Arylamine *N*-acetyltransferase activity in human cultured cell lines

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Many arylamine and hydrazine drugs and xenobiotics are acetylated by liver *N*-acetyltransferase (NAT; EC 2.3.1.5). Two loci, *mnat* and *pnat*, encode the enzymes designated monomorphic and polymorphic NAT (mNAT and pNAT) respectively. These isoenzymes have different substrate specificities. In addition, at the polymorphic locus a diversity of alleles is found, which differ by specific point mutations that may or may not result in amino acid substitutions. These point mutations result in the 'slow' acetylation of substrates of pNAT. The substrates for NAT include carcinogenic arylamines. Susceptibility to bladder cancer has been related to slow acetylation. NAT has been characterized in immortalized human cell lines to assess their use in studies of the metabolism or arylamines *in vitro*. A monocytic cell line (U937) and two hepatoma cell lines of parenchymal lineage (HepG2 and Hep3B) have been shown to catalyse acetylation of substrates of mNAT but do not acetylate sulphamethazine, a substrate specific for pNAT. Using PCR to amplify the alleles of pNAT, followed by restriction-enzyme digestion of the product, the cell lines have been genotyped: U937 cells are homozygous slow acetylators (S1a/S1a) and HepG2 cells are heterozygous slow acetylators (S1a/S2). Transcription of *pnat* was confirmed in the hepatoma cell lines, by amplification of cDNA generated from these cells. In addition, splicing of mRNA specific for pNAT has been demonstrated by using a primer which anneals to a region in the 5′ promoter region. Unlike the hepatoma cell lines, in U937 cells the pNAT gene is not transcribed. However, transcription of *mnat* was shown to occur in all three cell lines.

INTRODUCTION

In human liver cytosol, arylamine *N*-acetyltransferase (NAT; EC 2.3.1.5) catalyses the acetylation of the primary aromatic amine or the hydrazine group of xenobiotics. Variation in the human population occurs in the acetylation of several drugs such as sulphamethazine (SMZ), procainamide, isoniazid, hydralazine and caffeine, and certain carcinogens such as 2-aminofluorene, benzidine and aniline. This inter-individual variation in N-acetylation capacity was shown to be determined by a single autosomal gene locus (Evans and White, 1963), and compounds such as SMZ have been named polymorphic substrates (Glowinski et al., 1978) and found to be acetylated in liver and duodenum. A number of other arylamines, including *p*-aminobenzoic acid (PABA) and *p*-aminosalicylic acid (PAS), are also efficiently acetylated, but the acetylation of these compounds is not affected by the polymorphism (Pacifici et al., 1986), and they have been termed monomorphic substrates. These compounds are also acetylated in extrahepatic tissue (Pacifici et al., 1986; Coroneos and Sim, 1991), and in certain human cell lines (Coroneos et al., 1991). Anisidine, 2-aminofluorene and aniline, whose acetylation is polymorphic, appear to be acetylated both in the liver and in extrahepatic tissue.

The importance of the acetylator status of individuals lies in the predisposition to disease. Slow acetylators are at increased risk of benzidine-induced bladder cancer (Cartwright et al., 1982; Mommsen et al., 1985), whereas colonic cancer may be associated with the fast phenotype (Ilett et al., 1987). Hydralazine-induced immunotoxicity occurs almost exclusively in slow acetylators (Perry, 1973) and slow acetylators are at increased risk from procainamide-induced erythematosus (Rubin, 1989).

The genetic basis for NAT polymorphism has only recently begun to be unravelled. Two genes encoding functional NAT have been cloned from human genomic DNA (Blum et al., 1990) and from human liver cDNA (Ohsako and Deguchi, 1990). A pseudogene has also been cloned from genomic DNA (Blum et al., 1990). Both functional loci lie on chromosome 8. These genes have been expressed in eukaryotic expression systems and enzymic studies have allowed their identification. One locus, *mnat* (AAC1; Donis-Keller and Buckle, 1991), encodes an NAT that acetylates the monomorphic substrates PABA and PAS. The other locus, *pnat* (AAC2; Donis-Keller and Buckle, 1991), carries the isoenzyme responsible for the polymorphism initially described by Evans (1989). It is possible to separate the polymorphic and monomorphic isoenzymes by ion-exchange chromatography (Jenne, 1965; Grant et al., 1989, 1991; Coroneos et al., 1991). Many allelic variants at the *pnat* locus have been described, only one of which appears to catalyse acetylation efficiently (Vatsis et al., 1991; Hickman et al., 1992), and a summary is shown in Table 1. The variants arise from specific point mutations at several conserved locations, which may or may not result in amino acid substitutions, and which have allowed their characterization by restriction fragment polymorphism, and allele-specific PCR (Hickman et al., 1992). It is not clear how these mutations exert their deleterious effect on acetylation. Using genetically engineered NAT in eukaryotic cells it appears that the mutations result in production of less protein, either by increased instability of mRNA (Deguchi et al., 1990) or protein (Ohsako and Deguchi, 1990; Blum et al., 1991; Deguchi, 1992).

