105 USE OF TEMPERATURE TO ENHANCE THE SENSITIVITY OF THE DROSOPHILA WING SPOT ASSAY.
Katz AJ[1]. [1]Biological Sciences, Illinois State University, Normal, IL 61790.

Eight mutagens (5-azacytidine, cisplatin, diepoxybutane, diethylnitrosamine, dimethylnitrosamine, ethyl methanesulfonate, methyl methanesulfonate, and 3-methylcholanthrene) were tested at 1-3 concentrations in the wing spot assay of *Drosophila melanogaster* at both 25 and 27°C to learn the effects of a 2-degree increase in temperature on the mutagens' capacities to induce genetic alterations. A total of 13 mutagen/concentration treatments were evaluated at the 2 temperatures. For each treatment, the mean total number of spots per wing observed at 27°C was divided by that observed at 25°C to form a ratio. The range in value for the 13 ratios was 1.000 - 4.375 with a median value of 1.444 and a mean value of 1.653 (SE = 0.245). Calculation of a weighted mean (using a weight of 1/SE) yielded a value of 1.520 (SE = 0.124). Mean total number of spots per wing observed at 27°C (Y) was regressed upon that observed at 25°C (X) and resulted in a significant linear regression ($Y = 0.616 + 1.535X$; $r^2 = 0.937$). The observed increases in genotoxicity at the higher temperature may likely be due to enhanced cellular uptake of the mutagens by the target cells and/or enhanced mutagen-DNA reaction kinetics. For the 8 mutagens evaluated in this study, elevating the temperature at which the wing assay is conducted from 25 to 27°C appears to yield an approximate 50% increase in the total number of spots observed per wing without requiring any additional costs or effort. If this observed increase in the sensitivity of the wing assay at 27°C is representative of what can be expected with other mutagens, it is recommended that the wing assay be routinely conducted at the higher temperature.

106 TRINUCLEOTIDE REPEAT EXPANSIONS AND EVOLUTION.
Keats BJB[1], Justice CM[2], Limprasert P[3], Deininger PL[4], Batzer MA[1]. [1]Louisiana State University Health Sciences Center, New Orleans, LA 70112. [2]National Human Genome Research Institute, Baltimore, MD 21224. [3]Prince of Songkla University, Thailand. [4]Tulane University Medical Center, New Orleans, LA 70112.

Unstable and expanded trinucleotide repeat tracts are associated with several genetic diseases including the spinocerebellar ataxias and Friedreich ataxia. In the dominant disorders, spinocerebellar ataxia type 1 (SCA1) and Machado-Joseph disease (MJD), expansion of a CAG repeat is found within the coding region of the gene. The size of the expanded allele in SCA1 is usually greater than 40 CAG repeats and may be greater than 80, while the most frequent normal sized alleles have about 30 repeats. However, some overlap has been reported, with both normal and affected individuals having 39 repeats. For MJD the alleles fall into two distinct classes: normal sized alleles are generally less than 40 CAG repeats and expanded alleles have more than 60 repeats. The recessively inherited disorder, Friedreich ataxia (FRDA), is associated with a GAA repeat expansion within the first intron of the gene. The most frequent normal allele size is 9 GAA repeats, while the expanded allele usually has several hundred repeats. In order to analyze normal variation in repeat size at each of these loci we studied approximately 700 chromosomes from European, African, and Asian populations. Allele sizes ranged from 9-37 CAG repeats at SCA1, 14-40 CAG repeats at MJD, and 5-25 GAA repeats at FRDA. Average heterozygosity was .80. Additionally we examined repeat size in other primates including the chimpanzee, gorilla, orangutan, mangabey, and rhesus macaque, and also rodents. The results contribute to our understanding of the evolution of trinucleotide repeats that are associated with disease.

107 DECREASED APOPTOSIS IN TUMOR CELLS AND TRANSFORMED CELLS INDUCED BY CADMIUM CHLORIDE AND BERYLLIUM SULPHATE.
Keshava N[1], Lin F[1,2], Whong WZ[1], Ong T[1]. [1]Toxicology and Molecular Biology Branch, National Institute for Occupational Safety and Health, Morgantown, WV 26505. [2]Department of Toxicology, West China University of Medical Sciences, Chengdu, China 610041.

Imbalance in the process of cell proliferation, differentiation and programmed cell death (apoptosis) is involved in carcinogenesis. It is postulated that inhibition of apoptosis may help altered cells escape cell death and acquire a tumorigenic phenotype. In our previous study, BALB/c-3T3 cells were transformed with either cadmium chloride ($CdCl_2$) or beryllium sulphate ($BeSO_4$). Transformed cells were injected into nude mice and tumor cell lines were established. In the present study, we have induced apoptosis in non-transformed, transformed and tumor cells induced by $CdCl_2$ and $BeSO_4$ using actinomycin D. Apoptosis was measured using terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling [TUNEL] assay and enzyme-linked immuno-sorbent assay [ELISA]. Immuno-staining indicates that there was increased apoptosis in the non-transformed cells compared to transformed cells, and that transformed cells had a higher percentage of apoptotic cells compared to tumor cells. Similar results were obtained in the ELISA assay. It is possible that the neoplastically transformed cells induced by $CdCl_2$ and $BeSO_4$ may have undergone molecular changes allowing them to bypass apoptosis or they may not have accumulated enough genetic damage to induce an apoptotic response. Further studies are in progress to investigate the involvement of apoptosis-related genes (Bcl_2, Bax, Bcl-x_L, Bak) in the process of transformation and/or tumorigenesis.

