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Global Gene Profiling in Human Endometrium during the Window of Implantation

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Implantation in humans is a complex process that is temporally and spatially restricted. Over the past decade, using a one-by-one approach, several genes and gene products that may participate in this process have been identified in secretory phase endometrium. Herein, we have investigated global gene expression during the window of implantation (peak E2 and progesterone levels) in well characterized human endometrial biopsies timed to the LH surge, compared with the late proliferative phase (peak E2 level) of the menstrual cycle. Tissues were processed for poly(A^+) RNA and hybridization of chemically fragmented, biotinylated cRNAs on high density oligonucleotide microarrays, screening for 12,686 genes and expressed sequence tags. After data normalization, mean values were obtained for gene readouts and fold ratios were derived comparing genes up- and down-regulated in the window of implantation *vs.* the late proliferative phase. Nonparametric testing revealed 156 significantly ($P < 0.05$) up-regulated genes and 377 significantly down-regulated genes in the implantation window. Up-regulated genes included those for cholesterol trafficking and transport [apolipoprotein (Apo)E being the most induced gene, 100-fold], prostaglandin (PG) biosynthesis (PLA2) and action (PGE2 receptor), proteoglycan synthesis (glucuronyltransferase), secretory proteins [glycodelin, mammaglobin, Dickkopf-1 (Dkk-1, a Wnt inhibitor)], IGF binding protein (IGFBP), and TGF-β superfamilies, signal transduction, extracellular matrix components (osteopontin, laminin), neurotransmitter synthesis (monoamine oxidase) and receptors (γ aminobutyric acid A receptor π subunit), numerous immune modulators, detoxification genes (metallothioneins), and genes involved in water and ion transport [*e.g. Clostridia Perfringens Enterotoxin* (CPE) 1 receptor (CPE1-R) and K^+ ion channel], among others. Down-regulated genes included intestinal trefoil factor (ITF) [the most repressed gene (50-fold)], matrilysin, members of the G protein-coupled receptor signaling pathway, frizzled-related protein (FrpHE, a Wnt antagonist), transcription factors, TGF-β signaling pathway members, immune modulators (major histocompatibility complex class II subunits), and other cellular functions. Validation of select genes was conducted by Northern analysis and RT-PCR using RNA from endometrial biopsies obtained in the proliferative phase and the implantation window and by RT-PCR using RNA from cultured endometrial epithelial and stromal cells. These approaches confirmed up-regulation of genes corresponding to IGFBP-1, glycodelin, CPE1-R, Dkk-1, mammaglobin, and ApoD and down-regulation for PR membrane component 1, FrpHE, matrilysin, and ITF, as with the microarray data. Cultured endometrial epithelial cells were found to express mRNAs for glycodelin, CPE-1R, Dkk-1, the γ aminobutyric acid A receptor π subunit, mammaglobin, matrilysin, ITF and PR membrane component 1. The expression of IGFBP-1, CPE1-R, Dkk-1, and ApoD mRNAs increased upon decidualization of stromal cells *in vitro* with progesterone after E2 priming, whereas FrpHE decreased, consistent with the microarray results. Overall, the data demonstrate numerous genes and gene families not heretofore recognized in human endometrium or associated with the implantation process. Reassuringly, several gene products, known to be differentially expressed in the implantation window or in secretory endometrium, were verified, and the striking regulation of select secretory proteins, water and ion channels, signaling molecules, and immune modulators underscores the important roles of these systems in endometrial development and endometrial-embryonic interactions. In addition, the current study validates using high density oligonucleotide microarray technology to investigate global changes in gene expression in human endometrium. (*Endocrinology* 143: 2119–2138, 2002)

IMPLANTATION IN HUMANS involves complex interactions between the embryo and the maternal endometrium. Histologic examination of early human pregnancies reveals distinct patterns of blastocyst attachment to the endometrial surface and the underlying stroma (1, 2), supporting a model of implantation in humans in which the embryo apposes and attaches to the endometrial epithelium, traverses adjacent cells of the epithelial lining, and invades into the endometrial stroma. The endometrium is receptive to embryonic implantation during a defined window that is temporally and spatially restricted. Temporal definition of the window of implantation in human endometrium derives from several sources. Early studies suggest that the window resides in the midsecretory phase because embryos identified in secretory phase hysterectomy specimens were all free-floating before d 20 of the cycle and were all attached when specimens were obtained after d 20 (1). In addition, the

Abbreviations: Apo, Apoliprotien; CPE, *Clostridia Perfringens Enterotoxin*; CPE-1R, *Clostridia Perfringens Enterotoxin* 1 receptor; Dkk-1, Dickkopf-1; ECM, extracellular matrix; ESTs, expressed sequence tags; ets, erythroblastosis virus E26 oncogene; FGF, fibroblast growth factor; FrpHE, frizzled-related protein; $GABA_AR$, γ aminobutyric acid A receptor π subunit; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IDO, interferon γ-inducible indoleamine 2,3-dioxygenase; IGFBP, IGF binding protein; ITF, intestinal trefoil factor; MHC, major histocompatibility complex; MMP-7, matrix metalloproteinase 7; NF-AT, nuclear factor of activating T cells; PAN, Protein and Nucleic Acid Facility; PGRMC1, PR membrane component 1; VEGF, vascular endothelial growth factor.

TABLE 1. Oligonucleotide primers with predicted respective PCR product sizes

Gene	Sense primers	Antisense primers	bp
IGFBP-1	5′-ACTCTGCTGGTGCGTCTAC-3′	5′-TTAACCGTCCTCCTTCAAAC-3′	499
Glycodelin	5′-AAGTTGGCAGGGACCTGGCACTC-3′	5′-ACGGCACGGCTCTTCCATCTGTT-3′	420
CPE-1 R	5′-TACTCCGCCAAGTATTCTG-3′	5′-ATTACAGTGATGAATAGCTCTT-3′	900
Dkk-1	5′-AGGCGTGCAAATCTGTCTCG-3′	5′-TGCATTTGGATAGCTGGTTTAGT-3′	502
$GABA_A$ R π subunit	5′-GCTGGGGCTATGATGGAAATG-3′	5′-CTAGCAAGGCCCCAAACACAAAG-3′	429
Mammaglobin	5′-AGTTGCTGATGGTCCTCATG-3′	5′-AGAAGGTGTGGTTTGCAGC-3′	358
ApoD	5′-AAAAGCTCCAGGTCCCTTC-3′	5′-AGGGTTTCTTGCCAAGATCC-3′	498
PGRMC-1	5′-CTTCCTGCTCTACAAGATCG-3′	5′-CCTCATCTGAGTACACAGTG-3′	408
FrpHE	5′-CCGTGCTGCGCTTCTTCTTCTGTG-3′	5′-GCGGGACTTGAGTTCGAGGGATGG-3′	461
Matrilysin	5′-CTCTCAATAGGAAAGAGAAG-3′	5′-TGAATAAGACACAGTCACAC-3′	230
ITF	5′-TTGCTGTCCTCCAGCTCTG-3′	5′-CAGGCTCCAGATATGAAC-3′	322
GAPDH	5′-CACAGTCCATGCCATCACTGC-3′	5′-GGTCTACATGGCAACTGTGAG-3′	609

temporal and spatial appearance of epithelial dome-like structures (pinopodes) support a receptive phase of embryonic implantation because they appear on cycle d 20–24, correlate with implantation sites, and are believed to participate in attachment of the embryo to the epithelium (3). A recent report (4) demonstrates a high success (84%) of continuing pregnancy for embryos that implant between cycle d 22–24 (postovulatory d 8–10), compared with 18% when implantation occurred 11 d or more after ovulation. Together these data suggest that the window of implantation in humans spans cycle d 20–24 and involves the epithelium and subsequently underlying stroma.

Molecular definition of the window of implantation in human endometrium has been more difficult to define and derives primarily from animal models and clinical specimens (see Refs. 5 and 6 for reviews). These studies have revealed a limited number of potential molecular markers of the implantation window and of uterine receptivity to embryonic implantation. Animal models of homologous recombination and gene knockouts that demonstrate an implantation-based infertility phenotype provide important insight into potential markers for uterine receptivity and participants in the molecular mechanisms occurring during embryonic implantation into the maternal endometrium (5–7). By translation from such models and building upon a literature of known expressed genes and proteins and uniquely expressed secretory proteins in human endometrium, the expression of several molecules has been found to be specifically and temporally expressed within and framing the window of implantation in humans (7), suggesting their functionality in the implantation process. The molecular dialogue that occurs between the endometrium and the implanting conceptus involves cell-cell and cell-extracellular matrix interactions, mediated by lectins, integrins, matrix degrading enzymes and their inhibitors, and a variety of growth factors and cytokines, their receptors and modulatory proteins. Of note are molecules that participate in attachment of an embryo to the maternal endometrial epithelium, including carbohydrate epitopes (*e.g.* H-type 1 antigen), heparan sulfate proteoglycan, mucins, integrins (especially $\alpha_v\beta_3$, $\alpha_4\beta_1$), and the trophin-bystin/tastin complex (reviewed in Refs. 5 and 6). Molecules that participate in embryonic attachment to the epithelium and subsequent signaling between epithelium and stroma have been deduced from knockout studies of a given gene in mice that result in absence of embryonic attachment to the epithelium and loss of decidualization of the stroma. These molecules include leukemia inhibitor factor, the homeobox genes, HoxA-10 and HoxA-11, and cyclooxygenase 2 (reviewed in Ref. 7).

In the pregenomic era, a one-by-one approach has been useful to reveal select candidates for uterine receptivity. In the current study, we report global gene profiling, using high density oligonucleotide microarray technology, of human endometrium during the window of implantation. In addition, we have pursued, in well characterized human endometrial tissue and isolated endometrial stromal and epithelial cells, validation of select gene expression by RT-PCR and Northern analysis. This combined effort has resulted in the identification of genes, gene families, and signaling pathways that are candidates for uterine receptivity and molecular mechanisms underlying the process of human implantation. The endometrial signature of genes during the window of implantation provides an opportunity to design diagnostic (screening) tests for patients with infertility and endometrial disorders and for targeted drug discovery for treating implantation-based infertility, other endometrial disorders, and endometrial-based contraception.

Materials and Methods

Tissue specimens and cell culture

Tissues. Endometrial biopsies were obtained from normally cycling women after informed consent, under an approved protocol by the Stanford University Committee on the Use of Human Subjects in Medical Research, and the Human Subjects Committees at the University of North Carolina, Vanderbilt University, and the University of California at San Francisco. All specimens were obtained in accordance with the Declaration of Helsinki. A total of 28 biopsy samples were obtained from two time points of the menstrual cycle and used in this study: 10 in the late proliferative phase (peak circulating E2 levels; cycle d 8–10), and 18 during the window of implantation [midsecretory phase (peak E2 and progesterone)], which were timed to the LH surge (LH + 8 to LH +10, where LH = 0 is the day of the LH surge). Timing to the LH surge assured sampling during the window of implantation. Of the 28 biopsies, 11 (4 in late proliferative phase and 7 window of implantation) were used for microarray studies, 5 secretory specimens were used exclusively for cell isolation and culture, and 12 were used for Northern analysis and RT-PCR validation (*vide infra*). Different samples were used for the microarrays and the validation studies. Subjects ranged in age between 28 and 39 yr of age, had regular menstrual cycles (26–35 d), were documented not to be pregnant, and had no history of endometriosis. Endometrial biopsies were performed with Pipelle catheters under sterile conditions, from the uterine fundus. A portion of each sample was processed for histologic confirmation, and the remainder was processed for cell culture or immediately frozen in liquid nitrogen for