With the increasing number of alleles being discovered, methods have been refined which make it possible to determine the genotype of an individual based on a combination of PCR, restriction-fragment polymorphisms and allele-specific PCR (Kelly and Sim, 1991; Hickman and Sim, 1991; Blum et al., 1991; Hickman et al., 1992; Deguchi, 1992).

In this study, the expression of pNAT in three human cultured cell lines has been investigated. The advantage of using cultured cells is that these present a simplified system for effectively

Abbreviations used: mNAT, monomorphic *N*-acetyltransferase; pNAT, polymorphic *N*-acetyltransferase; PABA, *p*-aminobenzoic acid; PAS, *p*-aminosalicylic acid; PBS, 145 mM NaCl/10 mM $NaHPO_4$; PMA, phorbol 12-myristate 13-acetate; SMZ, sulphamethazine.

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Table 1 Summary of *pnat* alleles

Allele	Nucleotide		Amino acid		Identification (Restriction site)		Reference
	Position	Substitution	Position	Identity			
F1 (wild type)	282	C	94	Tyr	*Fok*I	Present	Blum et al., 1990; Ohsako and Deguchi, 1990
	341	T	114	Ile	None		
	481	C	161	Leu	*Kpn*I	Present	
	590	G	197	Arg	*Taq*I	Present	
	803	A	268	Lys	*Dde*I	Not present	
	857	G	286	Gly	*Bam*HI	Present	
S1a	341	T → C	114	Ile → Thr	None		Vatsis et al., 1991; Hickman et al., 1992
	481	C → T	161	Leu	*Kpn*I	Removed	
	803	A → G	268	Lys → Arg	*Dde*I	Added	
S1b	341	T → C	114	Ile → Thr	None		Hickman et al., 1992; Blum et al., 1991
	481	C → T	161	Leu	*Kpn*I	Removed	
S1c	341	T → C	114	Ile → Thr	None		Hickman et al., 1992
	803	A → G	268	Lys → Arg	*Dde*I	Added	
S2	282	C → T	94	Tyr	*Fok*I	Removed	Blum et al., 1991; Ohsako and Deguchi, 1990; Vatsis et al., 1991
	590	G → A	197	Arg → Gln	*Taq*I	Removed	
S3	282	C → T	94	Tyr	*Fok*I	Removed	Hickman et al., 1992; Deguchi et al., 1990; Hickman et al., 1992
	857	G → A	286	Gly → Glu	*Bam*HI	Removed	

studying variability of drug metabolism and toxicity in different humans. The expression of pNAT and mNAT activities was measured by the substrate specificity of N-acetylation and compared with the genotype of the cells, determined by PCR and restriction-fragment analysis. The transcription of the alleles was determined by PCR of cDNA prepared from the same cells.

MATERIALS AND METHODS

Cells

All cultured cells were obtained from the Cell Bank of the Sir William Dunn School of Pathology, Oxford, U.K. The human monocytic cell line, U937, and the hepatoma cell line, HepG2, were grown as described previously (Coroneos et al., 1991). The second hepatoma cell line Hep3B was grown in minimal essential medium (MEM, Gibco) containing non-essential amino acids, 10% (v/v) foetal-calf serum, 2 mM L-glutamine, 100 μM sodium pyruvate, penicillin (100 units/ml) and streptomycin (100 μg/ml).

Cells were either used to prepare cytosol as previously described (Coroneos et al., 1991), or harvested by centrifugation (1000 *g* for 10 min at 20 °C) washed with PBS and used immediately for the preparation of genomic DNA or RNA.