108 A MICROSCALE MICRONUCLUES TEST (MNT) IN VITRO SCREENING ASSAY WITH L5178Y TK+/- MOUSE LYMPHOMA CELLS TO PREDICT THE OUTCOME OF GLP-ASSAYS DETECTING CLASTOGENIC ACTIVITY.
Kirchner S[1], Albertini S[1], Chètelat AA[1], Gocke E[1], Muster W[1]. [1]Pharma Research Nonclinical-Development - Safety PRNS, F. Hoffmann La-Roche Ltd, CH-4070 Basel, Switzerland.

Screening assays capable to detectgenotoxic potential of new drug candidates in early phases of drug discovery will greatly help to improve the selection and optimization of lead compounds. Usually only limited amounts of a test compound are available in this phase and a large number of test chemicals has to be handled. To predict the clastogenic and aneugenic activity of potentially new drug candidates we have developed and validated a modified MNT *in vitro* protocol in our laboratory requiring very small amounts of test chemical (< 10 mg) and only a minimum of time (< 1 week). The predominant goal of the protocol is to scale down the amount of test material needed by reducing the volume of the incubation mix but keeping other changes to a minimum and thereby to allow best possible prediction of the outcome of standard GLP-assays needed for registration. For evaluation of micronuclei slides are stained with bisbenzimide H 33258 and visualized using fluorescence microscopy (the cytoplasm of individual cells is monitored by simultaneous use of phase contrast). Routinely relative cell counts are calculated to measure cytotoxicity and additional determination of the adapted relative survival allows comparing directly with cytotoxicity levels achieved in a corresponding ML/TK assay. Test strategies recommended by the outcome of the validation experiments and the value of this assay to predict clastogenic and aneugenic activity in the HCA and ML/TK assays will be discussed as well as comparison of toxicity levels between MNT in vitro screening assays and GLP assays.

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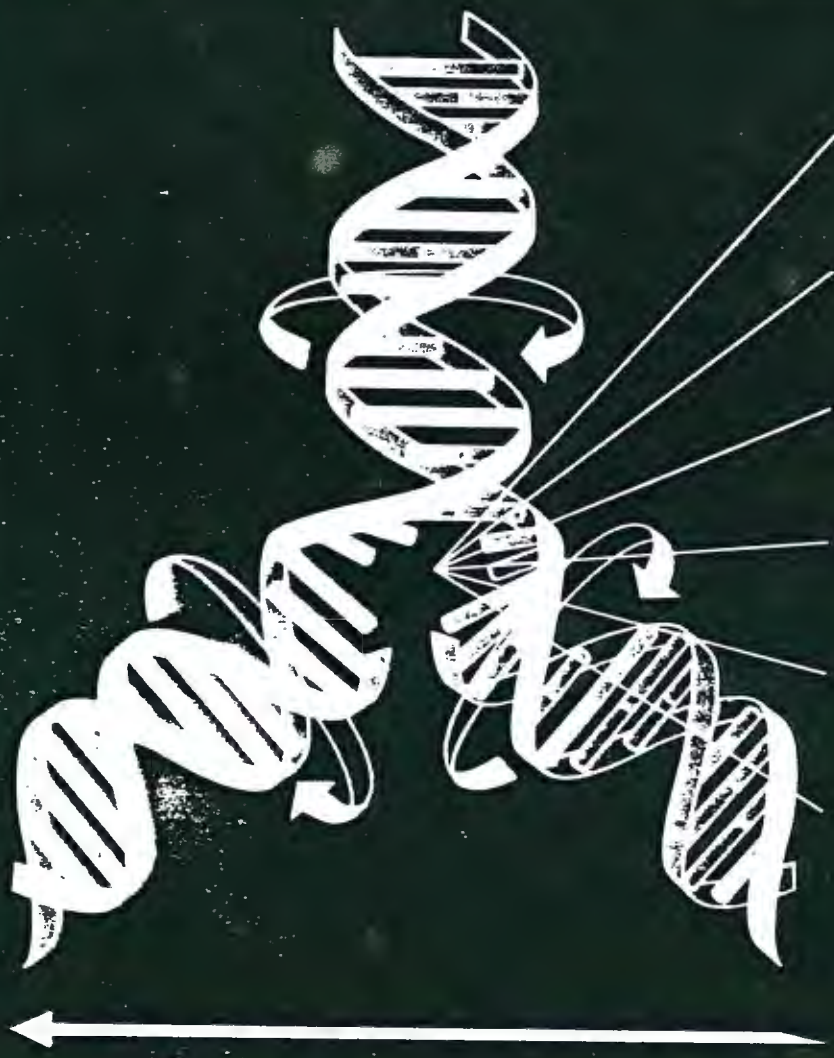

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