Identification of N-Acetyltransferase 2 (NAT2) Transcription Start Sites and Quantitation of NAT2-Specific mRNA in Human Tissues

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Abstract:

Human N-acetyltransferase 2 (NAT2) genetic polymorphism is associated with drug toxicity and/or carcinogenesis in various tissues. Knowledge of NAT2 gene structure and expression is critical for understanding these associations. Previous findings suggest that human NAT2 expression is highest in liver and gut but expressed at functional levels in other tissues. A sensitive and specific TaqMan reverse transcriptase-polymerase chain reaction (RT-PCR) assay with intron-spanning primers was developed and used, together with a second TaqMan RT-PCR assay based on amplification of a NAT2 open reading frame (ORF) exon segment, to measure NAT2 mRNA in 29 different human tissues. Cap-dependent amplification of mRNA 5' termini and review of public database information were done to more precisely define the NAT2 promoter(s) and to validate the quantitative RT-PCR assay design. The great majority (40/41) of NAT2 liver cDNAs had 5' termini between 8682 and 8752 nucleotides upstream of the NAT2 ORF exon, and 34 of 40 5' termini were at the −8711 and −8716 adenines. All 59 NAT2 cDNAs with 5' termini in this vicinity, including 40 of the liver isolates and 19 cDNAs in public databases from liver and other sources, showed direct splicing to the ORF exon, with no other noncoding exon detected. NAT2 mRNA was highest in liver, small intestine, and colon and was readily detected in most other tissues, albeit at much lower levels. NAT2 expression in diverse human tissues provides further mechanistic support underlying associations between NAT2 genetic polymorphism, drug toxicity, and/or chemical carcinogenesis.

Genetic polymorphism of the N-acetyltransferase 2 gene (NAT2) is strongly implicated in differential susceptibility to adverse drug reactions (Weber and Hein, 1985; Butcher et al., 2002) and to various diseases (Boukouvala and Fakis, 2005), especially cancers of the urinary bladder (Garcia-Closas et al., 2005; Carreon et al., 2006; Hein, 2006) and colon (Lilla et al., 2006; Moslehi et al., 2006), on exposure to carbocyclic and heterocyclic-aromatic amine carcinogens. A large number of studies have also examined the possible involvement of NAT2 in the etiology of cancers of various other organs (reviewed in Hein et al., 2000a,b). A critical question is whether vulnerability to neoplastic transformation is related to specific expression of NAT2 in the target organ (Williams, 2001), and studies have investigated NAT2 expression in various human (reviewed in Boukouvala and Fakis, 2005) and recently in Syrian hamster (Hein et al., 2006) and mouse (Loehle et al., 2006) tissues. We hypothesized that human NAT2 expression is highest in liver and gut but is also present in other human tissues. To test this hypothesis, sensitive, specific, and well characterized quantitative mRNA assays were used to quantitate relative expression of NAT2 in 29 different human tissue types.

Accurate quantitative measurements of NAT2 mRNA and protein pose a particular technical challenge because of the presence of the paralogous gene, N-acetyltransferase 1 (NAT1), which is ubiquitously expressed in human tissues and shares 87% nucleic acid homology within the protein coding region and 80% amino acid identity to NAT2 (Blum et al., 1990; Ohnsako and Deguchi, 1990). The high degree of nucleic acid homology between NAT1 and NAT2 is a potential confounder of general microarray-based analyses of mRNA in normal human tissues. Thus, the reliability of NAT2 and NAT1 expression data in public databases such as GEO Profiles (http://www.ncbi.nlm.nih.gov/) or from genome-wide scans (Shyamsundar et al., 2005) is dependent on factors that may not always be consistent, including the homology of the chosen probe to both NAT1 and NAT2 mRNAs and the experimental hybridization stringency. Although the NAT1 and NAT2 enzymes are distinguishable by differences in substrate selectivity and affinity, the utility of activity measurements for quantitative evaluation of multiple human tissues is constrained by assay sensitivity and the requirement for fresh tissue specimens with functionally intact enzyme from individuals with the same polymorphic genotype. The use of mRNA detection methods with validated specificity thus...