U937 cells (10^6 cells/ml) were stimulated with the phorbol ester, phorbol 12-myristate 13-acetate (PMA) (Kurata et al., 1989) at a concentration of 0.5 μg/ml and harvested after 17 h, when they had assumed a macrophage-like morphology by becoming adherent to the flask's surface.

Determination of N-acetylation

Acetylation of arylamines by the cytosolic fraction of the cell lines was measured using the polymorphic substrate SMZ, and the monomorphic substrates PABA and anisidine (*p*-methoxyaniline). A spectrophotometric assay was used which detects remaining substrate as described elsewhere (Coroneos et al., 1991). SMZ remaining in reaction mixtures after precipitation of protein by trichloroacetic acid was also separated by chromatography on a C18:0 reversed-phase column (Novapak: Waters, Watford, U.K.) using acetonitrile: H_2O (15:85, v/v) and a flow rate of 1 ml/min, which separates SMZ and acetyl-SMZ. The substrates and products are identified from their absorbance values at 256 nm. With this method, 0.04 nmol of acetyl-SMZ could be detected.

Preparation of DNA and RNA

Genomic DNA was prepared from cells by proteinase K digestion and phenol–chloroform extraction (DiLella and Woo, 1987).

Total RNA was extracted using guanidine isothiocyanate (Chirgwin et al., 1979) and CsCl density gradient centrifugation. After ethanol precipitation, RNA was digested with DNAase I (Boehringer–Mannheim, Lewes, Sussex, U.K.) to remove any contaminating DNA in 50 μl of 0.1 M sodium acetate (pH 5.0)/5 mM $MgSO_4$, with 35 units of enzyme, for 2 h at 25 °C. The mixture was then extracted twice with phenol:chloroform:isoamyl alcohol (25:24:1, by vol.), followed by extraction with chloroform:isoamyl alcohol (24:1, v/v), and after ethanol precipitation the RNA was resuspended in 20 μl of H_2O. First-strand cDNA was synthesized using RAV-2 reverse transcriptase (Amersham) on 10 μg of RNA in a total volume of 20 μl, according to the manufacturer's instructions, using oligo-dT to prime the reaction.

Oligonucleotides

The sequences of oligonucleotide primers used in the PCR are shown in Table 2. The regions to which they hybridize are shown in Figure 1 or Table 2. All the primers, apart from Nat-Hu19, Mono A and Mono C, have been described elsewhere (Kelly and Sim, 1991; Hickman and Sim, 1991). Nat-Hu19 was designed to recognize a region in the non-coding exon of polymorphic NAT (pNAT), which in the genome lies about 8 kb upstream from the

Table 2 Oligonucleotides used to prime PCR for amplification of polymorphic and monomorphic NAT genes

Nat-Hu7, Nat-Hu8, Nat-Hu14 and Nat-Hu16 are specific for *pnat* and anneal to residues 32 to 52, 850 to 870, 4 to 26 and 980 to 1002 respectively. Mono A and Mono C are specific for *mnat* and anneal to the regions −3 to −19, and 880 to 902 respectively. Nat-Hu19 corresponds to the 5′ non-coding exon (residues −22 to −41 of cDNA from Ebisawa and Deguchi, 1991) of *pnat*. Lower case letters refer to nucleotides added to the *nat* sequence in order to incorporate an *Sal*I restriction site.

Name	Nucleotide sequence
Nat-Hu7	tgtcgacGGCTATAAGAACTCTAGGAAC (sense)
Nat-Hu8	tgtcgacAATAGTAAGGGATCCATCACC (antisense)
Nat-Hu14	GACATTGAAGCATATTTTGAAAG (sense)
Nat-Hu16	GATGAAAGTATTTGATGTTTAGG (antisense)
Nat-Hu19	ATTGCATTCAGCCTAGTTCC (sense)
Mono A	ATCATGGACATTGAAGCATAT (sense)
Mono C	AAATAGACAAGATTGTTTCACTC (antisense)

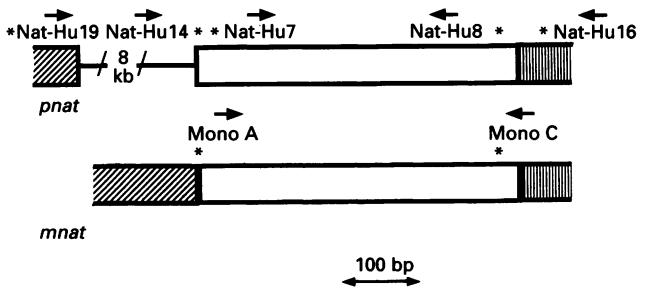

Figure 1 Schematic representation of the human polymorphic (*pnat*) and monomorphic (*mnat*) genes