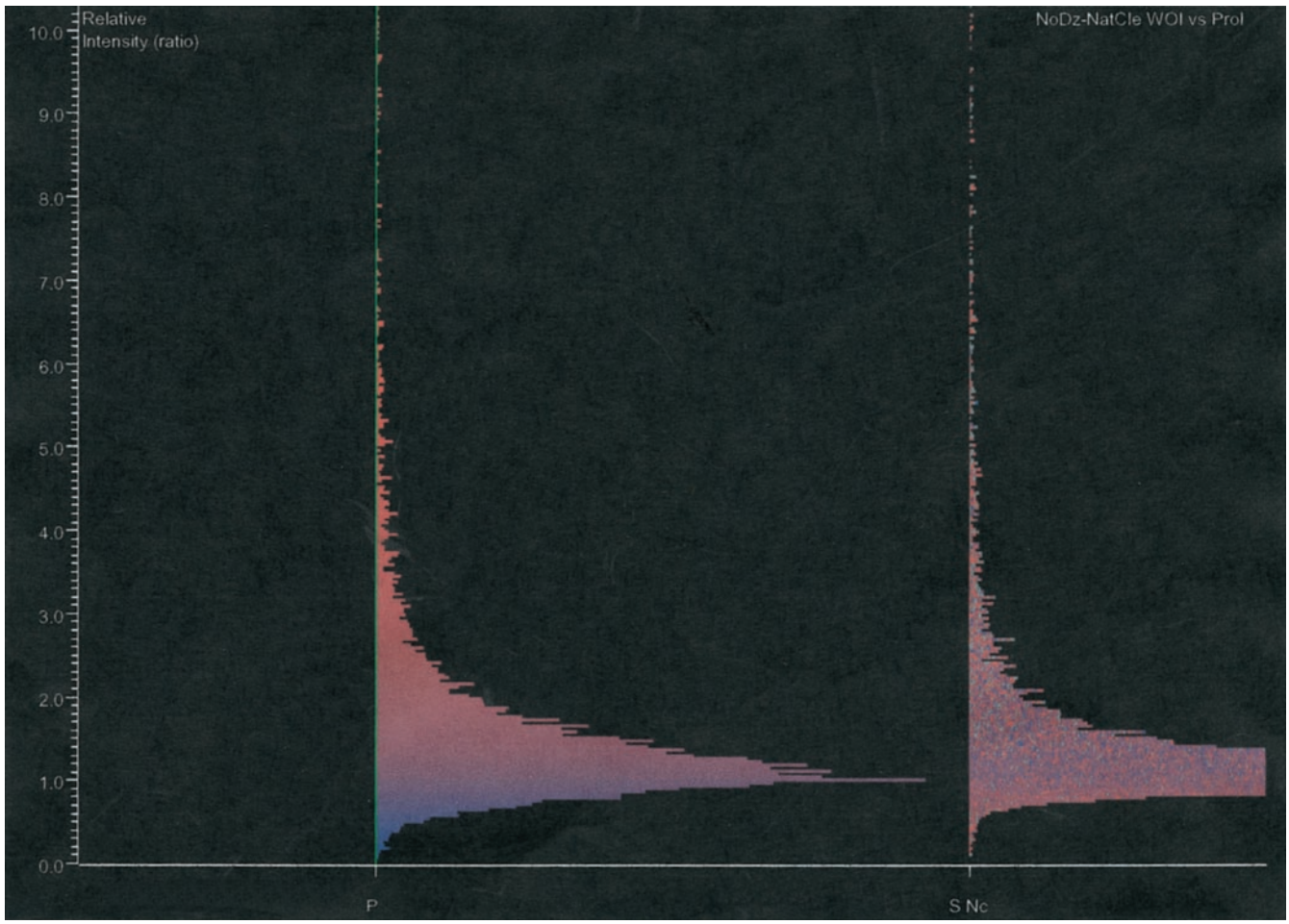

FIG. 1. Scatter plots of the composite normalized data, from all microarray experiments, were segregated into two groups: the late proliferative phase (P) and the window of implantation during the secretory phase (S Nc). Relative intensity of each gene, after normalization, is represented by the vertical axis. The colors are based on relative intensity of each gene, *red* being greater than the value of one and *blue* being less than one, during the proliferative phase.

subsequent RNA isolation. Cycle stages for all specimens (proliferative and midsecretory) were histologically confirmed (8) independently by three observers: L.C.G., B.A.L., and an independent pathologist.

Cell culture. Five midsecretory specimens were used for cell isolation and culture for this study. Tissue was subjected to collagenase (Sigma, St. Louis, MO) digestion, and stromal cells were separated from epithelium, as previously described (9). Initially, stromal cells were centrifuged and the resulting pellet was resuspended in DMEM/10% FBS. The cells were then preplated in 10-cm standard culture plates in DMEM/F12 media for 1 h at 37 C, and the media were then replaced with DMEM/10% FBS. Glands retained on the filter were backwashed into sterile tubes, washed with PBS three times, centrifuged and resuspended in MCDB-105. Endometrial stromal cells were plated and passaged in standard tissue culture plates at a density of 2–3 $\times$ 10^5/10 cm plate and cultured in phenol-red-free, high-glucose DMEM/MCDB-105 medium with 10% charcoal-stripped FBS, insulin (5 μg/ml), gentamicin, penicillin and streptomycin, as described (9). Stromal cells were used at passages 2–6 for these studies. Endometrial epithelial cells were plated in two chamber collagen type I-coated chamber slides (Costar, Cambridge, MA) and cultured in MEMα with 10% charcoal-stripped FBS at 37 C in 9% CO_2 for up to 1 wk (10). Purity was established by vimentin and cytokeratin immunostaining. The culture medium was renewed every 2 d, and the cells were harvested for RNA analysis at the end of the culture period.

Gene expression profiling

RNA preparation/target preparation/array hybridization and scanning. For microarray analysis, 4 late proliferative phase samples and 7 window of implantation samples were used. Each endometrial biopsy sample was processed individually for microarray hybridization (samples were not pooled) following the Affymetrix (Affymetrix, Santa Clara, CA) protocol. Poly(A)$^+$ RNA was initially isolated from the tissue samples using Oligotex Direct mRNA isolation kits (QIAGEN, Valencia, CA), following the manufacturer's instructions. Specimens (120–260 mg) yielded between 1–8 μg poly(A)$^+$ RNA and the purity of isolated mRNAs was evaluated spectrophotometrically by the A260/A280 ratio. A T7-(deoxythymidine)$_{24}$ oligo-primer was used for double-stranded cDNA synthesis by the Superscript Choice System (Life Technologies, Inc., Gaithersburg, MD). *In vitro* transcription was subsequently carried out with Enzo BioArray High Yield RNA T7 Transcript Labeling Kits (Enzo, Farmingdale, NY). Additional cRNA clean-up was performed using RNeasy spin columns (QIAGEN), before chemical fragmentation with 5$\times$ fragmentation buffer (200 mM Tris, pH 8.1; 500 mM KOAc; 150 mM MgOAc). After chemical fragmentation, biotinylated cRNAs were mixed with controls and were hybridized to Affymetrix Genechip Hu95A oligonucleotide microarrays [corresponding to 12,686 human genes and expressed sequence tags (ESTs)] on an Affymetrix fluidics station at the Stanford University School of Medicine Protein and Nucleic Acid (PAN) Facility. Fluorescent labeling and laser confocal scanning were conducted in the PAN Facility and generated the data for analysis.

Data analysis. One of the most critical steps in microarray profiling experiments is accurate assessment of the expression ratios between the sample and the reference, because most subsequent analyses depend on the accuracy of these ratios. The observed signal is comprised of the true expression level with noise due to background and noise due to exper-

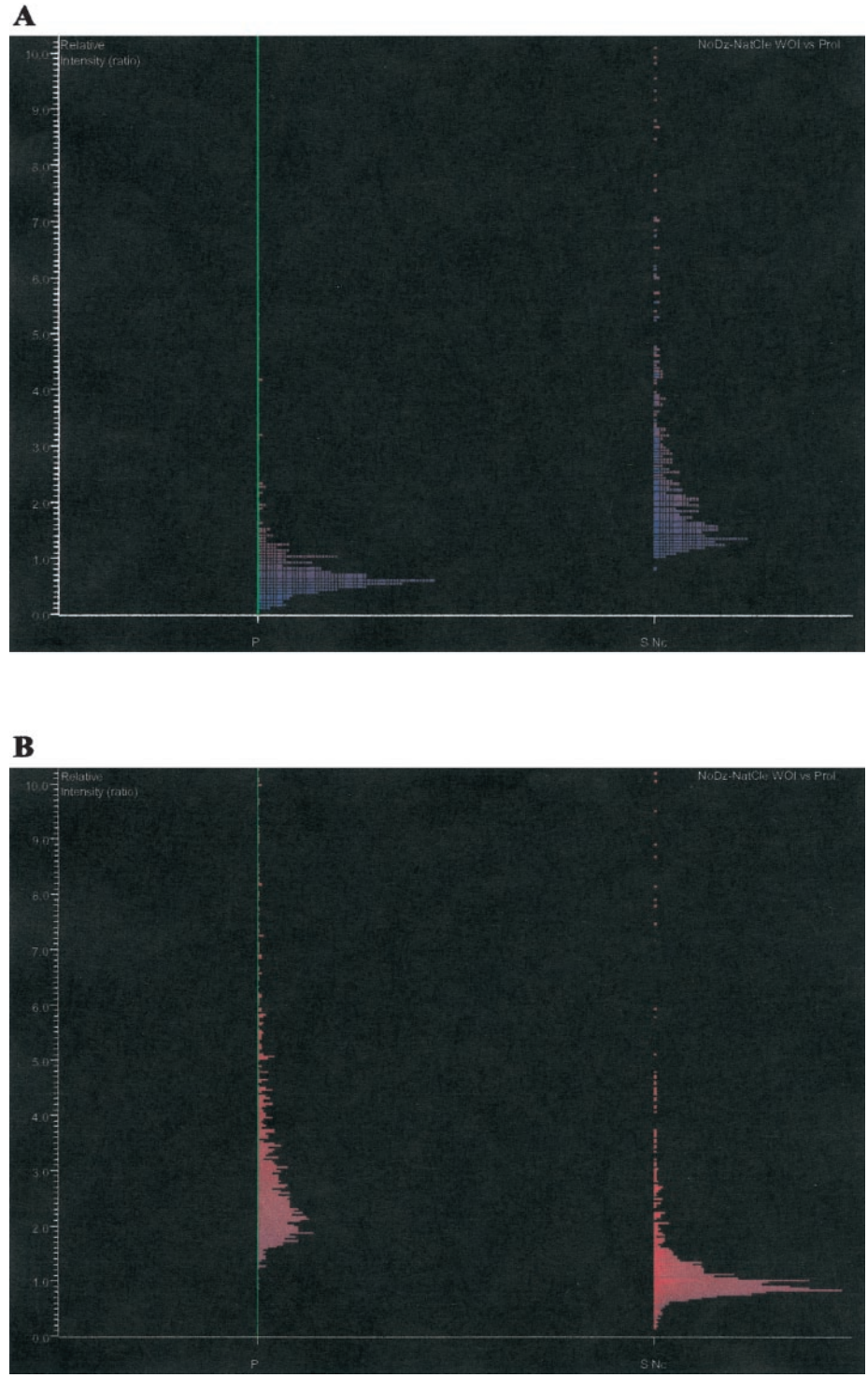

FIG. 2. Genes up- and down-regulated by at least 2-fold in the window of implantation *vs.* the late proliferative phase. A and B, Scatter plots of genes up-regulated (A) and down-regulated (B) before nonparametric testing. C and D, Line graphs of genes up-regulated (C) and down-regulated (D) after nonparametric testing. Relative intensity of each gene, after normalization, is represented by the *vertical axis*. The colors are based on relative intensity of each gene, *red* being greater than the value of one and *blue* being less than one, during the proliferative phase.

