A genomic view of estrogen actions in human breast cancer cells by expression profiling of the hormone-responsive transcriptome

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Abstract

Estrogen controls key cellular functions of responsive cells including the ability to survive, replicate, communicate and adapt to the extracellular milieu. Changes in the expression of 8400 genes were monitored here by cDNA microarray analysis during the first 32 h of human breast cancer (BC) ZR-75.1 cell stimulation with a mitogenic dose of 17 β -estradiol, a timing which corresponds to completion of a full mitotic cycle in hormone-stimulated cells. Hierarchical clustering of 344 genes whose expression either increases or decreases significantly in response to estrogen reveals that the gene expression program activated by the hormone in these cells shows 8 main patterns of gene activation/inhibition. This newly identified estrogen-responsive transcriptome represents more than a simple cell cycle response, as only a few affected genes belong to the transcriptional program of the cell division cycle of eukaryotes, or showed a similar expression profile in other mitogen-stimulated human cells. Indeed, based on the functions assigned to the products of the genes they control, estrogen appears to affect several key features of BC cells, including their metabolic status, proliferation, survival, differentiation and resistance to stress and chemotherapy, as well as RNA and protein synthesis, maturation and turn-over rates. Interestingly, the estrogen-responsive transcriptome does not appear randomly interspersed in the genome. In chromosome 17, for example, a site particularly rich in genes activated by the hormone, physical association of co-regulated genes in clusters is evident in several instances, suggesting the likely existence of estrogen-responsive domains in the human genome.

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Introduction

The identification of the gene expression patterns associated with cell responses to physiological stimuli, such as those represented by the female sex

hormones estrogen and progesterone, may provide the framework necessary for interpreting the biological significance of specific gene expression patterns observed in pathologies. In the case of estrogen, this is particularly true also for the

The entire data set from these experiments, including the full list of genes analyzed and hormone regulated, the fluorescence measurement data and corresponding images can be found at the Gene Expression Group web site: (<http://criseb.unina2.it/geneexpression/>), and will be deposited in the NCBI's Gene Expression Omnibus gene expression data repository (<http://www.ncbi.nlm.nih.gov/geo/>).

identification of gene sets useful to monitor the hormone signaling status in the cell and, in the case of breast cancer (BC), to subtype tumor specimens according to this functional phenotype. Indeed, hormone-responsive breast tumors are currently identified primarily by the presence of estrogen receptor- α (ER- α) and the estrogen-regulated progesterone receptors (PgRs). Characterization of the estrogen-responsive transcriptome would make it possible to go beyond individual genes or markers (in this case ER and PgR), allowing the analysis of hormone-related expression patterns across hundreds of genes (the estrogen-responsive transcriptome). This approach has the potential to lead to the identification of cancer-specific molecular 'signatures' characterizing previously undefined ER-positive tumor subtypes with distinctive biological and clinical behavior. Indeed, genome scale gene expression profiling is a promising new way to classify diseases in clinically relevant subtypes, according to multiple molecular parameters of the pathologic tissue. This can best be achieved when gene activity can be related to specific genetic or biological features of a given cell type (Alizadeh *et al.* 2000, Perou *et al.* 2000, Hedenfalk *et al.* 2001). Furthermore, identification of genes and whole genetic pathways responsive to estrogen can provide the necessary framework to understand the complex effects of these hormones and their receptors in target cells. Contrary to most other tumor promoters, estrogens do not act via 'classical' mitogenic pathways, but recognize intracellular receptors (ER- α and - β) which belong to the nuclear receptor family of transcription factors. In addition, direct and indirect effects of estrogen on extra-nuclear mitogenic signaling cascades have been proposed to contribute to the growth-promoting actions of these steroids (Moggs & Orphanides 2001), pointing to the fact that estrogen's actions in target cells might involve both 'genomic' and 'non-genomic' signaling. An open question concerning the biology and pharmacology of ERs concerns the extent of integration between the genomic and extra-genomic pathways, to permit, for example, promotion of cell proliferation by these steroids. A unifying way to clarify the issues listed above can be provided by new sets of estrogen target genes, each provided with information concerning its regulation by the hormone and

used to identify hormone-regulated processes in target cells.

Hormone-responsive BC cell lines represent one of the best experimental models available to elucidate estrogen's actions in target tissues. Their systematic study has so far led to the discovery of several hormone-responsive pathways, which have then been exploited to improve clinical management of breast and other hormone-responsive cancers. These include, for example, identification of PgR as a downstream target of estrogen as well as an in depth investigation of the mechanism of action of the ERs, antiestrogens and selective estrogen receptor modulators (SERMs). Thus, it can be reasonably assumed that genome-scale gene expression analyses of hormone-responsive BC cell response to estrogen will lead to a better understanding of the hormonal influences in this cell type not only *in vitro* but also *in vivo*. Indeed, Bouras *et al.* (2002), by analyzing the response of MCF-7 cells to estrogen by gene expression profiling with DNA microarrays, identified the *Stanniocalcin 2* gene as a hormone-responsive gene co-expressed with ER- α in BC lesions. Using a reverse approach, Finlin *et al.* (2001) found that expression of a novel gene, identified by microarray analysis in ER-positive breast tumors showing a slow tumor cell proliferation rate and a more favorable prognosis and named *RERG* (*Ras-related, estrogen-regulated and growth-inhibitory*), can be enhanced by estrogen in MCF-7 cells. Forced expression of this gene reduces the cell proliferation rate *in vitro* and inhibits their tumorigenic phenotype in nude mice (Finlin *et al.* 2001). Also, Inoue *et al.* (2002) and Soulez and Parker (2001) identified new estrogen target genes in BC cells which appear to be unaffected by antiestrogen treatment.

To provide a more detailed and comprehensive description of the gene expression patterns regulated by estrogen in BC cells, comparative cDNA microarray mRNA analysis was carried out before and after stimulation of hormone-responsive ZR-75.1 cells with a physiological dose of 17 β -estradiol (E2). Gene expression changes were monitored in hormone starved cells at 11 consecutive times ranging from 1 to 32 h into hormonal stimulation, in order to analyze gene expression changes in the cells during a whole mitotic cycle (Cicatiello *et al.* 2000). Validation of the microarray results was accomplished

systematically, by both experimental and *in silico* testing. The set of 344 hormone-responsive genes hereby identified depict a comprehensive genome-wide view of the genetic programs regulated by estrogen in this hormone-responsive cell type.

Materials and methods

Cell culture and RNA purification

Human breast cancer ZR-75.1 and MCF-7 cells were propagated in DMEM medium supplemented with 5% FBS and antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin and 250 ng/ml amphotericin-B). Before use, cells were plated at 20–40% confluence and maintained for 4 days in steroid-free medium (phenol red-free DMEM medium, with 5% charcoal-stripped FBS and antibiotics), before stimulation with 5×10^{-8} M E2. In the case of MCF-7 cells, hormone-deprived cultures were also maintained for 42 h in steroid-free medium containing 20 µM simvastatin, to achieve effective G₀–G₁ synchronization of the cultures and optimal response of cell cycle genes to the hormone (Altucci *et al.* 1996, Bonapace *et al.* 1996). At the indicated times, total cellular RNA was extracted from control (0, –E2) or E2-stimulated (+E2) cultures as described earlier (Bonapace *et al.* 1996). Six culture plates (150 mm) were used for each time-point, and the experiment was repeated three times within two weeks. Poly(A)⁺ RNA was isolated from pools of 2 mg total RNA, by batch-wise magnetic separation with Dynabeads (Oligo (dT)₂₅; Dynal, Oslo, Norway) according to the manufacturer's instructions, resuspended in diethyl pyrocarbonate-treated water, quantitated and diluted to a final concentration of 50 ng/µl. Before further use, total and poly(A)⁺ RNAs were tested by agarose gel electrophoresis. The response to estrogen of the cell cultures to undergo microarray testing was controlled by cytofluorimetric cell cycle kinetics analysis, performed as described earlier (Bonapace *et al.* 1996), as well as by analysis of cell cycle and estrogen-responsive gene mRNAs (cyclins, pS2 and *c-fos*) by Northern blotting, as described earlier (Altucci *et al.* 1996 and data not shown), or by multi-probe RNase protection assays (RPAs).

cDNA microarray probing

cDNA microarray mRNA analysis was carried out with UniGEM Human V 2.0 and Human

UniGene 1 (for ZR-75.1 cell mRNA analyses) and UniGEM Human V (for MCF-7 cell mRNA analyses) glass arrays (Incyte Genomics, St Louis, MO, USA) according to standard procedures (Schena *et al.* 1995, Iyer *et al.* 1999). Two hundred nanograms of each poly(A)⁺ RNA derived from estrogen-stimulated (+E2) cells were used to generate carbocyanine 5 (Cy5)-labeled cDNAs, that were mixed with common reference probes consisting of carbocyanine 3 (Cy3)-labeled cDNAs prepared from an equal amount of poly(A)⁺ RNAs purified from quiescent, hormone-deprived cultures (–E2), for competitive hybridization to a cDNA microarray grid containing 9182 human sequences of known genes and expressed sequence tags (representing 8372 unique gene/ESTs clusters), spotted in single, and 192 internal controls (for reverse transcription, absolute sensitivity and differential expression QCs: human UniGEM V 2.0 screening services; Incyte Genomics). cDNA synthesis, labeling and hybridization were generally repeated three times for each time-point (in 3 cases four times), in separate experiments. For at least one sample/set (time-point), the fluorescent labeling order was interchanged (control RNAs were labeled with Cy5 and sample RNAs with Cy3).

Statistical analysis of the microarray data

Data considered for analysis were, for each element in the array, absolute Cy3 or Cy5 fluorescence reading, signal vs background ratio (S/B) and 'balanced differential expression' value. This was calculated as the ratio between the fluorescence signal in the sample channel (+E2 probe) vs that in the reference channel (–E2 probe), after balancing (normalization) for differences in sample labeling efficiency and for laser performance which was based on the average hybridization signal of the whole array and on a set of housekeeping genes and other control elements present in multiple copies at different locations in the array grid. The data relative to elements of the array which did not pass the Incyte PCR quality controls, showing multiple bands or no amplification of the spotted DNA samples, as well as arrays that did not pass the Incyte microarray hybridization QCs, were discarded.

Identification of informative (expressed reproducibly to detectable levels) and hormone-regulated genes was carried out, in the case of the kinetics

measurements, according to three independent selection procedures, applied in parallel by different operators to help pinpoint possible methodological biases. With the first, a normalization step procedure was applied to correct for the possible differences in cDNA labeling, hybridization or detection efficiency which are observed during multiple analyses of the same mRNA sample by this technology, as described by Dudoit *et al.* (2001). In each array, all data showing a Cy3+Cy5 value lower than 500 (equal to 1/5 of the average Cy3+Cy5 value in all arrays) and a signal over background value (S/B) lower than 2 were discarded. This was necessary due to the lack of linear correlation between S/B vs Cy3+Cy5 observed for the vast majority of the analyzed data. In this way, 6080 genes were selected as informative, as they gave rise to reproducible hybridization signals. Subsequently, all the data where the ratio between the test and reference sample values diverged more than 15% within replicates were also discarded. For the remaining coherent replicates, the ratios between experimental time-points and their reference sample were calculated and averaged. All data showing a differential expression of at least ± 1.5 were first selected with this approach, as validation studies for the same microarray platform used for this study indicate that detection limits for reliable differential expression measurements are ± 1.4 -fold (Yue *et al.* 2001).

According to a second selection scheme, each array was first statistically evaluated for its performance. The fluorescence values from the -E2 sample channel of each element were compared between all arrays, and used for calculation of an average (mean) fluorescence value and the corresponding standard deviation (S.D.). The absolute deviation of each element's fluorescence value (F) from the corresponding mean (Fm) was then computed relative to the S.D., e.g. $\text{abs}(F-F_m)/\text{S.D.}$. The result represents the difference between the signal detected by each given element in the array, and the corresponding mean was used as the discriminant to discard unreliable data points, which were found to include all elements that did not pass Incyte QC's or showed a low S/B ratio. All data showing a Cy3+Cy5 fluorescence reading lower than 1/5 of the average Cy3+Cy5 value in each array were discarded. The remaining data sets were then used to identify differentially

expressed genes, which were selected when their balanced differential expression value showed consistent variations in at least three consecutive time-points. Genes showing an average balanced differential expression of at least ± 1.5 in at least two of three consecutive time-points were then selected as 'positive'.

The data relative to microarrays where the crude fluorescent values relative to the reference sample channel showed the highest S.D. (accounting for about one third of the total) were excluded from the third independent selection approach. All data were merged in this case in a single MS Excel file and the average balanced differential expression was calculated for each gene within 'time windows' including arrays covering three consecutive experimental time-points. Analysis was carried out sequentially over consecutive time windows overlapping one another by one time-point (e.g. window A: 1 h+2h+4h; window B: 4 h+6h+8h; etc.). In each case, genes showing an average balanced differential expression of at least ± 1.4 ($> 2 \times$ the mean balanced differential expression's S.D.)/time window were marked as positive. Once this section was completed, all results were merged in a single file, and genes scored positive when showing gradual and consistent changes over time, above the statistically set threshold of ± 1.5 in at least two time-points of the curve.

The results of the three selection procedures described above were then compared, and 372 genes, common to all three data sets, were considered positive and finally selected. This procedure led us to discard 120 genes that were not selected by at least one of the procedures described above. These genes were all manually re-controlled and found to show inconsistencies of differential expression value among replicates, or to fail the criterion of consistent variation over multiple consecutive time-points. Furthermore, 27 of the 372 positive genes were present in two or more copies in the array, generally under a different gene name or GenBank Accession number, and were identified as belonging to the same UniGene cluster by automatic database searches and BLAST sequence alignments. Interestingly, balanced differential expression data were found to be consistent between replicates, providing a further demonstration in support of the performance of the procedures adopted for data analysis and of the selection criteria set to mark differentially expressed

genes. In conclusion, 345 genes were identified in this way as estrogen regulated, one of which was later discarded during experimental validation of the microarray results (see below).

A statistical analysis of the gene expression changes defined in this way was further carried out by the method proposed by Tusher *et al.* (2001; significance analysis of microarrays – SAM), which provides a way to determine the significance of the gene expression changes detected by cDNA microarrays. SAM identifies genes showing statistically significant changes in expression between mRNA samples by assimilating a set of gene-specific *t*-tests and assigning a score to each gene on the basis of its changes in expression relative to the standard deviation for repeated measurements. Furthermore, it uses permutations of replicate measurements to estimate the ‘false discovery rate’ (FDR), e.g. the percentage of genes identified by chance. This method was applied to the sets of data generated as described above, by implementing the relative software (<http://www.stat-class.stanford.edu/SAM/SAMServlet>) following the recommendations provided by the A.s (Tusher *et al.* 2001). To enhance the robustness of the analysis, we selected to apply the SAM analysis also to samples grouped in 8 time-windows, each encompassing 4 h and overlapping with the neighboring windows by one time-point (e.g. 1~4 h, 4~8 h, 8~12 h, etc.). We found in this way that the number of significant genes decreased only slightly, when compared with single-point analyses, while the FDR value decreased significantly (to 0.2 to 0.5 in most cases). The data relative to 312 of the 344 genes selected initially (91%) were significant, according to this additional statistical analysis, in at least 6 of the 8 data sets considered. To account for the longitudinal nature of the experiment (estrogen stimulation of the cells was carried out for 32 h and measurements were performed at 11 time-points, grouped in 8 consecutive time-windows), the SAM differential expression data (fold-change) were compared with the raw differential expression data (average of multiple microarray measurements, as described above). This was performed by calculating, in each case, the corresponding Pearson’s correlation coefficient. All genes showed very similar differential expression profiles in the two data sets resulting from the two distinct analytical proce-

dures, providing a robust confirmation of the gene expression changes reported.

Differentially expressed genes were clustered according to their expression profiles by GeneCluster (Tamayo *et al.* 1999). The expression profiles obtained with these two approaches were compared and each gene cluster was visually inspected, to give rise to the final set of coherent clusters, with the aid of Cluster and TreeView tools (Eisen *et al.* 1998).

The data sets relative to these experiments, including the list of all genes analyzed and, separately, of those hormone regulated, the fluorescence measurements data and the corresponding scanning images can be viewed at the Gene Expression Group web site (<http://crisceb.unina2.it/geneexpression/>) and will be made available also through the NCBI’s Gene Expression Omnibus gene expression data repository (<http://www.ncbi.nlm.nih.gov/geo/>).

Real-time rtPCR

Real time, quantitative rtPCR analyses (Heid *et al.* 1994) were performed with an ABI Prism 7700 Sequence Detector and reagents provided by the same manufacturer (PE Applied Biosystems, Milan, Italy). cDNA sequences present in the arrays were obtained from Incyte Genomics and checked by comparison with the corresponding GenBank sequence files. Specific primers and probes for the mRNAs to be analyzed were designed using the dedicated software Primer Express. Amplicons of 64–101 bps were selected for testing. Melting temperatures of the primers were 59–61 °C and melting temperatures of the probes were 69–71 °C. rtPCR reactions were performed using amounts of cDNA corresponding to 0.025–0.125 µg DNase-treated total RNA, as recommended by the manufacturer. Each 25 µl of reaction mix contained 2 × Universal Master Mix, 100 nM TaqMan probe, 20 × PDAR 18S (pre-developed TaqMan assay reagent), 300 nM of each primer (except for AF022224, L16785, AF016050, U75285, D38553, AI568937 and AI741331: 300/600 nM, AA865269: 600/300 nM and U22376: 600/600 nM) (all these reagents were from PE Biosystems, Milan, Italy). After 2 min at 50 °C, to allow AmpErase uracil-N-glycosylase (UNG) to destroy potential contaminating PCR products, and 10 min at 95 °C to denature UNG

and activate Taq polymerase, amplification was then carried out for 40 cycles, each of 15 s at 95 °C and 1 min at 60 °C. All amplified samples were also resolved in a 2.5% agarose gel to verify the expected amplicon size. A dissociation curve was generated at the end of the PCR cycle to verify that a single product was amplified.

