

Transcriptome of Mouse Uterus by Serial Analysis of Gene Expression (SAGE): Comparison With Skeletal Muscle

M. LAROSE, J. ST-AMAND,* M. YOSHIOKA, P. BELLEAU, J. MORISSETTE, C. LABRIE, V. RAYMOND, AND F. LABRIE

Molecular Endocrinology and Oncology Research Center, Laval University Medical Center, Department of Anatomy and Physiology, Laval University, Quebec, Canada

ABSTRACT The aim of this study was to identify the transcriptome of the normal mouse uterus by Serial Analysis of Gene Expression method. mRNA was extracted from the uterus and also from the gastrocnemius muscle of mice. Short sequences (tags), each one usually corresponding to a distinct transcript, were isolated and concatemerized into long DNA molecules which were cloned and sequenced. We detected 44,484 tags for the uterus and 42,518 tags for the muscle, representing 14,543 and 14,958 potential transcript species, respectively. Seventy-five and sixty-nine genes were expressed at more than 0.1%, thus corresponding to 37 and 34% of the mRNA population detected in the respective tissues. In both cases, the most highly expressed genes are especially involved in muscle contraction, energy metabolism, and protein synthesis. Compared to skeletal muscle, some differentially expressed genes in the uterus are likely to correspond to its specific reproductive functions. The majority of these genes remain to be characterized. More than 70% of the different tags detected in the uterus did not match any sequence in the public databases and can represent novel or poorly identified genes. This study is the first quantitative description of the transcriptome of the uterus. *Mol. Reprod. Dev.* 68: 142–148, 2004.

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Key Words: uterus; muscle; SAGE; genomic profile

INTRODUCTION

The expression of numerous genes has been studied in the uterus under different conditions, including its development (Carlsson and Billig, 1991; Gu et al., 1999; Burroughs et al., 2002), different stages of the estrus cycle (Walmer et al., 1992; Kaneko et al., 2001; Burroughs et al., 2002; Moussad et al., 2002), at periimplantation and implantation (Reese et al., 2001; Kao et al., 2002), during pregnancy and postpartum (Ledingham et al., 2001; Uchide et al., 2001; Moussad et al., 2002), as well as when affected by various pathologies (Kodama et al., 2001; Burroughs et al., 2002; Fukuhara et al., 2002). The objective of the present study was to establish the genomic expression profile of

the whole uterus of intact nonpregnant mice. While some studies have described gene expression by microarrays (Popovici et al., 2000; Reese et al., 2001), we present here the transcriptome of the uterus studied by the serial analysis of gene expression (SAGE) technique.

SAGE is a powerful method to analyze a large number of transcripts in a given tissue, and it allows the quantitative cataloging and comparison of expressed genes under various developmental, physiological, and pathological states (Velculescu et al., 1995; Virlon et al., 1999; Welle et al., 1999; St-Amand et al., 2001). The technology is mainly based on two principles, namely, representation of mRNAs (cDNAs) by short sequence tags (15 bp) and concatenation of these tags to allow efficient sequencing. Determination of the abundance of distinct tags and identification of the genes corresponding to each of them provide a quantitative measure of the components of a population of mRNAs. Novel genes can also be discovered by this method. Knowledge of the genomic expression profile of the uterus obtained under normal conditions should provide much useful information for studies related to infertility, contraception, and various uterine pathologies.

MATERIALS AND METHODS

Female C57BL6 mice, 12–14 weeks old, were purchased from Charles River, Quebec, Canada, Inc. Animals received Lab Rodent Diet No. 5002 and water ad libitum. The animals were sham-operated at the flank's region 7 days before organ collection, and they received a subcutaneous injection (200 μ l) of a vehicle suspension (0.4% Methocel A15LV Premium/5% ethanol) 24 hr before sacrifice. After anesthesia under isoflurane, the animals were exsanguinated by cardiac

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*Correspondence to: Jonny St-Amand, PhD, SAGE Facility, Oncology and Molecular Endocrinology Research Center, Laval University Medical Center (CHUL), 2705, boul. Laurier–BlocT, Québec G1V 4G2, Canada. E-mail: Jonny.St-Amand@crchul.ulaval.ca

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puncture, the uterus was then collected, freed from adhering fat and connective tissue, emptied, rapidly frozen in liquid nitrogen, and stored at -80°C until analysis.