imental variations from the probe preparation and hybridization efficiency. Due to variations in the hybridization and scanning processes, several approaches for data analysis have been devised to compensate for these differences. Two major steps are: 1) to eliminate weak expressions that are statistically too close to the background estimate to avoid the detrimental effects on the ratios; and 2) to adjust the expression of each gene by the overall expression of signals on a specific chip. In the current study the data were analyzed with GeneChip Analysis Suite version 4.01 (Affymetrix), GeneSpring version 4.0.4 (Silicon Genetics), and Microsoft Corp. Excel/Mac2001 software. Expression profile data were first prepared using GeneChip Microarray Analysis Suite and subsequently exported to GeneSpring for further analysis. The GeneSpring version 4.0.4 software allows rank-sum normalization and statistical analysis. Initially, within each hybridization, the 50th percentile of all measurements was used as a positive control, and each measurement for each gene was divided by this control. The bottom tenth percentile was used for background subtraction. Between different hybridization outputs/arrays, each gene was normalized to itself by making a synthetic positive control for that gene comprised of the median of the gene's expression values over all samples of an experimental

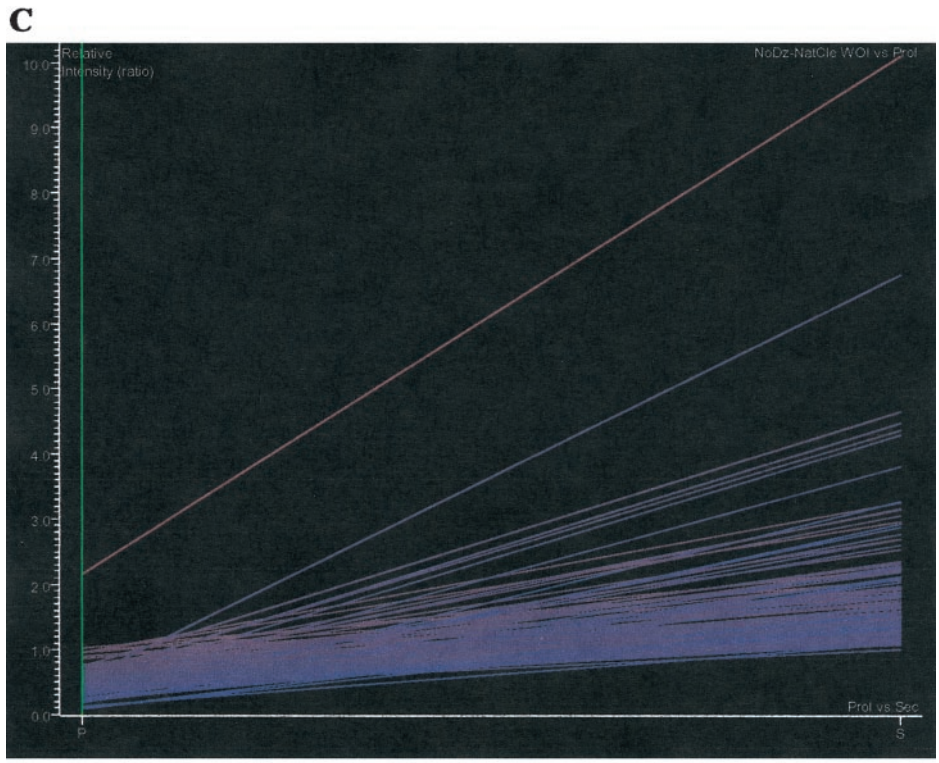

FIG. 2. Continued

group, and dividing the measurements for that gene by this positive control, as per the manufacturer's instructions. Mean values were then calculated among individual experimental groups for each gene probeset, and between-group fold-change ratios [*i.e.* window of implantation (n = 4) : late proliferative phase (n = 7) ratios] were derived. A difference of 2-fold was applied to select up-regulated and down-regulated genes, as described (11, 12). Because the data were not normally distributed, nonparametric testing was also conducted using the Mann-Whitney *U* test to calculate *P* values, and applying $P < 0.05$ to assign statistical significance between the two groups, as described (11). To assess chip-

to-chip variability, preliminary experiments were conducted in which RNA from one tissue sample was subjected to two independent hybridizations. Less than 2.7% of the total genes on the array showed more than 3-fold variation, providing a greater than 95% confidence level, consistent with the manufacturer's claims for chip-to-chip variability (12, 13).

Validation of gene expression data

RT-PCR. Genes of different expression fold changes were randomly selected for validation by RT-PCR and/or Northern analyses. Total RNA from cultured endometrial epithelial cells, stromal cells or whole endometrial tissue was isolated using Trizol (Life Technologies, Inc.) protocol, then treated with deoxyribonuclease (QIAGEN) and purified by RNeasy Spin Columns (QIAGEN). RT was first performed with Omniscript kit (QIAGEN) for 1 h at 37 C, followed by PCR in a 50-μl reaction volume with *Taq* polymerase (QIAGEN) and specific primer pairs using the Eppendorf Mastercycler Gradient (Brinkmann Instruments, Westbury, NY). The amplification cycle consisted of a hot start at 94 C for 2 min followed by 35 cycles of denaturation at 94 C for 1 min, annealing at 58 C for 1 min and extension at 72 C for 1 min. Specific primer pairs (Table 1) were synthesized by the PAN Facility, Stanford University School of Medicine, and were used at 25 pmol per reaction. Sequences were derived from public databases, and all PCR products were confirmed by the Stanford PAN Sequencing Facility. Subcloning by TA cloning into pGEM Teasy (Promega Corp., Madison, WI) or pDrive Cloning Vector (QIAGEN) were performed to generate specific probes for Northern analyses.

Northern analysis. Twelve endometrial biopsy samples were used for these studies, six from the late proliferative phase and six during the window of implantation. Total RNAs (10–20 μg) were electrophoresed on 1% formaldehyde agarose gels and transferred to nylon membranes for Northern analyses. Specific P^{32}-labeled cDNA probes, ranging 400–900 bp, were generated using Ready-to-Go random primer kit (Amersham Pharmacia Biotech, Peapack, NJ) and $^{32}\alpha$P-deoxy-CTP (NEN Life Science Products, Boston, MA). Membranes were prehybridized at 68 C for 30 min in ExpressHyb buffer (CLONTECH Laboratories, Inc., Palo Alto, CA) and hybridization carried out for another hour at 68 C using ExpressHyb buffer containing 1–2 $\times$ 10^6 cpm/ml of labeled probe. Washing was subsequently carried out according to the manufacturers' instructions. Membranes were exposed to Kodak (Rochester, NY) MS x-ray films, and densitometry performed with Bio-Rad Laboratories, Inc. (Hercules, CA) GS-710 Imaging Densitometer and analyzed by its accompanied software Quantity One, version 4.0.2. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA densitometric readings were used to normalize signals for genes expressed on a given Northern blot. These normalized data were used to calculate relative mRNA expression intensities between the window of implantation and proliferative phase endometrium samples on the same blot. Mean values of relative expression intensities from different Northern blots were used for final data presentation. Stripping and reprobing were performed using the same membranes.

Results

Data analysis

The data were analyzed with GeneChip Analysis Suite version 4.01, GeneSpring version 4.0.4, and Microsoft Corp. Excel/Mac2001 software, as described in *Materials and Methods*. Figure 1 shows the scatter plot of the normalized data for all genes and all experiments for samples in the proliferative phase and the secretory phase (window of implantation), underscoring that the data are not normally distributed. Fold-change ratios between groups (*i.e.* window of implantation:late proliferative phase ratios) were subsequently derived, and a difference of 2-fold, a generally adopted fold-change difference for oligonucleotide microarray profile analysis (11, 12), was applied to select up-regulated and down-regulated genes. Nonparametric testing was further applied, using a P value of 0.05 to identify statistical significance between the two groups (13). Figure 2, panels A and B, shows the scatter plots of genes up- and down-regulated by at least 2-fold before nonparametric testing, and panels C and D demonstrate individual genes up- and down-regulated by at least 2-fold after nonparametric testing. With this strategy, we identified, during the window of implantation, 156 genes that were significantly up-regulated, of which 40 were ESTs, and 377 genes that were significantly down-regulated, of which 153 were ESTs. Tables 2 and 3 show, in descending order, respectively, the fold increase and fold decrease, the P values ($P < 0.05$), and the GenBank accession numbers for the 116 specifically up-regulated genes and the 224 down-regulated genes in the window of implantation in human endometrium, compared with the late proliferative phase, according to clustering assignments (*vide infra*).

Clustering

The stringent data filtering for significant and consistent changes permitted identification of biologically relevant gene clustering in human endometrium during the window of implantation *vs.* the late proliferative phase. We performed unsupervised cluster analysis (14), based on National Center for Biotechnology Information/Entrez/Online Mendelian Inheritance in Man database search, which allowed grouping of genes into several categories (Tables 2 and 3). The most markedly up-regulated genes (categories in descending order of maximal fold change) include those involved in cholesterol trafficking and transport [apolipoprotein (Apo)E and D], PG biosynthesis and action (PLA2 and the PGE2 receptor), proteoglycan synthesis (glucuronyltransferase I), and a variety of secretory proteins, including glycodelin (pregnancy-associated endometrial α_2 globulin), mammaglobin (a member of the uteroglobin family), members of the Wnt regulation pathway [Dickkopf-1 (Dkk-1)], IGFBP family, and TGF-β superfamily. Additional genes were up-regulated, including G0S2 (a cell cycle switch protein), several genes involved in signal transduction, nitric oxide metabolism (arginase II), and extracellular matrix components/cell adhesion molecules, including osteopontin and laminin subunits. Also, of note are the marked up-regulation of genes for neuromodulator synthesis/receptors ($GABA_A$ receptor π subunit), immune modulators [*e.g.* natural killer-associated transcript 2, members of the complement family, and interferon-induced genes (interferon γ-inducible indoleamine 2,3-dioxygenase; IDO], genes involved in detoxification (several types of metallothioneins and glutathione peroxidase), phospholipid binding proteins (annexins), as well as some proteases, transcription factors and structural/cytoskeletal proteins. Among several gene families not heretofore known to exist in endometrium are members of water and ion transport that are common to the gastrointestinal epithelial mucosa and other mucosal surfaces [*e.g. Clostridia Perfringens Enterotoxin* (CPE) 1 receptor (CPE-1R) and the sulfonylurea receptor (K^+ ion channel)]. Several genes for other cellular functions were also up-regulated.

The most abundantly down-regulated genes involved secretory proteins, including ITF (a member of a family of proteins that maintains intestinal luminal epithelial cell in-