The sequences of the PCR primer pairs and of the fluorogenic probe (5' to 3'), respectively, that were used for each gene are as follows: AA194161: TGAGGAGCTCATGGTAGCCTGGGTTG, GGTCACAGATTCACCAAAGCTGA, and 6FAM-AGACACCGTGCTCCAGAGTAGGT-TAMRA; AA865269: CCGCCTTTTCTGGGTGGCGC, AGGAAACAGGGGAAACGTCTTG, and 6FAM-GTGGAGAGAGCCGCACACC-TAMRA; AF016050: TCTGGGTCCCACGTCGGCACA, GAACCTCTGCCCCACTGCATGAC, and 6FAM-TCCCTCTGGCTTCTGGTAGCG-TAMRA; AF022224: TTTACCTGATTTTCTTCAGGGCT-GCTGGG, CACCAGCTCTGCCGTCTCTG, and 6FAM-AAATTGGCAAATGGCCAGTTG-TAMRA; AF030424: CCAGACAAAACCCGGCCACGTGTA, TTGCGACCGTAGGCTACATGA, and 6FAM-ATGGAGTCAAAATCAGCATCTGACT-TAMRA; AF050110: TAACCACCA-GACCTGCCCAATGAAAGC, AGTGTGATTGCTCATACAGCTGATG, and 6FAM-AAGAATTGTTCTGATAGTTGAGGATGCT-TAMRA; AF068235: AAGGCAGCGCTTCCCGCGAA, GGCCTATCTCCCTCAGAACGG, and 6FAM-TAGTAGGCGCTCCTTTCCGG-TAMRA; AF109161: CCCTGCTGCAGGCCACCA-GATG, CCACTACATGCCGGATTTGC, and 6FAM-TCGGAAGTGCTGGTTTGTCC-TAMRA; AI310309: CTTCCAGACGCCATTTGGATTGGTT, AGACCTGTTTTTCTTGGCACA, and 6FAM-TCAGGCCAAGAAACGTCTTCTAAA-TAMRA; AI348071: CATCCGCTGACATCGCCACCG, TTCCCCGAACGCAGCTAAC, and 6FAM-CGCTAGATCCCTCCAAAGCAG-TAMRA; AI568937: CCACGCATCCGAGGTTTGGA, TAGCAGGAATGCTACAGCCAT-ATGAT, and 6FAM-GTAACTACCATGGCTCCCGAAGTTT-TAMRA; AI741331: ACTCAC-CCAAGGCCCCAGGGA, CTGGGCAAGGAGGAGCCTTAG, and 6FAM-TGCTCCCAA-CCCATTCATACTACA-TAMRA; D38553: CGCCCTGAAGCTCCGTGCTCA, TCCATCATAGTCTGGGTGCCA, and 6FAM-CTGCCCTCCCC-TCTCCTAGAT-TAMRA; L16785: TGGCCAT-

GAAGTTCCTCCGGGC, CGCTTCGAGCAG AAGGGATT, and 6FAM-AATGGTCCGTCTTTCAGGTCAA-TAMRA; M31732: TCTTGCTAGAGCGCGGTGCCGAC, AGTGAACACCGAGTGCCAAGAA, and 6FAM-CGCTCTTAATGTCCACTGCGTC-TAMRA; U22376: ACAGCTTCAGCAAAGAGAGGGCAATGG, AACATCACACAGGCCAAAGCCC, and 6FAM-CATACGTTTGTTCACCCTTGGG-TAMRA; U22970: CGTGCCAAACACCGTGCGAATCC, TCATGATATTGACATCTGCCGAC, and 6FAM-CTGCCCCAACCAGGTGG-TAMRA; U44427: TGGAGGCGCAGGCACAAGGTTTG, TCAGCTCGAAGTCAGCCACC, and 6FAM-TGCAACGGTTCAGTCTCCAAC-TAMRA; U75285: CTTTCCACCCTCATTGCTTCTTAAACAGCTG, AAAAGATTTGAGTTGCAAAGA CACTTAG, and 6FAM-GCATATAGAGAGGTATCCGTTTACAA-TAMRA; X55150: CCCACAGCTAAGGACATTTCCAGGGTT, ATTCCTGATCGCCAACCTTG, and 6FAM-TCCCA GGACCAGGCCCTAT-TAMRA.

Threshold cycle, C_t , which correlates inversely with the target cDNA levels, was measured as the cycle number at which the reporter fluorescent emission increased above a threshold level. To obtain a quantitation of target cDNA, as relative to the zero point, C_t of each sample was subtracted from the C_{t0} , and each of them was corrected for the C_t of the 18S ribosomal RNA, used as an internal reference and co-amplified with target, as described above. The relative quantity of the template (Q) was calculated as: $Q = 2^{-(C_t - C_{tr}) - (C_{t0} - C_{t0r})}$. Two independent RNA preparations were analyzed, all samples were run in triplicate and the mean and standard deviation calculated as the square root of: $Var(Q) = e^{-2\mu \ln 2 + 2\sigma^2 (\ln 2)^2} [e^{\sigma^2 (\ln 2)^2} - 1]$ where μ = the mean of $(C_t - C_{tr}) - (C_{t0} - C_{t0r})$ and σ its standard deviation. To compare measurements from replicate determinations, data from one experimental series were normalized by correcting for the mean ratio between series, and the data were finally averaged.

Multi-probe RNase protection assays (RPAs)

Human Apoptosis 2c and 5, and Human Cyclin 1 template sets (Pharmingen, San Diego, CA, USA) were used according to the manufacturer's instructions. Probes were labeled with α - ^{32}P uridine

triphosphate (NEN Italia, Milan, Italy) and the ribonuclease (RNase) protection assay was performed on total RNA (4 µg) with 6 to 8×10^5 d.p.m. of labeled probe mix. After RNase digestion, the protected probes were resolved on urea-5% polyacrylamide-bis-acrylamide standard sequencing gels. Dried gels were placed on Hyperfilm-MP autoradiographic films (Amersham Italia) in a cassette with an intensifying screen. Exposure time was between 12 and 48 h, depending upon the intensity of the individual signals to be visualized. Band quantitation was carried out by densitometric scanning of the autoradiograms.

***In silico* data analyses**

Each gene under study was assigned to its actual UniGene cluster (<http://www.ncbi.nlm.nih.gov/UniGene/>) and databases were searched by UniGene cluster and GenBank Accession numbers. A functional category was assigned to each gene according to Gene Ontology (<http://www.godatabase.org/cgi-bin/go.cgi>), from LocusLink (<http://www.ncbi.nlm.nih.gov/LocusLink/>), GeneCards (<http://bioinfo.weizmann.ac.il/cards/>) or GenAtlas (<http://bisance.citi2.fr/GENATLAS/>) databases of human genes. SAGE libraries representing mRNA tags expressed in estrogen deprived or stimulated ZR-75.1 (Seth *et al.* 2002) and MCF-7 (Charpentier *et al.* 2000) cells were queried with Xprofiler and Tag-to-Gene (<http://www.ncbi.nlm.nih.gov/SAGE/>). Alignment of the estrogen-responsive transcriptome identified here in ZR-75.1 cells with those identified previously in MCF-7 cells (Finlin *et al.* 2001, Bouras *et al.* 2002, Inoue *et al.* 2002, Lobenhofer *et al.* 2002, Hodges *et al.* 2003) was carried out by matching datasets by UniGene cluster number. Comparisons between estrogen-regulated genes in BC cells and serum-regulated genes in human fibroblasts (Iyer *et al.* 1999) or genes periodically expressed during the cell cycle in human cells (Whitfield *et al.* 2002) were carried out by matching datasets by UniGene cluster number. Where necessary, they were also performed according to gene description or GenBank Accession number. A cytogenetic location was assigned to each gene according to LocusLink, GeneCards and/or Ensembl (http://www.ensembl.org/Homo_sapiens/) databases. Estrogen-responsive chromosomal domains were investigated on the UC Santa Cruz 'Golden

Path' human genome browser (<http://genome.ucsc.edu/cgi-bin/hgGateway?org=Human>).

Results and discussion

cDNA microarray analysis of gene expression changes in estrogen-stimulated ZR-75.1 cells

ZR-75.1 cells express ER- α and show well characterized responses to estrogen and anti-estrogen both *in vitro* and *in vivo* (Cicatiello *et al.* 2000, Couillard *et al.* 2000), representing a reliable cellular model to study estrogen actions in target cells. Hormone deprivation of ZR-75.1 cells induces G₁ arrest, as shown in Fig. 1a by the low number of S and G₂/M cells in the culture (10.4 and 7.7% respectively), that results in growth arrest (Cicatiello *et al.* 2000 and data not shown). This quiescence status can be readily overrun by cell exposure to physiological concentrations of estrogen, that is followed by resumption of cell cycle progression (52.7% cells in S phase after 28 h of stimulation; Fig. 1a), and activation of the cyclins-CDKs (cyclin dependent kinases) cell cycle control pathway. This last effect was highlighted by the timed accumulation of G₁ cyclin D1 and D3 mRNAs during the first 1 to 12 h of stimulation, and of the S and G₂/M cyclin A and B mRNAs after 16 to 28 h (Fig. 1b,c).

Gene expression monitoring was carried out in hormone starved, quiescent ZR-75.1 cells before and at 11 time-points after stimulation with a mitogenic dose of E2 by cDNA microarray analysis. Cells were maintained under the same conditions used for the tests reported in Fig. 1. This experimental setting was selected to provide a comprehensive kinetic assessment of the gene responses to the hormone during a mitotic cycle (see Fig. 1b,c). The microarray platform used for this study included 9182 cDNA elements, representing 8372 randomly selected unique gene/ESTs clusters. cDNA was synthesized from mRNA extracted from cells stimulated with the hormone for the indicated time, labeled with the fluorescent Cy5 dye, mixed with an equal amount of a common reference target consisting of Cy3-labeled cDNA extracted from hormone deprived, quiescent cultures (time 0) and hybridized to a glass array as described in Materials and methods. Three to four independent hybridization reactions were carried out for each sample pair by independent cDNA synthesis, labeling and hybridization to different

microarrays. Furthermore, in at least one of these replicates the fluorescent labels were exchanged between the two cDNAs analyzed in parallel, to provide a further control. Six thousand and eighty gene transcripts were clearly detectable under the experimental conditions employed, and were further analyzed to identify estrogen target genes of this cell line. Based on statistical evaluation of the results, a gene scored positive (hormone regulated) when it showed consistent changes in differential expression (+E2/−E2) over multiple, sequential time-points and reached a threshold value of ± 1.5 within a time-window comprising two to three sequential measurements. This cutoff value was selected according to statistical parameters (see Materials and methods). In this way, 344 genes showing significant changes in activity in estrogen treated vs untreated cells were identified (Table 1).

As a first step to read the information on estrogen signaling and actions provided by the gene expression changes measured here, we used hierarchical clustering to organize the estrogen-regulated genes according to similarities in their respective inhibition or activation kinetics. The rationale for this analysis resides in the consideration that the function of a gene and its expression pattern are tightly connected, and that genes linked by coherent expression profiles are likely to respond in a comparable fashion and according to similar mechanisms to information concerning the cell environment, state or identity. Indeed, all available information on gene regulation in higher eukaryotes suggests that common expression patterns underscore shared functions and/or regulation. The estrogen-responsive genes were found to fall within eight clusters (Fig. 2a), which summarize the main kinetic patterns of gene expression changes detectable in estrogen-stimulated BC cells. The first three clusters (1 to 3) group all down-regulated genes, the following three (4 to 6) comprise genes

whose transient activation patterns relate kinetically to cell cycle phasing and the last two (7 and 8) include genes showing persistent activation by the hormone for up to 32 h. Interestingly, the main determinant for clustering of genes showing progressive changes in expression profiles (clusters 1–3 and 7–8) was the onset of their response to the hormone. The difference between the patterns of inhibition shown by the genes in clusters 1, 2 and 3, for example, is represented by the time-point where a progressive decrease in mRNA expression can first be detected (1 to 4 h for cluster 1 genes, 6 to 8 h for those of cluster 2 and ≥ 12 h for cluster 3). Similarly, clusters 7 and 8 are distinguishable from each other by the fact that mRNA expression levels start to increase significantly before (cluster 7) or after (cluster 8) 12 h stimulation. It is worth mentioning that the time clusters identified here correspond quite well with those recently described by Frasor *et al.* (2003) in estrogen-stimulated MCF-7 cells.

Figure 2b reports a graphical display of the results obtained and, in addition, a further subclustering of the genes belonging to each cluster, indicated by the correlation tree (Eisen *et al.* 1998) reported to the left, which points to higher similarities between expression profiles within subgroups of genes. This highlights how defined groups of genes, within each of the 8 kinetic clusters identified, are characterized by slightly different expression profiles in hormone-stimulated cells. The definition associated with each gene symbol, and more information concerning each of the 344 estrogen-responsive genes depicted in this Figure are reported in Table 1.

Microarray data validation

The differential expression threshold selected to identify estrogen-regulated genes (± 1.5 -fold

Figure 1 Mitogenic responses of ZR-75.1 cells to estrogen stimulation. (a) Cytofluorimetric cell cycle analysis of estrogen-deprived cell cultures before (−E2) and 28 h after stimulation with 5×10^{-8} M 17β -estradiol (+E2). (b) Cyclin mRNA expression was monitored by RPA (RNase protection assay) in total RNA extracted from cells in log-phase growth (C) or following estrogen deprivation, before (0) and at the indicated time-points after stimulation with 5×10^{-8} M 17β -estradiol (E2). Cyclins D1, D2 and D3 (cyl D1, cyl D2 and cyl D3) are G1-phase markers, whereas cyclins A and B (cyl A and cyl B) indicate S/G2-phase progression. L32 and GAPDH are cell cycle- and estrogen-unresponsive mRNAs, used as internal controls in each test. Arrows mark regulated genes; the gray arrow indicates that *CCND1* (cyl D1) mRNA also showed comparable changes by cDNA microarray analysis. (c) Graphic representation of data from mRNA expression monitoring by RPA. Average of results from multiple, independent measurements are reported only for regulated mRNAs and L32 control, following normalization with respect to GAPDH mRNA levels in each lane.

Table 1 Summary of the estrogen-mediated gene expression changes detected by gene expression profiling with cDNA microarrays in ZR-75.1 human breast cancer cells

Gene symbol	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
					ZR-75.1	MCF-7	
<i>FLJ14201</i>	Hs.193063	AA034414	<i>Homo sapiens</i> cDNA FLJ14201 fis, clone NT2RP3002955	1	A, C		Unnamed
<i>ESTs AA156287 MGC12335</i>	Hs.231518	AA156287	ESTs AA156287	1		C	Unnamed
	Hs.97411	AA426092	Hypothetical protein MGC12335	1			Unnamed
<i>ZNF217</i> <i>EST AA478625^f</i>	Hs.155040	AA460802	Zinc finger protein 217	1	A, C		Transcription
	Hs.433012	AA478625	ESTs, moderately similar to hypothetical protein FLJ20378 (<i>Homo sapiens</i>)	1	A		Unknown
<i>ESTs AA677934 RAB9B^f</i>	Hs.117864	AA677934	ESTs AA677934	1	A, C		Unnamed
	Hs.43005	AA699329	RAB9B, member RAS oncogene family	1			Unnamed
<i>FLJ13187</i> <i>MYO1B</i>	Hs.29724	AA713516	FLJ13187 Phafin 2	1	A, C		Unnamed
	Hs.121576	AA768869	Myosin IB; myosin-I alpha (<i>Homo sapiens</i>)	1	A		Cell communication, adhesion or motility
<i>ESTs AA814948</i>	Hs.96343	AA814948	ESTs, weakly similar to hypothetical protein FLJ20489 (<i>Homo sapiens</i>)	1		C	Unnamed
<i>RXRA</i>	Hs.20084	AA865269	Retinoid X receptor, alpha	1	PCR	A, F	Cell death/transcription
<i>ESTs AA909818</i>	Hs.241797	AA909818	EST, moderately similar to ALU6_HUMAN ALU (<i>Homo sapiens</i>)	1		C	Unnamed
<i>KIAA0321</i> <i>N4BP3</i>	Hs.8663	AB002319	KIAA0321 protein	1	SAGE	SAGE, A	Unnamed
	Hs.101761	AB002339	Nedd4 binding protein 3	1		C	Unknown
<i>HBP1</i>	Hs.10882	AF019214	HMG-box containing protein 1	1			Transcription
<i>TAPBP</i>	Hs.179600	AF029750	TAP binding protein (tapasin)	1	SAGE	A, F	Cell communication, adhesion or motility
<i>LIM</i>	Hs.154103	AF061258	LIM protein (similar to rat protein kinase C-binding enigma)	1		A	Unknown