Pools of 30 uteri and 30 gastrocnemius muscles were used to extract RNA. Total RNA was extracted with TRIzol Reagent (Invitrogen, Carlsbad, CA). Polyadenylated RNA was purified from uterus and muscle using the mRNA direct kit (Dyna, Oslo, Norway) and the Oligotex mRNA Mini Kit (Qiagen, Mississauga, Canada), respectively. The SAGE method was performed to quantify the global gene expression profile according to the strategy described by Velculescu et al. (1995, 1997) and the modifications and optimizations of Kenzelmann and Muhlemann (1999) and St-Amand et al. (2001). After the annealing of biotin-5'T₁₈-3' primers, the mRNA was converted to cDNA with the Invitrogen synthesis kit and cleaved with NlaIII. The 3' restriction fragment was isolated with streptavidin-coated magnetic beads (Dyna) and ligated to one of two annealed linker pairs. After extensive washing to remove unligated linkers, adjacent tags were released from the magnetic beads by cleavage with BsmFI for 3 hr at 37°C . The blunting kit of Takara Bio, Inc. (Otsu, Japan) was used for the blunting and ligation of tags since both reactions could be performed consecutively without phenol chloroform extraction and precipitation of short tags. The produced ditags were amplified by performing 80 PCR reactions with an initial denaturation step of 1 min at 95°C , followed by 20 cycles of 20 sec at 94°C , 20 sec at 60°C , and 2 sec at 72°C (St-Amand et al., 2001). In the processing of the uterus SAGE library, preliminary amplification (20 PCR reactions of 21 cycles) and gel purification were performed before generating the ditags by 40 PCR reactions of 10 cycles. It has already been established that this additional step does not influence the genomic expression profile (Virlon et al., 1999). The PCR products were analyzed by polyacrylamide gel electrophoresis (PAGE) and digested with NlaIII. The band containing the ditags was excised and self-ligated to produce long concatemers. The concatemers ranging from 500 bp to 2 kb were isolated by agarose gel electrophoresis and extracted using Gene-Clean Spin kit DNA purification (BIO 101, Vista, CA). These products were cloned into the SphI site of pUC19 and bacterial transformation was performed in Ultra-MAX DH5 α -FT competent cells (Invitrogen). White colonies were screened by PCR to select long inserts for automated sequencing. Sequence files were transferred from the sequencers to a Unix server (SUN3800) by FTP and were analyzed using different Perl scripts that we have developed. The SAGEseq.pl transformed the ABI files to FASTA format by calling the program `extract_seq` from the Staden package (Staden et al., 2000). Then, the SAGEparser.pl developed by Dr. Snyder and Dr. St-Amand was used to extract and count tags from concatemer sequences (`ftp://ftp.pbr.c.edu/public/eesnyder/SAGE/`). The quality of the concatemer sequences was evaluated according to the number of extracted tags. Tags corresponding to vector sequences

were discarded with the script `vectSAGE.pl`. We used a modified version of `xmatchdt.pl`, compatible with BIOPERL 0.7.2, to remove redundant concatemer sequences. To identify genes, a SAGEmap of 11 bp tags was generated by the script `SAGEmap.pl` using the NCBI 10 bp tag SAGEmap and the Unigene Cluster sequences and the mitochondrial mRNA sequences (`http://www.ncbi.nlm.nih.gov/`) (Lash et al., 2000; Wheeler et al., 2002). Tag sequences were required to contain the last NlaIII restriction site (CATG) at the 3' end of a given gene. SAGE libraries comparisons were done using the `SAGEana.pl` script. To overcome the lower quality of some EST sequences, the tags that did not identify a well-characterized mRNA were required to match at least two ESTs in the same UniGene Cluster including one EST with a known polyA tail. To estimate the statistical significance ($P \leq 0.05$) of twofold difference in tag abundance, the `SAGEana.pl` script calls the CCD program (Lash et al., 2000) which computes the posterior probability density function suggested by Chen et al. (1998).