The positions of the oligonucleotides used to amplify the specific alleles using PCR are indicated by asterisks. The precise position and sequences of primers are shown in Table 2. The cross-hatched bar denotes 5′ non-coding exon of *pnat* and the 5′ non-coding region of *mnat*. The open bar denotes coding sequence and the vertical lines denotes 3′ non-coding regions.

coding exon (Ebisawa and Deguchi, 1991). Nat-Hu19 was used with Nat-Hu16 to amplify pNAT cDNA sequences. The primer pair Mono A and Mono C was designed for specific amplification of the *mnat* gene.

The allele-specific primers used (DH1, DH2, DH3, and DH5) have been described (Hickman et al., 1992). The pair DH1 and DH5 amplifies F1 and S2 alleles. The pair DH2 and DH3 amplifies S1a, S1b and S1c alleles.

PCR

The DNA template for amplification was either 200 ng of genomic DNA or 20 % (v/v) of the cDNA mix. The reaction was performed in a final volume of 100 μl of a standard reaction mix using 10-fold concentrated stock buffer obtained from Boehringer–Mannheim (Lewes, Sussex, U.K.), containing 0.01 M Tris/HCl (pH 8.3), 0.05 M KCl, 1.5 M $MgCl_2$, 100 μg/ml gelatin, 50 pmol of each primer, 200 μM of each dNTP and 2.5 units of *Taq* DNA polymerase (same suppliers). The amplification conditions have been described previously (Hickman and Sim, 1991), except that the annealing temperature with the monomorphic-specific primers was 60 °C. The product was then used directly from the aqueous phase or precipitated with ethanol and resuspended in 20 μl of water.

For allele-specific PCR the template for the reaction was the product of a previous amplification of genomic DNA using primers Nat-Hu14 and Nat-Hu16, which amplify all *pnat* alleles. The allele-specific reaction was performed in a total volume of 50 μl of the standard reaction mix as described above containing 0.625 units of *Taq* DNA polymerase and using 10 μl of the DNA product from the first PCR as a template. The amplification conditions were 35 cycles: annealing 68 °C, 0.75 min; extension 72 °C, 0.75 min; and denaturation 94 °C, 0.75 min.

Restriction-enzyme digestion

To identify which *pnat* alleles are present in genomic NAT, the product of amplification of genomic DNA was digested with *Bam*HI, *Dde*I, *Fok*I, *Kpn*I and *Taq*I. Digestion of approx. 1 μg of amplified DNA was carried out in a total volume of 20 μl using the appropriate SuRE cut buffer (Boehringer–Mannheim) at the recommended temperature. Digested DNA was then separated by agarose-gel electrophoresis (1.4 %, 10 V/cm) along with DNA molecular-mass markers (1 kb DNA ladder, BRL, Uxbridge, Middlesex, U.K.) for comparison of size. DNA was revealed by ethidium bromide staining and u.v. transillumination.

RESULTS

Expression of NAT activities

All of the cell lines studied N-acetylate the monomorphic substrates PABA and anisidine but not the polymorphic substrate SMZ (Table 3). Therefore, the substrate specificity of the NAT activities indicates the presence of monomorphic NAT (mNAT) but not of pNAT. In order to increase sensitivity, h.p.l.c. was used (see the Materials and methods section) which would detect acetylation of 0.04 nmol of SMZ. Even under these conditions no acetylation was detected in the hepatoma cells.

Table 3 NAT in cultured cell lines

'w' denotes wild-type allele (F1), and 'm' denotes a mutant allele (see Table 1 for description of alleles). The genotyping was performed as previously described (Hickman et al., 1992) and is summarized under genotype.