Table 1 Continued

Gene symbol	Cont	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
						ZR-75.1	MCF-7	
FLJ31051		Hs.406199	A1079337	<i>Homo sapiens</i> cDNA FLJ31051 fis, clone HSYRA2000605, weakly similar to MYOSIN HEAVY CHAIN, CLONE 203	1			Unnamed
		Hs.79069	A1271688	Cyclin G2	1			Cell cycle
		Hs.20996	A1374654	ESTs A1374654	1		A, C, F	Unnamed
		Hs.57735	A1623132	Acetyl LDL receptor; SREC=scavenger receptor expressed by endothelial cells	1		C	low density lipoprotein metabolism
CEACAM6		Hs.73848	A1677998	Carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen)	1		A	Signal transduction
		Hs.93213	A1741331	BCL2-antagonist/killer 1	1			Cell death
BAK1	MGC10500	Hs.271599	A1769018	Hypothetical protein MGC10500	1	PCR/RNaseA, F	A	Unnamed
		Hs.92254	A1823970	Synaptotagmin-like 2	1		A, C	Unknown
HGS		Hs.24756	A1937435	Hepatocyte growth factor-regulated tyrosine kinase substrate	1			Signal transduction
		Hs.109059	AJ001189	Oligophrenin 1	1		C	Signal transduction
OPHN1	WDR10	Hs.70202	AL041643	WD repeat domain 10	1		SAGE, A	Unknown
		Hs.10706	AL048161	Epithelial protein lost in neoplasm beta	1		A, C	Unknown
ZNF33A		Hs.70617	D31763	Zinc finger protein 33a (K0X 31)	1		C	Transcription
		Hs.118174	D84294	Tetratricopeptide repeat domain 3	1		C	Unknown
PCM1	ERBB3	Hs.348501	H00568	Pericentriolar material 1	1		A, C	Unknown
		Hs.199067	M29366	V-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	1	SAGE	A, C	Signal transduction
EFNA1	COPA	Hs.399713	M57730	Ephrin-A1	1		A, B, F	Signal transduction
		Hs.75887	U24105	Coatomer protein complex, subunit alpha	1	SAGE	A	Transporters

Table 1 Continued

Gene symbol	Cont	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
						ZR-75.1	MCF-7	
SLC12A2		Hs.110736	U30246	Solute carrier family 12 (sodium/potassium/chloride transporters), member 2	1		A	Transporters
		Hs.23598	U47741	CREB binding protein (Rubinstein-Taybi syndrome)	1		A	Transcription
HSXIAPAF1	ATP2A3	Hs.139262	X99699	XIAP associated factor-1	1			Cell death
		Hs.5541	Z69881	ATPase, Ca ⁺⁺ transporting, ubiquitous	1		A	
MGC11034		Hs.103378	AA775792	Hypothetical protein	2		A, C	Unnamed
		Hs.5372	AB000712	MGC11034	2	SAGE	A	Cell communication, adhesion or motility
CLDN4				Claudin 4	2			Transcription
		Hs.82071	AF109161	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	2	PCR	SAGE	
CD24		Hs.375108	AI310309	CD24 antigen (small cell lung carcinoma cluster 4 antigen)	2	PCR/SAGE	SAGE, A, C	Signal transduction
		Hs.89603	AI922289	Mucin 1, transmembrane	2			Cell communication, adhesion or motility
IGFBP5	PGM5	Hs.180324	L27560	Insulin-like growth factor binding protein 5	2	SAGE	D	Signal transduction
		Hs.406261	L40933	Phosphoglucosyltransferase 5	2		A	Cell communication, adhesion or motility
ACHE	ISGF3G	Hs.154495	M55040	Acetylcholinesterase (YT blood group)	2		A, C	Signal transduction
		Hs.1706	M87503	Interferon-stimulated transcription factor 3, gamma (48 kDa)	2		A, B, C, F	Transcription
NMA	ANXA2	Hs.78776	U23070	Putative transmembrane protein	2			Signal transduction
		Hs.217493	AA164741	Annexin A2	3	SAGE		Cell communication, adhesion or motility

Table 1 Continued

Gene symbol	Cont	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
						ZR-75.1	MCF-7	
ATP9A KIAA0492 EGFL4		Hs.406434	AA620508	ATPase, Class II, type 9A	3			Transporters
		Hs.406707	AB007961	KIAA0492 protein	3			Unnamed
		Hs.158200	AB011541	EGF-like-domain, multiple	3			Unknown
BAG1		Hs.41714	AF022224	BCL2-associated athanogene	3	PCR		Cell death
IDS		Hs.172458	AF050145	Iduronate 2-sulfatase (Hunter syndrome)	3			Other enzymes
GTF2H2		Hs.191356	A1239815	General transcription factor IIH, polypeptide 2 (44 kDa subunit)	3		D	Transcription
TMSB10		Hs.76293	A1679377	Thymosin, beta 10	3		SAGE, F	Cell communication, adhesion or motility
ESTs A1807483 TNFRSF12A ¹		Hs.112222	Hs.365365	ESTs A1807483	3			Unnamed
		Hs.10086	Hs.355899	Tumor necrosis factor receptor superfamily, member 12A	3	SAGE		Cell communication, adhesion or motility
ESTs AL050331		Hs.237225	Hs.381074	<i>Homo sapiens</i> , clone MGC:24302 IMAGE:3996246, mRNA, complete cds	3			Unnamed
ANXA9		Hs.279928	AW016464	Annexin A9	3			Cell communication, adhesion or motility
APOD CPT2		Hs.75736	J02611	Apolipoprotein D	3		A	Transporters
		Hs.274336	U09648	Carnitine palmitoyltransferase II	3	SAGE	A	Lipid metabolism
HSU15552		Hs.85769	U15552	Acidic 82 kDa protein mRNA	3		A	Unknown
G1P3		Hs.265827	U22970	Interferon, alpha-inducible protein (clone IFI-6-16)	3			Unknown
IL1RL2		Hs.102865	U49065	Interleukin 1 receptor-like 2	3			Signal transduction
TCF7		Hs.169294	X59871	Transcription factor 7 (T-cell specific, HMG-box)	3			Transcription

Table 1 Continued

Gene symbol Cont FLJ11796	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
					ZR-75.1	MCF-7	
	Hs.284186	AA977661	<i>Homo sapiens</i> cDNA FLJ11796 fis, clone HEMBA1006158, highly similar to <i>Homo sapiens</i> transcription factor forkhead-like 7 (FKHL7) gene	4		A	Unnamed
SSI-3	Hs.345728	AB006967	STAT induced STAT inhibitor 3	4		C	Transcription
STC2	Hs.155223	AB012664	Stanniocalcin 2	4		SAGE, A, D, E	Hormones
HIP12	Hs.96731	AB014555	Huntingtin interacting protein 12	4			Unknown
MYH11	Hs.78344	AF001548	Myosin, heavy polypeptide 11, smooth muscle	4	SAGE		Cell communication, adhesion or motility
TIEG	Hs.82173	AF050110	TGFB inducible early growth response	4	PCR		Transcription
IER3	Hs.76095	A1185199	Immediate early response 3	4			Cell death
FLJ14299	Hs.288042	A1348071	Hypothetical protein FLJ14299	4	PCR		Unnamed
JUN	Hs.78465	A1598150	V-jun sarcoma virus 17 oncogene homolog (avian)	4		SAGE, A	Transcription
MYC	Hs.79070	K02276	V-myc myelocytomatosis viral oncogene homolog (avian)	4		A, B, C, E, F	Transcription
CYP1A1	Hs.72912	K03191	Cytochrome P450, subfamily I (aromatic compound-inducible), polypeptide 1	4			Xenobiotic metabolism
EGR1	Hs.326035	M80583	Early growth response 1	4		A, F	Transcription
TXNIP	Hs.179526	S73591	Thioredoxin interacting protein; upregulated by 1,25-dihydroxyvitamin D3	4		C	Unknown
SLC16A6	Hs.114924	U79745	Solute carrier family 16 (monocarboxylic acid transporters), member 6	4			Transporters

Table 1 Continued

Gene symbol	Cont	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
						ZR-75.1	MCF-7	
<i>FOS</i>		Hs.25647	V01512	V-fos FBJ murine osteosarcoma viral oncogene homolog	4		A	Transcription
<i>FLJ21425</i>		Hs.302738	AA194161	<i>Homo sapiens</i> cDNA: FLJ21425 fis, clone COL04162	5	PCR	A	Unnamed
<i>AKAP1</i>		Hs.78921	AA868550	A kinase (PRKA) anchor protein 1	5		SAGE, A, B, F	Signal transduction
<i>HSPA5</i>		Hs.75410	A1878886	Heat shock 70 kDa protein 5 (glucose-regulated protein, 78 kDa)	5			Protein folding
<i>HSPA8</i>		Hs.180414	AL044172	Heat shock 70 kDa protein 8	5		SAGE, A	Protein folding
<i>SDF1</i>		Hs.237356	L36033	Stromal cell-derived factor 1	5		A, B, C, F	Signal transduction
<i>SFRS7</i>		Hs.184167	L41887	<i>Homo sapiens</i> splicing factor, arginine/serine-rich 7 (SFRS7) gene, complete cds	5		F	RNA splicing
<i>RET</i>		Hs.350321	M31213	Ret proto-oncogene (multiple endocrine neoplasia and medullary thyroid carcinoma 1, Hirschsprung disease)	5		A, C	Signal transduction
<i>HSP70-1</i>		Hs.75452	M59828	Human MHC class III HSP70-1 gene (HLA), complete cds	5			Protein folding
<i>IGFBP4</i>		Hs.1516	M62403	Insulin-like growth factor binding protein 4	5	SAGE	D, E	Signal transduction
<i>EIF3S9</i>		Hs.57783	U78525	Eukaryotic translation initiation factor 3, subunit 9 (eta, 116 kDa)	5		C, D	Protein biosynthesis
<i>ACTG1</i>		Hs.14376	X04098	Actin, gamma 1	5		C	Cell communication, adhesion or motility
<i>ACTN1</i>		Hs.119000	X15804	Actinin, alpha 1	5			Cell communication, adhesion or motility

Table 1 Continued

Gene symbol Cont	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
					ZR-75.1	MCF-7	
CD44	Hs.169610	X55150	CD44 antigen (homing function and Indian blood group system)	5	PCR	A	Cell communication, adhesion or motility
CCND1	Hs.82932	X59798	Cyclin D1 (PRAD1: parathyroid adenomatosis 1)	5	RNase	SAGE, A, B, C, F	Cell cycle
NOLC1	Hs.75337	Z34289	Nucleolar and coiled-body phosphoprotein 1	5			Unknown
MST4	Hs.23643	AA191319	Mst3 and SOK1-related kinase	6		A, F	Other enzymes
COPS3	Hs.6076	AA287305	COP9 constitutive photomorphogenic homolog subunit 3 (<i>Arabidopsis</i>)	6			Signal transduction
VRK1	Hs.422789	AA312869	Vaccinia related kinase 1	6		C	Protein kinase
HAT1	Hs.13340	AF030424	Histone acetyltransferase 1	6	PCR	SAGE	Transcription
XPOT	Hs.85951	AF039022	Exportin, tRNA (nuclear export receptor for tRNAs)	6		SAGE, D	Transporters
GFRA1	Hs.105445	AF058999	GDNF family receptor alpha 1	6		A, C	Signal transduction
EXO1	Hs.47504	AF091754	Exonuclease 1	6			DNA replication and repair
FHX	Hs.120844	AF155132	FOXJ2 forkhead factor	6			Transcription
TULP1	Hs.93537	AI380477	Tubby like protein 1	6			Transcription
AGR2	Hs.413945	AI800451	Anterior gradient 2 homolog (<i>Xenopus laevis</i>)	6		A	Unknown
MYO38	Hs.374853	AI972375	Hypothetical brain protein my038	6			Unnamed
HNRPA2B1	Hs.232400	D28877	Heterogeneous nuclear ribonucleoprotein A2/B1	6			mRNA processing
OLFM1	Hs.74376	D82343	Olfactomedin 1	6		A	Unknown
PGAM1	Hs.181013	J04173	Phosphoglycerate mutase 1 (brain)	6			Glycid metabolism
DNAJA1	Hs.94	L08069	DnaJ (Hsp40) homolog, subfamily A, member 1	6		SAGE, F	Protein folding
HSPA9B	Hs.3069	L15189	Heat shock 70 kDa protein 9B (mortalin-2)	6			Cell death
HSPCB	Hs.74335	M16660	Heat shock 90 kDa protein 1, beta	6		SAGE	Protein folding

Table 1 Continued

Gene symbol	Cont	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
						ZR-75.1	MCF-7	
<i>HSPD1</i>		Hs.79037	M34664	Heat shock 60 kDa protein 1 (chaperonin)	6		SAGE	Protein folding
<i>NCL</i>		Hs.79110	M60858	Nucleolin	6			Transcription
<i>SNRPF</i>		Hs.105465	T24772	Small nuclear ribonucleoprotein polypeptide F	6		SAGE, A	mRNA processing
<i>EIF5</i>		Hs.433702	U49436	Eukaryotic translation initiation factor 5	6		SAGE, F	Protein biosynthesis
<i>KPNB2</i>		Hs.168075	U72069	Karyopherin (importin) beta 2	6		A	Transporters
<i>PLOD2</i>		Hs.41270	U84573	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase (lysine hydroxylase) 2	6		A	Other enzymes
<i>TRIP13</i>		Hs.6566	U96131	Thyroid hormone receptor interactor 13	6		A, B, F	Transcription
<i>PRC1</i>		Hs.344037	W02418	Protein regulator of cytokinesis 1	6		A	Cell cycle
<i>HMG2</i>		Hs.80684	X62534	High-mobility group (nonhistone chromosomal) protein 2	6			Transcription
<i>ABCE1</i>		Hs.12013	X76388	ATP-binding cassette, sub-family E (OABP), member 1	6	SAGE		RNAse L inhibitor
<i>CDC2</i>		Hs.334562	Y00272	Cell division cycle 2, G1 to S and G2 to M	6		A	Cell cycle
<i>FLJ20811</i>		Hs.83530	AA015605	FLJ20811 Hypothetical protein	7		A	Unnamed
<i>PSME3</i>		Hs.152978	AA100392	Proteasome (prosome, macropain) activator subunit 3 (PA28 gamma; Ki)	7		A	Protein degradation
<i>SKB1</i>		Hs.12912	AA152011	SKB1 homolog (S. pombe)	7		SAGE, A	Cell cycle
<i>BLVRB</i>		Hs.76289	AA280097	Biliverdin reductase B (flavin reductase (NADPH))	7		A, C	Xenobiotic metabolism
<i>MEST</i>		Hs.79284	AA305098	Mesoderm specific transcript homolog (mouse)	7		SAGE	Unknown

Table 1 Continued

Gene symbol	Cont	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
						ZR-75.1	MCF-7	
<i>EIF2S1</i>		Hs.151777	AA306546	Eukaryotic translation initiation factor 2, sub. 1 (alpha, 35kD)	7			Protein biosynthesis
<i>NP</i>		Hs.75514	AA311617	Nucleoside phosphorylase	7			Purine/pyrimidine metabolism
<i>CCT7</i>		Hs.108809	AA314436	Chaperonin containing TCP1, subunit 7 (eta)	7		SAGE	Protein folding
<i>DDX39</i>		Hs.311609	AA315491	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 39	7		SAGE	mRNA processing
<i>RNF3</i>		Hs.8834	AA403225	Ring finger protein 3	7	SAGE		Unknown
<i>RFC5</i>		Hs.171075	AA460085	Replication factor C (activator 1) 5 (36.5kD)	7		SAGE, F	DNA replication and repair
<i>UMPK</i>		Hs.95734	AA468735	Uridine monophosphate kinase	7			Purine/pyrimidine metabolism
<i>PWP2H</i>		Hs.79380	AB001517	PWP2 periodic tryptophan protein Homolog (yeast)	7		SAGE	Unknown
<i>ADCY3</i>		Hs.8402	AB011083	Adenylate cyclase 3	7		SAGE	Signal transduction
<i>FBP17</i>		Hs.301763	AB011126	Formin-binding protein 17	7			Unnamed
<i>STK12</i>		Hs.180655	AB011450	Serine/threonine kinase 12	7		B, F	Cell cycle
<i>SLC3A2</i>		Hs.79748	AB018010	Solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	7		SAGE, A, D	Transporters
<i>HNRPR</i>		Hs.15265	AF000364	Heterogeneous nuclear ribonucleoprotein R	7			mRNA processing
<i>EIF1AY</i>		Hs.155103	AF000987	Eukaryotic translation initiation factor 1A, Y chromosome	7			Protein biosynthesis
<i>STXBP1</i>		Hs.239356	AF004562	Syntaxin binding protein 1	7			Exocytosis
<i>SLC9A3R1</i>		Hs.184276	AF015926	Solute carrier family 9 (sodium/hydrogen exchanger), isoform 3 regulatory factor 1	7		A, C	Transporters
<i>NRP1</i>		Hs.69285	AF016050	Neuropilin 1	7	PCR		Signal transduction
<i>TCERG1</i>		Hs.13063	AF017789	Transcription elongation regulator 1 (CA150)	7			Transcription
<i>MTM1</i>		Hs.75302	AF020676	Myotubular myopathy 1	7			Signal transduction

