Accumulated recent evidence is indicating that alternative splicing represents a generalized process that increases the complexity of human gene expression. Here we show that mRNA production may not necessarily be limited to single genes, as human liver also has the potential to produce a variety of hybrid cytochrome P450 3A mRNA molecules. The four known cytochrome P450 3A genes in humans, CYP3A4, CYP3A5, CYP3A7, and CYP3A43, share a high degree of similarity, consist of 13 exons with conserved exon-intron boundaries, and form a cluster on chromosome 7. The chimeric CYP3A mRNA molecules described herein are characterized by CYP3A43 exon 1 joined at canonical splice sites to distinct sets of CYP3A4 or CYP3A5 exons. Because the CYP3A43 gene is in a head-to-head orientation with the CYP3A4 and CYP3A5 genes, bypassing transcriptional termination cannot account for the formation of hybrid CYP3A mRNAs. Thus, the mechanism generating these molecules has to be an RNA processing event that joins exons of independent pre-mRNA molecules, i.e. trans-splicing. Using quantitative real-time polymerase chain reaction, the ratio of one CYP3A43/3A4 intergenic combination was estimated to be ~0.15% that of the CYP3A43 mRNAs. Moreover, trans-splicing has been found not to interfere with polyadenylation. Heterologous expression of the chimeric species composed of CYP3A43 exon 1 joined to exons 2-13 of CYP3A4 revealed catalytic activity toward testosterone.

The ongoing genome projects are allowing the determination of the coding potential of several eukaryotic species at a rapidly increasing pace. However, as most eukaryotic genes are in pieces, consisting of discrete exonic sequences flanked by non-coding intronic segments, defining the expressed fraction of the genetic material is obviously of highest priority. The puzzling problem of identifying relatively short exons in a vast excess of intronic sequences is further complicated by recent results indicating that, during gene expression, an additional level of complexity also exists. Specifically, in several higher eukaryotes, processed RNA does not always represent a simple linear combination of exonic sequences. Thus, in addition to canonical transcripts, gene expression may also result in scrambled RNA molecules in which exons juxtapose in an order that is different to that in the gene (1–10). Moreover, the continuously increasing number of reports providing evidence for intergenic RNA molecules suggests that RNA splicing may also combine exons originating from more than one gene (Ref. 10 and references therein; see also Refs. 11–14). Because a majority of these hybrid transcripts is known to encompass exons from neighboring genes that have the same orientation, a plausible mechanism for intergenic mRNA formation would involve bypassing transcriptional termination and formation of a bicistronic transcript followed by alternative splicing. However, a number of in vivo and in vitro experiments indicated that the mammalian splicing apparatus also has the capability to join exons originating from distinct pre-mRNA molecules, a process termed trans-splicing (15–19). The recent findings of exon repetitions in some rat and human mRNAs provided the most convincing evidence that natural trans-splicing within transcripts of single genes may also occur in mammalian cells (20–23).

Cytochrome P450 3As (CYP3A) are heme-containing monooxygenases that catalyze stereospecific hydroxylation of a wide range of substrates, including endogenous steroids and more importantly various xenobiotics, such as drugs and environmental chemicals (24). There are four known CYP3A genes in humans, CYP3A4, CYP3A5, CYP3A7, and the recently discovered CYP3A43 (25–27). The proteins are very similar to each other bearing 71–88% amino acid identities. All four genes consist of 13 exons and form a cluster on chromosome 7q21–22.1. Interestingly, comparison of GenBank entries encompassing various CYP3A genomic sequences revealed a rather unique arrangement of the CYP3A locus, specifically that the CYP3A43 gene is in a head-to-head orientation with the other three, CYP3A4, CYP3A7, and CYP3A5, genes (Ref. 25; Fig. 1).

Recently we have shown that expression of members of another group of cytochrome P450s, the CYP2C, results, in addition to canonical mRNAs, in a variety of chimeric RNA molecules in human liver and epidermis (10, 28). Here we provide evidence that chimeric RNA production is not limited to the CYP2C genes on chromosome 10q24, but is also typical of the CYP3A genes. Specifically, in liver several hybrid mRNAs are produced that encompass the first exon of CYP3A43 joined to various sets of either CYP3A4 or CYP3A5 exons. In addition, because the CYP3A43 gene has an opposite orientation to the CYP3A4 and CYP3A5 genes, the mechanism of chimeric mRNA formation presumably involves splicing events between CYP3A43 and either CYP3A4 or CYP3A5 pre-mRNA molecules. Thus, these findings suggest that natural trans-splicing between distinct genes may occur in higher eukaryotes. Moreover, this intricate pattern of expressed mRNAs implies that novel alternative splicing pathways may significantly increase the complexity of human gene expression.

