STATENS ARBEIDSMILJØINSTITUTT

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Tittel: Nordisk arbeidsgruppe for utvikling av screening-metoder for bestemmelse av individuell eksponering for mutagener og kreftfremkallende stoffer.

Forfatter(e):

Prosjektansvarlig: Åge Haugen

Prosjektmedarbeidere:

Utgiver (seksjon): Toksikologisk seksjon
Åge Haugen og Steinar Øvrebø

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Sammendrag:

Dette dokument er en rapport fra det 3. nordiske møtet for utvikling av screening-metoder for bestemmelse av individuell eksponering for mutagene og kreftfrenkallende stoffer. Nordisk Ministerråd har gitt finansiell støtte til prosjektet. I de nordiskeland pågår et aktivt forskningsarbeid innen biologisk monitorering. Det er opprettet en arbeidsgruppe med deltagere som representerer ulike forskningsinstitutter i de nordiske land. Gruppen har medlemmer fra Danmark, Finland, Norge og Sverige, 3 medlemmer fra hvert land. Arbeidsgruppen møtes årlig. I gruppens arbeidsoppgaver inngår:

- kartlegge det aktuelle kunnskapsnivå og forskningsbehov
- koordinere våre prosjekter hvor ulike metoder anvendes
- harmonisere metodene
- foreslå problemstillinger og forskningsprosjekter ved egne eller andre institutter

Stikkord: Nordisk samarbeid
Mutagener
Kreftfremkallende stoffer
Biomonitorering

Key words: Nordic collaboration
Mutagens
Carcinogens
Biomonitorering

III MEETING OF THE NORDIC STUDY GROUP ON DEVELOPMENT OF METHODS FOR DETERMINATION OF INDIVIDUAL EXPOSURE TO MUTAGENS AND CHEMICAL CARCINOGENS

Hotel Seurahovi, Porvoo, Finland

October 3rd - 5th, 1990

INNHOLDSFORTEGNELSE

1. OPPSUMMERING

Onsdag 03.10. - fredag 05.10.90 møttes arbeidsgruppen i Finland. Møtet ble avholdt på Hotel Seurahovi i Porvoo med 21 deltagere. Hovedemnene denne gang var nye metoder i biologisk monitorering og genetisk predisposisjon. I den forbindelse var det invitert to eksterne foredragsholdere, Jeffrey R. Idle og Peter Farmer fra U K. De holdt følgende forelesninger:

P. Farmer: New and alternative methods in biological monitoring.

J. Idle: Interindividual variation in carcinogenesis.

Deretter orienterte deltagerne om forskningsprosjektene. Både foredragene og diskusjonene fungerte meget bra. Mange emner og problemstillinger innen biologisk monitorering ble tatt opp og flere samarbeidsprosjekter er under utvikling både innen gruppen og med andre forskningsgrupper. Avslutningsmøtet for dette prosjektet arrangeres i Norge om ett år og det skal skrives en sluttrapport.

Medlemmene av prosjektets styringsgruppe er:

Aage Haugen, Statens arbeidsmiljøinstitutt, Oslo. (Ansvarlig leder av prosjektet).

Herman Autrup, Institutt for Miljø og Arbejdsmedicin, Århus.

Kari Hemminki, Institutt for Arbeidshygiene, Helsinki.

Christer Hogstedt, Arbeidsmiljøinstituttet, Solna.

Oslo, 1. februar 1991

Aage Haugen

2. PROGRAM

Wednesday 03.10.90

	Arrival
13.00-14.00	Lunch
14.00-14.15	Welcome, **K. Hemminki**
14.15-17.15	**WORKSHOP ON INTERINDIVIDUAL VARIATION IN CARCINOGENESIS** **Chairman: H. Autrup**
14.15-15.15	Keynote lecture **J. Idle.** Interindividual variation in carcinogenesis
15.15-15.30	Discussion
15.30-15.45	Coffee Break
15.45-16.15	**K. Vähäkangas.** Different approaches to study interindividual and organ variation in human PAH-inducible P450 activities
16.15-16.45	**A. Haugen.** Rare Ha-*ras*-1 alleles and susceptibility to human lung cancer
16.45-17.15	Discussion
18.00	Dinner.