Table 1 Continued

Gene symbol	Cont	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
						ZR-75.1	MCF-7	
<i>SLC22A1LS</i>		Hs.300076	AF035407	Solute carrier family 22 (organic cation transporter), member 1-like antisense	7			Transporters
<i>NSAP1</i>		Hs.373499	AF037448	NS1-associated protein 1	7			mRNA processing
<i>ABCG1</i>		Hs.10237	AF038175	ATP-binding cassette, sub-family G (WHITE), member 1	7	SAGE		Transporters
<i>ITGB4BP</i>		Hs.406444	AF047433	Integrin beta 4 binding protein	7		C	Cell communication, adhesion or motility
<i>BUB1B</i>		Hs.36708	AF053306	BUB1 budding uninhibited by benzimidazoles 1 homolog beta (yeast)	7	SAGE		Cell cycle
<i>MGC19606</i>		Hs.91579	AF054996	MGC19606 Similar to hypothetical 34.0 kDa protein ZK795.3 in chromosome IV	7		C, D	Unnamed
<i>LOC51257</i>		Hs.132744	AF070529	Hypothetical protein LOC51257	7			Unnamed
<i>SQLE</i>		Hs.71465	AF098865	Squalene epoxidase	7	SAGE	A	Sterol metabolism
<i>PA2G4</i>		Hs.343258	AF104670	Proliferation-associated 2G4, 38kDa	7		SAGE, C	Cell cycle
<i>SNRPA</i>		Hs.173255	AI125327	Small nuclear ribonucleoprotein polypeptide A	7		F	mRNA processing
<i>RANBP1</i>		Hs.24763	AI160997	RAN binding protein 1	7		A, F	Cell cycle
<i>ALY</i>		Hs.241520	AI357591	Transcriptional coactivator	7	SAGE	C	Transcription
<i>VGF</i>		Hs.171014	AI520764	VGF nerve growth factor inducible	7		C	Signal transduction
<i>E2-EPF</i>		Hs.174070	AI571293	Ubiquitin carrier protein	7			Protein degradation
<i>SLC39A4</i>		Hs.352415	AI634653	Solute carrier family 39 (zinc transporter), member 4	7		C	Transporters
<i>GTF3A</i>		Hs.75113	AI686944	General transcription factor IIIA	7		F	Transcription
<i>ZWINT</i>		Hs.42650	AI739390	ZW10 interactor	7		A	Unknown
<i>GSTTLp28</i>		Hs.11465	AI752707	Glutathione-S-transferase like; glutathione transferase omega	7		A	Cell death

Table 1 Continued

Gene symbol Cont	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
					ZR-75.1	MCF-7	
<i>MCM2</i>	Hs.57101	A1814040	Minichromosome maintenance deficient 2, mitotin (<i>S. cerevisiae</i>)	7		F	DNA replication and repair
<i>REG</i>	Hs.21594	A1971219	RAS-like, estrogen-regulated, growth-inhibitor	7		A	Signal transduction
<i>TPBG</i>	Hs.82128	AJ012159	Trophoblast glycoprotein	7			Cell communication, adhesion or motility
<i>NXF1</i>	Hs.323502	AJ132712	Nuclear RNA export factor 1	7			Transporters
<i>RNP24</i>	Hs.75914	AL036166	Coated vesicle membrane protein	7	SAGE		Intracellular protein traffic
<i>HSPC242</i>	Hs.25199	AW006373	Hypothetical protein HSPC242	7	SAGE	C	Unnamed
<i>NSEP1^f</i>	Hs.74497	BF512252	Nuclease sensitive element binding protein 1	7		F	Transcription
<i>PTDSS1</i>	Hs.77329	D14694	Phosphatidylserine synthase 1	7			Lipid metabolism
<i>PFKP</i>	Hs.99910	D25328	Phosphofructokinase, platelet	7		A, F	Glycid metabolism
<i>KIAA0007</i>	Hs.90315	D26488	KIAA0007 protein	7			Unnamed
<i>KIAA0095</i>	Hs.155314	D42085	KIAA0095 gene product	7		A, E	Unnamed
<i>MCM7</i>	Hs.77152	D55716	Minichromosome maintenance deficient 7 (<i>S. cerevisiae</i>)	7		A, B, F	DNA replication and repair
<i>YWHAH</i>	Hs.349530	D78577	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide	7	SAGE	A, C	Signal transduction
<i>OAZIN</i>	Hs.223014	D88674	Ornithine decarboxylase antizyme inhibitor	7		SAGE	ornithine decarboxylase inhibitor
<i>PMAIP1</i>	Hs.96	D90070	Phorbol-12-myristate-13-acetate-induced protein 1	7		A, D	Unknown
<i>UMPS</i>	Hs.2057	J03626	Uridine monophosphate synthetase (orotate phosphoribosyl transferase and orotidine-5'-decarboxylase)	7			Purine/pyrimidine metabolism

Table 1 Continued

Gene symbol	Cont	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
						ZR-75.1	MCF-7	
<i>MTHFD1</i>		Hs.172665	J04031	Methylenetetrahydrofolate dehydrogenase (NADP+ dependent), methylenetetrahydrofolate cyclohydrolase, formyltetrahydrofolate synthetase	7		A	Folate metabolism
<i>C1QBP</i>		Hs.78614	L04636	Complement component 1, q subcomponent	7	SAGE		Unknown
<i>GNL1</i>		Hs.83147	L25665	binding protein	7			Signal transduction
<i>F12</i>		Hs.1321	M11723	Guanine nucleotide binding protein-like 1	7	SAGE		Other enzymes
<i>G6PD</i>		Hs.80206	M12996	Coagulation factor XII (Hageman factor)	7		SAGE	Glycid metabolism
<i>PGC</i>		Hs.1867	M18667	Glucose-6-phosphate dehydrogenase	7			Other enzymes
<i>AMD1</i>		Hs.262476	M21154	Progastricsin (pepsinogen C)	7			Other enzymes
<i>GOT2</i>		Hs.170197	M22632	S-adenosylmethionine decarboxylase 1	7		A, F	Other enzymes
				Glutamic-oxaloacetic transaminase 2, mitochondrial (aspartate aminotransferase 2)	7			Other enzymes
<i>AREG</i>		Hs.270833	M30704	Amphiregulin (schwannoma-derived growth factor)	7			Signal transduction
<i>HLX1</i>		Hs.74870	M60721	H2.0-like homeo box 1 (<i>Drosophila</i>)	7			Transcription
<i>TUBG1</i>		Hs.21635	M61764	Tubulin, gamma 1	7	SAGE	A, B, F	Cell communication, adhesion or motility
<i>CTSD</i>		Hs.343475	M63138	Cathepsin D (lysosomal aspartyl protease)	7	SAGE		Other enzymes
<i>SFRS1</i>		Hs.352256	M72709	Splicing factor, arginine/serine-rich 1 (splicing factor 2, alternate splicing factor)	7		A, B, C, D, F, SAGE, A, F	RNA splicing
<i>LTF</i>		Hs.105938	M73700	Lactotransferrin	7			Transporters

Table 1 Continued

Gene symbol Cont SLC7A5	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
					ZR-75.1	MCF-7	
	Hs.184601	M80244	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 5	7	SAGE	SAGE, A, C, D	Transporters
CSTF2	Hs.693	M85085	Cleavage stimulation factor, 3' pre-RNA, subunit 2, 64 kDa	7			mRNA processing
STIP1	Hs.355930	M86752	Stress-induced- phosphoprotein 1 (Hsp70/Hsp90-organizing protein)	7			Protein folding
FKBP4	Hs.848	M88279	FK506 binding protein 4 (59 kDa)	7	SAGE	SAGE, A, C	Protein folding
MVK	Hs.130607	M88468	Mevalonate kinase (mevalonic aciduria)	7			Sterol metabolism
VARS2	Hs.159637	M98326	Valyl-tRNA synthetase 2	7		C	Protein biosynthesis
HAX1	Hs.15318	N28312	HS1 binding protein	7		D	Cell death
SERPINE6	Hs.41072	S69272	Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 6	7		A	Protease inhibitors
CDC20	Hs.82906	U05340	CDC20 cell division cycle 20 homolog (<i>S. cerevisiae</i>)	7		B, F	Cell cycle
TMPO	Hs.406660	U09086	Thymopoietin	7		A	Unknown
ATP5G3	Hs.429	U09813	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9) isoform 3	7	SAGE	A	Electron transport and oxidative phosphorylation
TRAP1	Hs.376253	U12595	Heat shock protein 75	7		C, F	Signal transduction
FOXF1	Hs.155591	U13219	Forkhead box F1	7			Transcription
CENPA	Hs.1594	U14518	Centromere protein A (17kD)	7	SAGE		Cell cycle
MYB	Hs.1334	U22376	V-myb myeloblastosis viral oncogene Homolog (avian)	7	PCR	B, F	Transcription
SHMT2	Hs.75069	U23143	Serine hydroxymethyltransferase 2 (mitochondrial)	7		D	Other enzymes

Table 1 Continued

Gene symbol	Cont	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
						ZR-75.1	MCF-7	
SERPINA5		Hs.76353	U35464	Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5	7		A	Protease inhibitors
		Hs.211571	U36787	Holocytochrome c heme-lyase	7			Electron transport and oxidative phosphorylation
TPD52L1		Hs.16611	U44427	Tumor protein D52-like 1	7	PCR		Expressed in breast cancer
HSU53209		Hs.130829	U53209	Transformer-2 alpha (htra-2 alpha)	7			RNA splicing
DKC1		Hs.4747	U59151	Dyskeratosis congenita 1, dyskerin	7		SAGE, A, C, D	Ribosome biogenesis
SFRS10		Hs.30035	U61267	Splicing factor, arginine/serine-rich 10 (transformer 2 homolog, <i>Drosophila</i>)	7		SAGE, A, F	RNA splicing
ORP150		Hs.277704	U65785	Oxygen regulated protein (150 kDa)	7		A	Protein folding
FKBP5		Hs.7557	U71321	FK506 binding protein 5	7		A, F	Protein folding
YARS		Hs.239307	U89436	Tyrosyl-tRNA synthetase	7		A, D	Protein biosynthesis
LDHA		Hs.2795	X02152	Lactate dehydrogenase A	7		SAGE, B, F	Glycid metabolism
NRAS		Hs.260523	X02751	Neuroblastoma RAS viral (v-ras) oncogene homolog	7			Signal transduction
TFF1		Hs.350470	X05030	Trefoil factor 1 (breast cancer, estrogen-inducible sequence expressed in)	7		SAGE, C, D, E	Expressed in breast cancer
MRPL3		Hs.79086	X06323	Mitochondrial ribosomal protein L3	7			Ribosome biogenesis
TUBA1		Hs.75318	X06956	Tubulin, alpha 1 (testis specific)	7	SAGE	A	Cell communication, adhesion or motility
MYBL2		Hs.179718	X13293	V-myb myeloblastosis viral oncogene homolog (avian)-like 2	7		A, B, F	Transcription
GLA		Hs.69089	X14448	Galactosidase, alpha	7	SAGE	A	Other enzymes

Table 1 Continued

Gene symbol	Cont	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
						ZR-75.1	MCF-7	
<i>MTHFD2</i>		Hs.154672	X16396	Methylene tetrahydrofolate dehydrogenase (NAD+ dependent), methenyltetrahydrofolate cyclohydrolase	7		A, D	Folate metabolism
<i>HSPA6</i>		Hs.3268	X51757	Heat shock 70 kDa protein 6 (HSP70B)	7			Protein folding
<i>RRM1</i>		Hs.2934	X59543	Ribonucleotide reductase M1 polypeptide	7		A	Purine/pyrimidine metabolism
<i>WARS</i>		Hs.82030	X62570	Tryptophanyl-tRNA synthetase	7	SAGE	D	Protein biosynthesis
<i>EGR3</i>		Hs.74088	X63741	Early growth response 3	7		A, C	Transcription
<i>PMSC2</i>		Hs.75584	X66113	Polymyositis/scleroderma autoantigen 2 (100 kDa)	7			Protein kinase
<i>TPI1</i>		Hs.83848	X69723	Triosephosphate isomerase 1	7	SAGE		Glycid metabolism
<i>FEN1</i>		Hs.4756	X76771	Flap structure-specific endonuclease 1	7		SAGE, A, B, F	DNA replication and repair
<i>TSN</i>		Hs.75066	X78627	Translin	7			DNA replication and repair
<i>TUBB</i>		Hs.336780	X79535	Tubulin, beta polypeptide	7		B	Cell communication, adhesion or motility
<i>FXRD2</i>		Hs.19520	X86400	FXRD domain-containing ion transport regulator 2	7			Transporters
<i>PCK2</i>		Hs.75812	X92720	Phosphoenolpyruvate carboxykinase 2 (mitochondrial)	7		C, D	Glycid metabolism
<i>MARS</i>		Hs.279946	X94754	Methionine-tRNA synthetase	7			Protein biosynthesis
<i>SEC23B</i>		Hs.173497	X97065	Sec23 homolog B (S. cerevisiae)	7			Transporters
<i>NUP88</i>		Hs.172108	Y08612	Nucleoporin 88kDa	7		B, C, F	mRNA processing
<i>UNG</i>		Hs.78853	Y09008	Uracil-DNA glycosylase	7			DNA replication and repair
<i>COX5A</i>		Hs.296585	Y12065	Cytochrome c oxidase subunit Va	7	SAGE		Ribosome biogenesis
<i>LGALS1</i>		Hs.382367	AA035793	Lectin, galactoside-binding, soluble, 1 (galectin 1)	8		C	Cell communication, adhesion or motility

Table 1 Continued

Gene symbol	Cont	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
						ZR-75.1	MCF-7	
<i>ARL3</i>		Hs.182215	AA046366	ADP-ribosylation factor-like 3	8	SAGE	B, G	Signal transduction
<i>NPM1</i>		Hs.355719	AA173870	Nucleophosmin (nucleolar phosphoprotein B23, numatrin)	8	SAGE	SAGE	Ribosome biogenesis
<i>PPIA</i>		Hs.401787	AA304657	Peptidylprolyl isomerase A (cyclophilin A)	8			Protein folding
<i>SNRPB</i>		Hs.83753	AA313910	Small nuclear ribonucleoprotein polypeptides B and B1	8			mRNA processing
<i>SLC25A5</i>		Hs.79172	AA314562	Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5	8	SAGE	B, C, F	Transporters
<i>ITGAE</i>		Hs.851	AA425588	Integrin, alpha E (antigen CD103, human mucosal lymphocyte antigen 1; alpha polypeptide)	8		F	Cell communication, adhesion or motility
<i>PTTG1</i>		Hs.252587	AA430241	Pituitary tumor-transforming 1	8	SAGE	SAGE, A, D	Signal transduction
<i>PDCD5</i>		Hs.166468	AA452724	Programmed cell death 5	8		SAGE	Cell death
<i>RPA3</i>		Hs.1608	AA461254	Replication protein A3 (14 kDa)	8		A	DNA replication and repair
<i>ESTs AA528121</i>		Hs.121557	AA528121	ESTs, Weakly similar to DP1_HUMAN	8			Unnamed
<i>RPS3</i>		Hs.414990	AA593872	POLYPOSIS LOCUS PROTEIN 1 (<i>H. sapiens</i>)	8			Ribosome biogenesis
<i>HMGCR</i>		Hs.11899	AA602498	Ribosomal protein S3	8			Sterol metabolism
<i>LOC51321</i>		Hs.268122	AA85882	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	8			Unnamed
<i>FLJ32241</i>		Hs.352359	AA948729	Hypothetical protein LOC51321	8			Unnamed
<i>ESTs AA975298</i>		Hs.32058	AA975298	<i>Homo sapiens</i> cDNA FLJ32241 fis, clone PLACE6005231	8		SAGE, A	DNA replication and repair
<i>KNL4</i>		Hs.119324	AB017430	C1orf19 Chromosome 1 open reading frame 19	8		SAGE, A	Unnamed
				Kinesin-like 4	8			Cell cycle

Table 1 Continued

Gene symbol Cont	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
					ZR-75.1	MCF-7	
<i>PSMD11</i>	Hs.90744	AF001212	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 11	8		A	Protein degradation
<i>STK15</i>	Hs.250822	AF011468	Serine/threonine kinase 15	8	SAGE	A, B, F	Cell cycle
<i>ICAP-1A</i>	Hs.173274	AF012023	Integrin cytoplasmic domain-associated protein 1	8			Cell communication, adhesion or motility
<i>cig5</i>	Hs.17518	AF026941	Vipirin; similar to inflammatory response protein 6	8			Unknown
<i>RAD51C</i>	Hs.11393	AF029669	RAD51 homolog C (<i>S. cerevisiae</i>)	8		F	DNA replication and repair
<i>RAN</i>	Hs.10842	AF054183	RAN, member RAS oncogene family	8		SAGE	Cell cycle
<i>BCRP1</i>	Hs.433759	AF068235	Breakpoint cluster region protein, uterine leiomyoma, 1; barrier to autointegration factor	8	PCR	SAGE	Cell cycle
<i>H2AV</i>	Hs.301005	AF081192	Histone H2A.F/Z variant	8		A	Cell cycle
<i>CCT3</i>	Hs.1708	AI246667	Chaperonin containing TCP1, subunit 3 (gamma)	8			Protein folding
<i>SEC61B</i>	Hs.191887	AI281953	Protein translocation complex beta	8			Transporters
<i>TIMELESS</i>	Hs.118631	AI283089	Timeless homolog (<i>Drosophila</i>)	8			Unknown
<i>TKT</i>	Hs.89643	AI378884	Transketolase (Wernicke-Korsakoff syndrome)	8			Glycid metabolism
<i>PRO1855</i>	Hs.283558	AI421371	Hypothetical protein PRO1855	8			Unnamed
<i>NTRK1</i>	Hs.85844	AI471551	Neurotrophic tyrosine kinase, receptor, type 1	8			Signal transduction
<i>PAICS</i>	Hs.117950	AI524157	Phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase	8	SAGE	SAGE	Purine/pyrimidine metabolism

