Posttranscriptional regulation of UGT2B10 hepatic expression and activity by alternative splicing

Adrien Labriet, Eric P. Allain, Michèle Rouleau, Yannick Audet-Delage, Lyne Villeneuve and Chantal Guillemette

Drug Metabolism and Disposition

Supplemental Table and Figures

Table S1. Primers used in this study

Target	Primer	Sequence (5' to 3')
Cloning		
UGT2B10_ n9	4238F	TGTTCTCCATGCTGACTCGAGTCTAGAGGGCCC
	4239R	CAATGAGATCTCATCATGGAACCAGGTGAGGTTG
UGT2B10_n10	4240F	AACAATCCTCCCATTTTAGCATCCCAAAGGGATGAGATTACAGGTTTTGCTCGAGTCTAGAGGGCCC
	4241R	TGAGCCCAGGAGTTTGAAATCAGCATGGAGAACACAATGAGATCTCATCAACTTTGTGATGATAAATAGCACGG
	4242F	TGGACAAATTCTTCCATTTCCTACAGAGGAAAAACTTTCTTCATTATAACTCGAGTCTAGAGGGCCC
	4243R	GAGCTCTGATGAGGATGGTGTTGTTGTGTTTTCTCTGGAGTCAGTTGTGCAAAACCTGTAATCTCATCCCTTTG
Validation		
v1, n9, n10		
Exon 1	1894F	AGATTTGACATCGTTTTTGCAGATGCTTA
Exon 4	1895R	CCTTCATGTGAGCAATATTATCAGGTTGATCAAA
n9, n10		
Exon 1	4203F	GTTAAGAGATTGTCAGAAATTCA
Exon 6c	4211R	TCTCTGGAGTCAGTTGTGC
GAPDH	1904F	TGGGTGTGAACCATGAG
	1905R	CCCAGCGTCAAAGGTGG

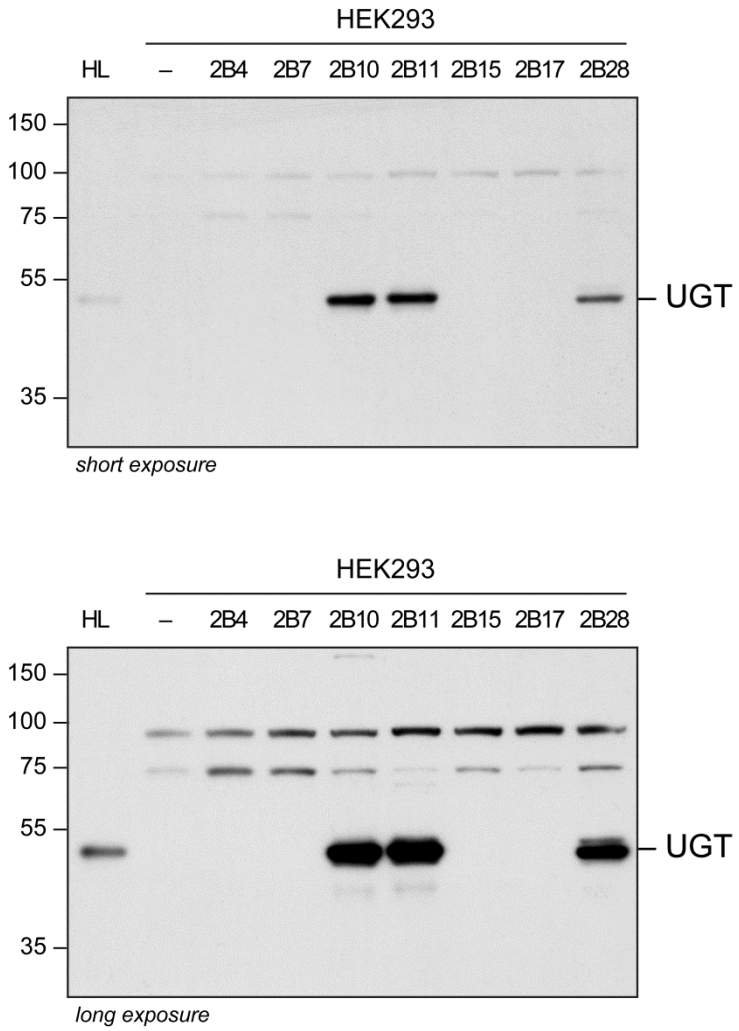


Fig. S1. Specificity of anti-UGT2B10 #1845 for human UGT enzymes. The microsomal fraction prepared from HEK293 cells (UGT negative) stably expressing each of the seven UGT2B enzymes or transfected with the empty vector pcDNA6 (-) was probed with the anti-UGT2B10 to demonstrate specificity. HL, human liver microsomes. Short and long exposures of the same immunoblot is provided to ascertain specificity.