Thursday 04.10.90

	Breakfast
9.00-12.30	**WORKSHOP ON NEW AND ALTERNATIVE METHODS IN BIOLOGICAL MONITORING** **Chairman: A. Haugen**
9.00-10.00	Keynote lecture **P.B. Farmer.** New and alternative methods in biological monitoring
10.00-10.15	Discussion
10.15-10.30	Coffee break
10.30-11.00	**K. Husgafvel-Pursiainen.** Detection of K-*ras* mutations in lung carcinoma by oligonucleotide hybridization and DGGE
11.00-11.30	**A. Önfelt.**
11.30-12.00	**A.L. Børresen.** Mutation analysis in human cancers using CDGE
12.00-12.30	Discussion
12.30-14.00	Lunch
14.00-18.00	**WORKSHOP ON MEASURES OF PRODUCTS OF EXPOSURE** **Chairman: K. Hemminki**
14.00-14.30	**R. Mustonen.** Analysis of 7-guanine methylated DNA by postlabeling
14.30-15.00	**C. Hansen.** Detection of carcinogen-DNA adducts by 32-P postlabeling

15.00-15.30	**K. Hemminki.** Quantitative aspects of 32-P postlabeling
15.30-15.45	**D. Segerbäck.** Planned and ongoing activities at the center for Nutrition and Toxicology, Karolinska Institute
15.45-16.00	Coffee break
16.00-16.30	**S. Øvrebø.** PAH adducts levels in WBC compared to industrial exposure
16.30-17.00	**K. Vähäkangas.** Current status of the Raahe coke oven study
17.00-17.30	**H. Autrup.** Exposure to aflatoxin B in Danish animal feed production workers
17.30-18.00	**M. Törnqvist.** Hemoglobin adducts: usefulness for identification and quantification of chemical risks
18.00-18.15	Discussion

18.30 Dinner

Friday 05.10.90

	Breakfast
9.00-9.30	**M. Sorsa.** Prediction of cancer risk by cytogenetics.
9.30-10.00	**K. Wassermann.** Genotoxicology at the Danish National Institute of Occupational Health
10.00-10.15	Coffee break
10.15-12.00	Interlaboratory collaboration
12.00-13.00	Lunch
	Departure

3. ABSTRACTS

DIFFERENT APPROACHES TO STUDY INTERINDIVIDUAL AND ORGAN VARIATION IN HUMAN PAH-INDUCIBLE P450 ACTIVITIES.

Kirsi Vähäkangas, Markku Pasanen, Hannu Raunio and Olavi Pelkonen, Department of Pharmacology and Toxicology, University of Oulu, Oulu, Finland.

It is not known to which extent the variation in the patterns and/or activities of P450 isozymes are responsible for the varying susceptibilities of different organs and individuals to e.g. chemical carcinogens. We are trying to find the best markers for relevant isozyme activities to correlate these in the future to the disease process. We have found for instance that AHH activity does not correlate with the formation of BPDE-DNA adducts in human placental microsomes or cultured blood lymphocytes. Since the adduct-formation is in association with the carcinogenicity of benzo(a)pyrene, it may be a more relevant measure than the AHH-activity which measures the formation of hydroxy metabolites. Isozyme-specific inhibitors or panels of inhibitors have proved useful in characterizing the human isozymes behind the enzyme activities. Using this approach we have shown that isozymes of P450 responsible for debrisoquine hydroxylation or some other activities do not contribute significantly to hepatic or placental AHH and ERDE activities. We also use monoclonal antibodies towards different isozymes of P450 to map isozyme paterns in human tissues. Unfortunately the availability of specific antibodies is rather restricted. Also, they differ greatly in their usability in inhibition and immunoblotting studies. Monoclonal antibody Mab 1-7-1, which has been raised towards the PAH-inducible P450 in rat liver by Park, Gelboin and co-workers, inhibits several activities in human placental microsomes, but does not detect any protein in Western blots. With this same antibody we have found large interindividual differences in the inhibition of activities when comparing different tissues and individuals.

New and alternative methods in biological monitoring

P.B. Farmer, MRC Toxicology Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF, U.K.