Table 1 Continued

Gene symbol	Cont	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
						ZR-75.1	MCF-7	
<i>COX6C</i>		Hs.351875	AI568937	Cytochrome c oxidase subunit VIc	8	PCR	A, F	Electron transport and oxidative phosphorylation
<i>NUDT1</i>		Hs.388	AI655586	Nudix (nucleoside diphosphate linked moiety X)-type motif 1	8		A	Unknown
<i>RAB6KIFL</i>		Hs.73625	AI739117	RAB6 interacting, kinesin-like (rabkinesin6)	8			Cell communication, adhesion or motility
<i>E1F4A1</i>		Hs.129673	AI755111	Eukaryotic translation initiation factor 4A, isoform 1	8	SAGE		Protein biosynthesis
<i>PSMD8</i>		Hs.78466	AI769251	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 8	8		SAGE	Protein degradation
<i>PSMC4</i>		Hs.211594	AI878823	Proteasome (prosome, macropain) 26S subunit, ATPase, 4	8			Protein degradation
<i>C5orf8</i>		Hs.75864	AI954986	Chromosome 5 open reading frame 8	8			Unnamed
<i>MAD2L1</i>		Hs.79078	AJ000186	MAD2 mitotic arrest deficient-like 1 (yeast)	8		B	Cell cycle
<i>ESTs AL034374</i>		Hs.250175	AL034374	Homolog of yeast long chain polyunsaturated fatty acid elongation enzyme 2	8			Lipid metabolism
<i>SIP</i>		Hs.27258	AL035305	Shah-interacting protein	8			Signal transduction
<i>ESTs AL036280</i>		Hs.356721	AL036280	ESTs, Highly similar to S04617 heterogeneous ribonuclear particle protein A1 (<i>H. sapiens</i>)	8	SAGE	SAGE	Unnamed
<i>ILF2</i>		Hs.75117	AL040837	Interleukin enhancer binding factor 2, 45 kDa	8		F	Transcription
<i>KIAA0102</i>		Hs.77665	AL047241	KIAA0102 gene product	8		A	Unnamed
<i>RAP1A</i>		Hs.184889	AL049557	RAP1A, member of RAS oncogene family	8	SAGE	A	Signal transduction
<i>MRPS14</i>		Hs.247324	AL049705	Mitochondrial ribosomal protein S14	8			

Table 1 Continued

Gene symbol	Cont	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
						ZR-75.1	MCF-7	
<i>SNRPG</i>		Hs.77496	AL110340	Small nuclear ribonucleoprotein polypeptide G	8	SAGE		mRNA processing
<i>PKM2</i>		Hs.198281	AW007619	Pyruvate kinase, muscle	8		F	Glycid metabolism
<i>NME1</i>		Hs.118638	AW024667	Non-metastatic cells 1, protein (NM23A) expressed in	8		SAGE, A	Nucleotide metabolism
<i>PSMA4</i>		Hs.251531	D00763	Proteasome (prosome, macropain) subunit, alpha type, 4	8	SAGE		Protein degradation
<i>RAD51</i>		Hs.343807	D14134	RAD51 homolog (RecA homolog, <i>E. coli</i>) (<i>S. cerevisiae</i>)	8		A	DNA replication and repair
<i>KIAA0101</i>		Hs.81892	D14657	KIAA0101 gene product	8		A	Unnamed Protein degradation
<i>PSMB3</i>		Hs.82793	D26598	Proteasome (prosome, macropain) subunit, beta type, 3	8			Protein degradation
<i>PSMB6</i>		Hs.77060	D29012	Proteasome (prosome, macropain) subunit, beta type, 6	8			Protein degradation
<i>KIAA0074</i>		Hs.1192	D38553	KIAA0074 protein	8	PCR	A	Unnamed Unknown
<i>MESDC2</i>		Hs.78871	D42039	Mesoderm development candidate 2	8		SAGE	Protein folding
<i>CCT5</i>		Hs.1600	D43950	Chaperonin containing TCP1, subunit 5 (epsilon)	8			RNA splicing
<i>SF3B3</i>		Hs.195614	D87686	Splicing factor 3b, subunit 3, 130 kDa	8			mRNA processing
<i>SNRPE</i>		Hs.334612	F20803	Small nuclear ribonucleoprotein polypeptide E	8		A	Heavy metal binding
<i>MT1L</i>		Hs.94360	F27787	Metallothionein 1L	8		A, F	Transporters
<i>ERP70</i>		Hs.93659	J05016	Protein disulfide isomerase related protein (calcium-binding protein, intestinal-related)	8		A	Purine/pyrimidine metabolism
<i>TK1</i>		Hs.105097	K02581	Thymidine kinase 1, soluble	8		SAGE, A	

Table 1 Continued

Gene symbol	Cont	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
						ZR-75.1	MCF-7	
<i>NME2</i>		Hs.433416	L16785	Non-metastatic cells 2, protein (NM23B) expressed in	8	PCR		Nucleotide metabolism
<i>DTYMK</i>		Hs.79006	L16991	Deoxythymidylate kinase (thymidylate kinase)	8		A, B, F	Purine/pyrimidine metabolism
<i>PRDX1</i>		Hs.180909	L19184	Peroxiredoxin 1	8		A, F	Other enzymes
<i>IMPDH2</i>		Hs.75432	L33842	IMP (inosine monophosphate) dehydrogenase 2	8			Purine/pyrimidine metabolism
<i>KPNB1</i>		Hs.180446	L38951	Karyopherin (importin) beta 1	8		SAGE	Transporters
<i>ADPRT</i>		Hs.177766	M29786	ADP-ribosyltransferase (NAD ⁺ ; poly (ADP-ribose) polymerase)	8		F	DNA replication and repair
<i>FDPS</i>		Hs.335918	M29863	Farnesyl diphosphate synthase (farnesyl pyrophosphate synthetase, dimethylallyltransferase, geranyltransferase)	8		SAGE	Sterol metabolism
<i>ARSB</i>		Hs.1256	M32373	Arylsulfatase B	8			Lipid metabolism
<i>CDC25C</i>		Hs.656	M34065	Cell division cycle 25C	8			Cell cycle
<i>PPIB</i>		Hs.394389	M63573	Peptidylprolyl isomerase B (cyclophilin B)	8		A, C	Protein folding
<i>DUT</i>		Hs.367676	R77436	dUTP pyrophosphatase	8			Purine/pyrimidine metabolism
<i>ESTs R99207</i>		Hs.36102	R99207	ESTs, Highly similar to SMHU1B metallothionein 1B (<i>H. sapiens</i>)	8			Unnamed
<i>SORD</i>		Hs.878	U07361	Sorbitol dehydrogenase	8			Glycid metabolism
<i>KPNA2</i>		Hs.159557	U09559	Karyopherin alpha 2 (RAG cohort 1, importin alpha 1)	8		A, B, F	Transporters
<i>TAF9</i>		Hs.60679	U21858	TAF9 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 32 kDa	8			Transcription
<i>D123</i>		Hs.82043	U27112	D123 gene product	8			Cell cycle
<i>DHRS2</i>		Hs.272499	U31875	Dehydrogenase/reductase (SDR family) member 2	8			Other enzymes

Table 1 Continued

Gene symbol	Cont	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
						ZR-75.1	MCF-7	
CCT4	LAMR1	Hs.79150	U38846	Chaperonin containing TCP1, subunit 4 (delta)	8	SAGE	SAGE, F	Protein folding
		Hs.181357	U43901	Laminin receptor 1 (67kD, ribosomal protein SA)	8	SAGE		Ribosome biogenesis
		Hs.69360	U63743	Kinesin-like 6 (mitotic centromere-associated kinesin)	8			Cell cycle
FOXN1	BIRC5	Hs.239	U74612	Forkhead box M1	8		A	Transcription
		Hs.1578	U75285	Baculoviral IAP repeat-containing 5 (survivin)	8	PCR	A	Cell death
ANP32B		Hs.84264	W73283	Acidic (leucine-rich) nuclear phosphoprotein 32 family, member B	8			Unknown
ATP5L		Hs.107476	W94335	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit g	8			Electron transport and oxidative phosphorylation
SERPINA3		Hs.234726	X00947	Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	8		A, C, F	Protease inhibitors
HMG1		Hs.6727	X12597	High-mobility group (nonhistone chromosomal) protein 1; High-mobility group box 1	8		A, B, F	Transcription
TRA1		Hs.82689	X15187	Tumor rejection antigen (gp96) 1	8		A, D, F	Protein folding
CKS2	EEF1B2	Hs.83758	X54942	CDC28 protein kinase 2	8		A, B, F	Cell cycle
		Hs.421608	X60489	Eukaryotic translation elongation factor 1 beta 2	8			Protein biosynthesis
ACLY	FDFT1	Hs.174140	X64330	ATP citrate lyase	8			Citrate metabolism
		Hs.48876	X69141	Farnesyl-diphosphate farnesyltransferase 1	8		SAGE, B, F	Sterol metabolism
SFPQ		Hs.180610	X70944	Splicing factor proline/glutamine rich (polypyrimidine tract binding protein associated)	8		SAGE, A, F	RNA splicing
PLK		Hs.433619	X75932	Polo-like kinase (Drosophila)	8	SAGE	A	Cell cycle

Table 1 Continued

Gene symbol	Cont	UniGene ^a	GenBank ^b	Gene description	Cluster ^c	Confirmation of microarray results ^d		Function ^e
						ZR-75.1	MCF-7	
ME1		Hs.14732	X77244	Malic enzyme 1, NADP(+)-dependent, cytosolic	8			Citrate metabolism
TMM17A		Hs.20716	X97544	Translocase of inner mitochondrial membrane 17 homolog A (yeast)	8			Transporters

^aUniGene cluster number; ^bGenBank Accession number; ^cestrogen-responsive gene cluster (Fig. 2); ^dgenes that showed a response to estrogen comparable to that reported in Fig. 2a by alternative mRNA assays (PCR: fluorogenic ('real-time') quantitative RT-PCR (Fig. 3a); RNase: RNase protection (Figs 1 and 3b); SAGE: serial analysis of gene expression (Seth *et al.* 2002, Charpentier *et al.* 2000)) or independent microarray analyses in either ZR-75.1 or MCF-7 cells (A: Finlin *et al.* 2001; B: Lobenhofer *et al.* 2002; C: our unpublished data; D: Inoue *et al.* 2002; E: Bouras *et al.* 2002; F: Hodges *et al.* 2003); ^e function assigned according to Gene Ontology categories from LocusLink and/or GeneCards databases; ^fEST AA478625=ESTs Incyte, RAB9B=ESTs AA699329 and TNFRSF12A=FN14 in Fig. 2a

change over multiple consecutive time-points) was lower than that generally applied in global gene expression studies (± 1.8 - to 2.0-fold change), although within the significance range experimentally determined for the microarray platform and protocols used for this study (Yue *et al.* 2001). We decided, however, to carry out wherever possible a verification of the gene expression changes summarized in Fig. 2. This was performed according to different strategies, including independent measurements of E2-induced mRNA expression changes in ZR-75.1 cells and functional analyses. Furthermore, we carried out comparative searches for genes showing similar regulation by estrogen in these and other BC cells within publicly available repositories of microarray and SAGE (serial analysis of gene expression) data.

First of all, the expression levels of 19 mRNAs encoded by genes included within the estrogen responsive gene set listed in Table 1 were measured also by fluorogenic quantitative polymerase chain reaction ('real time' rtPCR). The genes to be verified were selected to represent each of the clusters reported in Fig. 2 and to include mRNAs that showed different basal expression and/or magnitude of response to estrogen by microarray analysis. Starting from the sequence of the corresponding cDNA probes in the microarray, specific oligonucleotide primer pairs and probes were designed for each gene, and RNA quantitation was carried out in triplicate both on the same RNA samples used for microarray analysis and on replicate samples prepared thereafter. In this way, we were able to control microarray performance as well as biological reproducibility of the gene responses detected. For one of the genes controlled (*interferon alpha-inducible protein*, GenBank Acc. no. U22970) no amplification product could be detected, possibly due to wrong selection of the amplification target. For this reason, the microarray results relative to this mRNA were considered 'not confirmed' and the corresponding gene was eliminated from the set of 345 genes which scored positive during the selection procedures. For 18 other transcripts, PCR products of the expected size were obtained (data not shown) and fluorogenic rtPCR could thus be performed. The results of the corresponding quantitative mRNA assays are reported in Fig. 3a and show that rtPCR confirmed the expression profiles detected by microarray hybridization in all cases investigated. The same

(b)

was true for *CD44* antigen (*homing function and Indian blood group system*, GenBank Acc. no. X55150), whose mRNA fluorescent PCR reading was out of scale with respect to all other mRNAs, and for this reason was left out of the figure to optimize its graphical outlook.

A second validation approach consisted in verifying whether the biological information suggested by an increased, or decreased, expression of estrogen-regulated genes reflects known effects of these hormones in BC cells. To this aim, a functional classification of the genes from Fig. 2b was carried out whenever possible, starting from entries in publicly available databases on gene function and is reported in Table 2. Focusing on the 22 genes known to be involved in cell cycle control or progression, all but one of them code for either positive regulators of cell cycle progression or for components of the cell cycle machinery and are activated by the hormone. On the other hand, *Cyclin G2*, the only gene in this group to be down-regulated by estrogen, encodes a cell cycle inhibitor (Blondel & Mann 1996, Horne *et al.* 1997). Furthermore, the timed expression changes observed for these genes in hormone-stimulated cells are in agreement with their pattern of response to mitogens in BC or other cell types and the known roles of their products in defined cell cycle phases (see also below). This may be considered to represent a 'functional' validation of the microarray results for these genes, as it agrees with the fact that estrogens are potent mitogens, which stimulate cell cycle progression in ZR-75.1 cells (Fig. 1). A second functional group which was investigated in more detail is represented by genes involved in programmed cell death, or apoptosis, which is known to be prevented by estrogen in different cell types, including BC cells where these steroids act as survival factors (Kyprianou *et al.* 1991). Microarray analysis highlighted 11 genes directly involved in apoptotic processes (Tables 1

and 2). Two of them encode an apoptosis inducer (*Bcl2-antagonist/killer1/Bak1*) and a pro-apoptotic nuclear receptor (*Retinoid X receptor-alpha*) and are inhibited by the hormone. On the other hand, 6 genes which are activated by E2 (*Heat shock 70 kDa protein 10/HSC71*, *Heat shock 70 kDa protein 9B/mortalin 2*, *Glutathione transferase omega*, *HS1 binding protein*, *Immediate early response 3* and *Baculovirus IAP repeat-containing 5/survivin*) encode well known anti-apoptotic proteins. In the case of the apoptosis-related *Bcl2-associated athanogene/Bag1* gene, evaluation of the biological significance of its regulation by estrogen is made difficult by the existence of alternatively spliced mRNA variants in ZR-75.1 cells. These derive from a single transcription unit, give rise to proteins with divergent biological activities (Kudoh *et al.* 2002), but are indistinguishable by the microarray mRNA analysis carried out here. Hormonal regulation of the *Bcl2* partners *Bak1* and *Bag1* and of *Survivin* and *XIAP associated factor-1*, belonging respectively to the *Bcl2* and *IAP* pathways, suggests that these two functionally related genetic pathways could mediate some of the effects of estrogen on programmed cell death in BC cells. This possibility, suggested by the microarray results, was investigated by determining mRNA expression and hormonal regulation of other, related members of both these gene families in ZR-75.1 cells. The results of mRNA quantitations carried out by multi-probe RPAs are shown in Fig. 3b, where the data relative to *Bcl2* and its partners are reported on the left, and those related to *IAP* family genes are reported on the right. The results show that changes in *Bak1* mRNA expression detected by RPA (Fig. 3b-c), rtPCR (Fig. 3a) and microarrays (Fig. 2b) are consistent. Furthermore, they provide evidence of estrogen-induced inhibition of the pro-apoptotic *Bik* (*Bcl2-inhibitor killer*) gene and activation of the anti-apoptotic *Bcl2*, known to be a direct target for transcriptional regulation by ER- α in MCF-7 cells

Figure 2 (overleaf) Summary of gene expression changes detected in hormone-stimulated ZR-75.1 cells by cDNA microarray analysis. (a) Graphic representation of the 8 different gene expression clusters identified. The differential expression value (fluorescence reading of labeled cDNA from +E2 cells vs the same from -E2 cells) represents, in each case, the average value for all the genes of the cluster, as detected in multiple measurements. (b) Cluster image showing the 344 mRNAs that showed significant changes of expression in response to estrogen stimulation. The genes were clustered hierarchically into groups (clusters 1 to 8) on the basis of the similarity of their expression profiles, as described in the Materials and methods section. The expression pattern of each gene is displayed as a horizontal strip. For each gene, the ratio of mRNA levels in cells at the indicated time after estrogen stimulation vs the same in estrogen-deprived (time 0) cells is represented according to the scale reported at the bottom-right of the figure.

