polluted air appear to be radiomimetic, with head circumference at birth inversely correlating with levels of fetal PAH-DNA adducts. These responses are phenocopies of mendelian DNA repair deficiencies and emphasize the susceptibility of fetal brain to DNA damage. Ionizing radiation exposures as low as 0.01 Gy increase risks for childhood leukemia. Although maternal lifestyle and passive tobacco smoke exposures affect the frequencies of *HPRT, GPA* mutations in cord blood, it remains difficult to translate these results into risks for cancer. Maternal treatment with 3'-azido-3'-deoxythymidine (AZT) has raised concerns about targeting of mitochondrial DNA. Significant myopathy has been reported in some exposed infants. Genetic susceptibility also contributes to adverse outcomes. Animal models highlight the complexities of fetal and maternal xenobiotic metabolism and demonstrate how polymorphisms affecting bioactivation and detoxification can contribute to embryonic and fetal toxicity. In humans, induction of CYP1A1 in human placenta, which expresses fetal genotype appears to be protective against some birth defects but other genetic polymorphisms may increase risks for common anomalies such as cleft lip and cleft palate.