Over the past 15 years, biological monitoring techniques have been developed to quantify the exposure of man to genotoxic agents by measurement of the covalent adducts that these agents form with nucleic acid bases and amino acids in haemoglobin. Sensitivities in excess of those required to detect environmental exposure are now achievable for several electrophilic genotoxins. These methods, and the latest analytical developments will be reviewed critically. Examples of recently developed assays from our laboratory for determining exposure to styrene oxide and aromatic amines (e.g. 4,4'-methylene dianiline, 4,4'-methylene bis (2-chloroaniline)) will be illustrated and the problems that arise from the existence of 'background levels' of adducts described.

The detection of a protein adduct is now recognised to indicate the presence of a DNA adduct although the quantitative relationship varies from compound to compound. However the value of protein adduct measurements as an indication of genotoxic risk is still somewhat uncertain for human populations. It is hoped that current interlaboratory comparisons of monitoring techniques for DNA and protein adducts and of selected genetic end points will clarify this important relationship.

Detection of K-*ras* mutations in lung carcinoma by oligonucleotide hybridization and denaturing gradient gel electrophoresis (DGGE)

Kirsti Husgafvel-Pursiainen
Institute of Occupational Health, Helsinki, Finland

Point mutational activation of K-*ras* oncogene has been implicated to play an important role in the pathogenesis of non-small-cell lung cancer. We have studied the prevalence of point mutations in *c*-K-*ras*-2 in lung carcinoma. Genomic DNA was extracted from lung tumor biopsy samples and peripheral white blood cells obtained from 36 lung cancer patients before any treatment. The particular DNA sequences under study were amplified enzymatically, dot blotted and analyzed for activating point mutations in K-*ras* codons 12, 13 and 61 by hybridization with specific oligonucleotide probes. The 36 lung carcinomas under study included 17 squamous-cell carcinomas, 16 adenocarcinomas and 3 small-cell carcinomas. Altogether, 13 cases with point mutations in K-*ras* oncogene were found by oligonucleotide hybridization analysis. Codon 12 mutations were observed to be predominant in adenocarcinomas, only one adenocarcinoma case with codon 13 and one with codon 61 mutation was observed. Of the three squamous-cell carcinomas showing mutations, two had codon 12 mutations and one a codon 13 mutation. The white blood cell DNAs were all negative for point mutations in K-*ras*. We also applied denaturing gradient gel electrophoresis (DGGE) in analyzing the material. By this promising new technique were able to detect most of the mutations found by oligonucleotide hybridization analysis but not all of them. Furthermore, we were able to find an additional new mutation in the exon 1 region, since this technique is not limited to the previously known mutations of interest as is the case with the oligonucleotide probing.

Part of this work is done in collaboration with Børresen et al. (The Norwegian Radium Hospital, Oslo) and financially supported by the Nordic Industrial Fund.

ARBETS MILJÖ INSTITUTET
NATIONAL INSTITUTE OF OCCUPATIONAL HEALTH

Datum | Vår beteckning

Ert datum | Er beteckning

Handläggare

III Meeting of the Nordic study group on development of individual exposure to mutagens and chemical carcinogens.
October 1990

ABSTRACT

Activities at the National Institute of Occupational Health, Dpt Occupational Medicine, Solna, Sweden
Agneta Önfelt

Our work is divided into three main areas:

1. Cytogenetic studies of occupationally exposed groups and in vitro genotoxicity tests of chemical or physical agents.

2. Basic research on mitosis and mechanisms for induction of aneuploidy.

3. Molecular analysis of point mutations and establishment of conventional molecular techniques for evaluation of gene expression.

In collaboration with Benkt Högstedt, Halmstad hospital, we are presently investigating micronucleus formation in lymphocytes in samples from chimney sweeps. Within this study we compare two different protocols, with and without addition of cytochalasin B respectively.
The projects concerning mitosis represent the major part of our work at present. We are particularly interested in secondary signal systems and the possible role of primary signals. For the latter part we have found strong indications on the involvement of acetylcholin and its various receptors. The work with analytical methods for detection and characterization of point mutations is part of the inter-Nordic collaboration financed by Nordisk Industrifond. So far we have focused on the N-ras and HPRT loci utilising dot blotting, PCR and DGGE (see Børresen).

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III NORDIC STUDY GROUP MEETING.
Helsinki 3-5 October 1990

MUTATION ANALYSIS IN HUMAN CANCERS USING PCR AND CONSTANT DENATURING GEL ELECTROPHORESIS (CDGE). A.-L. Børresen, E. Hovig, B. Smith-Sørensen, S. Lystad and A. Brøgger. Dept. of Genetics, Inst. for Cancer Research, The Norwegian Radium Hospital, 0310 Oslo 3, Norway.