ABBREVIATIONS: NAT2, N-acetyltransferase 2; NAT1, N-acetyltransferase 1; kb, kilobase; ORF, open reading frame; 5'-RLM-RACE, 5'-RNA ligase-mediated rapid amplification of cDNA ends; q-RT-PCR, quantitative reverse transcriptase-polymerase chain reaction; TSS, transcription start site(s); UCSC, University of California, Santa Cruz; HEK, human embryonic kidney; DBTSS, Database of Transcriptional Start Sites; BD, BD Biosciences Clontech; bp, base pair(s).
offers the most practical and reliable means for sensitive quantitation of the relative expression levels of NAT2 in diverse human tissues. Recent reports have shown that NAT2 gene transcription involves at least two different promoters and more than 10 different 5′ noncoding exons (Husain et al., 2004; Boukouvala and Sim, 2005; Butcher et al., 2005; Barker et al., 2006). Two previous studies indicated that NAT2 mRNA synthesis is less complex, involving transcription from a single promoter located more than 8.7 kilobase (kb) upstream of the open reading frame (ORF) exon and removal of a single 8.7-kb intron during splicing (Ebisawa and Deguchi, 1991; Deguchi, 1992), but tentative evidence for an additional promoter site was recently described (Boukouvala and Sim, 2005). To critically test the major single-promoter model of NAT2, we have now conducted an extensive 5′-RNA ligase-mediated rapid amplification of cDNA ends (5′-RLM-RACE) analysis of liver RNA and compared the results of quantitative reverse transcriptase-polymerase chain reaction (q-RT-PCR) assays based on intron-spanning versus intronexon primer sets in diverse tissues.

Materials and Methods

5′-RLM-RACE Analysis of NAT2 mRNA. 5′-RLM-RACE analysis was performed starting with 10 µg of total RNA, using the First-Choice RLM-RACE kit (Ambion, Austin, TX) according to the manufacturer’s instructions. The final RT was performed with random decamer primers in a 20-µl reaction. The primers used for nested PCR were the adapter primers supplied by Ambion and the NAT2-specific outer, 5′-TCTCTTCTCCTCTGTCAGAAGCAGAAAAGTGC-3′, and inner, 5′-AAGGTCGAGCGTAGTTATGTTAAA-3′, primers. The NAT2 primers were designed to be specific for NAT2 (not NAT7) and to avoid the positions of known polymorphic sites. To obtain clones representing independently initiated transcripts, multiple independent nested PCR were carried out using as template 0.5 or 1 µl of product from the RT reaction stage of the 5′-RLM-RACE procedure with liver RNA. The outer reactions were each 25 µl. Two microliters of the resulting products were resolved on a 1% agarose gel, purified with Qiaex II (Qiagen, Valencia, CA), and cloned with the TOPO TA Cloning Kit for sequencing. PCR products for sequencing were resolved on a 1% agarose gel, purified with Qiaex II (Qiagen, Valencia, CA), and cloned with the TOPO TA Cloning Kit into the pcDNA 3.1/V5-His-TOPO vector (Invitrogen, Carlsbad, CA). Sequence reactions prepared with ABI Big Dye Terminators were analyzed on the ABI 310 Genetic Analyzer, as recommended by the manufacturer (Applied Biosystems, Foster City, CA). Transcription start sites (TSS) were inferred from sequence adjoining that of the 5′-RLM-RACE adapter, and mRNA splice locations were identified by alignment of cDNA sequences with NAT2 genomic sequences using the BLAT tool (Kent, 2002) at the University of California, Santa Cruz (UCSC) Gateway (http://genome.ucsc.edu/egi-bin/hgGateway).

Database Analyses. NAT2-specific cDNA and spliced expressed sequence tag sequences were identified by searches at the National Center for Biotechnology Information Unigene (http://www.ncbi.nlm.nih.gov/Unigene), Database of Transcriptional Start Sites (DBTSS) (http://dbtss.hgc.jp), and UCSC Gateway databases (Kent et al., 2002; Suzuki et al., 2002; Wheeler et al., 2006; Yamashita et al., 2006). Selected nonredundant cDNA sequences were aligned with genomic DNA using the BLAT tool (Kent, 2002) at the UCSC Gateway.