RESULTS

The uterus SAGE library contained 44,484 tags, 14,543 of them being distinct tags (Table 1A). We identified 3,774 distinct tags as well characterized genes or ESTs in the public databases. On the other hand, 10,769 distinct tags (74% of the distinct tags) did not match any known sequence. In the muscle, 42,518 tags were sequenced, 14,958 being distinct tags. More than 65% of the transcript species could not be identified in this tissue. Many distinct tags are detected either in the uterus only (10,667) or in muscle only (11,082). Table 1B presents the distinct tags detected more than once in the uterus and muscle libraries. In the uterus and muscle, 75 and 69 distinct tags, respectively, represented more than 0.1% of the total population (Table 1C). Among them, 37 in the uterus and 46 in the muscle correspond to well characterized genes, whereas 23 and 10, respectively, did not match any known sequence in the public databases. Only five and six distinct tags, respectively, matched with more than one transcript species.

Table 2 details the genes expressed more than 0.1% in the uterus, and the corresponding representation in muscle. Sequences of the detected distinct tags, number of times that one tag was detected, percent of the tag in the mRNA population, and gene description are presented. It can be seen that three major functions are represented in the uterus: muscle contraction (troponin I, T, and C; myosin light chain and heavy chain; tropomyosin 1 alpha; parvalbumin; myozenin 1; crystallin alpha B; myomesin 1), energy metabolism (aldolase 1; ATPase Ca⁺⁺ transport; glyceraldehyde-3-phosphate dehydrogenase; phosphoglycerate mutase 2; enolase 3; cytochrome c oxidase 1, 2, 6a; pyruvate kinase 3; creatine kinase; phosphofructokinase; ATP synthases; cytochrome b; glycogen phosphorylase), and protein synthesis (ribosomal protein S2, L35, S3a; eukaryotic translation elongation factor 1 and 2).

TABLE 1. Characterization of the SAGE Libraries Obtained in Mouse Uterus and Muscle (A), Including the Distinct Tags Detected More Than Once (B), and the Tags From Transcripts Present as More Than 0.1% (C)

		Uterus	Muscle
A	Sequenced tags	44,484	42,518
	Distinct tags	14,543	14,958
	Tissue-specific tags	10,667	11,082
	Identified tags (ESTs included) (%)	3,774 (26)	4,927 (33)
	No match (%)	10,769 (74)	10,031 (67)
B	Distinct tags detected more than once	3,449	3,649
	Tissue-specific tags	1,232	1,453
	Identified tags (%)	1,684 (49)	2,198 (60)
	No match (%)	1,765 (51)	1,451 (40)
C	Transcript species expressed $\geq 0.1\%$	75	69
	Tissue-specific tags	1	0
	Well characterized genes	37	46
	Partly characterized genes	9	7
	Multiple matches	5	6
	No match (%)	24 (32)	10 (14)

Comparison of the uterus and muscle genomic profiles reveals many similarities. In fact, the genes involved in the contractile apparatus and energy metabolism are largely predominant in both tissues. In addition, other genes are highly expressed, such as basigin, RNA binding protein regulatory subunit, receptor (calcitonin) activity modifying protein 2 (EST), N-myc downstream regulated 2, carbonic anhydrase 3, myc box dependent interacting protein 1, and glutathione peroxidase 4. Moreover, many novel genes are expressed more than 0.1% in the uterus. Notably, the most expressed tag having the sequence CATGGTGAC-CACGGG did not match any known gene in the public databases.

