31 EXPRESSION OF HUMAN AND MOUSE HPRT MINIGENES

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A hypoxanthine phosphoribosyltransferase (HPRT) minigene has been constructed from both human and mouse HPRT cDNA by the addition of proper transcription initiation and polyadenylation signals from genomic subclones of the HPRT gene. Calcium phosphate mediated gene transfer of these HPRT minigenes into HPRT deletion lines has shown stable transformation frequencies from 10^{-7} to $5x10^{-7}$. When an enhancer from the mouse HPRT is inserted into either the mouse HPRT minigene or human HPRT minigene, the stable transformation frequency approaches 10^{-5}. Microinjection of 5, 50, 250 linear or supercoil copies of each minigene/cell demonstrates enhancer as well as plasmid conformational importance for stable $HPRT^+$ colonies. Enzymatic, Northern, and Southern analyses on these lines have been performed. Expression of HPRT depends on both location and copy number of the transgene. These minigenes have been inserted into retroviral vectors and shown to stably express the HPRT gene by transfection as well as infection. Titers of these helper free virus vary from 10^2 to 10^5 cfu/ml. HPRT expression in virally infected cells have been monitored by enzymatic, Northern and Southern analysis. This work was supported by a NIH Fellowship to SMC, an Arthritis Foundation Fellowship to PIP, and NIH AM31428 and the Howard Hughes Medical Institute (ACC and CTC).

32 THE MOLECULAR BASIS FOR THE HEREDITARY OVERPRODUCTION OF ADENOSINE DEAMINASE.

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A marked increase in erythrocyte adenosine deaminase (ADA) activity has been associated with chronic hemolytic anemia and decreased erythrocyte ATP levels (Valentine et al., Science 1977). This defect is inherited as an autosomal dominant trait specific for erythroid cells. The kinetic and physicochemical properties of ADA are normal, suggesting the cell line-specific overproduction of a normal enzyme. We have investigated the molecular basis of this defect in erythrocytes and lymphoid cells from an affected individual. The proband's erythrocytes had a 70-fold increase in ADA specific activity and a 30-fold increase in immunologic cross-reacting material (CRM) as measured by a solid-phase radioimmunoassay. Southern blot analysis of genomic DNA using a full-length ADA cDNA probe revealed no evidence of ADA gene amplification or rearrangement. Northern blots of reticulocyte RNA did not demonstrate correspondingly increased levels of ADA-specific RNA. RNA from control reticulocytes hybridized with the ADA cDNA probe in three distinct bands at 5.8 kb, 3.0 kb, and 2.0 kb. The proband's reticulocyte RNA differed from controls in that the 3.0 kb band was replaced by a 2.0 kb band. Lymphoblast ADA specific activity, CRM, and RNA were similar to those in normal B lymphoblast controls. We conclude that there is a true overproduction of ADA protein which is specific for red cells and which may be mediated at the translational rather than the transcriptional level.

33 METABOLISM OF 5'-METHYLTHIOADENOSINE IN METHIONINE-DEPENDENT AND INDEPENDENT CELLS.

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Methionine (Met) dependency was investigated in a Met-dependent (CCL 39) and in a Met-independent (Raji) cell line. Met dependency was similarly observed with two Met precursors, L-homocysteine and 5'-deoxy-5'-methylthioadenosine (MTA), suggesting that this phenomenon represents, in fact, the inability of a cell to grow on a metabolic precursor of Met. The study of MTA metabolism in CCL 39 and Raji cells indicates that Met dependency is not due to the inability to synthetize Met from MTA, nor to incorporate newly synthetized Met into proteins, nor into S-adenosylmethionine (SAM). However, there are differences in SAM metabolism and disposition between CCL 39 and Raji cells. The synthesis of SAM from labelled MTA is higher in CCL 39 than in Raji cells, but SAM is much more excreted by CCL 39 than by Raji cells, with ratios of extracellular to intracellular SAM of 20.3 and 5.1, respectively. However, when the cells are incubated with labelled Met, the synthesis of SAM is similar in both types of cells, and the ratios of extracellular to intracellular SAM are much lower than in the previous case (0.8 and 1.0 for CCL 39 and Raji cells, respectively). These results, and the fact that exogenous unlabelled Met moderately affects the incorporation of labelled MTA into intracellular SAM by CCL 39 cells, and profoundly depresses it in Raji cells, support the hypothesis of a metabolic compartmentation of exogenous and endogenous Met in Met-dependent cells.