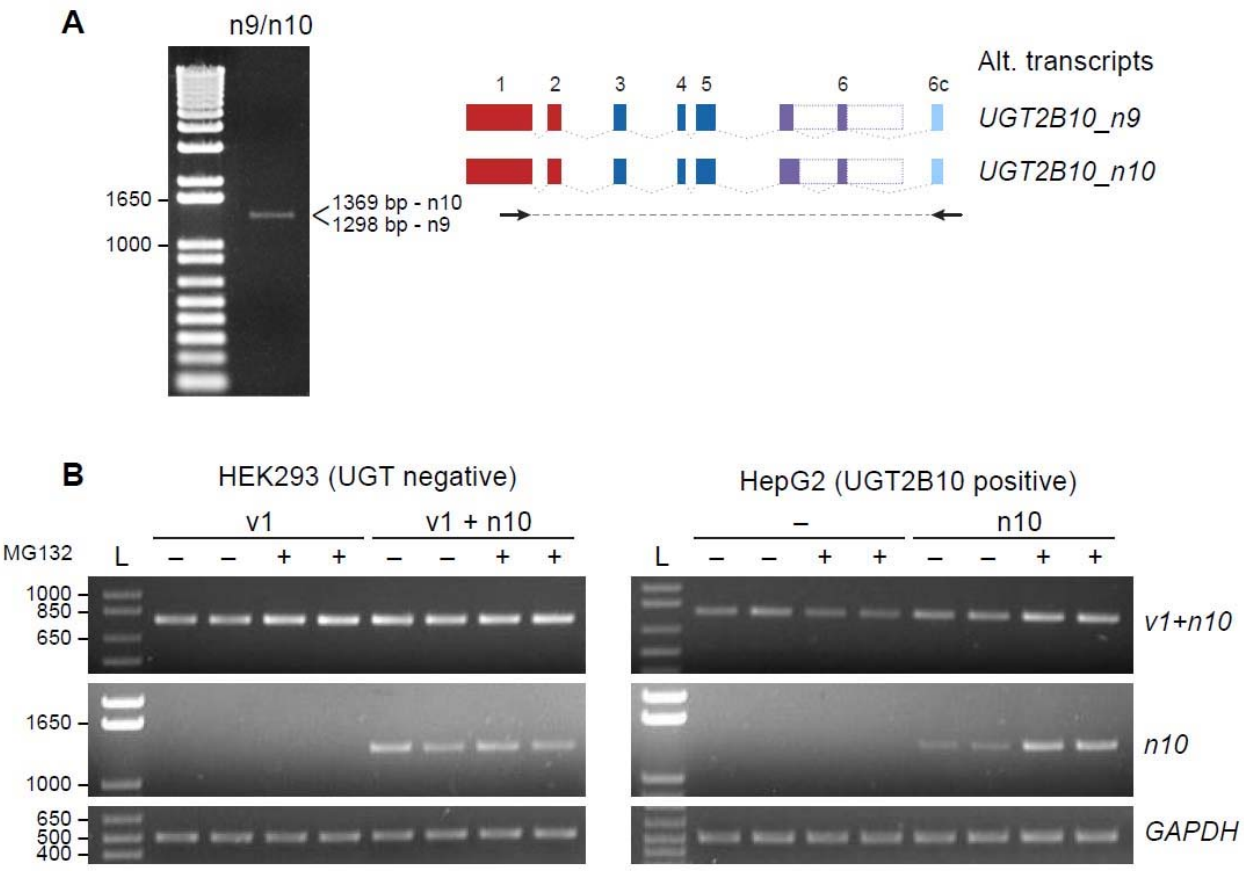


Fig. S2. RT-PCR amplification of *UGT2B10* transcripts. **A.** Validation of *UGT2B10_n9* and *UGT2B10_n10* expression in human liver. Primers (arrows; 4203F and 4211R) were located in exon 1 and in exon 6c common to both variants. PCR products corresponding to *n9* and *n10* could not be distinguished because they differed by only 71 base pairs (bp). The identity of PCR products as n9 and n10 was verified by Sanger sequencing. **B.** RT-PCR detection of transcripts in cell models exposed to MG132. PCR strategies specified on the right either amplified both *v1* and *n10* or only *n10*. GAPDH served as a positive and loading control. L: molecular weight ladder.