Table 3 presents the tags corresponding to the transcripts that are expressed at statistically significant higher levels in the uterus compared to muscle. One tag corresponds to troponin T3, five tags match with different ESTs corresponding to zinc finger protein 106, troponin I skeletal fast 2, adipsin, stearoyl-coenzyme A desaturase 1, and pM5 protein. Moreover, 51 tags do not correspond to any known sequence in the public databases. Of these, 21 of the tags were not detected and 11 were sequenced only once in muscle.

DISCUSSION

Using the SAGE technique, the present study has detected between 14,500 and 15,000 expressed transcript species in the uterus and muscle. In a first global characterization, the whole uterus, including the perimetrium, myometrium, and endometrium, has been studied. Under these conditions, it can be expected that the most highly expressed genes are from the muscular compartment, the myometrium representing the major part of the organ. Accordingly, the major functions of the muscle are detected (Welle et al., 1999; St-Amand et al., 2001). This genomic expression profile of the uterus underlines the primordial contractile function of this organ, being subject to contractions during sexual intercourse, pregnancy, delivery, the postpartum

period, with varying activity during the menstrual cycle. The SAGE method being very quantitative, comparison with the muscle library allows to 'subtract' the genes expressed in the muscle part of the uterus, and to point out genes potentially involved in the reproductive function of the organ. One tag corresponds to a clone identified as troponin T3, which could be a particular isoform of the uterine tissue. We have identified five tags matching to ESTs similar to known genes: troponin I could also be a specific form in the uterus, zinc finger protein 106 and adipsin, which are involved in the immune responses, stearoyl-coenzyme A desaturase 1 and pM5 protein. These differentially expressed genes may correspond to targets of steroid hormone action. However, only adipsin and stearoyl-coenzyme A desaturase 1 have null expression in the uterus of gonadectomized mice (unpublished results). Very interestingly, the majority of differentially expressed genes are novel genes ('no match').

The uterus is made of smooth muscles, while comparison is made with the gastrocnemius skeletal muscle. Thus, some differential expressions could result from the different muscle type. For example, the tag detected 63 times in the uterus and 289 times in the skeletal muscle, corresponds to the gene coding for myosin light chain, alkali, a typical protein of fast skeletal muscle. We do not report here all the genes that are more highly expressed in skeletal muscle, these being characteristic of that tissue. The genes in question are particularly involved in energy metabolism (cytochrome c oxidase, cytochrome b, NADH dehydrogenase, ATP synthase, etc.).

Some genes, which are highly expressed in mouse uterus, were not in one of the three major categories: muscle contraction, energy metabolism, and protein synthesis. Thus, basigin (Bsg) is a highly glycosylated transmembrane protein belonging to the immunoglobulin superfamily, a protein known to play pivotal roles in spermatogenesis, implantation, neural network formation, and tumor progression (Yoshida et al., 2000).

TABLE 2. Transcripts Highly Expressed in Uterus, and Their Representation in Muscle