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The abbreviations used are: CYP, cytochrome P450; RT, reverse transcription.
Reverse Transcription-Polymerase Chain Reaction—Human total RNA was purchased from Invitrogen (adult human liver) or purified from HepG2 cells. HepG2 cells (ATCC) were grown at 37°C, in an atmosphere of humidified air containing 5% CO₂, in RPMI 1640 with l-glutamine medium (Invitrogen) supplemented with 10% fetal calf serum (Invitrogen), 100 units/ml penicillin, and 100 μg/ml streptomycin (Invitrogen). Subculturing was performed at a subcultivation ratio of 1:6. Cells from confluent cultures were harvested by addition of trypsin, and subjected to RNA preparation using the SV Total RNA Isolation System (Promega).

cDNA synthesis was carried out with Moloney murine leukemia virus reverse transcriptase (Promega) in a 50-μl reaction mixture using oligo(dT) primers (25 ng/ml, Promega) with 10 μg of total RNA template. An aliquot of 1–5 μl of the reverse transcription reaction was directly subjected to the first amplification. Both the initial and nested polymerase chain reactions (PCRs) were performed in a 50-μl reaction mixture containing 20 pmol of each of the forward and reverse primers (Cybergene AB), 2 mM MgCl₂, 200 μM dNTPs, and 1.25 units of Taq DNA polymerase (Promega). The oligonucleotides used in the PCRs are listed in Table I. The reactions were carried out for 30 cycles, with 10 s at 92°C, 30 s at 52°C, and 2 min at 72°C. For the nested reaction, 1 μl of the first PCR was directly used. Alternatively, amplifications were done by using the Expand Long Template PCR System (Roche Molecular Biochemicals) for 30 cycles with 10 s at 92°C, 30 s at 52°C, and 2 min + 5 s/cycle at 68°C.

Analysis of PCR Products—The PCR products were cloned in Escherichia coli XL1-Blue cells using the pGEM-T Vector System (Promega). The cloned PCR products were sequenced using the BigDye Terminator kit (Applied Biosystems) with the pGEM-T Vector System (Promega). The PCR products were cloned in Escherichia coli XL1-Blue cells using the pGEM-T Vector System (Promega).

Real-time 5′ Exonuclease (TaqMan) PCR Assay—cDNA synthesis was carried out with Moloney murine leukemia virus reverse transcriptase (Promega) in a 13-μl reaction mixture using 20 pmol of consensus CYP3A exon 4-specific antisense primer, 5′-TAA CAT CT TCT TTC ACT A (Cybergene AB), with 15 μg of human liver total RNA template (Invitrogen). A 2-μl aliquot of the reverse transcription reaction was directly subjected to amplification. Quantitative real-time PCR (31) was performed using the TaqMan Universal PCR Master Mix (Applied Biosystems) with an Applied Biosystems Sequence Detector 7700. The amplification reaction was carried out for 40 cycles, with 15 s at 95°C and 1 min at 60°C in a volume of 30 μl containing 200 nm PCR primers and 200 or 225 nM fluorogenic probe for the canonical CYP3A4 cDNA or the CYP3A43/3A4 cDNA containing exon combination (1)3A43-(4-5-6-7-8-9-10-11-12-13)3A4 and (1)3A43-(4-5-6-7-8-9-10-11-12-13)3A4 in a volume of 30 μl containing 200 nm PCR primers and 200 or 225 nM fluorogenic probe for the canonical CYP3A4 cDNA or the CYP3A43/3A4 cDNA containing exon combination (1)3A43-(4-5-6-7-8-9-10-11-12-13)3A4 and (1)3A43-(4-5-6-7-8-9-10-11-12-13)3A4, respectively. Primers and probes (Cybergene AB) are listed in Table I. To create calibration curves, known amounts of plasmids (1 pg, 10 fg, 1 fg, and 100 ag) encompassing CYP3A43 and (1)3A43-(4-5-6-7-8-9-10-11-12-13)3A4, CYP3A43/3A4 cDNAs were used, respectively.