34 **APRT PARTIAL DEFICENCY IN A FAMILY.**

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A APRT partial deficency case in a 30 years old woman with gout, nephrolithiasis and renal failure had been, previously, described.(Acta Reuma. Port. 2,125,1974). We have, recently, studied the purine metabolism in this patient and in her family (Guido,17; Giorgia,16; Giulia,13 years-old). Giulia is apparently healty with normal uricemic value; Giorga showed an increasing serum urate levels (8 mg%) in 1980, when she was treted with purine free diet and henceforth she is normal uricemic. Guido shows several attacks of gout from the age of 12 years. In they all we have investigated HGPRT, APRT activity (nmol/mg prot./h) and erythocyte level of PRPP (nmol/mg prot.).HGPRT, and APRT, were determined as in Clinical Rheumat. 3, 229, 1984. Levels of PRPP were determined measuring the amount of IMP synthesized without adding any exogenous PRPP. The data are reported in table:

	PRPP	HGPRT	APRT		PRPP	HGPRT	APRT
M.Te.	12.6	141	8.6	Gior.	2	140	27
Guid.	4	148	13	Giul.	13.8	124.4	5
n.val.	1-5	111±13	28±6				

Turnover of [^{14}C] uric acid and its plasma disappearence has been evaluated in everyone.

35 ATP IN OXIDANT-INDUCED TISSUE INJURY.

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Oxidants, induce injury and death of neighboring cells. We have studied early changes following exposure of the cells to oxidants. Exposure of P388D1 cells to H_2O_2 (0.1 to 2.5 mM) induced a rapid (3-5 min) fall in ATP, turnover of components of the glutathione cycle, a slower (>15 min) rise in intracellular Ca^{++} and Na^+ and, after 4-5 hrs, cell lysis. Exposure to H_2O_2 produced a slight elevation of ADP at a time when ATP levels were low, and a stoichiometric increase in IMP and inosine. In determining the cause for the fall in ATP, we observed that P388D1 cells exposed to 0.04 to 2.5 mM H_2O_2 lost NAD at an initial rate of 0.26 nmoles/10^6 cells/min, leading to an 80 percent depletion of NAD within 20 min. This fall in NAD is coupled with inhibition of glycolysis. Poly(ADP-ribose)polymerase, the nuclear enzyme catalyzing conversion of NAD to nicotinamide and protein-bound ADP-ribose, was activated by exposure of the cells to H_2O_2. Inhibition of this enzyme by 2.5 mM 3-amionobenzamide prevented the oxidant induced fall in NAD. This also prevented the sustained fall in ATP, and the other events (above) except for glutathione cycle activity. H_2O_2 induced DNA strand breaks in target cells, potentially responsible for activation of the polymerase. These cells indicate that oxidant induced stimulation of poly(ADP-ribose)polymerase causes a fall in NAD leading to a sustained depletion of ATP, leading to cell death.

36 PURINE NUCLEOTIDE AND DEOXYNUCLEOTIDE CATABOLISM IN HUMAN T LYMPHOCYTES.

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In PNP and ADA deficiencies the abnormalities observed in intracellular deoxynucleotide levels are more severe than in the corresponding ribose derivatives. These observations indicate selective roles for ADA and PNP in adenine and guanine deoxynucleotide degradation as compared to the corresponding ribonucleotide catabolism. We have found that distinct pathways are used for the catabolism of these purine deoxynucleotides and ribonucleotides. Thus, while adenine ribonucleotides are deaminated primarily by adenylate deaminase, adenine deoxynucleotides are exclusively deaminated by adenosine deaminase, explaining the specific impairment of adenine deoxynucleotide catabolism observed in ADA deficiency. In parallel, two distinct catabolic pathways exist for guanine nucleotide and deoxynucleotide degradation, while guanine ribonucleotide deamination proceed primarily through guanylate reductase to IMP, guanine deoxyribonucleotides are exclusively dephosphorylated to deoxyguanosine and then phosphorylized by PNP to guanine. These observations emphasized the importance of ADA and PNP not only in nucleotide degradation but primarily in the conversion of purine deoxyribonucleotides to their corresponding ribonucleotide derivatives through the active purine nucleoside cycles. The apparent activities of the catabolic enzymes adenosine deaminase, adenylate deaminase and purine nucleoside phosphorylase as well as the purine nucleoside cycle change markedly in the course of human T-lymphocyte differentiation.