Tag sequence	Uterus		Muscle		Gene description (UniGene cluster and accession number)
	No	%	No	%	
GTGACCACGGG	1,810	4.07	88	0.21 ^a	NM
GAGGGCCGGAA	1,370	3.08	993	2.34	Troponin I, skeletal, fast 2 (Mm.39469, NM_009405)
GTGATGCTAAG	1,036	2.33	1,288	3.03	Myosin light chain, phosphorylatable, fast skeletal muscle (Mm.14526, NM_016754)
ACTGTCCGGGC	1,020	2.29	392	0.92 ^a	Troponin T3, skeletal, fast (Mm.14546, BC003747)
GGTGCCAACTA	970	2.18	598	1.41	Troponin T3, skeletal, fast (Mm.14546, NM_011620)
AAGATCAAGAT	901	2.03	768	1.81	Actin gamma 2, beta, alpha, alpha1, alpha2 (Mm.16562, BC002042; Mm.297, X03765; Mm.686, NM_009608; Mm.214950, BC014877; Mm.16537, AA710012); junction cell adhesion molecule 1 (Mm.20903, U89915)
TGACAGAAGAG	808	1.82	780	1.83	Troponin C, fast skeletal (Mm.1716, NM_009394)
CCTACTAACCA	783	1.76	552	1.30	Aldolase 1, A isoform (Mm.16763, NM_007438)
GAGCAGACCGT	715	1.61	881	2.07	Myosin, heavy polypeptide 4, skeletal muscle (Mm.35531, AJ278733)
CATCTTCAGCC	660	1.48	570	1.34	ATPase, Ca ⁺⁺ transporting, cardiac muscle, fast twitch 1 (Mm.35134, NM_007504)
GCCTCCAAGGA	520	1.17	551	1.30	Glyceraldehyde-3-phosphate dehydrogenase (Mm.5289, NM_008084)
ATTGACGTGGA	384	0.86	236	0.56	NM
GTAATCCTGCT	310	0.70	5	0.01 ^a	NM
AAAGTCATTGA	295	0.66	334	0.79	Tropomyosin 1, alpha (Mm.121878, NM_024427)
GAAGCTGTTGC	236	0.53	189	0.44	Phosphoglycerate mutase 2 (Mm.219627, NM_018870)
GACCTCATTCC	204	0.46	216	0.51	Enolase 3, beta muscle (Mm.29994, NM_007933)
GCTGCCCTCCA	169	0.38	397	0.93 ^a	Cytochrome c oxidase subunit 1 (MTG, NC_001569, Pos: 6816)
CCAGCCAGCGT	169	0.38	138	0.32	RIKEN (Mm.41421, AK004293)
GCCCCTCTCTT	161	0.36	77	0.18	EST Desmin (Mm.6712, AI159732)
CATCATAAAAA	159	0.36	0	0.00 ^a	NM
TGGGCCACCTC	154	0.35	214	0.50	RIKEN parvalbumin (Mm.2766, AK013561)
AGCAGTCCCT	146	0.33	292	0.69	Cytochrome c oxidase subunit 2 (MTG, NC_001569, Pos: 7500)
CCAGAGGCTGT	140	0.31	1	0.00 ^a	NM
GTAGGCACGGC	137	0.31	7	0.02 ^a	NM
TGCATCATTTT	113	0.25	82	0.19	Myozenin 1 (Mm.141702, NM_021508)
CTCAGTCTCC	111	0.25	112	0.26	EST (Mm.201606, BM123103)
AAGATCCCAAA	110	0.25	67	0.16	NM
ACTCTACCAGC	106	0.24	24	0.06 ^a	NM
CCTTTAATCCC	88	0.20	76	0.18	Multiple matches (22)
CCTCTCTCACT	87	0.20	64	0.15	NM
AACGGCTAAAC	87	0.20	37	0.09	NM
CAATAGAGACC	84	0.19	113	0.27	Pyruvate kinase 3 (Mm.2635, NM_011099)
CCCTGCCTTAA	83	0.19	116	0.27	Creatine kinase, muscle (Mm.2375, NM_007710)
GGTCAGTCGGT	81	0.18	9	0.02 ^a	NM
TCCCTATAAGC	80	0.18	1	0.00 ^a	NM
CAAGGTGACAG	79	0.18	71	0.17	Ribosomal protein S2 (Mm.1129, NM_008503)
GGGTCTGTCTG	79	0.18	63	0.15	EST (Mm.212927, AI536423)
GCAACAACACA	71	0.16	13	0.03 ^a	NM
GCCAAGTGGAG	69	0.16	51	0.12	Eukaryotic translation elongation factor 2 (Mm.27818, BC007152)
AAGGAGTCTCT	65	0.15	60	0.14	NM
CACGGGACCAC	65	0.15	39	0.09	RIKENs (Mm.181721, AK011734; Mm.196156, AK019085)
CCTACAGTTGA	63	0.14	289	0.68 ^a	Myosin light chain, alkali, fast skeletal muscle (Mm.1000, AK003182)
TTCTGGGTAGG	63	0.14	26	0.06	RIKEN (Mm.2060, NM_026061)
CCTGCAACCAG	60	0.13	97	0.23	Phosphofructokinase, muscle (Mm.26550, NM_021514)
GCCACCGTCTT	59	0.13	2	0.00 ^a	NM
GTGGCGGTGGC	58	0.13	186	0.44 ^a	NM
TCTGCACCTCC	58	0.13	45	0.11	Eukaryotic translation elongation factor 1 alpha 2 (Mm.2645, NM_007906)
GACAACGCCAG	58	0.13	38	0.09	ATP synthase, H ⁺ transp., mitoch. F1 complex, gamma polypept. 1 (Mm.12677, NM_020615)
GAGCCTGGGAT	58	0.13	15	0.04 ^a	NM
CCGACGGGCGC	56	0.13	4	0.01 ^a	NM
CTCTGACTTAC	55	0.12	37	0.09	Basigin (Mm.726, Y16256)
GAGGGGCAGGA	55	0.12	23	0.05	Cytochrome c oxidase subunit 6a, polypeptide 2 (Mm.43824, NM_009943)
GTGGTGGAGTG	55	0.12	7	0.02 ^a	NM
AGGCAAAATAT	53	0.12	282	0.66 ^a	Cytochrome b (MTG, NC_001569, Pos:14542)
GCCAACCAAGT	53	0.12	51	0.12	RNA binding protein regulatory subunit (Mm.2507, NM_020569)
AGTTGGAACA	53	0.12	45	0.11	EST Receptor (calcitonin) activity modifying protein 2 (Mm.218611, BE992028)
GATAGCTTGGG	52	0.12	109	0.26	NM