Human RNA Samples. Purified total RNAs from human tissues were obtained from two independent commercial sources, Ambion and BD Biosciences Clontech (BD) (Palo Alto, CA). The human tissue expression panel from Ambion included adipose, bladder, brain, cervix, colon, esophagus, heart, kidney, liver, lung, ovary, placenta, prostate, skeletal muscle, small intestine, spleen, testis, thymus, thyroid, and trachea. Each of the Ambion panel RNAs was a pool derived from three individuals. A sample of breast RNA, also obtained from Ambion, was derived from one female. The expression panel obtained from BD included RNA from brain, cerebellum, colon, fetal brain, fetal liver, heart, kidney, lung, placenta, prostate, salivary gland, skeletal muscle, small intestine, spinal cord, spleen, stomach, testis, thymus, trachea, and uterus. The BD panel RNAs generally were pooled from a variable number of individuals, ranging from 3 to 63; however, the brain, heart, placenta, and stomach RNAs were all derived from single individuals. RNAs representing adrenal gland, bladder, and liver were also obtained from BD. For the 5′-RLM-RACE analysis, the liver RNA sample from BD and a second liver RNA sample from Ambion, representing different individual males, were used. MCF-7 cells were grown as described previously (Husain et al., 2004). HepG2 human hepatocellular carcinoma cells were obtained from the American Type Culture Collection (HT-8065) and cultured in Dulbecco’s modified Eagle’s medium/Ham’s F-12 medium (1:1) (Invitrogen) supplemented with 10% fetal bovine serum (HyClone Laboratories, Logan, UT), penicillin (100 U/ml), and streptomycin (100 µg/ml) (Cambrex Bio Science Walkersville, Inc., Walkersville, MD). Cells were propagated in a 37°C humidified chamber containing 5% CO2. Cell culture medium was routinely changed every 2 or 3 days. Frozen samples of human colon tumor cell lines Caco-2 (J. C. States), SW480 (W. S. Zundel), and HT-29 (D. M. Miller), human lung adenocarcinoma cell line A549 (C. M. Kingle), and human embryonic kidney (HEK)-derived cell line HEK-293 (C. M. Kingle) were provided by the laboratories of the indicated University of Louisville investigators. RNAs were extracted as previously described (Barker et al., 2006).

Quantitative Real-Time RT-PCR. For quantitative real-time RT-PCR, TaqMan analysis was performed using the 7,700 sequence detection system from ABI (Applied Biosystems). The 20-µl PCR reactions were 1× TaqMan Universal Master Mix, with NAT2 forward and reverse primers (300 nM) and 100 nM probe. The primers, 5′-TGGAGAACAGAGGATTTGCTGAT-3′ and 5′-GATCTGGTGCTGCAAATGTGCAGT-3′, span the position of the 8.7-kb intron (Ebisawa and Deguchi, 1991; Deguchi, 1992; Boukouvala and Sim, 2005) and were used with the probe 6FAM 5′-TCTCTGTTGCTGCTGCCC-3′-MGBNFQ. A second NAT2 primer pair and probe set has been described (Zang et al., 2004) and is located entirely within the 3′ portion of the single exon that contains the entire NAT2 ORF. For PCR, initial incubations at 50°C for 2 min and 94°C for 10 min were followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Quantitation of the endogenous control 18S rRNA was performed using TaqMan Ribosomal RNA Control Reagents for 18S rRNA (Applied Biosystems). Four microliters of diluted cDNA, equivalent to 40 ng of the initial RNA template, was used in each PCR. Two independent RT reactions were prepared for each RNA, and for each cDNA product, real-time PCR were carried out in duplicate, for a total of four readings. Each assay plate included reaction mix controls with water in place of template. As an additional negative control, TaqMan quantitation of reactions prepared in parallel without addition of RT was also run at least once for each set of replicate RT reactions. Baselines and threshold levels were selected as recommended by ABI, and values of Ct, the cycle number at which the measured fluorescence reached the set threshold value, were recorded. For each real-time run, a ΔCt (NAT2 Ct – 18S rRNA Ct) was calculated for each sample, and a ΔΔCt value was then derived by subtracting the smallest ΔCt from all the ΔCt values. The formula 2−ΔΔCt was used to calculate an initial relative concentration value for all the samples, except that samples with a NAT2 Ct of 40 were assigned as zero. For each RT set, the measurements obtained from the two real-time runs were averaged and normalized with respect to the average of all the samples in the same RT group.