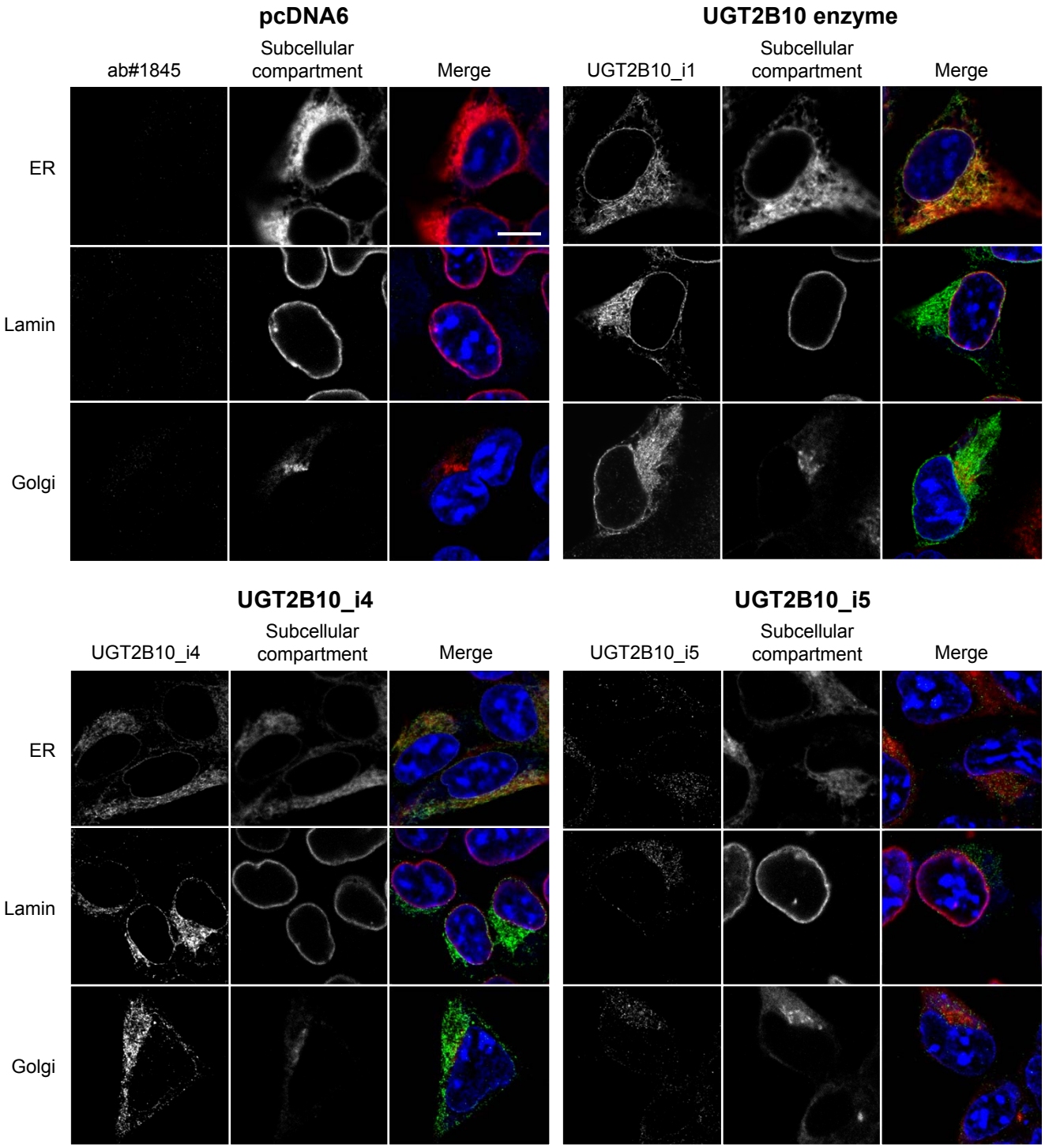


Fig. S3. Subcellular localization of UGT2B10 and alternative isoforms expressed in HEK293. UGT2B10 proteins were detected with #1845, and cellular compartments with specific markers as described in Materials and Methods. In merged images, nuclei are stained with DRAQ5. Bar represents 10 µm. Control HEK293 cells were transfected with the empty vector pcDNA6.