(Continued)

TABLE 2. (Continued)

Tag sequence	Uterus		Muscle		Gene description (UniGene cluster and accession number)
	No	%	No	%	
GTTGCTGAGAA	52	0.12	31	0.07	EST (Mm.196030, C76574); ribosomal protein 10 (Mm.100113, NM_052835)
TCGTCGATGGA	52	0.12	20	0.05	NM
GCTTCATCTCC	51	0.11	34	0.08	Crystallin, alpha B (Mm.178, NM_009964)
ACCGGTTTAAA	50	0.11	71	0.17	Muscle glycogen phosphorylase (Mm.27806, NM_011224)
GCCGAGCATAA	50	0.11	51	0.12	ATP synthase, H ⁺ transport., mitoch. F0 complex, sub. f, isoform2 (Mm.1674, NM_020582)
TAAAAAGAAAG	50	0.11	43	0.10	N-myc downstream regulated 2 (Mm.26722, NM_013864)
TCATCTTCAGC	50	0.11	26	0.06	NM
CGCCGCCGGCT	49	0.11	39	0.09	Ribosomal protein L35 (Mm.16423, NM_025592)
GATTCGGTGAG	49	0.11	31	0.07	RIKEN (Mm.10474, AK012544)
CCTATTAAAAA	47	0.11	67	0.16	Carbonic anhydrase 3 (Mm.300, AK003671)
CACAAACGGTA	47	0.11	33	0.08	RIKEN (Mm.29911, AK014294)
TTCAGGGCGGG	47	0.11	24	0.06	Myc box dependent interacting protein 1 (Mm.4383, NM_009668)
AGAGGTGTAGA	47	0.11	2	0.00 ^a	NM
AAGGTCTGCCT	46	0.10	29	0.07	Glutathione peroxidase 4 (Mm.2400, NM_008162)
GGGAAGGCGGC	46	0.10	26	0.06	Ribosomal protein S3a (Mm.6957, NM_016959)
GATCCCCGCAG	46	0.10	15	0.04	NM
AAGGATGTGCC	45	0.10	38	0.09	Ring finger protein 13 (Mm.9326, AK002999); genes associated with retinoid-IFN-induced mortality 19 (Mm.21162, NM_023312)
TGCCTGTAGGC	45	0.10	26	0.06	Myomesin 1 (Mm.4103, NM_010867)

^aDifferentially expressed genes ($P \leq 0.05$). NM, no match.