Detection and localization of single base differences in specific regions of genomic DNA are of great importance in analysis of mutations associated with human diseases. Both inherited and aquired mutations are of importance in analysis of malignant diseases. Denaturing gradient gels have the potential of revealing minor base changes in DNA. Coupled with PCR, this system represents a powerful tool in detection of mutations. The basis for this electrophoretic separation technique is strand dissociation of DNA fragments in discrete, sequence dependent melting domains followed by an abrupt decrease in mobility. We have modified the DGGE by using constant denaturant gels corresponding to the specific melting domains of certain DNA fragments. This leads to increased resolution of mutants as fragments differing in as little as one base pair migrate with a consistently different mobility through the whole gel allowing separations of several centimeters. We have also utilized a mini-gel-system for running these gels, effectively reducing running times, without losing resolution. As a model system we have used this technique to separate six out of seven exon 3 hypoxanthine phosphoribosyltransferase (HPRT) presequenced mutants, while using conventional DGGE we were able to separate three. We have now introduced this system to screen for mutations within the Rb gene and the p53 gene in different human cancers. The system seems to be rapid and reliable in screening for mutations in human cancers.

DETECTION OF CARCINOGEN-DNA ADDUCTS BY 32-P POSTLABELING

Hansen, C., Autrup, H.
Department of Environmental Carcinogenesis, The Fibiger Institute, Ndr. Frihavnsgade 70, DK-2100 Copenhagen

Carcinogen-DNA adducts are believed to be directly involved in the steps of initiation and malignant progression in carcinogenesis. We have investigated if carcinogen-DNA adducts could be detected and correlated to smoking or occupational exposure to styrene using 32-P postlabeling.

The influence of maternal smoking during pregnancy on adduct levels in placenta and umbilical cord vein and artery was investigated. Samples of placenta and umbilical cord were obtained from 20 women, 12 non-smokers and 8 smokers, giving birth to normal healthy children and adduct levels in the three tissues were compared. It was found that mean adduct levels in the three tissues were unlike, with placenta and umbilical cord artery at the same level, while the mean level in the vein were significantly lower, indicating a transplacental exposure to the fetus and that the higher mean adduct level in artery could be due to fetal metabolism of procarcinogens. A difference between mean adduct levels in the three tissues investigated was observed when the tissues were compared with respect to smoking behaviour. This difference, although not statistically significant using non-parametric statistics, showed that smokers mean adduct levels were higher in all three tissues as compared to mean levels in non-smokers.

We have investigated if other tissues, different from those normally utilizeed in human exposure studies could be of use in postlabeling studies. For this purpose, samples of human sperm DNA obtained from a group of smokers and non-smokers, was analyzed. However no correlation between adduct levels and smoking status was observed.

Styrene, a widely used compound in the reinforced plastics industry and in the production of polystyrene, is metabolized to the ultimate carcinogen, styrene-7,8-oxide, that has been shown to induce SCEs in humans as well as cancer in rats. Due to these observations it is suspected that styrene may constitute a potential occupational hazard. The aims of this part of our studies has been to develop methodologies to detect and quantitate adducts formed between DNA and the ultimate carcinogenic form of styrene. So far we have focused on calf thymus DNA reacted in vitro with styrene-7,8-oxide, and used the postlabeling technique to detect and quantitate the adducts formed in order to establish the proper experimental conditions for maximal sensitivity of the technique. It has been possible to detect and quantitate 5 adducts with dose-response properties. In experiments where mice were given a single intragastric dose (0.01 to 10 mg/kg) no styrene related adducts have so far been detected in liver, colon or kidney DNA. Since it is assumed that the N^7-guanine adduct of styrene-oxide is unstable, an animal system, in which urine obtained from rats treated with radiolabeled guanine and styreneoxide were collected. These preliminary data show that a double-labeled fraction can be isolated after elution through C-18 Seppak and HPLC suggesting that this could be repair products of N^7-guanine and styreneoxide.