For cloning purposes, a 1-μl aliquot of the PCR reaction was subjected to a 10 cycle reamplification with 10 s at 92°C, 30 s at 60°C, and 1 min at 72°C. The PCR was performed in 50 μl using 2.5 units of Taq polymerase with 2 mM MgCl₂, 200 μM dNTPs, and 120 μM of each primer.

Polyadenylated RNA was isolated by subjecting 120 μg of human liver total RNA (Invitrogen) to two round purification with Poly(A) Spin mRNA Isolation Kit (New England Biolabs). Poly(A)+ RNA and flow-through were precipitated with ethanol and reverse-transcribed as described for total RNA (above). Two μl of the RT reaction were directly used in quantitative real-time PCR.

Heterologous Expression of CYP3A4 and CYP3A43/3A4 in COS-7 Cells—CYP3A4 and chimeric proteins encoded by (1)3A43-(2-3-4-5-6-7-8-9-10-11-12-13)3A4 and (1)3A43-(4-5-6-7-8-9-10-11-12-13)3A4 RNAs were expressed under the control of human cytomegalovirus promoter in expression vector pcDNAI.1 (Invitrogen). CYP3A4 cDNA was isolated by RT-PCR on human liver RNA using the Advantage High Fidelity PCR system (CLONTECH) with CYP3A4 exon 1, forward, nested, and

was performed on a denaturing 10% acrylamide, 8 M urea gel and visualized after exposure to Fuji Super RX film.
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| PCR primers and TaqMan probes | CYP3A4 exon 13, reverse, nested |
|-------------------------------|--------------------------------|
| PCR primers                   | CYP3A4 exon 13, reverse, nested |
| CYP3A4 exon 1, forward         | CYP3A4 exon 13, reverse, nested |
| CYP3A4 exon 1, forward, nested | CYP3A4 exon 13, reverse, nested |
| CYP3A4 exon 13 reverse         | CYP3A4 exon 13, reverse, nested |
| CYP3A4 exon 13, forward         | CYP3A4 exon 13, reverse, nested |
| CYP3A5 exon 1, forward         | CYP3A4 exon 13, reverse, nested |
| CYP3A5 exon 1, forward, nested | CYP3A4 exon 13, reverse, nested |
| CYP3A5 exon 13, reverse        | CYP3A4 exon 13, reverse, nested |
| CYP3A5 exon 13, reverse, nested | CYP3A4 exon 13, reverse, nested |
| CYP3A7 exon 1, forward         | CYP3A4 exon 13, reverse, nested |
| CYP3A7 exon 1, forward, nested | CYP3A4 exon 13, reverse, nested |
| CYP3A7 exon 13, reverse        | CYP3A4 exon 13, reverse, nested |
| CYP3A43 exon 1, forward        | CYP3A43 exon 13, reverse, nested |
| CYP3A43 exon 1, forward, nested | CYP3A43 exon 13, reverse, nested |
| CYP3A43 exon 13, reverse       | CYP3A43 exon 13, reverse, nested |
| CYP3A43 exon 13, reverse, nested | CYP3A43 exon 13, reverse, nested |

CYP3A43 exon 13 reverse primers (Table I). The RT-PCR product was cloned using the pGEM-T System (Promega), sequenced, and subsequently subcloned on an pcDNA1.1 (Invitrogen). cDNAs of (CYP3A43) exon 1 and (CYP3A4) exon 7, are in the same phase as exon 2. Therefore, all three detected mRNA molecules are characterized by the potential to encode for chimeric CYP3A proteins. Worth noting is that, in the other two combinations, the CYP3A exon 13 fragments are spliced immediately after the CYP3A43 exon 3, i.e. exon 4 and exon 7, are in the same phase as exon 2. Therefore, all three detected mRNA molecules are characterized by open reading frames that start at the canonical ATG initiation codon and terminate at the canonical TGA termination codon (Table I).

Nest RT-PCR amplification using 3A4 exon 1 forward and 3A4 exon 13 reverse primers resulted in no products (data not shown). This suggests directionality in the formation of CYP3A43/3A4 intergenic mRNAs.