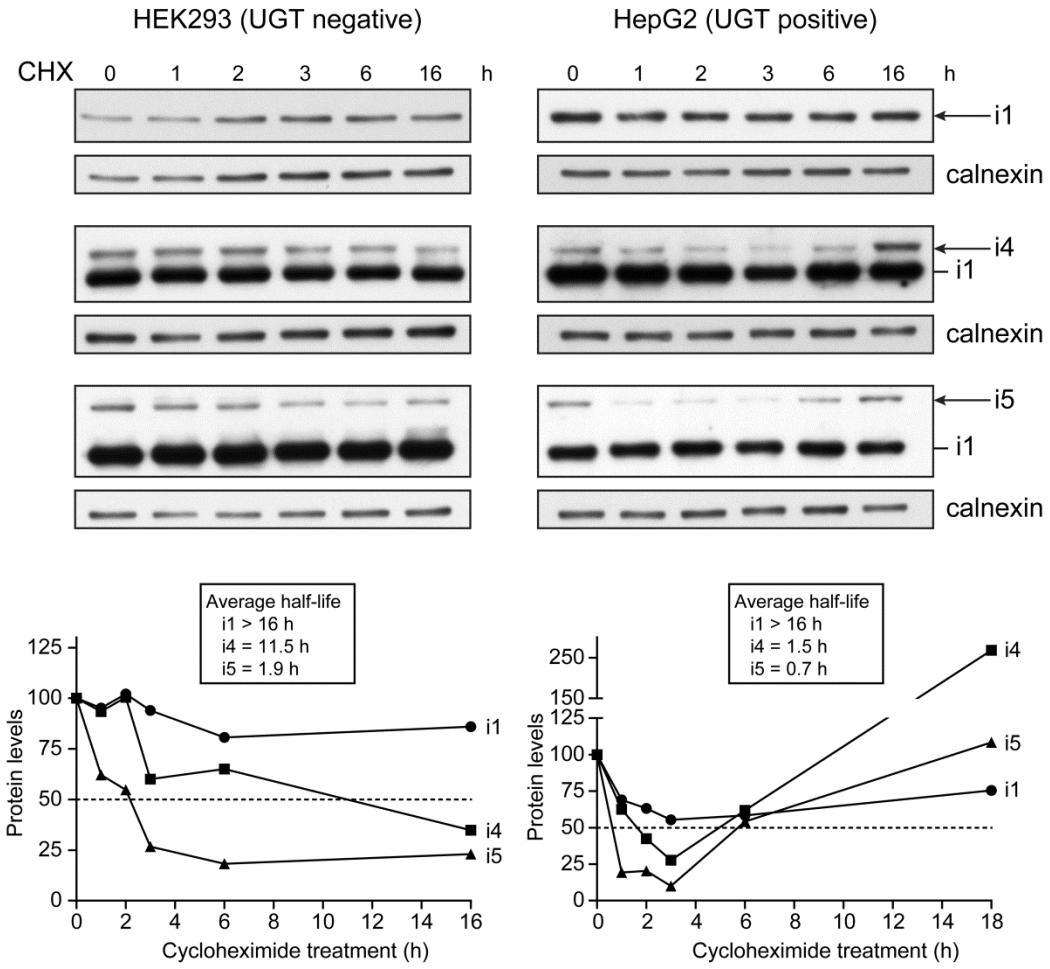
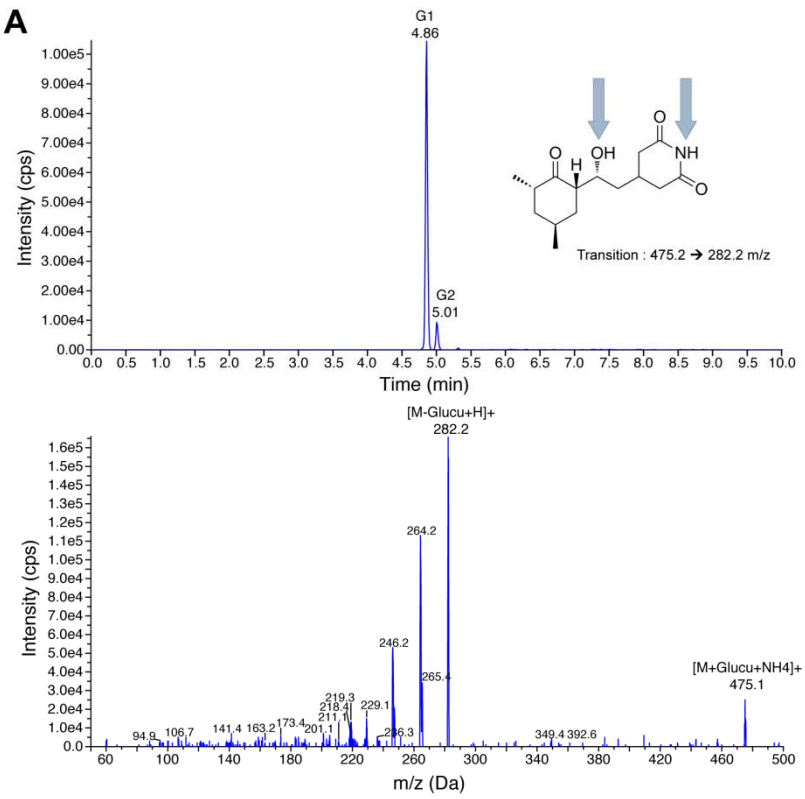


