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