TABLE 2. Genes up-regulated during the window of implantation

Families/GenBank accession no.	Fold up	*P* value	Description (N = 156)
Cholesterol transport/trafficking			
M12529	100.0	0.013	ApoE
J02611	5.6	0.0013	ApoD
PG biosynthesis			
M22430	18.2	0.0300	RASF-A PLA2 (phospholipase A2)
U19487	3.6	0.0300	PGE2 receptor
Carbohydrate/glycoprotein synthesis			
AB009598	15.6	0.03	Glucuronyltransferase I
AB014679	6.4	0.0066	*N*-Acetylglucosamine-6-*O*-sulfotransferase (GlcNAc6ST)
Secretory proteins			
M61886	14.6	0.0272	Pregnancy-associated endometrial alpha2-globulin (glycodelin)
U33147	12.4	0.0255	Mammaglobin
AB020315	12.1	0.0057	Dickkopf-1 (hdkk-1)
M31452	7.0	0.0272	Proline-rich protein (PRP)
M57730	4.9	0.0057	B61
X16302	2.7	0.0130	IGFBP-2
M93311	2.4	0.0049	Metallothionein-III
AB000584	2.4	0.0057	TGF-β superfamily protein
Cell cycle			
M69199	9.2	0.0184	G0S2 protein
M14752	6.4	0.0300	c-abl
M60974	3.9	0.0057	Growth arrest and DNA-damage-inducible protein (gadd45)
AF002697	2.2	0.0130	E1B 19K/Bcl-2-binding protein Nip3
U66469	2.0	0.0418	Cell growth regulator CGR19
Proteases/peptidases			
M17016	9.0	0.0130	Serine protease-like protein
M30474	5.2	0.0343	γ-Glutamyl transpeptidase type II
L12468	4.0	0.0279	Aminopeptidase A
AL008726	2.5	0.0013	Lysosomal protective protein precursor, cathepsin A, carboxypeptidase C
Nitric oxide synthesis			
U82256	8.3	0.0057	Arginase type II
Extracellular matrix/cell adhesion molecules			
J04765	8.1	0.0013	Osteopontin
U17760	4.1	0.0017	Laminin S B3 chain
M61916	2.6	0.0184	Laminin B1 chain
Neuromodulators/synthesis/receptors			
M68840	7.5	0.0013	Monoamine oxidase A (MAOA)
U95367	2.6	0.0437	GABA-A receptor pi subunit
Immune modulators/cytokines			
L41268	7.2	0.0082	Natural killer-associated transcript 2 (NKAT2)
M84526	6.7	0.0272	Adipsin/complement factor D
M31516	5.9	0.0013	Decay-accelerating factor
AF031167	5.9	0.0013	IL-15 precursor
D63789	4.5	0.0300	SCM-1 β precursor (lymphotactin)
M85276	4.0	0.0437	NKG5 NK and T cell specific gene
U14407	3.7	0.0300	IL-15
M34455	3.7	0.0049	Interferon-γ-inducible indoleamine 2,3-dioxygenase (IDO)
U31628	3.3	0.0066	IL-15 receptor α chain precursor (IL15RA)
AC006293	2.9	0.0082	Chromosome 19, cosmid F15658
D87002	2.4	0.0130	Immunoglobulin lambda gene locus
L09708	2.1	0.0279	Complement component 2 (C2)
M14058	2.0	0.0130	Complement C1r
Detoxification			
J03910	5.9	0.0013	Metallothionein-IG (MTIG)
M10943	3.8	0.0049	Metallothionein-If
R93527	3.6	0.0049	Homo sapiens cDNA similar to metallothionein
M13485	3.5	0.0013	Metallothionein I-B
H68340	3.5	0.0049	Homo sapiens cDNA similar to metallothionein-If
K01383	3.0	0.0279	Metallothionein-I-A
X71973	2.9	0.0130	Phospholipid hydroperoxide glutathione peroxidase
Structural/cytoskeletal proteins			
M88338	5.2	0.0418	Serum constituent protein (MSE55)
M34175	4.3	0.0212	β Adaptin
M19267	3.7	0.0300	Tropomyosin
X06956	3.4	0.0082	α-Tubulin

TABLE 2. Continued

Families/GenBank accession no.	Fold up	*P* value	Description (N = 156)
Phospholipid binding proteins			
D28364	4.7	0.0300	Annexin II
M82809	2.2	0.0279	Annexin IV (ANX4)
Cell surface proteins/receptors			
L78207	4.3	0.0013	Sulfonylurea receptor (SUR1) (K^+-channel)
U11863	3.4	0.0418	HP-DAO2 diamine oxidase, copper/topa quinone containing mRNA
J03779	2.7	0.0184	Common acute lymphoblastic leukemia antigen (CALLA)
D50683	2.6	0.0437	TGF-β II-R α
X97324	2.1	0.0272	Adipophilin
Transporters			
AB000712	3.9	0.0272	hCPE-R (*Clostridia Perfringens Enterotoxin* receptor-1)
U81800	3.4	0.0057	Monocarboxylate transporter (MCT3)
U36341	2.9	0.0255	Creatine transporter (SLC6A8)
AJ131182	2.5	0.0130	Epsilon COP
AB000714	2.2	0.0057	hRVP1 (splice variant of CPE-R)
X57522	2.1	0.0437	RING4
Transcription factors			
J04102	3.9	0.0255	Erythroblastosis virus oncogene homolog 2 (ets-2)
V00568	3.1	0.0437	c-myc
U51127	2.8	0.0300	Interferon regulatory factor 5 (Humirf5)
AL022726	2.8	0.0130	ID4 Helix-loop-helix DNA binding protein
L32164	2.4	0.0117	Zinc finger protein
Signal transduction			
Y10032	3.6	0.0066	Putative serine/threonine protein kinase
D87953	3.5	0.0013	RTP
X69550	2.9	0.0272	rho GDP-dissociation inhibitor 1
L76200	2.8	0.0130	Guanylate kinase (GUK1)
U67156	2.7	0.0013	Mitogen-activated kinase kinase kinase 5 (MAPKKK5)
D38305	2.5	0.0130	Tob
tigr:HG162-HT3165	2.4	0.0066	Tyrosine kinase, receptor Axl, Alt. Splice 2
M54915	2.1	0.0013	h-pim-1 protein (h-pim-1)
L12535	2.1	0.0279	RSU-1/RSP-1
Other cellular functions			
U07919	7.3	0.0057	Aldehyde dehydrogenase 6
U12778	3.7	0.0130	Acyl-CoA dehydrogenase
M94856	3.5	0.0130	Fatty acid binding protein homolog (PA-FABP)
U80184	3.4	0.0272	FLII
U09196	3.4	0.0013	1.1-kb mRNA up-regulated in RA treated HL-60 neutrophilic cells
AF042800	3.4	0.0300	Suppressor of white apricot homolog 2 (SWAP2)
D83198	3.3	0.0013	mRNA expressed in thyroid gland
X79882	3.2	0.0057	lrp
M62896	3.2	0.0057	Lipocortin (LIP) 2 pseudogene mRNA
D38047	3.0	0.0013	26S proteasome subunit p31
U90551	3.0	0.0057	Histone 2A-like protein (H2A/l)
AJ223352	2.8	0.0130	Histone H2B
L38928	2.8	0.0437	5,10-Methenyltetrahydrofolate synthetase
L33799	2.7	0.0130	Procollagen C-proteinase enhancer protein (PCOLCE)
U20938	2.7	0.0255	Lymphocyte dihydropyrimidine dehydrogenase
S72370	2.6	0.0066	Pyruvate carboxylase
X00737	2.6	0.0437	Purine nucleoside phosphorylase
X59960	2.6	0.0279	Sphingomyelinase
X15573	2.6	0.0300	Liver-type 1-phosphofructokinase (PFKL)
D26535	2.5	0.0300	Dihydrolipoamide succinyltransferase
U02556	2.6	0.0279	RP3
U78190	2.5	0.0300	GTP cyclohydrolase I feedback regulatory protein (GFRP)
AF090421	2.5	0.0255	Ribosome S6 protein kinase
AF054825	2.4	0.0437	VAMP5
J04444	2.4	0.0130	Cytochrome c-1
X02152	2.3	0.0049	Lactate dehydrogenase-A (LDH-A)
M61832	2.3	0.0437	*S*-Adenosylhomocysteine hydrolase (AHCY)
U61263	2.2	0.0130	Acetolactate synthase homolog
Z80779	2.2	0.0388	H2B/g
X13973	2.2	0.0279	Ribonuclease/angiogenin inhibitor (RAI)
AF000573	2.2	0.0437	Homogentisate 1,2-dioxygenase
X93086	2.1	0.0212	Biliverdin IX α reductase
AF042386	2.2	0.0255	Cyclophilin-33B (CYP-33)
AF020736	2.0	0.0130	ATPase homolog
ESTs/Unknown function	N = 40		

TABLE 3. Genes down-regulated during the window of implantation

Families/GenBank accession no.	Fold down	*P* value	Description (N = 377)
Secretory proteins			
L08044	49.8	0.0418	Intestinal trefoil factor
AF026692	19.8	0.0017	Frizzled related protein frpHE
AF056087	6.3	0.0013	Secreted frizzled related protein FRP
AB000220	5.8	0.0047	Semaphorin E
X78947	2.9	0.0279	Connective tissue growth factor
U38276	2.6	0.0130	Semaphorin III family homolog
AF020044	2.2	0.0130	Lymphocyte secreted C-type lectin precursor
Proteases			
L22524	24.1	0.0082	Matrilysin
M96859	10.8	0.0213	Dipeptidyl aminopeptidase like protein
X51405	9.7	0.0117	Carboxypeptidase E
AF071748	3.1	0.0117	Cathepsin F (CATSF)
Signal transduction			
L15388	23.5	0.0213	G protein-coupled receptor kinase (GRK5)
M29551	7.6	0.0464	Calcineurin A2
AB007972	5.3	0.0130	Chromosome 1 specific transcript KIAA0503
L06139	5.1	0.0279	Receptor protein-tyrosine kinase (TEK)
U31384	4.7	0.0212	G protein γ-11 subunit
L07592	3.9	0.0213	PPAR
S62539	3.5	0.0013	Insulin receptor substrate-1
U02390	3.4	0.0274	Adenylyl cyclase-associated protein homolog CAP2 (CAP2)
D87116	3.4	0.0212	MAP kinase kinase 3b
AB015019	3.2	0.0274	BAP2-α
AB009356	3.2	0.0049	TGF-β activated kinase 1a
U61167	3.1	0.0130	SH3 domain-containing protein SH3P18
AF015254	3.1	0.0117	Serine/threonine kinase (STK-1)
U59863	2.9	0.0212	TRAF-interacting protein I-TRAF
U36764	2.8	0.0300	TGF-β receptor interacting protein 1
D50863	2.6	0.0017	TESK1
L33881	2.5	0.0212	PKC iota isoform
U59912	2.4	0.0049	Smad1
Y18046	2.4	0.0117	FOP (FGFR1 oncogene partner)
S59184	2.4	0.0212	RYK-related to receptor tyrosine kinase
AF042081	2.3	0.0279	SH3 domain binding glutamic acid-rich-like protein
X56468	2.2	0.0049	mRNA for 14.3.3 protein, a protein kinase regulator
U94905	2.2	0.0013	Diacylglycerol kinase ζ
D10522	2.2	0.0130	80K-L protein
U37139	2.2	0.0025	β 3-endonexin
L36870	2.2	0.0386	MAP kinase kinase 4 (MKK4)
U02570	2.2	0.0279	CDC42 GTPase-activating protein
U85245	2.1	0.0049	Phosphatidylinositol-4-phosphate 5-kinase type II β
X02596	2.0	0.0013	bcr (breakpoint cluster region) gene in Philadelphia chromosome
Cell surface proteins/receptors			
D10925	11.3	0.0082	HM145
L78132	4.8	0.0133	Prostate carcinoma tumor antigen (pcta-1)
AB011542	3.5	0.0177	MEGF9
M34641	3.4	0.0013	FGF receptor-1
M87770	3.2	0.0212	FGF receptor (K-sam)
U09278	3.2	0.0130	Fibroblast activation protein
AB015633	3.0	0.0017	Type II membrane protein
X83425	2.7	0.0388	Lutheran blood group glycoprotein
Y00264	2.6	0.0130	Amyloid A4 precursor
L20852	2.2	0.0212	Leukemia virus receptor-2 (GLVR2)
Extracellular matrix/cell adhesion molecules			
M92642	11.2	0.0013	α-1 type XVI collagen (COL16A1)
AL049946	10.1	0.0017	DKFZp564I1922
M34064	6.0	0.0013	Human N-cadherin
U69263	5.6	0.0117	Matrilin-2 precursor
J04599	4.2	0.0013	hPGI mRNA encoding bone small proteoglycan I (biglycan)
X78565	3.9	0.0130	Tenascin-C
U19718	3.0	0.0066	Microfibril-associated glycoprotein (MFAP2)
D13666	2.8	0.0117	Osteoblast specific factor-2 (OSF-2os)
X53002	2.4	0.0049	Integrin β-5 subunit
X53586	2.3	0.0013	Integrin α-6
X17042	2.1	0.0279	Hematopoetic proteoglycan core protein