Fig. S4. Stability of UGT2B10 proteins - Replicate assay. The half-life of UGT2B10 proteins were determined by translational inhibition with cycloheximide (CHX) in HEK293 and HepG2 cell models. Protein levels were measured by densitometry scanning of immunoblots from two independent assays. Half-lives were averaged from the two biological replicates. Related to Fig.6A.



B

	Cycloheximide (area/h/µg protein)	
	G1	**G2**
Human Liver	202917	10234
HepG2	5901	582
UGT1A1	177	1
UGT1A3	68	-
UGT1A4	8	-
UGT1A6	-	-
UGT1A9	215208	1247
UGT2B4	278	5
UGT2B7	135573	8016
UGT2B10	-	-
UGT2B11	-	-

G : glucuronide;
- : below limit of detection

Fig. S5. Analysis of cycloheximide glucuronidation by high-performance liquid chromatography tandem mass spectrometry. **A**. Human liver microsomes produced two cycloheximide glucuronides (G1 and G2). Representative chromatograms of cycloheximide glucuronides (top) and their fragmentation pattern (bottom). Putative glucuronidation sites are indicated by blue arrows in the chemical structure of cycloheximide. **B**. Glucuronidation activity of microsomes from human liver, HepG2 pcDNA6, HEK-UGT2B11 and of commercial UGT supersomes. Two independent assays were conducted in triplicate.

```
UGT2B10_i5              510 lmrSHcVlhAdfKLLGSnNPPiLASQrdEITGfaqltpektqq 48
SP|P51790-5|CLCN3_i4      6 lgeSHyVvQAGLqLLGSSNPPaLASQvAEITGthytmtnggsi 47
SP|Q96EP1-3|CHFR_i3     135 emvpccVAQAGLKLLGSSdPPTLASQSIvITGsggggispkgs 57
NP_001339349|ALG9_ii    401 asrSqfVAQAGLKLLGSSNPPTsASQnIGITGlshrawpltsp 60
XP_011508885|MITD1_iX5  150 qtgfHhVAQAGLKLLGSSsPPTLASQSAGITGMshctqphyly 52
                                * :* ::****  **  ***   ***
```

Fig. S6. Sequence similarity of the UGT2B10_i5 unique sequence with other alt. human proteins. A BLASTp search with the unique C-terminal UGT2B10_i5 amino acid sequence revealed a conserved region encoded by the frame shift in exon 6 (boxed). asterisk: conserved amino acid; colon: conservative substitution. Alignment was produced with the Clustal Omega tool available at www.Uniprot.org.