To obtain additional evidence by a non-PCR-based method for the presence of intergenic CYP3A mRNAs in human liver, the RNAase protection analysis was employed. Specifically, a riboprobe was generated (see "Materials and Methods") that spans 68 bases of the CYP3A43 exon 1 to CYP3A4 exon 2 splice junction. The CYP3A43 exon 1 overlap was chosen to be of sufficient length (43 bases) that would allow efficient hybridization under the conditions used; however, the CYP3A4 exon 2 overlap was minimized to only 25 bases, to destabilize formation of RNA duplexes with the highly abundant CYP3A4 mRNA. As anticipated, protected fragments of a size of 68 and 43 bases were clearly visible (Fig. 1). Moreover, the intensity of the protected fragments increased with increasing amounts of input human liver RNA. The ratio of the two protected fragments was calculated to be 1.5, which indicates that the joining of CYP3A43 exon 1 to CYP3A4 exon 2 is a much more rare event than the canonical joining of CYP3A43 exons 1 and 2.

Intergenic Splicing between CYP3A43/3A4 Genes—In similarity with the CYP3A43/3A4 chimeric mRNA molecules, CYP3A transcripts encompassing CYP3A43 and CYP3A5 exons are also formed in human liver. Specifically,
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Fig. 2. CYP3A43/3A4 intergenic transcripts. The products of a nested RT-PCR amplification with Taq polymerase, using CYP3A43 exon 1 and CYP3A4 exon 13 primers on human liver total RNA, were separated on an agarose gel and are shown on the left. Lane M, molecular weight markers with size of DNA fragments (base pairs) indicated; lane +, amplification products with addition of human liver RNA during reverse transcription; lane −, amplification products without addition of human liver RNA during reverse transcription. Schematic structures of the amplified fragments are shown on the right. Black boxes denote CYP3A43 exon 1; white boxes denote CYP3A4 exons.

### Table II

**Correlation between translational reading frame and trans-splicing**

| Exon | Reading frame | Intergenic species |
|------|---------------|--------------------|
| 2    | −1            | +                  |
| 3    | 0             | −                  |
| 4    | −1            | +                  |
| 5    | 0             | −                  |
| 6    | 0             | −                  |
| 7    | −1            | +                  |
| 8    | +1            | −                  |
| 9    | 0             | −                  |
| 10   | +1            | −                  |
| 11   | 0             | +                  |
| 12   | −1            | +                  |
| 13   | 0             | −                  |

Note that all but one of the CYP3A exons that were found to juxtapose to the CYP3A43 exon 1 have a reading frame that is identical to the reading frame of exon 2.

The mechanism for trans-splicing—An interpretation of the mechanism that generates CYP3A43/3A4 and CYP3A43/3A5 intergenic mRNAs could be the existence of a duplicated CYP3A43 exon 1 sequence, having the same orientation as the CYP3A4 and the CYP3A5 genes, that is used in a cis-splicing process. To test this possibility, Southern hybridization was performed on human DNA digested with various restriction endonucleases. A CYP3A43 exon 1-specific oligonucleotide was used as the probe. Fig. 5 shows that hybridization resulted in single bands in all four DNA samples. Moreover, the estimated sizes for the DNA fragments fit well with those deduced from the genomic sequence, i.e. 4240, 1224, 4928, and 1961 bp for SpeI-, NdeI-, EcoRI-, and BglII-digested DNA, respectively. These results provide strong evidence that there is only a single CYP3A43 exon 1 in the human genome and thus indicate that the CYP3A43 gene is indeed the source of the 3A4 exon 1 sequences in the CYP3A43/3A4 and CYP3A43/3A5 intergenic mRNA molecules. The absence of a duplicated CYP3A43 exon 1 is also supported by the available genomic GenBank® entries that cover the CYP3A loci. Therefore, because the CYP3A43 gene is encoded by a different DNA strand than the other members of the CYP3A cluster, including the genes for CYP3A4 and CYP3A5, the formation of the identified intergenic CYP3A transcripts can only be rationalized by splicing between distinct pre-mRNA molecules, i.e. trans-splicing.