TABLE 3. Continued

Families/GenBank accession no.	Fold down	*P* value	Description (N = 377)
Transcription factors			
D89377	9.0	0.0213	MSX-2
L11672	7.2	0.0343	Kruppel related zinc finger protein (HTF10)
M21535	6.1	0.0386	erg protein (ets-related gene)
V01512	4.9	0.0464	Oncogene c-fos
U09848	4.8	0.0343	Zinc finger protein (ZNF139)
M68891	4.0	0.0418	GATA-binding protein (GATA2)
AJ222700	4.0	0.0049	TSC-22 protein
X62534	3.8	0.0130	HMG-2
AF003540	3.1	0.0343	Kruppel family zinc finger protein (znfp104)
X07384	3.0	0.0117	GLI protein
M31523	3.0	0.0184	Transcription factor (E2A)
L13689	3.0	0.0279	Proto-oncogene (BMI-1)
AF045451	2.9	0.0049	Transcriptional regulatory protein p54
D63874	2.8	0.0017	HMG-1
AL096880	2.8	0.0049	mRNA containing zinc finger C2H2 type domains
AC004774	2.8	0.0057	BAC clone RG300E22
X59871	2.8	0.0213	T cell factor 1 (TCF-1, splice form C)
M97676	2.7	0.0388	Homeobox protein (HOX7)
L19314	2.7	0.0047	HRY
X84373	2.7	0.0130	Nuclear factor RIP140
X53390	2.6	0.0025	Upstream binding factor (hUBF)
X17360	2.5	0.0279	HOX 5.1
AF071309	2.5	0.0013	OPA-containing protein
AJ223321	2.5	0.0017	RP58
U80760	2.4	0.0130	CAGH1 alternate open reading frame
D28118	2.4	0.0464	DB1
M16937	2.3	0.0057	Homeobox c1 protein
U31814	2.3	0.0049	Transcriptional regulator homolog RPD3
AF104913	2.3	0.0300	Eukaryotic protein synthesis initiation factor
D13969	2.3	0.0049	Mel-18 protein
AL031668	2.3	0.0049	EIF2S2 [eukaryotic translation initiation factor 2, subunit 2 (β, 38 kD)]
X59268	2.3	0.0117	Transcription factor IIB
AF031383	2.2	0.0049	hMed7 (MED7)
AB006572	2.2	0.0130	RMP mRNA for RPB5 mediating protein
M27691	2.1	0.0212	Transactivator protein (CREB)
X72889	2.1	0.0130	hbrm
D85939	2.1	0.0274	p97 homologous protein
M62831	2.1	0.0130	Transcription factor ETR101
X95525	2.1	0.0013	TAFII100 protein
Apoptosis/inhibitors			
AF001294	5.6	0.0117	IPL
AF036956	4.4	0.0047	Neuroblastoma apoptosis-related RNA binding protein (NAPOR-1)
M96954	3.0	0.0213	Nucleolysin
M77142	2.7	0.0130	Polyadenylate binding protein (TIA-1)
AF005775	2.3	0.0212	Caspase-like apoptosis regulatory protein 2 (clarp)
AF016266	2.2	0.0013	TRAIL receptor 2
M59465	2.0	0.0464	TNFα inducible protein A20
Immune modulators/receptors			
M83664	4.7	0.0049	MHC class II lymphocyte antigen (HLA-DP) β chain
M60028	4.6	0.0017	MHC class II HLA-DQ-beta (DQB1, DQw9)
X94232	3.5	0.0386	T cell activation protein
J00194	2.9	0.0130	HLA-dr antigen α-chain
M24594	2.6	0.0213	Interferon-inducible 56Kd protein
Vasoactive substances			
J05081	4.7	0.0418	Endothelin 3 (EDN3)
AF022375	3.4	0.0279	Vascular endothelial growth factor
Cell cycle			
X77494	4.1	0.0279	MSSP-2
AF017790	4.1	0.0117	Retinoblastoma-associated protein HEC
AF059617	3.5	0.0212	Serum-inducible kinase
M68520	3.4	0.0049	cdc2-related protein kinase
AB000449	3.2	0.0418	VRK1
D38073	2.7	0.0343	hRlf β subunit (p102 protein)
U37359	2.6	0.0213	MRE11 homolog hMre11
M25753	2.5	0.0213	Cyclin B
L20046	2.5	0.0133	ERCC5 excision repair protein

TABLE 3. Continued

Families/GenBank accession no.	Fold down	*P* value	Description (N = 377)
U50535	2.4	0.0300	BRCA2
X59798	2.4	0.0013	PRAD1 mRNA for cyclin
L78833	2.2	0.0274	BRCA1, Rho7 and vatI genes
Structural/cytoskeletal proteins			
L10678	3.0	0.0049	Profilin II
AF027299	2.6	0.0279	Protein 4.1-G
S78296	2.1	0.0418	Neurofilament-66
U03057	2.1	0.0013	Actin bundling protein (HSN)
Transport proteins			
L04569	2.8	0.0213	L-type voltage-dependent calcium channel a1 subunit (hHT)
U83993	2.5	0.0057	P2X4 purinoreceptor
U07139	2.0	0.0130	Voltage-gated calcium channel β subunit
Ion binding proteins			
X72964	2.5	0.0013	Caltractin
AF070616	2.1	0.0049	BDP-1 protein
M81637	2.1	0.0388	Grancalcin
U29091	2.0	0.0279	Selenium-binding protein (hSBP)
Steroid hormone action			
Y12711	2.4	0.0057	Putative progesterone binding protein
AJ000882	2.1	0.0212	Steroid receptor coactivator 1e
Neuromodulators/receptors			
U29195	2.2	0.0418	Neuronal pentraxin II (NPTX2)
Other cellular functions			
AF041210	7.1	0.0013	Midline 1 fetal kidney isoform 3 (MID1)
M97815	5.6	0.0274	RA-binding protein II (CRABP-II)
M90656	5.1	0.0130	γ-Glutamylcysteine synthetase (GCS)
U16954	5.1	0.0386	AF1q
X69838	5.1	0.0082	G9a
U90268	4.6	0.0418	Krit1
AJ000644	4.5	0.0279	SPOP
U79299	4.2	0.0418	Neuronal olfactomedin-related ER localized protein
M14539	4.1	0.0025	Factor XIII subunit a
U57646	4.0	0.0047	Cysteine and glycine-rich protein 2 (CSRP2)
U03911	3.8	0.0049	Human mutator gene (hMSH2)
AJ001381	3.7	0.0279	myh-1c
AL031230	3.6	0.0117	NAD+-dependent succinic semialdehyde dehydrogenase (SSADH)
U78027	3.5	0.0057	Brutons tyrosine kinase (BTK), α-D-galactosidase A (GLA), L44-like ribosomal protein (L44L) and FTP3 (FTP3)
J02683	3.5	0.0049	ADP/ATP carrier protein
AC004770	3.3	0.0057	hFEN1
D89053	3.3	0.0212	Acyl-CoA synthetase 3
L35594	3.2	0.0279	Autotaxin
U42360	3.2	0.0279	N33
U46689	3.1	0.0013	Microsomal aldehyde dehydrogenase (ALD10)
U39067	3.1	0.0418	Translation initiation factor eIF3 p36 subunit
S71018	3.0	0.0184	Cyclophilin C
X96752	3.0	0.0013	L-3-hydroxyacyl-CoA dehydrogenase
U90030	3.0	0.0076	Bicaudal-D (BICD)
S79639	3.0	0.0388	EXT1=putative tumor suppressor/hereditary multiple exostoses candidate gene
AF000416	3.0	0.0130	EXT-like protein 2 (EXTL2)
AJ131244	2.9	0.0130	Sec24 protein (Sec24A isoform)
D38076	2.9	0.0279	RanBP1 (Ran-binding protein 1)
AF058718	2.9	0.0386	Putative 13 S Golgi transport complex
U84011	2.9	0.0279	Glycogen debranching enzyme isoform 6 (AGL)
D38524	2.8	0.0279	5′-Nucleotidase
AF043325	2.8	0.0017	*N*-Myristoyltransferase 2
X97335	2.7	0.0437	Kinase A anchor protein
M37721	2.7	0.0279	Peptidylglycine α-amidating monooxygenase
Y00757	2.7	0.0013	Polypeptide 7B2
X95592	2.6	0.0130	C1D protein
AF051321	2.6	0.0076	Sam68-like phosphotyrosine protein α (SALP)
K03000	2.6	0.0013	Aldehyde dehydrogenase 1
M96860	2.6	0.0418	Dipeptidyl aminopeptidase-like protein
U35451	2.6	0.0013	Heterochromatin protein p25
tigr:HG4074–HT4344	2.5	0.0057	Rad2

TABLE 3. Continued

Families/GenBank accession no.	Fold down	*P* value	Description (N = 377)
U03634	2.5	0.0418	P47 LBC oncogene
U14518	2.5	0.0213	Centromere protein-A (CENP-A)
J04031	2.5	0.0133	Methylenetetrahydrofolate dehydrogenase-methenyltetrahydrofolate cyclohydrolase-formyltetrahydrofolate synthetase
X59543	2.4	0.0049	M1 subunit of ribonucleotide reductase
AJ236876	2.4	0.0076	Poly(ADP-ribose) polymerase-2
AF093774	2.4	0.0082	Type 2 iodothyronine deiodinase
U74324	2.4	0.0117	Guanine nucleotide exchange factor mss4
U73737	2.4	0.0076	hMSH6
X06745	2.4	0.0025	DNA polymerase α-subunit
AF060219	2.4	0.0130	RCC1-like G exchanging factor RLG
AF005043	2.3	0.0049	Poly(ADP-ribose) glycohydrolase (hPARG)
D61391	2.3	0.0013	Phosphoribosypyrophosphate synthetase-associated protein 39
AF068754	2.3	0.0049	Heat shock factor binding protein 1 HSBP1
Y10746	2.3	0.0013	MBD 1
U31930	2.3	0.0013	Deoxyuridine nucleotidohydrolase
M97287	2.3	0.0025	MAR/SAR DNA binding protein (SATB1)
U84720	2.2	0.0049	mRNA export protein (RAE1)
AF000993	2.2	0.0117	Ubiquitous TPR motif, X isoform (UTX)
U04840	2.2	0.0117	Onconeural ventral antigen-1 (Nova-1)
D14041	2.2	0.0130	H-2K binding factor-2
D55654	2.2	0.0049	Cytosolic malate dehydrogenase
U36336	2.2	0.0279	Lysosome-associated membrane protein-2b (LAMP2)
L36140	2.1	0.0130	DNA helicase (RECQL)
AF047442	2.1	0.0279	Vesicle trafficking protein sec22b
AF084481	2.1	0.0082	Transmembrane protein (WFS1)
U53209	2.1	0.0279	Transformer-2 alpha (htra-2 α)
L24521	2.1	0.0049	Transformation-related protein mRNA
L42572	2.1	0.0130	p87/89 gene
L37043	2.1	0.0013	Casein kinase I epsilon
AJ001258	2.1	0.0279	NIPSNAP1 protein
AJ005896	2.1	0.0437	JM4 protein
U87459	2.1	0.0117	Autoimmunogenic cancer/testis antigen NY-ESO-1
M30938	2.0	0.0130	Ku (p70/p80) subunit
U59151	2.0	0.0279	Cbf5p homolog (CBF5)
AB010882	2.0	0.0279	hSNF2H
AJ132917	2.0	0.0130	Methyl-CpG-binding protein 2
U96915	2.0	0.0049	sin3-associated polypeptide p18 (SAP18)
ESTs/Unknown function	N = 153		

tegrity) and proteases, such as matrilysin [matrix metalloproteinase 7 (MMP)], dipeptidyl aminopeptidase, and carboxypeptidase E. Also markedly down-regulated genes included those for G protein-coupled receptor signaling: G protein-coupled receptor kinase and G protein γ-11 subunit. Also, marked down-regulation of calcineurin (a protein involved in Ca^{2+} signaling) was observed, as well as some members of the Wnt pathway [frizzed related protein (FrpHE) and secreted frizzled-related protein], genes for TGF-β signaling (homolog of the *Drosophila* mothers against decapentaplegic 1), the PPAR and members of the fibroblast growth factor receptor family. Select extracellular matrix/cell adhesion molecules were down-regulated, including α-1 type XVI collagen and the extracellular matrix protein, tenascin-C. Numerous genes corresponding to transcription factors were found to be down-regulated during the implantation window, including homeobox genes (muscle segment homeobox-2 and homeobox-7), Kruppel family of zinc finger proteins, the early response gene protein [erythroblastosis virus E26 oncogene (ets)-related gene], several proto-oncogenes (c-*fos*, B lymphoma Mo-MLV insertion region, and others), apoptosis/inhibitors (TNF-related apoptosis-inducing ligand receptor 2), and immune modulators (MHC class II subunits). Of interest is the observed down-regulation of vasoactive substances [endothelin 3 and vascular endothelial growth factor (VEGF)], several cell cycle regulators, and genes whose products have relevance to steroid hormone actions [putative progesterone binding protein/PR membrane component 1 (PGRMC1) and steroid receptor coactivator 1e]. Down-regulation of several transporters and calcium channel subunits, structural and cytoskeletal proteins, and ion binding proteins were observed, as well as genes for other cellular functions.