Results

Molecular Analysis of the 5′ Structure of NAT2 mRNA. Two human male liver RNA samples, obtained from independent commercial sources, were each submitted to 5′-RLM-RACE for identification of NAT2 TSS and determination of 5′-mRNA splicing patterns. Clones of 41 independent 5′-RLM-RACE products were sequenced. The location of 40 of the TSS was between 8682 and 8753 base pairs (bp) upstream of the NAT2 ORF exon (Fig. 1A), with 9 TSS at the −8711 adenine and 25 TSS at the −8716 adenine. These two adenosines were located at positions −59 and −64 with respect to the splice donor sequence utilized in all the corresponding mRNAs. The only atypical 5′-RLM-RACE clone originated from a start site located a further 4.6 kb upstream from the −8.7-kb promoter, and the spliced structure did not include any sequences from the −8.7-kb promoter region (data not shown).
adenocarcinoma (BX095770, AI792606) and hepatocellular carcinoma (AV684197) are shown as X, and the 5' ends of the transcripts are indicated by a and b. The positions of the most frequent TSS locations at -59 and -64 are located 8711 and 8716 nucleotides upstream of the 5' ends of the transcripts identified by 5'-RLM-RACE, as shown in Fig. 1A. The TSS identified by 5'-RLM-RACE, defines the position of the intron-spanning forward q-RT-PCR primer as shown in italics. The sequence corresponding to the intron-spanning forward q-RT-PCR primer is shown in italics. The sequence of the primer marked IS-for is shown in italics.

Figure 1B summarizes the positions of the 5' termini of 19 different NAT2 cDNA clone sequences retrieved from the public databases described under Materials and Methods. Although the cloning of most of these cDNAs was not specifically intended to detect TSS, every observed 5' terminus lies within the same ~50-bp region as the TSS identified by 5'-RLM-RACE, and a similar pattern of clustering of 5' ends in or near the -59 to -64 segment is apparent. The three clones from DBTSS, HSI05750, HSI08034, and HS115615, were isolated from small intestine RNA with an “oligo-capping” method (Suzuki et al., 2002), which, like 5'-RLM-RACE, defines the position of capped 5' nucleotides. The 5' termini of two of the small intestine DBTSS clones are at -64, which is also the most common TSS in liver, and the third is at -61.

The region containing the NAT2 TSS has 85% sequence identity to the major TSS region of the paralogous NAT1 gene (Fig. 1C). Although there was more diversity in the locations of NAT1 TSS identified in breast tissue and a breast tumor cell line (Husain et al., 2004), one of the frequent NAT1 TSS occurred at an adenine that corresponds to the primary NAT2 TSS adenine at the -64 position (Fig. 1).

For all 40 of the liver 5'-RLM-RACE cDNAs with 5' termini in the -8.7-kb promoter region, the mRNA splicing pattern conformed to that shown in Fig. 2, utilizing the consensus splice donor sequence located 8658 nucleotides upstream of the NAT2 ORF exon (Fig. 1). All 19 of the NAT2 public database cDNAs also utilize this splice donor sequence, and 18 of 19 are spliced to the typical NAT2 ORF exon acceptor site. One cDNA, BG569272, exhibits splicing that eliminates 339 nucleotides of the NAT2 ORF exon and may represent an aberrant transcript form or cloning artifact.

**q-RT-PCR Analysis of NAT2 mRNA in Diverse Human Tissues.**

Two different TaqMan primer and probe sets were utilized for quantitative analysis of NAT2 mRNA (Fig. 2). In genomic DNA, the primers of the intron-spanning assay set, IS-for and IS-rev (Fig. 2), are separated by the 8.7-kb intron, eliminating any measurement error caused by possible DNA impurities in RNA samples. The IS-for primer sequence is entirely 3' of the TSS locations NAT2 mRNA identified in liver RNA (Fig. 1) and all three of the authentic TSS from small intestine (Fig. 1B). The TSS from the intron-spanning assay results, on a logarithmic scale, are shown in Figs. 3A and 4A for two sets of human RNA samples representing 29 different tissue types, including 15 tissues represented in both panels. In each set, the highest NAT2 mRNA level was found in liver, with similar or slightly less expression found in small intestine and about 5- to 10-fold lower expression in colon. For several tissues, including kidney, lung, spleen, and testis, which are represented in both the Ambion (Fig. 3A) and the BD series (Fig. 4A), NAT2 expression levels were consistently about 1% of that detected in liver. Lower or more variable levels of expression occurred in several other tissues, and consistently low levels of NAT2 expression were found in both of the samples representing heart, skeletal muscle, thymus, and trachea (Figs. 3A and 4A).