Table 2 Functional classification of the estrogen (E) regulated genes identified in ZR-75.1 human breast cancer cells by gene expression profiling with cDNA microarrays according to their predominant functional role and/or to known activities of the products they encode

Function ^a	Number of E-regulated genes
Signal transduction	25
Cell cycle	22
DNA replication and repair	13
Cell death/apoptosis	11
Cell communication, adhesion or motility	19
Gene transcription	31
Messenger RNA processing	12
RNA splicing	6
Ribosome biogenesis	7
Protein biosynthesis	10
Protein folding/heat shock response	16
Protein degradation	8
Transporter	25
Electron transport and oxidative phosphorylation	4
Citrate metabolism	2
Glycid metabolism	10
Lipid metabolism	3
Low-density lipoprotein metabolism	1
Sterol metabolism	5
Xenobiotic metabolism	2
Folate metabolism	2
Purine/pyrimidine metabolism	10
Enzyme (other than above)	13
Ornithine decarboxylase inhibitor	1
Protease inhibitor	2
RNAse L inhibitor	1
Heavy metals binding	1
Hormone	1
Oxidative stress	1
(Expressed in breast cancer) ^b	2
Not defined ^c	78

^aA functional category was assigned to each gene according to Gene Ontology guidelines from LocusLink and/or GeneCards;

^binclude: 'Trefoil factor 1/pS2' and 'Tumor protein D53-like 1/hD53' genes; ^cinclude both poorly characterized and unnamed (ESTs) genes.

LDL; ODC,.

(Perillo *et al.* 2000). Similarly, in the case of the *IAP* pathway *XIAP*, *cIAP1* and of the *IAP*-associated tumor necrosis factor receptor-associated factors *Traf2* and *Traf3* genes are all activated (right image of Fig. 3b). These data, when combined, support the anti-apoptotic effects of the hormone and can thus be considered a further functional confirmation of the indications provided by the microarray results reported in Fig. 2b.

The third validation approach consisted in searching for entries matching genes identified in the present study within publicly available sets of estrogen-regulated genes identified by genome-wide expression profiling in hormone-responsive

BC cells. The datasets considered included gene expression data from microarray (Finlin *et al.* 2001, Bouras *et al.* 2002, Inoue *et al.* 2002, Lobenhofer *et al.* 2002, Hodges *et al.* 2003) and SAGE (Charpentier *et al.* 2000, Seth *et al.* 2002) analyses carried out in E2-stimulated ZR-75.1 or MCF-7 cells. Alignment of microarray and SAGE data obtained in the two cell lines was possible here since they show comparable responses to estrogen *in vitro* (Cicatiello *et al.* 2000). Furthermore, similar experimental conditions, including conditions for hormone deprivation and stimulation, were applied in all cases considered. In the case of SAGE data, only mRNAs represented by ≥ 60 tags, following

normalization, in either of the matched SAGE libraries were considered for comparison; a transcript scored positive when it showed $\geq$ twofold change in hormone treated vs control cells. The results of these comparative analyses are summarized in the 6th and 7th columns of Table 1. In ZR-75.1 cells, 107 mRNAs from the SAGE libraries were found to match genes from the microarray set of Fig. 2b, including 48 transcripts showing $\geq$ twofold change in expression following cell exposure to the hormone. Concerning the MCF-7 cell SAGE libraries, 116 matches with estrogen-regulated transcripts from Table 1 were found, including 55 mRNAs whose expression changed significantly in response to estrogen also under these conditions. On the other hand, analysis of the microarray data from MCF-7 cells provided confirmation of the expression changes reported in Fig. 2b for 206 genes. These include 76 genes whose hormone responsiveness was confirmed in two or more independent microarray tests and 33 genes which showed a similar response to the hormone in this same cell line also by SAGE (Table 1). When combined, the results of the different tests and comparative analyses described above indicate that estrogen responsiveness appears reproducible for at least 228 genes among those listed in Fig. 2b and Table 1 ($\sim 2/3$). Hormonal regulation was evident for 148 such genes in two or more experimental settings and, for 45 of them, in two BC cell lines. Considering the latter results, and the fact that direct mRNA measurements performed with 'real time' rtPCR yielded $>94\%$ confirmations (Fig. 3), we can reasonably assume that the vast majority of the changes in gene expression observed in this study reflect true actions of estrogen on the transcriptome of hormone-responsive BC cells.

Comparative analysis of the gene expression changes induced by estrogen and serum growth factors during mitogenic stimulation of human cells

Given the stimulatory effects of estrogen on cell cycle progression of ZR-75.1 cells, it is likely that some of the gene responses identified in this study are part of the automatic cellular responses of human cells to mitogens. These, occurring in all cells independent of the nature of the stimulus, represent the so called cell cycle 'clock'. To verify this possibility, and to highlight at the same time potential estrogen and/or BC cell-specific gene responses, the hormone-induced gene expression program was compared with that identified in serum-stimulated human fibroblasts (Iyer *et al.* 1999). Comparison was made possible since serum stimulation of fibroblasts and estrogen stimulation of ZR-75.1 cells were carried out at comparable time points and the experimental design, including the cDNA microarray platform, was similar. Data were compared for identical time points, from 0 to 24 h of stimulation in each case, and genes were matched in the two databases by name, UniGene cluster and GB Accession number. One hundred and forty-eight estrogen-regulated genes were found in the serum-stimulated fibroblasts database and their expression profiles were compared in the two stimulated cell types by calculating, in each case, the Pearson's correlation coefficient, accompanied by manual inspection of the data for further confirmation. Fifty-two such genes displayed similar expression profiles in both cell lines (Pearson's >0.40 ; Table 3), suggesting that they are likely to be part of a cell type-independent genomic response to extracellular stimuli and/or that, at least in some cases, they reflect similarities

Figure 3 Experimental validation of the microarray results. (a) Real time, fluorogenic quantitative rtPCR assays were carried out as described in the Materials and methods section. Average PCR data were plotted in each case relative to the values detected in RNA from estrogen-deprived cells (time 0). Genes are grouped according to clusters (from top: clusters 1 to 3, clusters 4 to 6, cluster 7 and cluster 8) and microarray results (left) are reported side-by-side with the corresponding rtPCR results (right), to allow direct comparisons. (b) RPAs (RNase protection assays) were carried out in the same RNA samples as described in the Materials and methods section. For details concerning gene names, see the text. L32 and GAPDH are internal controls, as reported in the legend to Fig. 1. Bars mark regulated genes, the gray bar indicates that *BAK1* mRNA showed comparable changes by cDNA microarray analysis. (c) Graphic representation of data from apoptosis-related gene expression monitoring by RPA. Average of results from three independent measurements are reported only for regulated mRNAs and for L32 control mRNA, following normalization with respect to GAPDH mRNA levels in each lane.

Table 3 List of genes characterized by similar expression profiles in estrogen-stimulated ZR-75-1 cells and serum stimulated human fibroblasts^a

Symbol	UniGene ^b	GenBank ^c	Gene description	Cluster ^d	Pearson's ^e	Function ^f
<i>CCND1</i>	Hs.82932	X59798	Cyclin D1 (PRAD1: parathyroid adenomatosis 1)	5	0.50	Cell cycle
<i>PA2G4</i>	Hs.343258	AF104670	Proliferation-associated 2G4, 38kDa	7	0.64	Cell cycle
<i>RANBP1</i>	Hs.24763	AI160997	RAN binding protein 1	7	0.94	Cell cycle
<i>MAD2L1</i>	Hs.79078	AJ000186	MAD2 mitotic arrest deficient-like 1 (yeast)	8	0.78	Cell cycle
<i>KSK2</i>	Hs.83758	X54942	CDC28 protein kinase 2	8	0.76	Cell cycle
<i>RAN</i>	Hs.10842	AF054183	RAN, member RAS oncogene family	8	0.75	Cell cycle
<i>IER3</i>	Hs.76095	AI185199	Immediate early response 3	4	0.91	Cell death
<i>MCM2</i>	Hs.57101	AI814040	MCM2 minichromosome maintenance deficient 2, mitotin (<i>S. cerevisiae</i>)	7	0.54	DNA replication and repair
<i>MCM7</i>	Hs.77152	D55716	MCM7 minichromosome maintenance deficient 7 (<i>S. cerevisiae</i>)	7	0.72	DNA replication and repair
<i>FEN1</i>	Hs.4756	X76771	Flap structure-specific endonuclease 1	7	0.77	DNA replication and repair
<i>FLJ32241</i>	Hs.352359	AA948729	<i>Homo sapiens</i> cDNA FLJ32241 fis, clone PLACE6005231	8	0.78	DNA replication and repair
<i>PFKP</i>	Hs.99910	D25328	Phosphofructokinase, platelet	7	0.77	Glycid metabolism
<i>LDHA</i>	Hs.2795	X02152	Lactate dehydrogenase A	7	0.78	Glycid metabolism
<i>MT1L</i>	Hs.94360	F27787	Metallothionein 1L	8	0.71	Heavy metal binding
<i>HNRP2B1</i>	Hs.232400	D28877	Heterogeneous nuclear ribonucleoprotein A2/B1	6	0.93	mRNA processing
<i>NME2</i>	Hs.275163	L16785	Non-metastatic cells 2, protein (NM23B) expressed in	8	0.81	Nucleotide metabolism
<i>IDS</i>	Hs.172458	AF050145	Iduronate 2-sulfatase (Hunter syndrome)	3	0.66	Other enzymes
<i>AMD1</i>	Hs.262476	M21154	S-adenosylmethionine decarboxylase 1	7	0.72	Other enzymes
<i>PLOD2</i>	Hs.41270	U84573	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase (lysine hydroxylase) 2	6	0.55	Other enzymes
<i>E1F3S9</i>	Hs.57783	U78525	Eukaryotic translation initiation factor 3, subunit 9 (eta, 116kDa)	5	0.56	Protein biosynthesis
<i>HSPA5</i>	Hs.75410	AI878886	Heat shock 70kD protein 5 (glucose-regulated protein, 78kDa)	5	0.72	Protein folding
<i>DNAJA1</i>	Hs.94	L08069	DnaJ (Hsp40) homolog, subfamily A, member 1	6	0.62	Protein folding
<i>HSPCB</i>	Hs.74335	M16660	Heat shock 90kDa protein 1, beta	6	0.66	Protein folding
<i>STIP1</i>	Hs.75612	M86752	Stress-induced-phosphoprotein 1 (Hsp70/Hsp90-organizing protein)	7	0.92	Protein folding
<i>FKBP4</i>	Hs.848	M88279	FK506 binding protein 4 (59kDa)	7	0.78	Protein folding
<i>CCT5</i>	Hs.1600	D43950	CCT5 Chaperonin containing TCP1, subunit 5 (epsilon)	8	0.41	Protein folding
<i>PPIB</i>	Hs.699	M63573	Peptidylprolyl isomerase B (cyclophilin B)	8	0.89	Protein folding
<i>HSPD1</i>	Hs.79037	M34664	Heat shock 60kDa protein 1 (chaperonin)	6	0.59	Protein folding
<i>NP</i>	Hs.75514	AA311617	Nucleoside phosphorylase	7	0.82	Purine and pyrimidine metabolism
<i>RRM1</i>	Hs.2934	X59543	Ribonucleotide reductase M1 polypeptide	7	0.88	Purine and pyrimidine metabolism

Table 3 Continued

Symbol Cont	UniGene ^b	GenBank ^c	Gene description	Cluster ^d	Pearson's ^e	Function ^f
<i>IMPDH2</i>	Hs.75432	L33842	IMP (inosine monophosphate) dehydrogenase 2	8	0.63	Purine and pyrimidine metabolism
<i>DKC1</i>	Hs.4747	U59151	Dyskeratosis congenita 1, dyskerin	7	0.67	Ribosome biogenesis
<i>COX5A</i>	Hs.296585	Y12065	Cytochrome c oxidase subunit Va	7	0.86	Ribosome biogenesis
<i>SFRS1</i>	Hs.352256	M72709	Splicing factor, arginine/serine-rich 1 (splicing factor 2, alternate splicing factor)	7	0.65	RNA splicing
<i>GNL1</i>	Hs.83147	L25665	Guanine nucleotide binding protein-like 1	7	0.45	Signal transduction
<i>HBP1</i>	Hs.10882	AF019214	HMG-box containing protein 1	1	0.89	Transcription
<i>EGR1</i>	Hs.326035	M80583	Early growth response 1	4	0.46	Transcription
<i>FOS</i>	Hs.25647	V01512	V-fos FBJ murine osteosarcoma viral oncogene homolog	4	0.45	Transcription
<i>MYC</i>	Hs.79070	K02276	V-myc myelocytomatosis viral oncogene homolog (avian)	4	0.42	Transcription
<i>HAT1</i>	Hs.13340	AF030424	HAT1 Histone acetyltransferase 1	6	0.46	Transcription
<i>NCL</i>	Hs.79110	M60858	Nucleolin	6	0.72	Transcription
<i>ATP9A</i>	Hs.70604	AA620508	ATPase, Class II, type 9A	3	0.52	Transporters
<i>APOD</i>	Hs.75736	J02611	Apolipoprotein D	3	0.88	Transporters
<i>SEC23B</i>	Hs.173497	X97065	Sec23 homolog B (<i>S. cerevisiae</i>)	7	0.54	Transporters
<i>SLC25A5</i>	Hs.79172	AA314562	Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5	8	0.85	Transporters
<i>SEC61B</i>	Hs.77028	A1281953	Protein translocation complex beta	8	0.46	Transporters
<i>NOLC1</i>	Hs.75337	Z34289	Nucleolar and coiled-body phosphoprotein 1	5	0.71	Unknown
<i>C1QBP</i>	Hs.78614	L04636	Complement component 1, q subcomponent binding protein	7	0.77	Unknown
<i>KIAA0321</i>	Hs.8663	AB002319	KIAA0321 protein	1	0.45	Unnamed
<i>MGC19606</i>	Hs.91579	AF054996	MGC19606 Similar to hypothetical 34.0 kDa protein ZK795.3 in chromosome IV	7	0.68	Unnamed
<i>ESTs R99207</i>	Hs.36102	R99207	ESTs, Highly similar to SMHU1B metallothionein 1B (<i>H. sapiens</i>)	8	0.65	Unnamed
<i>KIAA0074</i>	Hs.1192	D38553	KIAA0074 protein	8	0.71	Unnamed

^aThe expression data from serum-stimulated fibroblasts were from Iyer *et al.* (1999) (<http://genome-www.stanford.edu/serum/>); ^bUniGene cluster number; ^cGenBank Accession number; ^destrogen-responsive gene cluster (Fig. 2); ^ePearson's correlation coefficient, describing the degree of correlation between expression profiles in the two mitogen-stimulated cell lines within the first 24 h of stimulation. A threshold value of 0.40 was selected arbitrarily, following visual inspection of the data; ^ffunction assigned according to Gene Ontology categories from LocusLink and/or GeneCards databases.

between signaling by serum factors and estrogen. This last possibility is suggested by a number of reports indicating 'non-genomic' actions of estrogen in target cells leading to direct hormonal regulation of multiple cytoplasmic signal transduction pathways (reviewed by Kelly & Levin 2001). In agreement with this last possibility, the 'immediately-early' *EGR1* (*early growth response 1*) gene, which is among the genes showing comparable changes in BC cells and fibroblasts (Table 3), has been reported to be activated by estrogens in cardiomyocytes via serum-responsive DNA elements present in its promoter (de Jager *et al.* 2001). On the other hand, genome-scale gene expression analysis in growth factor-stimulated cells has revealed that diverse signaling pathways activated by different mitogen receptors induce broadly overlapping, rather than independent, sets of genes (Fambrough *et al.* 1999). This suggests that individual signal transduction pathways evoke in a given cell redundant patterns of gene expression, which are independent from the nature of the signaling pathway involved (Pawson & Saxton 1999). Indeed, some of the genes co-regulated by estrogens and serum mitogens (Table 3) belong to the recently identified transcriptional program of the cell division cycle of human cancer cells, which includes genes undergoing automatic, periodic changes in expression linked to specific cell cycle phases (see below). On the other hand, eighteen genes among those common to the two datasets compared show opposite changes in BC vs fibroblasts (Pearson's < -0.40 ; Table 4), while the remaining seventy eight estrogen targets are unresponsive to serum in fibroblasts (Table 5). These two gene sets are more likely to include hormone and/or BC-specific genomic targets, used to understand the nature of specific estrogen effects in BC cells.

Identification of the estrogen-regulated genes which belong to the transcriptional program of the cell division cycle

Assignment of genes to known genetic programs, such as, for example, cell regulatory circuits and metabolic or functional pathways, may greatly help the functional interpretation of genome-wide gene expression profiling data (Ashburner *et al.* 2000). In the case of the gene expression changes induced by estrogen this analysis is particularly important, since it may help provide logical frameworks to highlight key aspects of hormone-responsive BC

cell biology and to elucidate the mechanism of action of hormones and antihormones.