Because clinical endometrial biopsy samples contain a mixture of different cell populations, including glandular and surface epithelial cells, stromal cells, and vascular, smooth muscle, and blood cell components, it is anticipated that many genes and gene families participating in different processes would be represented in the microarray data. In addition, previously documented genes in these cellular components would be anticipated to be detected in the GeneChip analysis. Reassuringly, many genes known to be significantly up-regulated in human endometrium during the secretory phase were up-regulated more than 2-fold and included (Table 2): pregnancy-associated endometrial α_2-globulin [glycodelin, an exclusively endometrial epithelial

cell product predominantly expressed in the secretory phase of the cycle (15)]; IGFBP-2, which is exclusively an endometrial stromal cell product up-regulated in the secretory phase (16); osteopontin, an endometrial epithelial-specific protein (17); PGE2 receptor; TGFβ-type II receptor; and IL-15 (18, 19) and its receptor (20, 21). Others (reviewed in Refs. 5 and 6), such as IGF-II, plasminogen activator inhibitor, urokinase receptor, tissue inhibitor of metalloproteinase-3, fibroblast growth factor (FGF)-6, and FGF-8, and IGFBP-1 were also up-regulated, although they did not reach statistical significance by nonparametric testing. For the genes down-regulated in the window of implantation (Table 3), significant down-regulation of matrilysin (MMP-7) and tenascin-C was detected, consistent with previous studies demonstrating their decreased expression in secretory, compared with proliferative phase, endometrium (22, 23).

Validation of gene expression

We validated expression of select up-regulated and down-regulated genes using two approaches: RT-PCR and Northern analyses with RNAs from late proliferative and window of implantation endometrial biopsy tissue samples and RT-PCR using RNA isolated from cultured human endometrial glandular and stromal cells. The results are shown in Figs. 3–5. For the RT-PCR studies, the primer sets are shown in Table 1. Although quantitative PCR was not performed, the RT-PCR data in Fig. 3 demonstrate clearly in the implantation window compared with late proliferative phase endometrium, up-regulation of IGFBP-1, glycodelin, CPE-1R, Dkk-1, $GABA_A$receptor π subunit, mammaglobin, and ApoD, and down-regulation of PGRMC1, frpHE, matrilysin, and intestinal trefoil factor (ITF). These data are consistent with the observations from the microarray data (Tables 2 and 3).

Northern analyses were conducted to validate further select changes in gene expression in the implantation window *vs.* the late proliferative phase, and a representative set of Northern blots is demonstrated in Fig. 4. Densitometric analyses were conducted and signals were normalized to GAPDH for samples on the same blots. Ratios between the window of implantation *vs.* proliferative endometrium gene expression were calculated for each mRNA on each blot. Mean values of relative expression intensities from n = 3 different blots were then calculated. These data demonstrate up-regulation of Dkk-1 (3.2-fold), IGFBP-1 (2.8-fold), $GABA_A$ receptor π subunit (24.6-fold), and glycodelin (3.1-fold), and the down-regulation of PGRMC-1 (1.3-fold), matrilysin (20.0-fold), and FrpHE, 50-fold. The data are consistent with and validate those obtained through the microarray expression profiling analysis, although the levels of fold-change are not the same as in the microarray analysis (Tables 2 and 3) and would not be expected to be the same (see *Discussion*).

RT-PCR experiments using RNA from cultured endometrial epithelial and stromal cells and the primers listed in Table 1, revealed the following (Fig. 5): PCR products corresponding to glycodelin (positive control), the CPE-1R, Dkk-1, the $GABA_A$ receptor π subunit, mammaglobin, matrilysin, ITF, and PGRMC-1 were all expressed in human endometrial epithelial cells (panel A), demonstrating their expression in this cell type in human endometrium. With cultured human endometrial stromal cells (Fig. 5B), up-regulation of the CPE-1R, Dkk-1, ApoD, and IGFBP-1 (positive control) and down-regulation of frpHE upon *in vitro* decidualization with progesterone after E2 priming were observed. The $GABA_A$ receptor π subunit, mammaglobin, matrilysin, ITF, and PGRMC-1 were not detected in isolated and cultured endometrial stromal cells before or after decidualization (data not shown).

Discussion

Molecular mechanisms that involve apposition, attachment, and intrusion of an implanting embryo into human endometrium are beginning to be appreciated. Most of what is believed to occur during human implantation is derived from animal models that have been invaluable, especially when the reproductive phenotype involves implantation failure (7). A limiting factor in research with human endometrium has been the availability of appropriately characterized clinical specimens. Herein, we have presented global gene profiling of well characterized human endometrial bi-

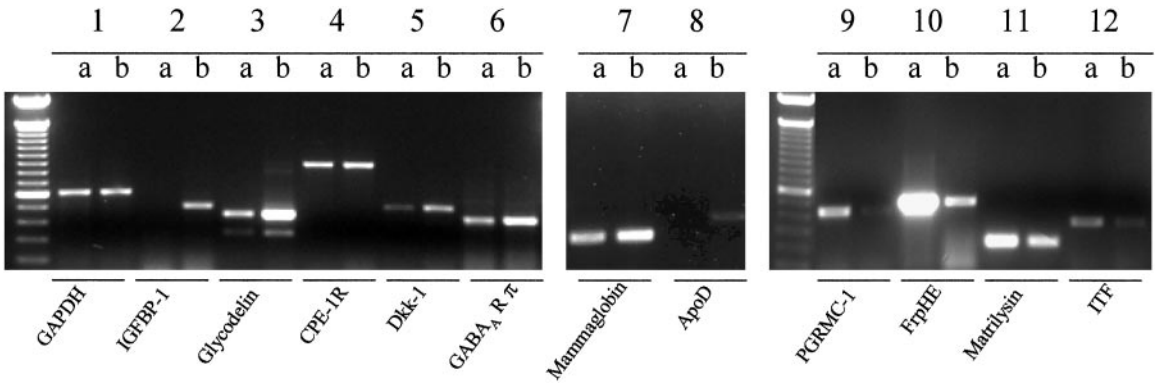

FIG. 3. Validation of selected genes more than 2-fold up- or down-regulated during the window of implantation in human endometrium by RT-PCR. Endometrial biopsy samples from late proliferative phase (n = 3) and the window of implantation (n = 3) were processed for total RNA, and representative results are shown. RT-PCR was conducted with specific primer sets shown in Table 1, using the samples from the proliferative phase (lanes a) or window of implantation (lanes b). Appropriate-sized products corresponding to GAPDH (lanes 1a, 1b), IGFBP-1 (lanes 2a, 2b), glycodelin (lanes 3a, 3b), CPE-1R (lanes 4a, 4b), Dkk-1 (lanes 5a, 5b), $GABA_A$ R π (lanes 6a, 6b), mammaglobin (lanes 7a, 7b), ApoD (lanes 8a, 8b), PGRMC-1 (lanes 9a, 9b), FrpHE (lanes 10a, 10b), matrilysin (lanes 11a, 11b), and ITF (lanes 12a, 12b) are shown.

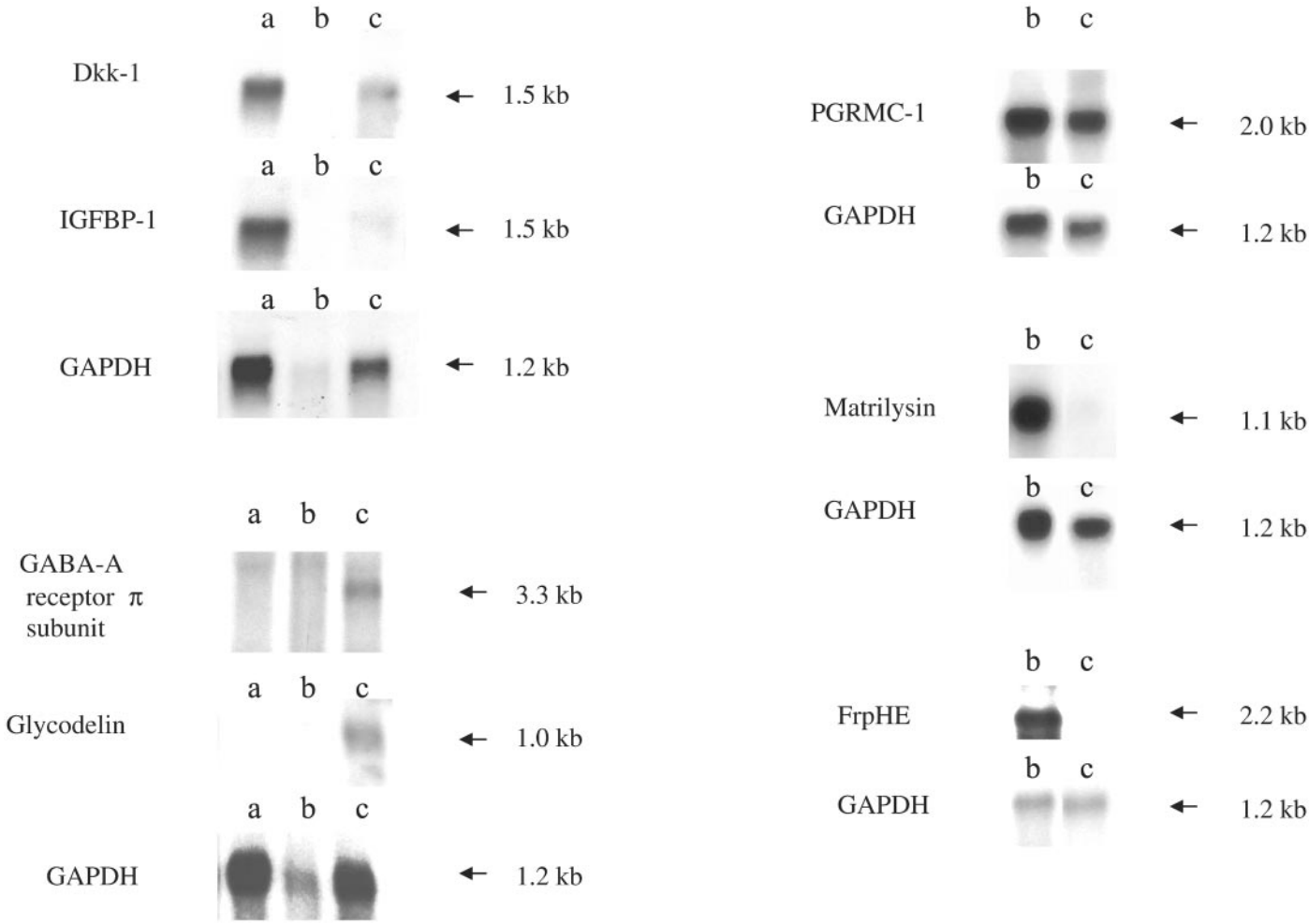

FIG. 4. Northern analysis demonstrating up-regulation of Dkk-1, IGFBP-1, $GABA_A$ R π subunit, glycodelin, and down-regulation of PGRMC-1, matrilysin and FrpHE in the secretory phase (implantation window, lane c), compared with the proliferative phase (lane b). Placental basal plate with decidua (lane a) is shown as a positive control for Dkk-1 and IGFBP-1 on the *left panel*. GAPDH hybridization of respective blots are shown for comparison.

opsy samples that were obtained during the window of implantation, defined by timing to the LH surge and histologically confirmed (8). About one-third of the samples we collected had to be discarded because their histology was not consistent with normal temporal development in the cycles in which they were obtained. This observation underscores the need for precise histologic confirmation of endometrial samples before analysis. The approach taken in this study also demonstrates that reproducible and sensitive experimental methodology for global gene analysis can be applied to small quantities of human endometrial tissue. Similar approaches have been successfully pursued with whole tissues [*e.g.* developing rat kidney (24)] and breast cancer biopsies (25). The current study used high-density oligonucleotide microarray expression profiling that allowed profiling and interrogation of expression of 12,686 full-length genes and ESTs in human endometrial biopsies. The microarray technologies and the data presented herein highly support the use of this powerful approach to investigate molecular candidates involved in human uterine receptivity. Results from the human genome project suggest that we have interrogated about one third to one half of existing human genes, and thus, investigation of additional genes, as well as their validation, present a formidable task and await further investigation.