Studies of Bsg knockout mice reveal that many mutant embryos are lost around the time of implantation. In female mutants, the ovaries and genital tract are morphologically normal and the defects are probably in the capability of implantation in the uterus and the maturation of oocytes (Igakura et al., 1998; Kuno et al., 1998). The RNA-binding protein regulatory subunit gene has large identity (95%) with the fertility protein SP22 from the rat. The localization of SP22 over a discrete region of the sperm head suggests a pivotal role in sperm-egg interactions (Welch et al., 1998). SP22 homologues have been studied in the mouse and human, but a definitive role for the protein has not yet been established. This uterine molecule could play a role in the implantation process, allowing, for example, interactions between the uterine matrix and the fertilized egg. N-myc downstream-regulated gene 2 (*NDRG2*) is a novel early mineralocorticoid-specific induced gene, which is known to be highly expressed in the muscle (Boulkroun et al., 2002). Carbonic anhydrase 3, a major protein of red skeletal muscle, was localized immunohistochemically in smooth muscle cells of the human uterus (Vaananen and Autio-Harmanen, 1987). This protein is abundant in adult skeletal muscle (Tweedie and Edwards, 1989). We have recently found in our laboratory that carbonic anhydrase 3 is the most highly expressed gene in retro peritoneal adipose tissue of normal male mice (Bolduc et al., 2002). This protein catalyzes the reversible hydration of CO₂ to generate bicarbonate and H⁺ ions, an important reaction in many physiological processes such as acid-base homeostasis and ions exchange (Tweedie and Edwards, 1989). Myc box dependent interacting protein 1, also called Bin1, is

also highly expressed in skeletal muscle and potentially plays a functional role in the muscle differentiation process (Wechsler-Reya et al., 1998). It is known that Bin1, a tumor suppressor and pro-apoptotic molecule, can regulate progression of prostate cancer (Gurumurthy et al., 2001). The expression of this gene in the uterus is an interesting observation. Glutathione peroxidase 4 (GPX4), also named phospholipid hydroperoxide glutathione peroxidase (PHGPx), encodes a selenoprotein that plays a role in eukaryotic defense against potentially lethal peroxidative injury, protecting membrane lipids. Beyond its mere antioxidant potential, this protein exerts cell- and tissue-specific roles in metabolic regulation (Brigelius-Flohe, 1999).

A very exciting observation resulting from this experiment is the large number of nonidentified genes expressed in the tissue, even among the most expressed genes. The SAGE method is certainly time-consuming, but it permits to obtain original information and to reveal unexpected data. It would be of great interest to further characterize the novel genes, particularly those being differentially expressed in the uterus.

In conclusion, we have presented for the first time a quantitative description of the transcriptome of the uterus, and such information should lead to the cloning of genes encoding new proteins playing an important role in the physiology of the uterus. Such data obtained in the normal uterus should be very useful for basic knowledge as well as for future studies on steroid-sensitive genes and patho-physiological conditions. Such a characterization of the transcriptome of the whole uterus shows that contraction, energy metabolism, and protein expression correspond to the most

TABLE 3. List of Tags Corresponding to Genes More Highly Expressed in the Uterus Than in the Muscle ($P \leq 0.05$)