As an initial step in this direction, each gene was assigned, where possible, to a functional category according to the Gene Ontology Consortium guidelines (last column of Table 1). One of the observations made possible by this analysis is that a significant number of estrogen-regulated genes appear to relate to functions associated with the cell division cycle. This led us to pursue our quest for gene expression changes which may represent predominantly cell cycle responses to the mitogenic stimulus exerted by E2, and for this reason are less likely to include estrogen- or BC-specific gene responses. With this in mind, we searched within our list of estrogen-responsive genes for members of the recently identified cell cycle transcriptional program of human cells (Whitfield *et al.* 2002). This analysis yielded the 51 estrogen-responsive genes listed in Table 6, which are among those characterized by periodic expression during the mitotic cycle in synchronized cells. For twenty-four such genes, all activated by the hormone, the inferred function supports their inclusion in this category. Indeed, sixteen of them encode proteins functionally involved in cell cycle phase control/accomplishment, seven are involved in DNA replication and repair and one in nucleotide metabolism, while two encode for tubulins (alpha 1 and beta). The functions of six other genes are unknown, while those of the remaining nineteen, suggesting their involvement in gene transcription, mRNA processing, splicing and transport, ribosome biogenesis, protein folding and degradation or metabolism, are compatible with a role of these gene products during cell cycle progression. It is worth mentioning that ten genes among those listed in Table 6 (i.e. *CDC28 protein kinase 2/CKS2*, *Cyclin D1/PRAD1*, *Dyskeratosis congenita 1-dyskerin/DKC1*, *Flap structure-specific endonuclease 1/FEN1*, *Member of the RAS oncogene family/RAN*, *Minichromosome maintenance deficient 2-mitotin/MCM2*, *Mitotic arrest deficient-like 1/MAD2 L1*, *Ribonucleotide reductase M1 polypeptide/RRM1*, *S-adenosylmethionine decarboxylase 1/AMD1* and *KIAA0074 protein*) show comparable expression patterns in estrogen-stimulated ZR-75.1 cells and serum-stimulated fibroblasts (see Table 3). This finding supports the view that regulation of these genes in estrogen-stimulated cells is part of the anabolic

Table 4 List of genes characterized by divergent expression profiles in estrogen-stimulated ZR-75.1 cells and serum stimulated human fibroblasts^a

Symbol	UniGene ^b	GenBank ^c	Gene description	Cluster ^d	Pearson's ^e	Function ^f
<i>CLDN4</i>	Hs.5372	AB000712	Claudin 4	2	-0.40	Cell communication, adhesion or motility
<i>ITGAE</i>	Hs.851	AA425588	Integrin, alpha E (antigen CD103, human mucosal lymphocyte antigen 1; alpha polypeptide)	8	-0.88	Cell communication, adhesion or motility
<i>BAK1</i>	Hs.93213	A1741331	BCL2-antagonist/killer 1	1	-0.41	Cell death
<i>ME1</i>	Hs.14732	X77244	Malic enzyme 1, NADP(+)-dependent, cytosolic	8	-0.62	Citrate metabolism
<i>TPD52L1</i>	Hs.16611	U44427	Tumor protein D52-like 1	7	-0.82	Expressed in breast cancer
<i>G6PD</i>	Hs.80206	M12996	Glucose-6-phosphate dehydrogenase	7	-0.78	Glycid metabolism
<i>PCK2</i>	Hs.75812	X92720	Phosphoenolpyruvate carboxykinase 2 (mitochondrial)	7	-0.46	Glycid metabolism
<i>TKT</i>	Hs.89643	A1378884	Transketolase (Wernicke-Korsakoff syndrome)	8	-0.50	Glycid metabolism
<i>CTSD</i>	Hs.343475	M63138	Cathepsin D (lysosomal aspartyl protease)	7	-0.51	Other enzymes
<i>WARS</i>	Hs.82030	X62570	Tryptophanyl-tRNA synthetase	7	-0.90	Protein biosynthesis
<i>AKAP1</i>	Hs.78921	AA868550	A kinase (PRKA) anchor protein 1	5	-0.72	Signal transduction
<i>SQLE</i>	Hs.71465	AF098865	Squalene epoxidase	7	-0.87	Sterol metabolism
<i>HMGCR</i>	Hs.11899	AA602498	3-Hydroxy-3-methylglutaryl-Coenzyme A reductase	8	-0.73	Sterol metabolism
<i>FDFT1</i>	Hs.48876	X69141	Farnesyl-diphosphate farnesyltransferase 1	8	-0.89	Sterol metabolism
<i>RXRA</i>	Hs.20084	AA865269	Retinoid X receptor, alpha	1	-0.47	Transcription
<i>BLVRB</i>	Hs.76289	AA280097	Biliverdin reductase B (flavin reductase (NADPH))	7	-0.57	Xenobiotic metabolism
<i>PCM1</i>	Hs.75737	H00568	Pericentriolar material 1	1	-0.53	Unknown
<i>MGC11034</i>	Hs.103378	AA775792	Hypothetical protein MGC11034	2	-0.57	Unnamed

^aThe expression data from serum-stimulated fibroblasts were from Iyer *et al.* 1999 (<http://genome-www.stanford.edu/serum/>); ^bUniGene cluster number; ^cGenBank Accession number; ^destrogen-responsive gene cluster (Fig. 2); ^ePearson's correlation coefficient, calculated as described in the legend of Table 3. A threshold value of -0.40 was selected arbitrarily, following visual inspection of the data; ^ffunction assigned according to Gene Ontology categories from LocusLink and/or GeneCards databases.

Table 5 List of estrogen-regulated genes which were found unresponsive to serum stimulation in human fibroblasts by cDNA microarray gene expression analysis^a

	UniGene ^b	GenBank ^c	Gene description	Cluster ^d	Function ^f
Symbol					
<i>MYH11</i>	Hs.78344	AF001548	Myosin, heavy polypeptide 11, smooth muscle	4	Cell communication, adhesion or motility
<i>ITGB4BP</i>	Hs.5215	AF047433	Integrin beta 4 binding protein	7	Cell communication, adhesion or motility
<i>TMSB10</i>	Hs.76293	AI679377	Thymosin, beta 10	3	Cell communication, adhesion or motility
<i>MUC1</i>	Hs.89603	AI922289	Mucin 1, transmembrane	2	Cell communication, adhesion or motility
<i>TPBG</i>	Hs.82128	AJ012159	Trophoblast glycoprotein	7	Cell communication, adhesion or motility
<i>PGM5</i>	Hs.167473	L40933	Phosphoglucosmutase 5	2	Cell communication, adhesion or motility
<i>TUBG1</i>	Hs.21635	M61764	Tubulin, gamma 1	7	Cell communication, adhesion or motility
<i>ACTG1</i>	Hs.14376	X04098	Actin, gamma 1	5	Cell communication, adhesion or motility
<i>ACTN1</i>	Hs.119000	X15804	Actinin, alpha 1	5	Cell communication, adhesion or motility
<i>SKB1</i>	Hs.12912	AA152011	SKB1 homolog (<i>S. pombe</i>)	7	Cell cycle
<i>CCNG2</i>	Hs.79069	AI271688	Cyclin G2	1	Cell cycle
<i>D123</i>	Hs.82043	U27112	D123 gene product	8	Cell cycle
<i>BAG1</i>	Hs.41714	AF022224	BCL2-associated athanogene	3	Cell death
<i>HSPA9B</i>	Hs.3069	L15189	Heat shock 70 kDa protein 9B (mortalin-2)	6	Cell death
<i>ACLY</i>	Hs.174140	X64330	ATP citrate lyase	8	Citrate metabolism
<i>RPA3</i>	Hs.1608	AA461254	Replication protein A3 (14 kDa)	8	DNA replication and repair
<i>TSN</i>	Hs.75066	X78627	Translin	7	DNA replication and repair
<i>UNG</i>	Hs.78853	Y09008	Uracil-DNA glycosylase	7	DNA replication and repair
<i>ATP5G3</i>	Hs.429	U09813	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit c (subunit 9) isoform 3	7	Electron transport and oxidative phosphorylation
<i>ATP5L</i>	Hs.107476	W94335	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit g	8	Electron transport and oxidative phosphorylation
<i>PKM2</i>	Hs.198281	AW007619	Pyruvate kinase, muscle	8	Glycid metabolism
<i>PGAM1</i>	Hs.181013	J04173	Phosphoglycerate mutase 1 (brain)	6	Glycid metabolism
<i>SORD</i>	Hs.878	U07361	Sorbitol dehydrogenase	8	Glycid metabolism
<i>TPI1</i>	Hs.83848	X69723	Triosephosphate isomerase 1	7	Glycid metabolism
<i>PTDSS1</i>	Hs.77329	D14694	Phosphatidylserine synthase 1	7	Lipid metabolism
<i>CPT2</i>	Hs.274336	U09648	Carnitine palmitoyltransferase II	3	Lipid metabolism
<i>SNRPB</i>	Hs.83753	AA313910	Small nuclear ribonucleoprotein polypeptides B and B1	8	mRNA processing
<i>HNRPR</i>	Hs.15265	AF000364	Heterogeneous nuclear ribonucleoprotein R	7	mRNA processing
<i>SNRPE</i>	Hs.334612	F20803	Small nuclear ribonucleoprotein polypeptide E	8	mRNA processing
<i>MST4</i>	Hs.23643	AA191319	Mst3 and SOK1-related kinase	6	Other enzymes
<i>GOT2</i>	Hs.170197	M22632	Glutamic-oxaloacetic transaminase 2, mitochondrial (aspartate aminotransferase 2)	7	Other enzymes
<i>SHMT2</i>	Hs.75069	U23143	Serine hydroxymethyltransferase 2 (mitochondrial)	7	Other enzymes

Table 5 Continued

	UniGene ^b	GenBank ^c	Gene description	Cluster ^d	Function ^f
Symbol Cont					
<i>SERPINB6</i>	Hs.41072	S69272	Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 6	7	Protease inhibitors
<i>SERPINA5</i>	Hs.76353	U35464	Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5	7	Protease inhibitors
<i>EIF2S1</i>	Hs.151777	AA306546	Eukaryotic translation initiation factor 2, subunit 1 (alpha, 35 kDa)	7	Protein biosynthesis
<i>EIF4A1</i>	Hs.129673	AI755111	Eukaryotic translation initiation factor 4A, isoform 1	8	Protein biosynthesis
<i>PSMD8</i>	Hs.78466	AI769251	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 8	8	Protein degradation
<i>CCT3</i>	Hs.1708	AI246667	Chaperonin containing TCP1, subunit 3 (gamma)	8	Protein folding
<i>HSP70-1</i>	Hs.8997	M59828	Human MHC class III HSP70-1 gene (HLA), complete cds	5	Protein folding
<i>FKBP5</i>	Hs.7557	U71321	FK506 binding protein 5	7	Protein folding
<i>TRA1</i>	Hs.82689	X15187	Tumor rejection antigen (gp96) 1	8	Protein folding
<i>PMSCL2</i>	Hs.75584	X66113	Polymyositis/scleroderma autoantigen 2 (100 kDa)	7	Protein kinase
<i>UMPK</i>	Hs.75939	AA468735	Uridine monophosphate kinase	7	Purine and pyrimidine metabolism
<i>DTYMK</i>	Hs.79006	L16991	Deoxythymidylate kinase (thymidylate kinase)	8	Purine and pyrimidine metabolism
<i>DUT</i>	Hs.367676	R77436	DUTP pyrophosphatase	8	Purine and pyrimidine metabolism
<i>MRPL3</i>	Hs.79086	X06323	Mitochondrial ribosomal protein L3	7	Ribosome biogenesis
<i>HSU53209</i>	Hs.24937	U53209	Transformer-2 alpha (htra-2 alpha)	7	RNA splicing
<i>SFRS10</i>	Hs.30035	U61267	Splicing factor, arginine/serine-rich 10 (transformer 2 homolog, <i>Drosophila</i>)	7	RNA splicing
<i>ABCE1</i>	Hs.12013	X76388	ATP-binding cassette, sub-family E (OABP), member 1	6	RNAse L inhibitor
<i>ADCY3</i>	Hs.8402	AB011083	Adenylate cyclase 3	7	Signal transduction
<i>MTM1</i>	Hs.75302	AF020676	Myotubular myopathy 1	7	Signal transduction
<i>CEACAM6</i>	Hs.73848	AI677998	Carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen)	1	Signal transduction
<i>RAP1A</i>	Hs.865	AL049557	RAP1A, member of RAS oncogene family	8	Signal transduction
<i>IGFBP5</i>	Hs.180324	L27560	Insulin-like growth factor binding protein 5	2	Signal transduction
<i>IGFBP4</i>	Hs.1516	M62403	Insulin-like growth factor binding protein 4	5	Signal transduction
<i>NMA</i>	Hs.78776	U23070	Putative transmembrane protein	2	Signal transduction
<i>CITED2</i>	Hs.82071	AF109161	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	2	Transcription
<i>GTF3A</i>	Hs.75113	AI686944	General transcription factor IIIA	7	Transcription
<i>ZNF33A</i>	Hs.70617	D31763	Zinc finger protein 33a (KOX 31)	1	Transcription
<i>ISGF3G</i>	Hs.1706	M87503	Interferon-stimulated transcription factor 3, gamma (48 kDa)	2	Transcription

Table 5 Continued

	UniGene ^b	GenBank ^c	Gene description	Cluster ^d	Function ^f
Symbol Cont					
MYB	Hs.1334	U22376	V-myb myeloblastosis viral oncogene homolog (avian)	7	Transcription
SLC3A2	Hs.79748	AB018010	Solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	7	Transporters
ABCG1	Hs.10237	AF038175	ATP-binding cassette, sub-family G (WHITE), member 1	7	Transporters
LTF	Hs.105938	M73700	Lactotransferrin	7	Transporters
COPA	Hs.75887	U24105	Coatomer protein complex, subunit alpha	1	Transporters
SLC12A2	Hs.110736	U30246	Solute carrier family 12 (sodium/potassium/chloride transporters), member 2	1	Transporters
ATP2A3	Hs.5541	Z69881	ATPase, Ca ⁺⁺ transporting, ubiquitous	1	Transporters
RNF3	Hs.8834	AA403225	Ring finger protein 3	7	Unknown
PWP2H	Hs.79380	AB001517	PWP2 periodic tryptophan protein homolog (yeast)	7	Unknown
MESDC2	Hs.78871	D42039	Mesoderm development candidate 2	8	Unknown
PMAIP1	Hs.96	D90070	Phorbol-12-myristate-13-acetate-induced protein 1	7	Unknown
HSU15552	Hs.85769	U15552	Acidic 82 kDa protein mRNA	3	Unknown
G1P3	Hs.265827	U22970	Interferon, alpha-inducible protein (clone IFI-6-16)	3	Unknown
FLJ20811	Hs.83530	AA015605	FLJ20811 Hypothetical protein	7	Unnamed
FLJ13187	Hs.29724	AA713516	FLJ13187 Phafin 2	1	Unnamed
C5orf8	Hs.75864	AI954986	Chromosome 5 open reading frame 8	8	Unnamed
KIAA0102	Hs.77665	AL047241	KIAA0102 gene product	8	Unnamed
KIAA0007	Hs.90315	D26488	KIAA0007 protein	7	Unnamed

^aThe expression data from serum-stimulated fibroblasts were from Iyer *et al.* (1999) (<http://genome-www.stanford.edu/serum/>). Differential expression data were compared between the two experimental data sets by calculating the Pearson's correlation coefficient value for the data relative to the first 24 h of stimulation, as described in the legend of Table 3. The data were then further controlled by visual inspection; ^bUniGene cluster number; ^cGenBank Accession number; ^destrogen-responsive gene cluster (Fig. 2); ^efunction assigned according to Gene Ontology categories from LocusLink and/or GeneCards databases.

effect of these steroids on the cell cycle machinery.

In conclusion, the results of all these comparative analyses indicate that the gene expression program activated by estrogen in BC cells reflects more than a simple mitogenic response of the cell to the hormone, since as many as 293 among its genes do not appear to belong to the transcriptional program of the cell division cycle.