Endometrial biopsy specimens contain several cell populations and may differ in their complement of such populations. This heterogeneity may contribute to differences observed in relative expression of select genes between the implantation window and the late proliferative phase as assessed by the microarray approach *vs.* Northern analysis or RT-PCR approaches. In addition, because different samples were used for the microarrays and the validation studies, subject-to-subject biologic variation in samples obtained in the same phase of would be anticipated. Also, in the microarray analysis, the mean of an individual gene readout from the samples in the window of implantation was compared with the mean of the same gene readout of the proliferative phase samples; whereas, in calculating the fold-change for a given gene analyzed by Northern analysis, the mean of the densitometric OD readings were calculated after normalization to GAPDH. We speculate that differences in

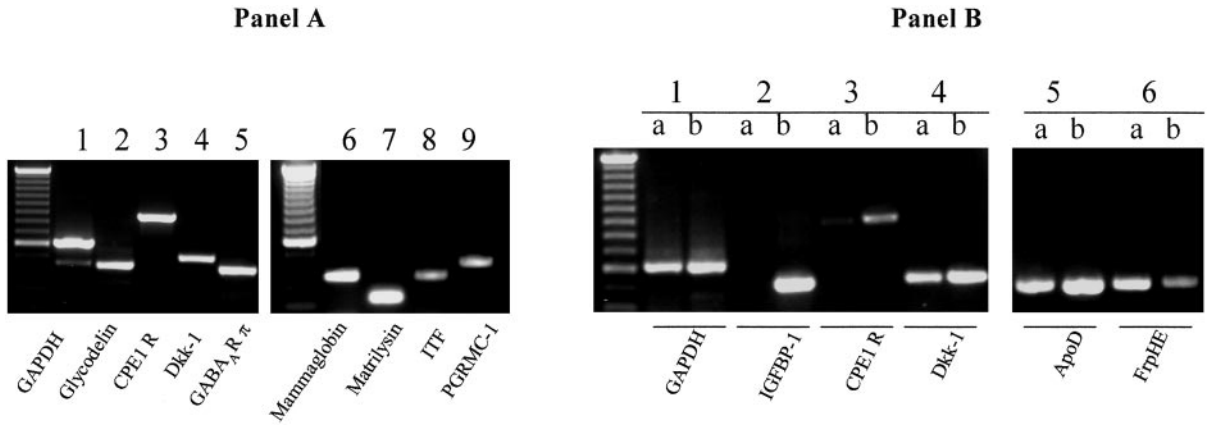

FIG. 5. Expression of selected genes in cultured human endometrial epithelial (A) and stromal (B) cells by RT-PCR. A, Lane 1, GAPDH (control); lane 2, Glycodelin; lane 3, CPE-1R; lane 4, Dkk-1; lane 5, $GABA_A$ R π subunit; lane 6, Mammaglobin; lane 7, Matrilysin; lane 8, ITF; lane 9, PGRMC-1. B, Demonstrates RT-PCR products using endometrial stromal cells nondecidualized (lanes "a") or decidualized (lanes "b") with progesterone after E2 priming, as described in *Material and Methods*. Lanes 1a and 1b, GAPDH (control); lanes 2a and 2b, IGFBP-1; lanes 3a and 3b, CPE-1R; lanes 4a and 4b, Dkk-1; lanes 5a and 5b, ApoD; lanes 6a and 6b, FrpHE. Experiments were conducted with isolated cells from five different samples. Representative results are shown.

the fold-change values between the two methodologies may also be due to the lower abundance of specific mRNAs in relation to the highly abundant GAPDH mRNA, especially because the microarray profile represents true abundance of each mRNA species globally within the tissue, whereas Northern analysis reflects mRNAs of higher abundance and is poor in detecting very low abundance transcripts.

While this heterogeneity among samples may influence the relative expression of some genes, we confirmed previously documented genes within the window of implantation, such as the endometrial glandular-specific glycodelin and the stromal cell-specific, IGFBP-1 and IGFBP-2. Other molecules, such as the PGE2 receptor, IL-15, and the TGF-β type II receptor have all been reported to be up-regulated in human endometrium during the secretory phase in various cell types (5–7), further validating the approach taken herein. Down-regulation of matrix metalloproteinase-7 (matrilysin) has been demonstrated previously (22), as has the down-regulation of tenascin-C, a multifunctional extracellular matrix glycoprotein that is regulated by multiple soluble factors, integrins, and mechanical forces, and known to be highly expressed in the proliferative phase of the menstrual cycle compared with the secretory phase (23). While the data presented contribute to the molecular signature of the endometrium that defines the state of receptivity to embryonic implantation, localization of cell type expression for select genes is clearly needed and is underway in our laboratories. Also, while the validation studies presented herein support cell-specific expression for a few, selected genes and validate in a limited fashion the microarray data, they do not represent the full spectrum of cell types in the endometrium, underscoring the need for *in situ* hybridization studies and subsequent protein demonstration. In addition, endometrial proteins that require posttranslational modifications for their activity are not revealed by gene profiling techniques and require alternative methods of investigation.

The choice to compare gene expression profiles in the window of implantation to the late proliferative phase was made to focus on the comparison of genes expressed during peak exposure of the endometrium to E2 and progesterone (window of implantation) *vs.* peak E2 (late proliferative phase). While many of the genes are known to be regulated by progesterone directly, *e.g.* glycodelin, IGFBP-1 and tissue inhibitor of metalloproteinase-3, regulation of others during the window of implantation likely derive from progesterone-induced (or suppressed) paracrine products that are mediators of the progesterone response. The finding of unique gene families, not previously known to be expressed in human endometrium or to be regulated by progesterone provides new avenues of investigation, which is an advantage of this unbiased technique and which transcends the goals of the current investigation to other fields. In addition, why more genes are down-regulated than up-regulated is an interesting observation, and we speculate that because during the implantation window, compared with the late proliferative phase, ERs are down-regulated in endometrial epithelial cells, genes that were up-regulated by E2 in the proliferative phase are now down-regulated due to the loss of E2 action. In addition, direct down-regulation by progesterone and multiple progestomedins during the implantation window may result in more down-regulated genes compared with up-regulated genes.

Up-regulated genes

Several genes and gene families that are up-regulated in the window of implantation warrant further discussion. ApoE is the most abundantly (100-fold) up-regulated gene in the window of implantation. It binds hydrophobic molecules and is important in cholesterol transport and trafficking (26). Local production of ApoE in steroidogenic tissues, particularly the ovary, has been reported, through mechanisms involving the low density lipoprotein receptor family (27). The high expression of ApoE (and ApoD) in the endometrium suggests an important role for it in cholesterol transport in this tissue, perhaps for steroid hormone biosynthesis or steroid hormone binding.

PLA2, the second most abundantly (18-fold) up-regulated

gene in the window of implantation, belongs to a family of enzymes (secreted, membrane bound, Ca^{2+} dependent) that catalyze the hydrolysis of membrane glycerophopholipids, resulting in the release and metabolism of arachadonic acid and generation of lipid signals: platelet-activating factor, lysophosphatidic acid, PG and leukotrienes (see Ref. 28 for review). The importance of PG action during the window of implantation is underscored by the concomitant (4-fold) up-regulation of the PGE2 receptor (Table 2). PLA2 is also involved in calcium influx into nonexcitable cells (29) and in the modulation of TNF-α and IL-1β-induced NF-κB activation (30), which is important in endometrial function (31). PGs are important for vascular permeability and endometrial decidualization (see Refs. 5 and 6 for reviews). Further definition of mechanisms underlying PLA2 and PG actions during the implantation window are major challenges for further investigation.

Of interest in the implantation window is the finding of expression and marked (12-fold) up-regulation of mammaglobin, classically known as a breast-specific uteroglobin family member (32). Mammaglobin B has been identified in rat uterus (33), and uteroglobin has been well characterized in rabbit and human endometrium and is known to be regulated by progesterone (reviewed in Ref. 34). Several properties of members of the uteroglobin family have been identified, including serving as a substrate for transglutaminases and acting as an antiinflammatory agent by inhibiting PLA2 (35). It is striking that both PLA2 and mammaglobin, a putative inhibitor of this enzyme, are so markedly up-regulated during the window of implantation in human endometrium. Of course, mammaglobin, a member of the secretory lipophilin family of proteins that are prominent in glandular secretions and hormone-responsive tissues (36), may have other functions, yet to be identified in the implantation window in human endometrium.

Another inhibitor of PLA2 is annexin IV, a member of the annexins or lipocortin family of calcium-dependent phospholipid-binding proteins (37). Annexin IV, also known as placental anticoagulant protein II, has anticoagulant activity, as well. The up-regulation of annexin IV, annexin II, and lipocortin-2 in the implantation window underscores the importance of regulating PLA2 activity and maintaining an environment for anticoagulation during implantation.

Pregnancy-associated endometrial α_2 globulin, also known as glycodelin, is an endometrial epithelial-specific protein and is up-regulated in human endometrium during the periimplantation period and in the late secretory phase (15). Data in this study support these well established observations. Glycodelin belongs to a family of lipocalins that participate in regulation of the immune response that also includes α_1 microglobulin and the γ chain of complement factor 8 (38). The lipocalins typically bind small hydrophobic molecules, like retinol and RA, although glycodelin does not bind these molecules (15).

The finding of members of the Wnt family is surprising. Of particular interest is the marked up-regulation of Dickkopf-1 (an inhibitor of Wnt signaling) and of LRP (low density lipoprotein receptor-like protein), and the down-regulation of frizzled related protein (FrpHE), also an inhibitor of Wnt signaling (39). Dickkopf-1 inhibits Wnt signaling by binding LRP5/6 (40), and FrHPE inhibits Wnt action by competitive binding to Wnt ligand(s). Wnt7A (−/−) null mice are infertile and have complete absence of uterine glands and a reduction in mesenchymally derived uterine stroma (41). We have localized Wnt7A exclusively to epithelium and frizzled receptor to epithelium and stroma in human endometrium (Tulac, S., L. C. Kao, and L. C. Giudice, manuscript in preparation). It is possible that the Wnt family may play a role in epithelial-embryo and/or epithelial-stromal interactions and thus in uterine receptivity. The role of the Wnt family in human endometrium and implantation is currently under investigation in our laboratories.

Proteoglycans, extracellular matrix (ECM) proteins, and cell surface glycoproteins function in epithelial-embryonic interactions. The ECM is also a reserve of many peptide growth factors and angiogenesis modulators, underscoring the importance of its regulation in events occurring in the endometrium. Of particular interest is the marked (16-fold) up-regulation of glucyronyltransferase I, a central enzyme in heparan/chondroitin sulfate and other proteoglycan biosynthesis (42, 43). Also, significantly up-regulated (8-fold) during the window of implantation is the ECM protein, osteopontin, known to be progesterone-regulated and up-regulated in the midsecretory phase in human endometrium (17). Osteopontin has been postulated to bridge embryo-epithelial attachment. Also, we found up-regulation of laminin B, and proline-rich protein, an ECM protein commonly found in intestinal epithelium (44).