Tags	Uterus		Muscle		Description
	No	%	mb	%	
ACTGTCCGGGC	1,020	2.293	392	0.922	Troponin T3, skeletal, fast (Mm.14546, BC003747)
CAGTCTCCAGC	41	0.092	9	0.021	EST zinc finger protein 106 (Mm.27653, AW414184)
GAGGGCCGGGA	22	0.049	3	0.007	EST troponin I, skeletal, fast 2 (Mm.39469, AV011886)
CATCTGAAAAA	16	0.036	0	0.000	EST adipsin (Mm.4407, AW215391)
GCAGTGGGTAG	15	0.034	0	0.000	EST stearoyl-Coenzyme A desaturase 1 (Mm.140785, BB768467)
GCGTTGGTCTG	13	0.029	0	0.000	EST pM5 protein (Mm.22121, BE951548)
GTGACCACGGG	1,810	4.069	88	0.207	NM
GTAATCCTGCT	310	0.697	5	0.012	NM
CATCATAAAAA	159	0.357	0	0.000	NM
CCAGAGGCTGT	140	0.315	1	0.002	NM
GTAGGCACGGC	137	0.308	7	0.016	NM
ACTCTACCAGC	106	0.238	24	0.056	NM
GGTCAGTCGGT	81	0.182	9	0.021	NM
TCCCTATAAGC	80	0.180	1	0.002	NM
GCAACAACACA	71	0.160	13	0.031	NM
GCCACCGTCCT	59	0.133	2	0.005	NM
GAGCCTGGGAT	58	0.130	15	0.035	NM
CCGACGGGCGC	56	0.126	4	0.009	NM
GTGGTGGAGTG	55	0.124	7	0.016	NM
AGAGGTGTAGA	47	0.106	2	0.005	NM
CGCGTCACTAA	44	0.099	0	0.000	NM
GACCTGAGGGC	43	0.097	7	0.016	NM
ATCCCCGCGCA	41	0.092	6	0.014	NM
CAGGGACGACC	39	0.088	0	0.000	NM
TATTAGCTCTA	37	0.083	3	0.007	NM
ATGTGTTTGAC	36	0.081	5	0.012	NM
AAGGAGACCCA	32	0.072	4	0.009	NM
GTGGCAGTTCA	32	0.072	4	0.009	NM
TCCTATTAAGC	32	0.072	2	0.005	NM
ACTCTAGGTGT	29	0.065	1	0.002	NM
ATGGAGGGAGC	29	0.065	1	0.002	NM
GTCAACAGTTA	29	0.065	3	0.007	NM
ATATCATATTT	28	0.06	5	0.012	NM
ACTGTGAGACA	27	0.06	2	0.005	NM
TTGGAACAATG	25	0.06	0	0.000	NM
GCACTGGATGA	24	0.05	2	0.005	NM
TCTCTTCACCG	23	0.05	0	0.000	NM
CCTGAGGCCAG	22	0.05	1	0.002	NM
GTGGACCACGG	22	0.05	0	0.000	NM
GAAGATGCCAG	21	0.05	0	0.000	NM
ACTGTCCAGGC	20	0.04	1	0.002	NM
GAACCCTTCTC	19	0.04	1	0.002	NM
CATCATAACATC	19	0.04	0	0.000	NM
CATCATAAAAAG	18	0.04	0	0.000	NM
TACCTATTAAG	17	0.04	0	0.000	NM
GCGACCACGGG	17	0.04	0	0.000	NM
TACGGAATCAC	16	0.04	1	0.002	NM
CTGGAACTCAA	16	0.04	1	0.002	NM
CCCTATTAAGC	16	0.04	1	0.002	NM
CACTAAGGGCT	15	0.03	1	0.002	NM
ACCGTCCGGGC	15	0.03	0	0.000	NM
GTGGCCACGGG	15	0.03	0	0.000	NM
GCCTCAGTTCC	13	0.03	0	0.000	NM
TGCCTATTAAG	13	0.03	0	0.000	NM
TCACTATTAAG	12	0.03	0	0.000	NM
TCCCTGTTAAG	12	0.03	0	0.000	NM
TGACAGAGGAG	12	0.03	0	0.000	NM

NM, no match.

highly expressed genes in this tissue, a finding common with the skeletal muscle. The present study has also permitted the identification of many new genes not yet described in the public databases.

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