	Cell line		
Property	U937	HepG2	Hep3B
NAT activity (nmol/min per mg of cytosol)			
PABA	10.38 ± 0.86*	0.99 ± 0.12*	11.1†
Anisidine	8.92 ± 0.98*	0.92 ± 0.13*	7.5†
SMZ	0.003 ± 0.001*	< 0.002	< 0.008
Genotype	*S1a/S1a*	*S1a/S2*	*S4/S4*
Specific mRNA	*mNAT*	*mNAT* *pNAT*	*mNAT* *pNAT*
Genotype determination			
*Bam*HI	w/w	w/w	w/w
*Dde*I	m/m	w/m	w/w
*Fok*I	w/w	w/m	m/m
*Kpn*I	m/m	w/m	w/w
*Taq*I	w/w	w/m	w/w

* Data have been published in Coroneos et al., 1991.
† The average of two assays is given.

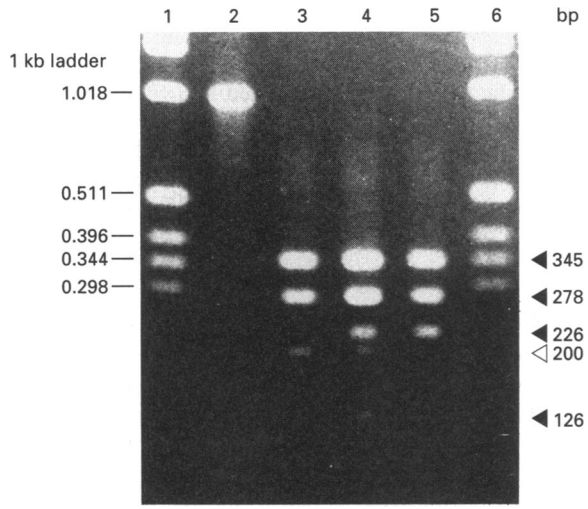

Figure 2 Identification of the mutation at position 803

Genomic DNA from U937 cells (lane 3), HepG2 cells (lane 4) and Hep3B cells (lane 5) was amplified with Nat-Hu7 and Nat-Hu14 and digested with *Dde*I. The 970 bp uncut product from U937 DNA is shown in lane 2. The products of digestion were separated on a 1.5% (w/v) agarose gel. The 200 bp informative band is indicated by the open arrow. The 226 bp band results when the nucleotide at position 803 is 'A' as in the alleles F, S1b, S2, S3 (Table 1). Molecular-mass markers (1 kb ladder) were run concurrently in lanes 1 and 6.

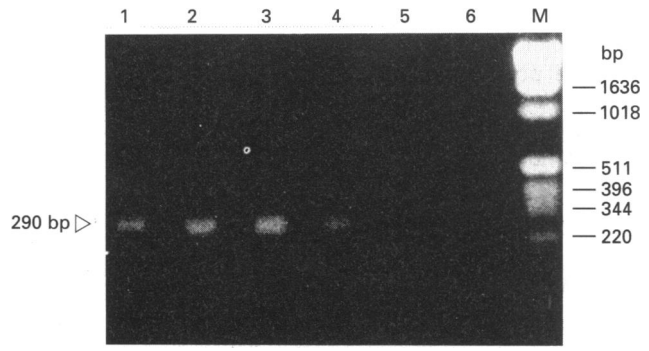

Figure 3 Allele-specific PCR of genomic DNA from U937, HepG2 and Hep3B cells

Genomic DNA from U937 cells (lanes 1 and 2), HepG2 cells (lanes 3 and 4) and Hep3B cells (lanes 5 and 6) was amplified with Nat-Hu14 and Nat-Hu16 and the product was then amplified with allele-specific primers DH2 and DH3 as described in the Materials and methods section. This primer pair will amplify specifically alleles S1a, S1b and S1c in which the nucleotide at position 341 is 'C' to generate a 290 bp band (indicated by the open arrow). Molecular-mass markers are shown in lane M.

Genotyping of the cell lines

The absence of pNAT activity in U937 cells has been shown previously to be due to a lack of mRNA encoding *pnat*, although the gene is present (Kelly and Sim, 1991). In order to determine whether the polymorphic gene was present in the hepatoma cell lines, genomic DNA was amplified with the polymorphic-specific primer pairs Nat-Hu14 and Nat-Hu16, Nat-Hu7 and Nat-Hu16, and Nat-Hu7 and Nat-Hu8. Products of the expected sizes were obtained with each primer pair. The specificity of the amplification was confirmed since all products could be cleaved

