structure. Protection by NAC against cyto- and genotoxic effects of tobacco smoke in human bronchial epithelium may have clinical relevance.

EXTINCTION OF PROVIRAL EXPRESSION IN CELL HYBRIDS: APPROACHES TO THE ISOLATION OF A HUMAN SUPPRESSOR GENE

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The fusion of rat fibroblasts transformed by a single integrated copy of Rous sarcoma virus to normal mouse or human cells results in hybrids which are morphologically normal. The provirus is retained and is intact but transcriptionally inactive. Karylogical examination of normal and transformed hybrids suggests that chromosome 11 may carry the suppressor gene. In an attempt to isolate the suppressor gene we are pursuing several strategies including:

(1) the use of a retroviral vector as an insertional mutagen since the normal hybrids are often haploid with respect to their human chromosomes;

(2) DNA mediated co-transfection with an HPRT cDNA clone or pSV2neo using either back selection or fusion with the transformed parental line to distinguish spontaneous revertants from suppressed transfectants.

EXPRESSION OF FUNCTIONAL EGF RECEPTORS IN INSECT CELLS USING A BACULOVIRUS VECTOR

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To obtain large amounts of functional epidermal growth factor (EGF) receptors for biochemical and biophysical studies, we have subverted the natural life cycle of the Autographia californica Nuclear Polyhedrous Virus (AcNPV) in an insect cell line (Spodoptera Frugiperda) to express the human EGF receptor cDNA. The cDNA for the full length of EGFR was cloned into an expression vector which when cotransfected with wild type AcNPV formed recombinant AcNPV. Insect cells infected with this virus produced a membrane protein which was recognised by the monoclonal antibodies R_1 and F_4 which bind to the external and cytoplasmic domains of the human receptor respectively. EGF bound to whole cells with a kd of 10^{-8} mol showing approximately 10^6 binding sites per cell. Auto-phosphorylation of the immunoprecipitated recombinant protein showed that it possessed an active tyrosine kinase which like the natural receptor phosphorylated the three C terminal tyrosine residues designated P_1, P_2 and P_3 (1) SDS page analysis revealed that this insect cell protein was slightly smaller (160 kd) than that of EGF receptor protein found in A431 cells. Biosynthetic studies showed this size disparity was accounted for by differences in glycosylation.

It is hoped to increase the productivity of our system by using suspension-perfusion culture systems and also to compare the structure and functions of this protein with other similarly produced mutant/trancated EGF receptors.

(1)Nature, 311: 483-485, 1984.

rRNA MATURATION AS AN INDICATOR OF CYTOTOXICITY OF THE ANTINEOPLASTIC DRUG 5-FLUOROURACIL

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Subclones of the human rDNA transcriptional unit were prepared and used as probes for blot hybridisation of fractionated RNA species isolated from human colonic tumour cells growing in vitro. The results established that 5-Fluorouracil (5-FU) affected rRNA maturation and led to the accumulation of rRNA precursors. The effects correlated with cytotoxicity of 5-FU. The implications of these findings for the mode of action of 5-FU and the development of novel chemotherapeutic strategies have been evaluated.

IN VIVO AND IN VITRO BINDING OF PERCHLOROETHYLENE (PCE) TO NUCLEIC ACIDS

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PCE is hepatocarcinogenic in mice, but the evidence of its genotoxicity in short-term assays is as yet limited. Therefore, we attempted to measure covalent binding of PCE to DNA both in vivo, by

injecting ip ^{14}C-PCE (specific activity-14.6 mCi/mmol, 127 μCi/Kg) to male Wistar rats and BALB/c mice, and in vitro as previously described (Turina et al, Res. Comm. Chem. Pathol. Pharmacol., 52: 305, 1986). In contrast to a previous report (Schumann et al, Toxicol. Appl. Pharmacol., 55: 207, 1980), we found that PCE covalently binds to nucleic acids both in vivo and in vitro. Specific activity of mouse liver DNA was higher than that of DNA from kidney, lung and stomach. CBI values calculated according to Lutz (Mutat. Res., 65: 289, 1979) were 10.5 and 76 for rat and mouse liver DNA, respectively, i.e. similar to those of weak initiators and lower than those of the saturated analogue 1,1,2,2-tetrachloroethane. This fact agrees with the finding that chlorine substitution results in a destabilization in alkanes and in a stabilization in alkenes (Henschler, J. Environ. Pathol. Toxicol., 1: 125, 1977).

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STRUCTURE AND EXPRESSION OF PROVIRUS IN ASV-TRANSFORMED MAMMALIAN CELLS

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In the present study TWERC cells (derived from PR-RSV-transformed rat cells) SAM IV cells (derived from a spontaneously arisen rat tumour) and SAM B77 cells (supertransformed in vitro) were investigated to determine the type of changes that had been induced in their genomes after treatment with bromodeoxyuridine and avian sarcoma virus B77, respectively.

THE ROLE OF THE c-abl ONCOGENE IN PHILADELPHIA CHROMOSOME POSITIVE CHRONIC MYELOGENOUS LEUKAEMIA

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Chronic myelogenous leukaemia (CML) is characterized by the presence of the Philadelphia (Ph1) chromosome in the leukaemic cells of 96% of all CML patients. The Ph1 chromosome (22q-) is the result of a reciprocal translocation between chromosome 22 and chromosome 9, t(9q34,22q11). Previously we described the localization of the human c-abl oncogene on chromosome 9 and demonstrated its translocation to the Ph1 chromosome in CML patients. The cloning and analysis of breakpoint fragments revealed that the breakpoints on chromosome 22 all cluster in a very limited area, the breakpoint cluster region, bcr. Breakpoints on chromosome 9, however, are scattered over a large area which may vary from zero to more than 100 kb upstream of the v-abl homologous sequences of the c-abl gene. A unique 8.5 kb chimeric bcr-abl RNA is detected in the leukaemic cells of all CML patients. Cloning of chimeric c-DNAs (5'bcr and 3'abl) from a CML derived cell line strongly indicates that bcr and c-able coding sequences are linked in frame by RNA splicing, independent from the highly variable distance between these two genes on the Ph1 chromosome. The specific presence of the chimeric bcr-able RNA (and protein) in CML cells suggests the involvement of this hybrid product in the development of CML. Recent cloning of full-length hybrid c-DNAs will help to test this hypothesis.

METHIONINE BIOSYNTHESIS IN EHRLICH ASCITES (EAC) CELLS IN RELATION TO THE AGE OF THE HOST

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In EAC cells grown in Swiss female mice at various stage os their postnatal development (1, 3, 12, 18 months) only one of two possible routes of the last steps of methionine biosynthesis was found to be active. Betaine methyltransferase (EC 2.1.1.5) utilizing betaine for homocysteine methylation was not detected in tumour cells. Both enzymes of the operative, folate cofactor and B12 dependent route i.e. methylenetetrahydrofolate reductase (EC 1.1.99.15) and methionine synthase (EC 2.1.1.13) had the lowest activities in EAC cells grown in the youngest mice. In this age group the statistically significant tumour effect on two examined enzymes in liver of EAC-bearing mice was lower than in the older hosts. The relation between methionine synthase and methylenetetrahydrofolate reductase activities in EAC cells and tumour effect in uninvolved host organs has been