QUANTITATIVE ASPECTS OF ^{32}P-POSTLABELING

Kari Hemminki, Krzysztof Szyfter, Pavel Vodicka, Pertti Koivisto, Riitta Mustonen and Anne Reunanen
Institute of Occupational Health, Topeliuksenkatu 41 a A.
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Summary

Two parameters of the ^{32}P-postlabeling assay were evaluated using 19 synthesized adduct standards, some with two diastereomeric forms. One parameter was the efficiency of labeling, i.e. the proportion of the adduct added phosphorylated by T_4 polynucleotide kinase. While some adducts labeled well, low labeling was noted for N-7 guanine adducts, in particular. The reasons were likely to be steric. The other parameter scrutinized was 3'-dephosphorylation by nuclease P1, a technique used to enrich the adducts before postlabeling. Usually, bulky adducts were resistant towards dephosphorylation.

PAH adduct levels in WBC compared to industrial PAH exposure.

S. Øvrebø, A. Haugen, H. Barstad and P. E. Fjeldstad
National Institute of Occupational Health, POB 8149 Dep,
N-0033 Oslo 1, Norway.

Exposure to polycyclic aromatic hydrocarbons (PAH) is found in several different industries. Best known are coke oven plants, aluminum plants, foundry works, electrode production and gas works. The level of PAH exposure is highly variable both with respect to job categories and industry type.

Literature data shows that benzo[a]pyrene concentrations in air vary considerably in different factories with PAH exposure, but is typically highest in coke oven plants, aluminum plants and among roofers. We have recently studied exposure in an aluminum plant and an electrode factory were the levels of benzo[a]pyrene (B[a]P) exposure was medium.

Based on literature data there is no consistent relation between B[a]P exposure and adduct levels in industrial exposure to PAH, for example in aluminum plants high levels of exposure is found but few adduct level determination have been reported. However in foundry works relatively high adduct levels have been published although low air values are found.

Relations between air measurements of PAH and adduct levels will be discussed based on literature data and our own measurements.

CURRENT STATUS OF THE RAAHE COKE OVEN PLANT STUDY

Kirsi Vähäkangas[1], Olavi Pelkonen[1], Steinar Øvrebø[2], Aage Haugen[2], Anne Reunanen[3], Anneli Alhonen-Raatesalmi[3], Kari Hemminki[3], Lauri Pyy[4], Erkki Yrjänheikki[4].
Department of Pharmacology and Toxicology, University of Oulu[1] and Regional Institute of Occupational Health, Oulu, Finland[4]; Institute of Occupational Health, Helsinki, Finland[3]; Department of Toxicology, National Institute of Occupational Health, Oslo, Norway[2].

A coke oven plant started its function in Raahe, Finland in October 1987. Samples for biomonitoring were taken from September 1987 on. This way we are able to follow individually, whether and how much the available biodosimetry methods reflect the change from a non-exposure situation to an occupational exposure. We have taken four sets of samples, which are referred to as ^{A}(before exposure), ^{B}(6 months after the start), ^{C}(1,5 years) and ^{D}(2,5 years). Each time stationary and personal air samples have been collected, as well as blood and urine samples from the workers. They have also filled a questionnaire about e.g. smoking habits. Several parameters of potential value for biomonitoring of PAH-exposure are being studied, including 1-OH-pyrene in urine and PAH-DNA adducts in blood lymphocyte DNA.

The major PAH-compounds in the air were phenanthrene, pyrene, chrysene, benzo(a)pyrene and benzo(e)pyrene. Naphtalene was found to be the main compound in the gasous samples. The highest air levels were measured on top and on the sides of the coke oven. There was a significant correlation between the pyrene concentrations in the air and the urinary 1-OH-pyrene levels. BPDE-DNA adducts in peripheral blood lymphocytes are being measured by three different methods:
Synchronous fluorescence spectrophotometry (SFS), USERIA immunoassay and ^{32}p-postlabeling. According to the samples measured so far, the adduct levels have been very low or undetectable for the most part. We have found no difference between the smokers and non-smokers in the adduct levels either by SFS or USERIA in sets A and B. The mean fluorescence value was highest in set B, lower in set C (only partly analyzed) and lowest in set A. Set D is being analyzed. By USERIA there were a couple of more values over 0.8 fmol/µg in set B (11%) than in set A (5,5%). By ^{32}p-postlabeling there seem to be higher levels of adducts in the samples from set C than from A and B. The results are being analyzed currently.