Figures 3B and 4B show results of a second NAT2-specific TaqMan q-RT-PCR assay, which utilizes primers and a probe all located within the single NAT2 ORF exon (Fig. 2), for the same tissue RNA samples assayed with the intron-spanning set. The NAT2 ORF assay results...
showed a somewhat poorer signal/noise ratio, as indicated by a
general tendency to higher Ct values, and the presence of genomic
DNA contamination interfered with measurements of a few samples
(Fig. 4B). The detected hierarchy of tissue-specific NAT2 mRNA
expression was very similar for the intron-spanning and the ORF
TaqMan assays in the tissue RNA panels from Ambion (Fig. 3, A and
B) and BD (Fig. 4, A and B). Thus, there was no evidence for any
alternative NAT2 promoter(s) expressing NAT2 mRNA with a differ-
ent tissue-specific profile or of sufficient quantity to substantially alter
the relative level of NAT2 mRNA established by transcription at the
−8.7-kb promoter locus.

**q-RT-PCR Analysis of NAT2 mRNA in Human Cell Lines.**
Figure 5 shows NAT2 TaqMan measurements for five tumor cell lines
from breast (MCF-7), colon (Caco-2, SW480, and HT-29), liver
(HepG2), and lung (A549) and a virally transformed embryonic
kidney cell line (HEK-293). None of the lines, including those derived
from liver or colon, showed a level of NAT2 expression that corre-
sponded to that of the high-expressing normal tissues. The two cell
lines with the highest NAT2 expression, HepG2 and Caco-2, exhibited
only about 1% and 5 to 10%, respectively, of the NAT2 expression in
the corresponding tissue.

**Discussion**
Accurate measurement of NAT2 expression in different human
tissues is an important aspect of assessing its role in drug toxicity or
carcinogenesis at various organ sites. In this study, a NAT2-specific
q-RT-PCR TaqMan assay with intron-spanning primers was designed
based on the structure of NAT2 cDNAs in public databases, and the
design was validated by an extensive 5′-RLM-RACE analysis of
NAT2 mRNA in liver. The accuracy of the assay for assessing relative
mRNA levels in diverse tissues was verified by comparison with an
alternative q-RT-PCR assay based on primers within the NAT2 ORF.
A recent study suggested that NAT2 transcripts also may be initi-
ated immediately 5′ to the ORF-containing exon based on detection of
RT-PCR products corresponding to such transcripts, most promi-
nently in liver (Boukouvala and Sim, 2005). We have detected similar
products by RT-PCR of liver and placental RNA, but RT-dependent
PCR products of equal or greater prominence were also found using
primer pairs from entirely within the 8.7-kb intron or from segments including the NAT2 promoter exon and immediately adjacent intronic sequences (data not shown). These results suggested that unspliced or incomplete primary NAT2 transcripts from the −8.7-kb promoter are present in RNA from liver and other tissues and that functional activity of an ORF-adjacent promoter cannot be directly inferred from demonstration of RT-PCR products derived from that region. The location of any NAT2 promoter immediately 5' to the NAT2 ORF also is inconsistent with the 5' structure of cDNAs in the public databases, and our finding that 0 of 41 of the NAT2 cDNAs characterized by 5'-RLM-RACE of liver mRNAs had a 5' terminus at that position. Together, the findings that NAT2 transcripts in liver originate predominantly at the −8.7-kb promoter and that both the intron-spanning (Figs. 3A and 4A) and ORF-only (Figs. 3B and 4B) TaqMan assays show similar relative levels of NAT2 mRNA in liver and other tissues suggest that any other possible alternative promoter in the nonhepatic tissues must be relatively very weak. These results also suggest that the pattern of NAT2 TSS at the −8.7-kb promoter in tissues other than liver is not substantially shifted to a location 3' to the forward primer used for the q-RT-PCR assay because such a pattern of transcription initiation would also be detected as a discrepancy between the reported intron-spanning and ORF-only measurements. Thus, in the absence of evidence for any alternative NAT2 TSS or splicing pattern, the intron-spanning TaqMan assay design shown in Fig. 2 appears to be superior for comparing NAT2 mRNA levels in different human tissue samples because of its high sensitivity and the elimination of measurement errors caused by contaminating genomic DNA.

The reliability of the NAT2 measurements is also dependent on specificity of the assay, which is important to verify empirically because of the high homology of the NAT2 and NAT1 sequences in the regions that include the TaqMan assay primers. The MCF-7 cell line is known to express a high level of NAT1 mRNA (Husain et al., 2004), but only minimal levels of NAT2 mRNA were detected in MCF-7 with the intron-spanning assay (Fig. 5) or the ORF assay (data not shown), showing that each assay is highly specific for NAT2.