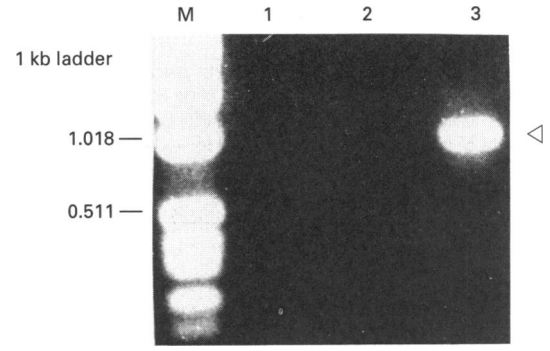

Figure 4 Amplification of mNAT-specific sequences from first-strand cDNA of U937 cells

First-strand cDNA from U937 cells (10^7 cells) was amplified with the primers Mono A and Mono C (lane 3). Two controls were included: no template in the PCR mixture, lane 1; and total DNAase-treated RNA as template in the PCR mixture, lane 2. Molecular-mass markers are shown in lane M. The open arrow indicates the 900 bp amplified product in lane 3.

by *Hin*cII and were resistant to *Hin*dIII which are restriction sites diagnostic of the *pnat* and *mnat* genes respectively.

Having confirmed the presence of the *pnat* in these cells, the genotype of the cells was examined. The cells were genotyped using the amplified products for restriction-fragment analysis. The PCR products were digested with *Kpn*I, *Dde*I, *Bam*HI, and *Fok*I which cleave specific alleles. The ease of distinction between different alleles is illustrated clearly in Figure 2 for *Dde*I digestion (see Table 1 for summary). U937 cells (Figure 2, lane 3), possess the mutation in both alleles with the resulting generation of 345, 278, 200 and 126 bp fragments. HepG2 cells (Figure 2, lane 4), possess one allele with the mutation, with the resulting 345, 278, 226, 200 and 126 bp fragments. In contrast, Hep3B cells lack the mutation entirely, resulting in 345, 278, 226 and 126 bp fragments. A summary of the results obtained with the series of digests is shown in Table 3.

HepG2 cells are heterozygous slow acetylators genotyped as S1a/S2 (Table 3). The S2 allele was inferred from the lack of the *Taq*I restriction site at nucleotide 590, and the lack of a *Fok*I digestion at position 282, and S1a was inferred from the introduction of a *Dde*I restriction site at position 803 and the lack of *Kpn*I digestion at position 481.

Hep3B cells are phenotypically slow acetylators, but the pattern obtained does not match that of any known slow alleles. Therefore the allele has been assigned S4 (Table 3). Digestion with *Fok*I showed that a mutation exists at position 282 in both alleles. None of the other restriction enzymes, nor the allele-specific PCR, identified any mutations in either of the alleles. Figure 3 illustrates that the Hep3B cells do not possess any S1 allele, as indicated by the lack of PCR product with the primer pair DH2 and DH3 (which only amplify DNA if the base at 341 is mutated).

The genotype of U937 cells was determined and these were shown to be homozygous for S1a, by the lack of *Kpn*I digestion and the introduction of an additional *Dde*I restriction sited at position 803 (Table 3).

Expression of NAT genes

The lack of pNAT in the hepatoma cells could thus be explained by the fact that they were derived from individuals who were slow acetylators. Hepatocytes from rabbits have been shown to express NAT activity (McQueen et al., 1983). In order to determine

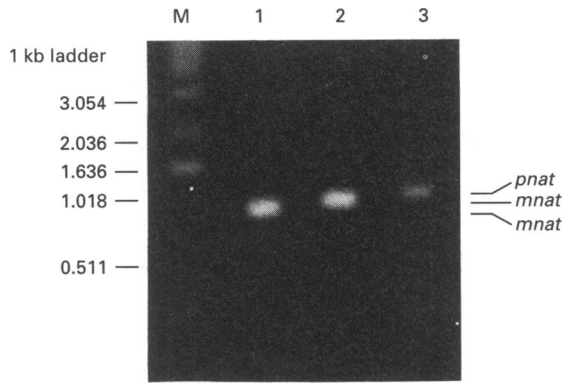

Figure 5 Amplification of NAT sequences from first-strand cDNA of Hep3B cells

First-strand cDNA from Hep3B cells was amplified with Mono A and Mono C (lanes 1 and 2) or Nat-Hu7 and Nat-Hu16 (lane 3). The products were digested with *Hin*dIII (lanes 1 and 3) or *Hin*cII (lane 2). The digested mNAT-specific DNA after digestion with *Hin*cII is shown in lane 2. The pNAT-specific DNA remaining uncut by *Hin*dIII is shown in lane 3. The molecular-mass markers are shown in lane M.