A number of genes involved in immune modulation deserve special mention, although their cellular expression has not yet been determined. These include (also see Table 2): natural killer-associated transcript 2, members of the complement family (including adipsin, which is the same as complement D (45), decay-accelerating factor, and complement 1r), IL-15 (18, 19) and its receptor (20, 21), NKG5 [an NK and T-cell specific gene strongly up-regulated upon cell activation (46)], interferon γ-inducible IDO, interferon regulatory factor 5, and lymphotaxin/SCM1β (expressed in NK cells; Ref. 47). Some of these immune modulators are well characterized in human endometrium and have functions related to NK cell differentiation [*e.g.* IL-15 (18–21, 48)] and complement action (49–52), and may play key roles in immune tolerance of an implanting embryo [*e.g.* IDO may have a role in the prevention of allogeneic fetal rejection by tryptophan catabolism (53)]. The impressive regulation of immune modulators underscores the need for further investigation into this important group of gene families in the implantation process, especially in view of the controversies currently surrounding immune-based therapies for some infertility patients.

Of interest are transport proteins for water and ions that are common to kidney and gastrointestinal epithelium (Table 2). It is reasonable that mechanisms are conserved for water and ion transport—whether they be in the gut, the kidney, or the endometrium. Finding expression of these transporters and their marked up-regulation during the implantation window likely reflects the importance of water (and ion) shifts that take place across the epithelium and the importance of endometrial stromal edema that occur during the window of implantation (8, 54). The gene for the CPE-1R, for

example, was up-regulated 4-fold in the implantation window. This receptor is a tight junction protein component that forms pores for water transport in the gut (55), and in response to CPE results in massive water shifts into the intestinal lumen. It has been found to be abundantly expressed in the gastrointestinal tract and in the uterus (56). Whether this receptor is involved in water transport that occurs during the midsecretory phase, is unknown. Further, its endogenous ligand in the endometrium (and its true function) await definition. The finding of the CPE-1R and of a membrane protein potassium channel—the sulfonyl urea receptor (57), open new avenues of investigation in endometrial biology, focusing on, *e.g.* signaling from an embryo involving ion fluxes, with appropriate channels in place for such interactions.

Genes for members of the metallothionein family of proteins that are involved in detoxification and zinc binding are up-regulated 2.3- to 5.8-fold during the implantation window. In zinc deficiency and in metallothionein knockout mice, there is an alteration of Th1 and Th2 cytokines (58). Because the ratio of Th1 to Th2 is believed to be important for successful implantation in humans (see Ref. 59 for review), this gene family may provide a mechanism to regulate the immune balance for embryonic tolerance during implantation. Also of note is the up-regulation of genes governing intracellular Ca^{2+} signaling and Ca^{2+} homeostasis [annexin II (60)], underscoring the importance of Ca^{2+} in the implantation window (5, 6). Genes whose products are involved in G protein-coupled receptor desensitization, *e.g.* β-arrestin, β-adaptin, and clathrin (61), are up-regulated, supporting attenuated G protein-coupled receptor signaling in the implantation window. Cyclophilins are up-regulated during the implantation window, and since they bind with Hsp 90 to inactivate steroid hormone receptors (62), they may contribute to the observed down-regulation of the estrogen receptor in endometrial epithelium between cycle d 20–24 (63).

Up-regulation of the $GABA_A$ receptor π subunit and documentation of its epithelial origin in human endometrium during the implantation window (Table 2 and Figs. 3–5) raise the issue of the role of neurotransmitters and of progesterone metabolism in this tissue. The $GABA_A$ receptor has been reported in rat uterus (64) and is important in the binding of reduced metabolites of progesterone in this tissue. Whether this is important in human endometrium remains to be determined. The observations of up-regulation of monoamine oxidase (important in norepinephrine synthesis) and diamine oxidase, well recognized in human endometrium (28, 65–67), underscore the need to reach beyond conventional thinking about mechanisms operating in endometrial development and perhaps embryo-endometrial interactions. Cellular localization of these genes and their ligands (*e.g.* for the $GABA_A$ receptor π subunit) clearly need further definition. However, these findings and our recent findings of neuromodulators and their receptors in decidualized human endometrial stromal cells (68) underscore further consideration of neurotransmitter receptors participating in signals from an implanting embryo during nidation into the endometrium. Some of these receptors may have other functions, as has been shown for dopamine and morphine, stimulating nitric oxide production by human endometrial glandular epithelial cells in culture (69).

Down-regulated genes

ITF, a member of a family of secreted proteins that are expressed in the epithelial mucosal layer of the small intestine and colon, is the most markedly (50-fold) down-regulated gene in human endometrium during the window of implantation (Table 3). Studies with ITF null (−/−) mice support a central role for ITF in maintenance and repair of the intestinal mucosa (70). Whether an analogous role is present in endometrium warrants further investigation.

Other markedly down-regulated genes include some that are involved in G protein-coupled receptor signaling: G protein-coupled receptor kinase (23-fold reduction); HM145 (a G protein-coupled receptor for leukocyte chemoattractants (71), 11-fold reduction), and the G protein γ 11 subunit (4.7-fold reduction). Down-regulation of this signaling pathway raises questions of identifying ligand/receptor complexes using this pathway and why their down-regulation is important during the implantation window. This is notable, especially because this apparently is coordinated with up-regulation of G protein receptor inhibitory factors (*vide supra*).

Several peptidases were also found to be down-regulated during the implantation window (Table 3), including, matrilysin (24-fold), dipeptidyl amino peptidase (10-fold), carboxypeptidase E (9.7-fold), and cathepsin F (3-fold), suggesting that proteolysis is minimized during this part of the menstrual cycle. As has been shown for MMPs (reviewed in Ref. 72), inhibition of MMPs may be critical to the maintenance of endometrial tissue architecture, very important during the implantation window. Dipeptidyl amino peptidase is a brush-border membrane-bound enzyme in the kidney proximal tubule and has been implicated in regulation of the biologic activity of multiple hormones and chemokines. Carboxypeptidase E is a regulated secretory pathway sorting receptor which regulates hormone, neuropeptide, and granin secretion in a calcium-dependent manner, important in prohormone processing, including proinsulin and neurotransmitters (73). Down-regulation of these enzymes may be part of a local control mechanism for regulating peptide activity within the endometrium.

Several other genes were also markedly down-regulated, including MSX-2 (a homeobox gene, 9-fold), genes involved in calcium and ion transport, and calcineurin, a protein involved in Ca^{2+} signaling (7.5-fold). Calcineurin is important in the activation of T cells (74). Antigen recognition by T cell receptors initiates signal transduction resulting in activation of tyrosine kinases, followed by PLC phosphorylation. This causes phosphatidyl inositol phosphate phosphorylation to phosphatidyl inositol phosphate 3, elevating intracellular Ca^{2+} and 1,2-diacylglycerol. Through the increased level of free Ca^{2+}, a complex of calmodulin and calcineurin is formed. Calcineurin is a Ca-/calmodulin-dependent ser-thr phosphatase and dephosphorylates the nuclear factor of activating T cells (NF-AT). In the dephosphorylated form, NF-AT crosses into to the nucleus to function as a transcription activator for IL-2 expression. Down-regulation of calcineurin in endometrium would suggest limitation of NF-AT activation in this tissue. In addition, several transcription factors are down-regulated. Of note is the erg protein, a

member of the ets family, important in regulation of extracellular matrix (75). With the dynamic changes in the extracellular matrix that occur in endometrium during the window of implantation and during early pregnancy, ets family members may play an important role.

Semaphorin E and semaphorin III family homologs were found to be down-regulated (6- and 3-fold, respectively) during the implantation window. Semaphorin III, interacting with its receptor, can result in either chemorepulsion or chemoattraction of developing axons, depending on levels of cellular cGMP (76). Finding the semaphorins and neurotransmitter receptors, as described above, suggests that perhaps we should be looking at other systems, such as ion signaling and chemoattractants/repellants for mechanisms to guide an embryo within the endometrium, analogous to neurotransmitter and semaphorin action in the neuronal system.

Of note also is down-regulation during the implantation window of the vasoactive factor, endothelin 3, and the angiogenic factor, VEGF (Table 3). Minimizing vasoconstriction is teleologically sound during a period that requires enhanced blood flow to the conceptus. Why VEGF is down-regulated is not clear, and conflicting reports have been reported on cyclic variations of this angiogenic factor in human endometrium (see Ref. 77 for review). However, Semaphorin III and VEGF compete for the same receptor, neuropilin-1 and this interaction results in inhibition of aortic endothelial cell migration (78). Interactions between the angiogenic system and the neuronal guidance system suggest potential new mechanisms for regulation of cellular motility in the endometrium during the implantation window, if indeed this extrapolation can be made.

The current study opens new conceptual approaches to mechanisms involved in the steroid hormone-dependent differentiation of the endometrium in the secretory phase of the menstrual cycle and mechanisms underlying endometrial development optimal for embryonic implantation and for embryo-endometrial interactions. While much validation of cell-specific expression of various genes described herein remains to be determined, the classes of molecules described herein support the following model. As an embryo attaches to the endometrial epithelium, bridging to cell surface carbohydrates and proteins is important, and mechanisms must be in place in the maternal endometrium for synthesis of these molecules. Once attachment occurs, a set of mechanisms is likely to be put into motion for endometrial-stromal interactions, intrusion of the trophoblast into the stromal compartment, and guidance of the trophoblast to the maternal spiral arteries, while maintaining integrity of the ECM and anticoagulation. It is envisioned that embryo-endometrial interactions involve ion transport and signaling through paracrine mechanisms via growth factors and cytokine families, as well as adaptation of guidance mechanisms similar to those used in angiogenesis and neuronal migration to target the trophoblast through the stroma to reach to the maternal vasculature. The immune system must facilitate tolerance of the implanting allograph and other protective mechanisms (*e.g* antibacterial, detoxification) are likely to be important to maximize viability of the implanting conceptus. While this model is rudimentary at best, it provides a framework for the role of the genes identified in this study in these processes. It is important to note that despite the anticipated interactions between the endometrium and the conceptus, based on gene expression in the endometrium during the implantation window described herein, the microarray approach provides a static snapshot of gene expression in the endometrium in the absence of an embryo and does not reveal the dynamic dialog that occurs minute-to-minute during embryonic implantation between the endometrium and the embryo. Nonetheless, it does provide insight into the molecular pathways, molecular signals, and physiologic processing that await an embryo should nidation occur.

Validation of functions for genes in the window of implantation will derive, in the future, from animal models of homologous recombination and gene knockouts, transgenic mice, studies in nonhuman primates, and other species whose endometrium and implantation processes are similar to those in humans, and further studies in human endometrial disorders related to implantation-based infertility. We believe that the current study provides the basis for defining markers of uterine receptivity during the window of implantation in human endometrium. Recent applications of global gene profiling relevant to implantation include a study by Reece *et al.* (79) in which uterine genes and gene families were characterized in mice during implantation in a variety of pregnancy models, and by Aronow *et al.* (80) on genes involved in human trophoblast differentiation. Information from these studies and the current study in human endometrium should further advance our knowledge about implantation in humans. However, just because a gene is regulated in the implantation window does not necessarily mean that it is important in the implantation process, and differences between coincidence and function await further validation.

The data presented herein offer the opportunity to develop an endometrial database of genes expressed during the window of implantation. The current study validates using microarray technology to investigate global changes in gene expression in human endometrium and can be extrapolated to defining the genetic profiles during the proliferative phase, periovulatory phase, and during the late secretory phase in the absence of implantation and in preparation for menstrual desquamation. Such an approach sets the stage for further investigation of global changes in gene expression in disorders of the endometrium, including implantation-related infertility (as in women with endometriosis), evaluation of the endometrium for normalcy in women with hyperandrogenic disorders, in normovulatory women in response to therapeutics in which the endometrium is targeted (or as a side effect of other therapies), as well as endometrial hyperplasia and endometrial cancers. Finally, this study sets the stage to develop a screen for candidate genes in patients with infertility and for targeted drug discovery for enhancing (or inhibiting) implantation for infertility treatment (or contraception).

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