On basis of these findings it can already be concluded that smokers can not be differentiated from non-smokers by the means of the PAH-DNA adducts in lymphocyte DNA. At this point it seems that the urinary 1-OH-pyrine and/or detection of carcinogen-DNA adducts in peripheral blood lymphocyte DNA may have value as biomonitoring methods in workers occupationally exposed to PAH.

Exposure to Aflatoxin B in Danish Animal Feed Production Workers.
Autrup, J, Schmidt J., Seremet, T. and Autrup, H.

Workers in animal feed production have an increased risk of developing liver cancer, and it is assumed that aflatoxin B (AFB) play an important role as a causative agent.

A competitive ELISA assay has been developed using a monoclonal antibody that recognizes AFB. The albumin fraction is isolated from blood serum by precipitation and is digested with pronase (0.67 mg/1 mg albumin) for 18 hrs. at 37°. The digest is purified on a C18 suppla column, and the AFB-containing fraction is eluted by 80% methanol. This fraction is concentrated on an Aflatest affinity column, and eluted by 50% methanol. The content of aflatoxin was determined by a competetive ELISA. The sensitivity of the assays is 5 ng AFB/mg albumin. The level of detectability can be increased by increasing the amount of albumin used for digestion.

Blood sample have been collected from 35 workers, working at 3 different sites after their return from 4 weeks of vacation, and samples after 2 months at work in the plants will be collected early October.

HEMOGLOBIN ADDUCTS: USEFULNESS FOR IDENTIFICATION AND QUANTIFICATION OF CHEMICAL CANCER RISKS

Margareta Törnqvist, Department of Radiobiology, Stockholm University, S-106 91 STOCKHOLM, SWEDEN

Determination of hemoglobin adducts in human populations give an adequate measure of dose, because of the well-defined life-spun of the red cells. This technique solves most of the problems involved in risk assessment on the basis of disease-epidemiological data or experimental data with observations of biological end-points:

- Sensitivity is sufficient for the detection of risks down to the level where risks are becoming acceptably low.
- Measurement can be done shortly after onset of exposure, i.e. the long latency times are overcome.
- The causative agent is identified.
- Adduct levels measured are useful for risk quantification.
- The need for animal experiments is restricted to short-term tests with a few individuals for dose-distribution studies etc.

This will be exemplified with studies on epoxides and alkenes under various exposure conditions and developments of analytical techniques will be discussed.

Genotoxicology at The Danish National Institute of Occupational Health. Current and future activities.

Karsten Wassermann, Bjørn A. Nexø, Lisbeth Knudsen and Mona-Lise Binderup, Section of Biotechnology and Genotoxicology, Laboratory of Biology and Toxicology, The Danish National Institute of Occupational Health, Copenhagen, Denmark.

The section of biotechnology and genotoxicology is in the process of establishing commonly accepted genotoxic and cytogenetic tests in our newly build laboratories. These tests, among which are unscheduled DNA synthesis (UDS), the micronucleus test, Ames' test, and yeast assay, will serve as a basis for environmental and biological monitoring. Typically, Ames' test is suitable for measurements of point mutations, whereas the use of *S. Cerevisiae* D7 and D61M also may be applicable for studies of recombination and aneuploidy induction. A study of cytogenetic monitoring including analysis of chromosomal integrity and overall DNA repair in lymphocytes from a population of stainless steel welders has now been concluded; the study showed a.o.t. an increased frequency of chromosomal aberrations and a decrease in UDS upon exposure to stainless welding. A validation of the molecular aspects of Na-AAF induced UDS, which was used as a measure of overall DNA repair in the total population of lymphocytes, is now being pursued. Thus, studies of chemical-DNA adduct formation and repair at the level of the gene in the context of heterogeneous DNA damage and repair will be performed. Here we have already developed suitable techniques for the analysis of alkylation damage and repair in specific genomic regions from cells in culture. Finally, the section is in the process of developing a retrovirus-based mutagenicity assay for in vivo studies. This new test is based on an endogenous provirus, Emv-3, in the DBA/2 mouse, which has an inherent point mutation in codon 3 of the *gag*-gene. Young animals do not have replicating virus, which, however, may be stimulated upon exposure to e.g. 7,12-dimethylbenzanthracene (DMBA). This stimulation may be measured as an increase of a specific gene product shortly after exposure.

4. DELTAGERE

KEYNOTE LECTURES

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