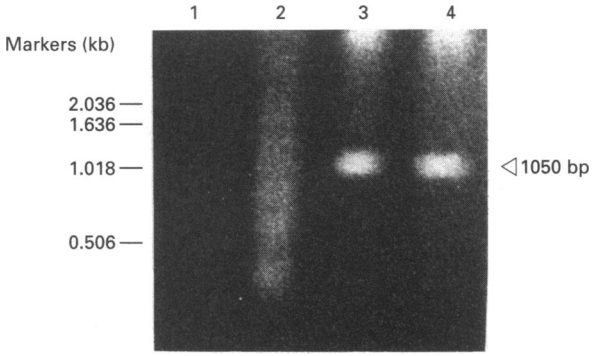

Figure 6 Amplification of pNAT-specific sequences from first-strand cDNA from HepG2 and Hep3B cells to show that splicing occurs

Genomic DNA (lane 2) or cDNA (lanes 3 and 4) from HepG2 cells (lanes 2 and 3) or Hep3B (lane 4) was amplified with the primers Nat-Hu19 and Nat-Hu16. The band indicated with the open arrowhead is the product, indicative of correct splicing (1050 bp). A control containing no template DNA is shown in lane 1. Molecular-mass markers were run concurrently. The products of the amplification were separated on a 1.2% (w/v) agarose gel.

whether these immortalized cells are also capable of transcribing *pnat*, cDNA was generated and investigated for the presence of *pnat* sequences. This was then followed by amplification of *pnat* cDNA with primers Nat-Hu14 and Nat-Hu16, and *mnat* cDNA with primers Mono A and Mono C. In the U937 cells, in agreement with a previous study (Kelly and Sim, 1991), only *mnat* sequences could be detected in the cDNA (Figure 4); negative results with Nat-Hu14 and Nat-Hu16 as primers are not shown. Stimulation of the cells with PMA resulted in differentiation of the cells into a macrophage-like morphology, but again no *pnat* cDNA was detected although *mnat* cDNA was present. On the other hand in the hepatoma cell lines both *pnat* and *mnat* cDNA were present, as shown in Figure 5 for Hep3B cells.

Mature *pnat* mRNA consists of a 5′ non-coding exon which is spliced to a site adjacent to the coding exon (Figure 1, and Ebisawa and Deguchi, 1991). To determine whether this region was being correctly spliced in the cultured hepatoma cells, a primer was designed derived from this region: Nat-Hu19 (Figure 1). This was used together with the downstream primer Nat-Hu16 and products were obtained with both the HepG2- and Hep3B-derived cDNAs, whereas a similar band was not amplified up with genomic DNA (Figure 6).

DISCUSSION

A summary of the alleles for the *pnat* locus is shown in Table 1 with the corresponding identifying restriction digestion sites. It appears that the various mutant alleles are generated by specific point mutations at conserved sites, although not all mutations lead to amino acid substitutions. When the amino acid sequence of mNAT is compared with that of pNAT only one of the substitution positions (286) corresponded to any of the 'slow-allele' amino acid substitution positions. It is interesting to note that the different amino acids are clustered at three regions (Figure 7). Therefore it is possible that the alterations may act to destabilize the mRNA or the protein, rather than alter the substrate specificity of the enzyme. In support of this are the observations on protein stability of the mutant alleles. Blum et al. (1991) and Ohsako and Deguchi (1990) showed that protein was destabilized, but that mRNA levels were not affected in studies on the cloned cDNA species expressed *in vitro* and from chimeric mutant constructs (Blum et al., 1991; Deguchi, 1992).

It has been shown that the monocytic cell line, U937, and the hepatoma cell lines, HepG2 and Hep3B, all express mNAT. This has been demonstrated both at the protein level and the cDNA level. The cells were genotyped and it was shown that U937 cells are homozygous slow acetylators (S1a/S1a), HepG2 cells are heterozygous for two slow alleles (S1a/S2) and Hep3B cells are likely to be homozygous for a different allele, although the presence of two unusual alleles cannot be totally discounted. A mutation in the Hep3B allele was identified by loss of digestion with *Fok*I. A mutation at position 282 has been reported to be insufficient alone to have a deleterious effect on the protein (Blum et al., 1991; Deguchi, 1992). It is likely that other mutations exist in this allele. The introduction of mutations on culturing may be discounted, as the *pnat* alleles in U937 cells which were fully sequenced were identified and matched the ethnic origin from which they were derived. The rare allele in Hep3B cells has not yet been described in a human population. This does not exclude its existence since less than 20 individual *pnat* sequences have been determined and there are distinct *pnat* alleles in different ethnic populations (Blum et al., 1991; Deguchi, 1992; Hickman et al., 1992). The Hep3B cell line was derived from an American of African origin and none of the reported sequences are derived from this ethnic group.