The steady state ratio of the trans-spliced mRNA molecules—To obtain a quantitative measurement of the abundance of the trans-spliced CYP3A mRNA, the hybrid mRNA molecules containing exon combination (1)3A43-(4-5-6-7-8-9-10-11-12-13)3A4 was analyzed by real-time 5′ exonuclease (TaqMan) PCR. Aliquots of human liver cDNA were subjected to quantitative PCR to measure the absolute amount of the canonical CYP3A43 cDNA and the (1)3A43-(4-5-6-7-8-9-10-11-12-13)3A4 CYP3A43/3A4 cDNA in the same sample. The identity of the fragments amplified during the real-time PCR assay was verified by cloning and DNA sequence analysis. The quantity of the CYP3A43 and the CYP3A3/3A4 cDNAs was measured in separate experiments by the use of two independent standard curves. These were generated with known amounts of plasmid DNAs encompassing the cloned CYP3A43 and (1)3A43-(4-5-6-7-8-9-10-11-12-13)3A4 CYP3A43/3A4 cDNAs, respectively. The real-time PCR assay revealed that the ratio between the canonical CYP3A43 mRNAs and the (1)3A43-(4-5-6-7-8-9-10-11-12-13)3A4 hybrid mRNA molecules is 6501; thus, this CYP3A43/3A4 chimeric species represents ~0.15% of the canonical CYP3A43 transcripts (Fig. 6).

The trans-spliced CYP3A mRNA molecules are polyadenylated—To address the question whether the trans-spliced mRNAs were polyadenylated, real-time 5′ exonuclease (TaqMan) PCR quantitation assay on the poly(A)+ fraction of human liver RNA was performed. Specifically, the ratio between the canonical CYP3A43 mRNA and the (1)3A43-(4-5-6-7-8-9-10-11-12-13)3A4 hybrid mRNA molecule was determined in isolated poly(A)+ liver RNA. The strategy for quantitation was as in the case of the total RNA described above. The real-time PCR assay revealed that the ratio is essentially the same in the poly(A)+ fraction as in the total RNA, i.e. the (1)3A43-(4-5-6-7-8-9-10-11-12-13)3A4 CYP3A43/3A4 chimeric species represents ~0.16% of the canonical CYP3A43 transcripts (data not shown). This also means that the hybrid (1)3A43-(4-5-6-7-8-9-10-11-12-13)3A4 CYP3A43/3A4 mRNA molecule has the same polyadenylation state as the canonical CYP3A43 mRNA, i.e. both are polyadenylated.

The full-length hybrid CYP3A43/3A4 is catalytically active—A majority of the detected trans-spliced CYP3A mRNAs

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has open reading frames that start at the canonical ATG codon and end at the canonical TGA termination codon (Table II). Therefore, the potential of coding hybrid, full-length or partial length, CYP3As exists. To address the question whether the encoded chimeric CYP3A proteins are enzymatically active, we measured testosterone 6β-hydroxylase activity, a typical hu-
FIG. 4. CYP3A43/3A5 intergenic transcripts. The products of a nested RT-PCR amplification with Taq polymerase, using CYP3A43 exon 1 and CYP3A5 exon 13 primers on human liver total RNA, were separated on an agarose gel and are shown on the right. Individual lanes are as in Fig. 2. Schematic structures of the amplified fragments are shown on the left. Black boxes denote CYP3A43 exon 1; gray boxes denote CYP3A5 exons.

FIG. 5. Southern hybridization experiment on human genomic DNA using a CYP3A43 exon 1-specific probe (see “Materials and Methods”). The restriction enzymes used are shown. Arrows on the left indicate the size of molecular weight markers (base pairs).

natural hybrid mRNA formation may be a generalized process and is not only limited to the CYP2C genes on chromosome 10q24. Moreover, by taking advantage of the unique arrangement of the CYP3A locus, we predict that the underlying mechanism of chimeric CYP3A43/3A4 and CYP3A43/3A5 mRNA formation is trans-splicing. Although trans-splicing is a widespread phenomenon among lower eukaryotes (34), only a few isolated cases have been reported in mammals (11, 12, 35–37). A majority of these hybrid mRNA molecules contain exons from DNA segments located on different chromosomes. Therefore, the most likely mechanism for the formation of these transcripts was proposed to be trans-splicing; however, DNA rearrangements have not been conclusively ruled out.

The map of the CYP3A locus provides an indisputable example that DNA duplications may result in a variety of solitary exons or sets of exons within gene clusters (Ref. 38; Fig. 1). Duplicated exons may even be identical to the original ones of the corresponding gene (38, 39). However, the genomic hybridization experiment performed, as well as the sequence of the CYP3A3 locus, provides solid evidence that the CYP3A43 exon 1 is a single copy exon, and thus the CYP3A43 gene is the source of that exon in the intergenic CYP3A mRNA. Therefore, the mechanism generating the hybrid CYP3A mRNA, containing sequences from both DNA strands, has to be trans-splicing.