None of the cell lines studied here, including ones derived from liver and colon, was found to have the high level of NAT2 mRNA expression characteristic of those tissues. This finding is consistent with previous reports of very low or undetectable NAT2 enzyme activity in liver-derived cell lines (Coroneos and Sim, 1993).
observed differences between human tissues and cultured immortalized cell lines are not unexpected because gene expression patterns in transformed and cancer cell lines do not always reflect the tissue from which they are derived and can vary with culture conditions.

For the 45 human tissue RNA samples representing 29 different organ types, NAT2 mRNA levels (Figs. 3A and 4A) were highest in liver, with slightly lower amounts in small intestine and colon. The expression of NAT2 in liver has long been recognized as the primary determinant of the human acetylator phenotype (Grant et al., 1990; Bendriss et al., 1998), and NAT2 enzyme is most readily detected in liver, colon, and intestine (Turesky et al., 1991; Sim et al., 2000). NAT2 mRNA was also readily detected in both tested samples of kidney, lung, spleen, and testis and single samples from fetal liver, spinal cord, stomach, and uterus at a level about 1% compared with liver. NAT2 mRNA was also detected in heart, skeletal muscle, thymus, and trachea, albeit at 10-fold lower levels. Widespread expression of NAT2 in human tissues has been previously reported (Windmill et al., 2000). The low level of NAT2 mRNA in fetal liver compared with adult liver suggests that NAT2 expression in humans may follow a similar developmental pattern as in mice (McQueen and Chau, 2003).

NAT2 mRNA expression in bladder is of particular interest because very strong epidemiological associations have been established between the NAT2 slow acetylation phenotype and increased risk of cancer of the urinary bladder caused by arylamines (Garcia-Closas et al., 2005; Carreon et al., 2006; Hein, 2006). The NAT2 rapid acetylation phenotype has also often been associated with increased risk of colorectal cancer (Lilla et al., 2006; Mosleh et al., 2006). Although NAT2 mRNA was relatively high in colon, the NAT2 mRNA level in bladder was very low compared with other tested tissues, about 0.1% of the liver level. This result suggests caution in assuming that a gene modifying cancer susceptibility is more likely to be highly expressed in the affected tissue. The association of urinary bladder cancer with NAT2 genetic polymorphism could be the result of impaired detoxification of carcinogens in the liver, where NAT2 mRNA expression is high. Alternatively, factors such as the concentrations of procarcinogens, the presence of competing or cooperating enzyme activities, or other cell- or organ-specific features could magnify the effects of NAT2 enzyme activity in the bladder. A third possibility is that mRNA levels and enzyme activities measured with extracts from whole organs do not reflect the activity of the specific population of cells in which the carcinogenic mutations arise.

The identification of the precise location of NAT2 TSS by 5'-RLM-RACE permits a detailed comparison of the corresponding NAT1 P1 (Husain et al., 2004) and NAT2 promoter sequences. In the immediate vicinity of the NAT2 TSS, there is 85% nucleotide identity with the NAT1 P1 promoter (Fig. 1), similar to the 87% identity found in the coding region of these paralogous genes. Within this region, the spectrum of NAT2 TSS in liver is relatively limited, with 34 of 40 of the 5′ termini located at one of two closely spaced adenine residues. In contrast, the spectrum of NAT1 TSS, previously determined in RNA from breast and the MCF-7 cell line (Husain et al., 2004), is more diffuse, with only a partial overlap with the location of the NAT2 TSS (Fig. 1). It is notable that the NAT1 P1 promoter is actively transcribed in essentially all the human tissues (Barker et al., 2006), whereas high level NAT2 expression is limited to very few tissues. The more limited spectrum of NAT2 transcription initiation sites may thus reflect a transcription initiation complex that has a greater degree of structural definition because of the required binding of tissue-specific transcription factors. Another distinction of the NAT2 promoter that likely contributes to its different expression profile is the absence of the consensus Sp1 site that occurs in the NAT1 P1 promoter immediately upstream of the sequence shown in Fig. 1.

In conclusion, NAT2-specific TSS were identified. A sensitive and specific TaqMan RT-PCR assay with intron-spanning primers was developed and used, together with a second TaqMan RT-PCR assay based on amplification of a NAT2 ORF exon segment, to measure NAT2 mRNA in 29 different human tissues. NAT2 mRNA was highest in liver, small intestine, and colon and readily detected in most other tissues, albeit at levels 1% or less than in liver. Expression of NAT2 in various human tissues may be relevant to associations between NAT2 genetic polymorphism and drug toxicity or arylamine-induced cancers at these sites.

References


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