U937 cells do not express *pnat* mRNA, whereas both the hepatoma cell lines expressed *pnat* mRNA. The fact that no acetylation of the polymorphic substrate SMZ was observed in the hepatocyte-derived cell lines can therefore be explained by the lack of an F1 allele. The liver is the site of polymorphic N-acetylation. The presence of *pnat* mRNA in the hepatoma cells supports the hypothesis that hepatocytes are the cells responsible for N-acetylation in human liver. Tissue macrophages are the other major cell type in the liver, but stimulation of U937 cells into a macrophage-like morphology, using PMA (Kurata et al., 1989), did not induce expression of *pnat* mRNA.

Mature mRNA for the synthesis of pNAT is formed by the splicing of an upstream promoter region, which is required for efficient translation (Ebisawa and Deguchi, 1991).

To investigate whether the mRNA species that was detected in the hepatoma-derived cells was likely to be spliced a primer, Nat-

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Human  pNAT  MDIEAYFERIGYKNSRNKLDLETLTDILEHQIRAVPFENLNMHCGQAMELGLEAIFDHIVRRNRGGWCLQVNQLL 75
Human  mNAT        L      K                Q              I   D  D        QV              H
Rabbit pNAT        YQ      NP       S      FQ   T  Y    SI   ES E D       Q               Y
Rabbit mNAT

                                                      T
Human  pNAT  YWALTTIGFQTTMLGGYFYIPPVNKYSTGMVHLLLQVTIDGRNYIVDAGSGSSSQMWQPLELISGKDQPQVPCI 149
Human  mNAT           E       V ST AK       I                    F R Y                    V
Rabbit pNAT        T  E       FV GSNND      I  IV     N          F R Y      V             S
Rabbit mNAT                     C  HT

                                                                Q
Human  pNAT  FCLTEERGIWYLDQIRREQYITNKEFLNSHLLPKKKHQKIYLFTLEPRTIEDFESMNTYLQTSPTSSFITTSFC 223
Human  mNAT   R    N F        TQ   P E   H D  EDS YR   S   K                    S V TSK
Rabbit pNAT   R R  GET         Q HVPDQ   N E  EKKIY  L C   Q      E   A      E  S V LDK I
Rabbit mNAT                                  R T R                             I    P

                                                          R                  E
Human  pNAT  SLQTPEGVYCLVGFILTYRKFNYKDNTDLVEFKTLTEEEVEEVLKNIFKISLGRNLVPKPGDGSLTI 290
Human  mNAT       D  H      T  H R          I      S    I K       N   Q K     H  RFF
Rabbit pNAT       E  H     LT  S TY    E          V T      G   T   N   GKK  S N HL F
Rabbit mNAT                    F                                                  F
```

Figure 7 Comparison of the amino acid sequences of NAT genes

Deduced amino acid sequences were taken for human NAT from Blum et al. (1990), and for rabbit fNAT (NAT found in the liver of fast-acetylator rabbits) and for sNAT (NAT found in the liver of slow-acetylator rabbits) from Sasaki et al. (1991). Only amino acids differing from pNAT are indicated. The amino acids above the pNAT sequence indicate the substitutions in the human allelic variants of pNAT.

Hu19, which lies in this upstream non-coding region, was designed. When PCR was performed on cDNA from both the hepatoma cells, products were obtained, indicating that correct splicing had occurred (Figure 5). When PCR using this primer pair was performed on genomic DNA, no similar product was obtained. Therefore pNAT mRNA is being spliced in the hepatoma cell lines. Although both of the transformed cell lines described do not have fast alleles for pNAT, these studies are being extended to search for hepatoma cell lines carrying the F allele. Hepatoma cell lines of different NAT genotypes could prove useful as human model systems for toxicity and metabolism studies.

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