The recent finding of repetitions of exons in mRNAs from the rat carnitine octanoyltransferase (20), sodium channel SNS (21), hypertension-related SA (22), and the human Sp1 (23) genes are also likely to be the result of trans-splicing. However, the CYP3A mRNAs characterized in this report represent significantly more complex products of trans-splicing, as these are not limited to single genes.

The exclusive usage of CYP3A43 exon 1 as the 5’ exon during these trans-splicing reactions may indicate the existence of sequences in the CYP3A43 exon 1 and/or its flanking intron 1 that promote the intermolecular reaction of CYP3A pre-mRNAs. The fact that CYP3A43 intron 1 is the longest intron in the human CYP3A locus (see GenBank® entries in Fig. 1), is reminiscent of an earlier finding that exon scrambling, an additional nontypical alternative splicing process, most frequently occurs with exons that are flanked by large introns (2).

trans-Splicing CYP3A mRNAs—Taking advantage of real-time PCR (31), we extended the qualitative RT-PCR data to quantitative measurements. This resulted in the finding that the abundance of the trans-spliced mRNA molecules is low, nearly 10 orders of magnitude less than that of the canonical CYP3A43. It must be noted, however, that the quantified (1)3A43-(4-5-6-7-8-9-10-11-12-13)3A4 mRNA is not the most abundant chimeric species. The data of Fig. 2, as well as the use of the processive Expand amplification system,2 provide clear evidence that the 13 exon-containing molecule, i.e. the (1)3A43-(4-5-6-7-8-9-10-11-12-13)3A4 mRNA, is the most frequently occurring combination. This is also substantiated by the results of the RNase protection analysis (Fig. 3), which suggest that the abundance of this mRNA is about 1% that of CYP3A43.

During recent years, a large amount of data favors a model in which in vivo synthesis of mature mRNAs is the product of an “RNA factory” in which transcription, capping, splicing and polyadenylation occur in a mutually regulated way (40). However, during the formation of the CYP3A43/3A4 and the CYP3A43/3A5 trans-spliced mRNA molecules, transcription and trans-splicing are likely to occur apart from each other, both spatially and in time. The finding that the (1)3A43-(4-5-6-7-8-9-10-11-12-13)3A4 CYP3A43/3A4 mRNA molecule is poly-
denylated indicates that trans-splicing does not interfere with polyadenylation. This observation is consistent with a previous finding that in vivo trans-splicing and polyadenylation may occur independently (41).

Function of the trans-Spliced CYP3As—The exact biological significance of the chimeric CYP3A mRNAs remains to be elucidated. Low abundance may suggest that these transcripts only represent “biological noise” of gene expression. However, there is a non-randomness in this process, as only combinations with the CYP3A43 exon 1 upstream of either CYP3A4 or CYP3A5 exons, but not vice versa, were detected. Moreover, there is a clear preference in the choice of the trans-spliced

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**Fig. 6. Results of the real-time PCR quantitation.** Measured C<sub>T</sub> values (cycle threshold, i.e. the cycle number at which the measured fluorescent emission that reflects the amount of amplified products reaches an arbitrary threshold value, Ref. 31; y axis) were plotted against initial template copy number (x axis). Note that the x axis is at logarithmic scale. The linear regression equations that fit the data points obtained in triplicate with known amounts of CYP3A43 (panel A) or CYP3A43/3A4 (panel B) cDNAs (see “Materials and Methods”) are shown in the top right corner. The least squares fit method, taking advantage of a Microsoft Excel 98 program, was used. The C<sub>T</sub> values obtained for CYP3A43 and CYP3A43/3A4 from the human liver cDNA samples, in combination with the corresponding equations, allowed the calculations of the respective copy number shown in the bottom left corner.
indicate that the number of unique mRNA molecules may by far exceed the number of genes (46). Relevant calculations have led to the conclusion that the main underlying process, alternative splicing, is a more frequent phenomenon than previously anticipated, and thus represents "more the rule than the exception" (47). The unusually highly complicated pattern of hybrid CYP3A transcripts together with the recent findings of various chimeric CYP2C RNAs (10, 28) collectively suggest that intergenic mRNA formation may represent a generalized splicing pathway that deepens the complexity of splicing patterns in gene families. Moreover, the identified hybrid CYP3A transcripts indicate that an underlying mechanism for intergenic mRNA synthesis in mammalian cells may involve trans-splicing.

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