Analysis of the distribution of estrogen-regulated genes among chromosomes

Genome-wide analyses of human transcriptome maps consistently indicate that the genes expressed

in each given cell type often cluster in defined chromosomal regions, which have been called RIDGEs (regions of increased gene expression; Caron *et al.* 2001). Genes expressed in skeletal muscle, for example, have been found selectively concentrated in certain human chromosomes (Bortoluzzi *et al.* 1998). Similar evidence of a gene order in the human genome has also been provided by analysis of housekeeping genes (Lercher *et al.* 2002), which suggests how the expression neighborhood may serve to regulate the functions of these genes (Oliver *et al.* 2002). Similarly, Spellman and Rubin (2002) observed in *Drosophila* that neighboring genes are often expressed in similar patterns. Such a phenomenon is not limited to the genomes

Table 6 List of genes regulated by estrogen in ZR-75-1 cells which are characterized by periodic expression during the cell cycle in human cells

	Symbol	UniGene ^c	Gene description	Cluster ^d	Function ^e
Phase^b					
M/G1	<i>CCND1</i>	Hs.82932	Cyclin D1 (PRAD1: parathyroid adenomatosis 1)	5	Cell cycle
M/G1	<i>PRC1</i>	Hs.344037	Protein regulator of cytokinesis 1	6	Cell cycle
M/G1	<i>RAN</i>	Hs.10842	RAN, member RAS oncogene family	8	Cell cycle
M/G1	<i>NSAP1</i>	Hs.155489	NS1-associated protein 1	7	mRNA processing
M/G1	<i>AMD1</i>	Hs.262476	S-adenosylmethionine decarboxylase 1	7	Other enzymes
M/G1	<i>E2-EPF</i>	Hs.174070	Ubiquitin carrier protein	7	Protein degradation
M/G1	<i>HSPA8</i>	Hs.180414	Heat shock 70 kDa protein 8	5	Protein folding
M/G1	<i>DKC1</i>	Hs.4747	Dyskeratosis congenita 1, dyskerin	7	Ribosome biogenesis
M/G1	<i>PTTG1</i>	Hs.252587	Pituitary tumor-transforming 1	8	Signal transduction
M/G1	<i>HMGCR</i>	Hs.11899	3-Hydroxy-3-methylglutaryl-Coenzyme A reductase	8	Sterol metabolism
M/G1	<i>ILF2</i>	Hs.75117	Interleukin enhancer binding factor 2, 45 kDa	8	Transcription
M/G1	<i>NXF1</i>	Hs.323502	Nuclear RNA export factor 1	7	Transporters
M/G1	<i>KPNB1</i>	Hs.180446	Karyopherin (importin) beta 1	8	Transporters
G1/S	<i>MCM2</i>	Hs.57101	MCM2 minichromosome maintenance deficient 2, mitotin (<i>S. cerevisiae</i>)	7	DNA replication and repair
G1/S	<i>UNG</i>	Hs.78853	Uracil-DNA glycosylase	7	DNA replication and repair
G1/S	<i>SFRS7</i>	Hs.184167	<i>Homo sapiens</i> splicing factor, arginine/serine-rich 7 (SFRS7) gene, complete cds	5	RNA splicing
G1/S	<i>HSU53209</i>	Hs.24937	Transformer-2 alpha (htra-2 alpha)	7	RNA splicing
S	<i>EXO1</i>	Hs.47504	Exonuclease 1	6	DNA replication and repair
S	<i>FEN1</i>	Hs.4756	Flap structure-specific endonuclease 1	7	DNA replication and repair
S	<i>RAD51</i>	Hs.343807	RAD51 homolog (RecA homolog, <i>E. coli</i>) (<i>S. cerevisiae</i>)	8	DNA replication and repair
S	<i>RRM1</i>	Hs.2934	Ribonucleotide reductase M1 polypeptide	7	Purine and pyrimidine metabolism
S	<i>ZNF217</i>	Hs.155040	Zinc finger protein 217	1	Transcription
S	<i>ZWINT</i>	Hs.42650	ZW10 interactor	7	Unknown
S	<i>ESTs AL050331</i>	Hs.237225	<i>Homo sapiens</i> , clone MGC:24302 IMAGE:3996246, mRNA, complete cds (<i>hypothetical protein HT023</i>)	3	Unnamed
S	<i>KIAA0101</i>	Hs.81892	KIAA0101 gene product	8	Unnamed
G2	<i>MUC1</i>	Hs.89603	Mucin 1, transmembrane	2	Cell communication, adhesion or motility
G2	<i>TUBA1</i>	Hs.75318	Tubulin, alpha 1 (testis specific)	7	Cell communication, adhesion or motility
G2	<i>TUBB</i>	Hs.336780	Tubulin, beta polypeptide	7	Cell communication, adhesion or motility
G2	<i>STK12</i>	Hs.180655	Serine/threonine kinase 12	7	Cell cycle
G2	<i>KNSL4</i>	Hs.119324	Kinesin-like 4	8	Cell cycle
G2	<i>MAD2L1</i>	Hs.79078	MAD2 mitotic arrest deficient-like 1 (yeast)	8	Cell cycle
G2	<i>CDC25C</i>	Hs.656	Cell division cycle 25C	8	Cell cycle
G2	<i>PSMD11</i>	Hs.90744	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 11	8	Protein degradation
G2	<i>HMG2</i>	Hs.80684	High-mobility group (nonhistone chromosomal) protein 2	6	Transcription

Table 6 Continued

	Symbol	UniGene ^c	Gene description	Cluster ^d	Function ^e
Phase^b Cont					
G2	<i>KPNA2</i>	Hs.159557	Karyopherin alpha 2 (RAG cohort 1, importin alpha 1)	8	Transporters
G2	<i>TMPO</i>	Hs.11355	Thymopoietin	7	Unknown
G2	<i>KIAA0074</i>	Hs.1192	KIAA0074 protein	8	Unnamed
G2/M	<i>BUB1B</i>	Hs.36708	BUB1 budding uninhibited by benzimidazoles 1 homolog beta (yeast)	7	Cell cycle
G2/M	<i>CDC20</i>	Hs.82906	CDC20 cell division cycle 20 homolog (<i>S. cerevisiae</i>)	7	Cell cycle
G2/M	<i>CENPA</i>	Hs.1594	Centromere protein A (17 kDa)	7	Cell cycle
G2/M	<i>STK15</i>	Hs.250822	Serine/threonine kinase 15	8	Cell cycle
G2/M	<i>KNSL6</i>	Hs.69360	Kinesin-like 6 (mitotic centromere-associated kinesin)	8	Cell cycle
G2/M	<i>CKS2</i>	Hs.83758	CDC28 protein kinase 2	8	Cell cycle
G2/M	<i>PLK</i>	Hs.77597	Polo-like kinase (<i>Drosophila</i>)	8	Cell cycle
G2/M	<i>BIRC5</i>	Hs.1578	Baculoviral IAP repeat-containing 5 (survivin)	8	Cell death
G2/M	<i>TSN</i>	Hs.75066	Translin	7	DNA replication and repair
G2/M	<i>RAD51C</i>	Hs.11393	RAD51 homolog C (<i>S. cerevisiae</i>)	8	DNA replication and repair
G2/M	<i>SFPQ</i>	Hs.180610	Splicing factor proline/glutamine rich (polypyrimidine tract binding protein associated)	8	RNA splicing
G2/M	<i>TRIP13</i>	Hs.6566	Thyroid hormone receptor interactor 13	6	Transcription
G2/M	<i>FOXM1</i>	Hs.239	Forkhead box M1	8	Transcription
G2/M	<i>ANP32B</i>	Hs.84264	Acidic (leucine-rich) nuclear phosphoprotein 32 family, member B	8	Unknown

^aIdentification of genes periodically expressed during the cell cycle was according to the gene expression data of Whitfield *et al.* (2002) (<http://genome-www.stanford.edu/Human-CellCycle/HeLa/>); ^bcell cycle phase of maximum gene expression change in synchronously cycling HeLa cells (Whitfield *et al.* 2002); ^cUniGene cluster number; ^destrogen-responsive gene cluster (Fig. 2); ^efunction assigned according to Gene Ontology categories from LocusLink and/or GeneCards databases.

of higher eukaryotes, as it has also been observed in yeast cells, where genes with similar functions localize in adjacent positions along chromosomes (Cohen *et al.* 2000). These, and similar observations on locus control regions (LCRs) (Dekker 2003), point to the involvement of chromatin domains in the control of gene activity within genomic neighborhoods. At the same time, they suggest a higher order organization of genomes, according to which gene positioning in the chromosomes often reflects their expression in specific cell types, as well as their responsiveness to signals from the environment and the differentiation status of the cell. To our knowledge, this possibility has not been evaluated yet in the case of genes responsive to a hormone. We thus searched the cytogenetic location of each estrogen-regulated gene identified

here, and then asked whether hormone-responsive gene distribution among chromosomes showed any imbalance. The results of this analysis are summarized in Tables 7 and 8, which showed (i) the predicted number of genes/chromosome and the number of genes within the chip which could be assigned to a specific cytogenetic location, identified manually on genome browsers (Table 7) or automatically by the web-based Onto-Express V2 software (Draghici *et al.* 2003, Table 8); (ii) the chromosomal distribution of the 344 hormone-regulated genes, obtained as above; (iii) a comparison between the predicted distribution of these genes among chromosomes, by random partitioning based exclusively on chromosomal gene density, and that determined experimentally, including statistical evaluation of these data by the

Table 7 Chromosomal location of the estrogen responsive genes identified by genome-wide gene expression profiling in human breast cancer ZR-75.1 cells

Chromosome	Genome ^a	Analyzed ^b	Estrogen-regulated genes			
			A. Predicted ^c	B. Observed ^d	B/A ^e	χ^2 test ^f
1	3533	702	34.7	35	1.01	0.9613
2	2834	495	24.5	24	0.98	0.9188
3	2191	386	19.1	12	0.63	0.0957
4	1780	283	14.0	9	0.64	0.1703
5	2021	352	17.4	21	1.21	0.3785
6*	2106	351	17.4	26	1.49	0.0336
7	2073	349	17.3	14	0.81	0.4198
8	1604	251	12.4	12	0.97	0.9029
9	1582	262	13.0	12	0.92	0.7837
10	1654	261	12.9	17	1.32	0.2435
11	2221	388	19.2	16	0.83	0.4541
12	1855	399	19.7	23	1.17	0.4520
13	865	133	6.6	2	0.30	0.0670
14	1237	231	11.4	16	1.40	0.1656
15	1365	213	10.5	13	1.24	0.4368
16	1532	291	14.4	15	1.04	0.8708
17*	1731	391	19.3	33	1.71	0.0015
18	719	114	5.6	4	0.71	0.4786
19*	1934	404	20.0	10	0.50	0.0219
20	973	176	8.7	7	0.80	0.5527
21	457	84	4.2	4	0.95	0.9373
22	833	139	6.9	4	0.58	0.2604
X	1524	247	12.2	12	0.98	0.9482
Y	248	10	0.5	1	2.00	0.4613
U ^g	242	981	—	2	—	—
Total	39 114	7893	(342)	344	—	1.0000

^aGene assignment to specific chromosomes according to The Weizmann Institute's Unified Database for human genome mapping (http://bioinformatics.weizmann.ac.il/udb_25/); ^bgenes analyzed (spotted DNAs which pass QC tests during microarray fabrication and fluorescence image analysis); ^ccalculated considering the number of genes analyzed, their partitioning among chromosomes and the number of estrogen-regulated genes detected experimentally; ^dnumber of estrogen regulated genes per chromosome (according to GeneCards); ^eratio of the number of genes observed vs number of genes predicted; ^fprobability associated with χ^2 test (chromosomes where results suggest the highest probability of non-random density of estrogen-regulated genes are marked by asterisks); ^gchromosomal assignment uncertain.

χ^2 test (Table 7); (iv) significance analysis of the chromosomal distribution of estrogen-regulated genes among chromosomes, calculated by hypergeometric probability distribution (Table 8). When combined, the results of these studies show that hormone-responsive gene density is significantly higher in chromosomes 6 and 17 ($P=0.0342$ and 0.002 respectively) and lower in chromosome 19 ($P=0.0219$). This, which was confirmed by direct computation of data within each chromosome (Table 7) as well as by comparative evaluations across all chromosomes (Table 8), provides strong evidence supporting the possibility of a higher order organization of the hormone-regulated transcriptome within the genome. Accordingly, genes regulated either directly or indirectly by

estrogen in BC cells might cluster together in discrete chromosomal domains. It is worth mentioning that this is not unprecedented, as several examples of coordinated gene regulation within defined chromosomal domains are known. This is the case, for example, of the rRNA, histone, homeobox or globin gene clusters, where colinearity reflects important features of the physiological role of the genes involved and the genetic mechanisms allowing their regulation. Interestingly, expression of certain Hox gene clusters is controlled also by retinoids (Boncinelli *et al.* 1991), which – like estrogen – act via nuclear receptors (RARs and RXRs). A considerable fraction of the hormone-regulated genes identified here (close to 10% of the total) are localized on the relatively small

Table 8 Significance analysis of estrogen responsive gene distribution among chromosomes observed in ZR-75.1 cells^{a,b}

Chromosome	Genome	Analyzed genes ^c		Expressed genes ^d		Estrogen-regulated genes ^e					
		n (A)	A/T (%) ^f	n (E)	E/A (%) ^g	P-value	n (R)	R/A (%) ^h	P-value	R/E (%) ⁱ	P-value
1	4635	702	15.15	527	75.07	0.3937	33	4.70	0.3833	6.26	0.3136
2	3693	495	13.40	390	78.79	0.4986	22	4.44	0.4857	5.64	0.4131
3	2940	386	13.13	272	70.47	0.3192	10	2.59	0.0183	3.68	0.0584
4	2253	283	12.56	177	62.54	0.2264	8	2.83	0.1445	4.52	0.1638
5	2713	352	12.97	252	71.59	0.4252	20	5.68	0.0656	7.94	0.0880
6*	2833	351	12.39	267	76.07	0.4458	22	6.27	0.0238	8.24	0.0342
7	2771	349	12.59	266	76.22	0.3999	15	4.30	0.4377	5.64	0.4856
8	2167	251	11.58	190	75.70	0.3852	11	4.38	0.3803	5.79	0.3910
9	2060	262	12.72	202	77.10	0.3592	13	4.96	0.2775	6.44	0.2855
10	2378	261	10.98	197	75.48	0.4364	17	6.51	0.0824	8.63	0.4337
11	2858	388	13.58	292	75.26	0.4449	19	4.90	0.3302	6.51	0.2664
12	2354	399	16.95	298	74.69	0.4908	19	4.76	0.1646	6.38	0.2989
13	1355	133	9.82	88	66.17	0.2098	2	1.50	0.0752	2.27	0.0945
14	1603	231	14.41	178	77.06	0.4186	14	6.06	0.0359	7.87	0.0776
15	1597	213	13.34	156	73.24	0.4248	10	4.69	0.2844	6.41	0.3441
16	1916	291	15.19	235	80.76	0.4059	14	4.81	0.2475	5.96	0.3121
17*	2202	391	17.76	330	84.40	0.1981	30	7.67	0.0007	9.09	0.0022
18	1204	114	9.47	74	64.91	0.3366	3	2.63	0.2732	4.05	0.2987
19*	2361	404	17.11	334	82.67	0.2320	10	2.48	0.0576	2.99	0.0230
20	1276	176	13.79	133	75.57	0.4470	8	4.55	0.3438	6.02	0.3731
21	616	84	13.64	57	67.86	0.4839	4	4.76	0.4648	7.02	0.3055
22	1047	139	13.28	113	81.29	0.2757	3	2.16	0.1351	2.65	0.1183
X	2062	247	11.98	180	72.87	0.3602	12	4.86	0.3618	6.67	0.3139
Y	330	10	3.03	7	70.00	0.4291	2	20.00	—	28.57	—
U ^j	—	981	—	865	88.18	0.4237	23	2.34	0.1883	2.66	0.1642
Total	51 224	7893	15.41	6080	77.03	—	344	—	—	4.36	—

^aComparisons and statistical analyses performed with Onto-Express Version 2 microarray data analysis software (Draghici *et al.* 2003; <http://vortex.cs.wayne.edu:8080>);^bgene assignment to specific chromosomes performed automatically by Onto-Express software; ^canalyzed (spotted DNAs which passed QC tests during microarray fabrication and fluorescence image analysis); ^ddetectable by microarray hybridization (fluorescence signal/background ratio >3.0 and absolute fluorescence values above threshold); ^enumber of estrogen regulated genes per chromosome (according to Onto-Express); chromosomes where the number of the hormone regulated genes identified differs significantly from predictions based on random distribution modeling are marked by asterisks; ^fratio between the number of genes analyzed (A) and the total number of genes in the genome (T); ^gratio between the number of genes expressed in ZR-75.1 cells (E) and the total number of genes analyzed (A) and the ratio between the number of estrogen-regulated genes (R) and the total number of genes analyzed (A); ^hratio between the number of genes expressed in ZR-75.1 cells (E) and the total number of genes analyzed (A); ⁱratio between the number of genes of estrogen-regulated genes (R) and the number of genes expressed in ZR-75.1 cells (E); ^jchromosomal assignment uncertain.

Table 9 Distribution of estrogen (E) responsive genes from breast cancer cells within chromosome 17

Interval from pter (Mb)	No. of genes ^a n (T)	No. of E responsive genes ^b n (R)	R/T (%)	Cytogenetic ^a
0–4	104	4	3.85	17p13.1–p13.3
4–8	141	13	9.22	
8–12	74	5	6.76	
12–16	45	1	2.22	
16–20	114	3	2.63	
20–25	45	1	2.22	
centromere				
25–29	107	2	1.87	17q21.2–p21.3
29–33	49	3	6.12	
33–37	105	2	1.90	
37–41	179	4	2.23	
41–45	133	13	9.77	
45–49	112	5	4.46	
49–53	20	7	35.00	17q21.33
53–57	56	0	0.00	17q22–q23
57–61	60	7	11.67	
61–65	62	3	4.84	
65–69	55	3	5.45	
69–73	59	2	3.39	
73–77	117	5	4.27	
>77	94	12	12.77	17q25.2–q25.3
Total	1731	95	100.00	

^aFrom Ensembl and/or UCSC Golden Path databases; ^bresponse to estrogen in breast cancer cells known and/or assessed by microarray analysis.

chromosome 17, despite the fact that this carries only ~5% of the genes expressed at detectable levels in ZR-75.1 cells (Tables 7 and 8). Interestingly, this chromosome frequently shows gross abnormalities in BC (deletions and, more often, amplifications in 15 to 25% of the cases; Forozan *et al.* 2000), which are thought to be involved in malignant transformation of these cells and/or to be determinant for the tumor phenotype. We thus focused our attention on this chromosome and determined the physical localization of its estrogen-regulated genes, including those identified in this study and nine additional ones known to be regulated by E2 in BC cells (*BRCA1*, *ERBB2*, *KRT19*, *MPO*, *TP53*, *TRIM16*, *SHBG*, *WNT3* and *ζNF147*; Yamada *et al.* 1993, Inoue *et al.* 1995, Xu *et al.* 1997, Liu *et al.* 1998, Murayama *et al.* 1999, Choi *et al.* 2000, Perissi *et al.* 2000, Qin *et al.* 2002, Katoh 2003). In addition, we searched for more such genes in the published datasets from microarray and/or SAGE analyses of the hormone-regulated transcriptome of BC cells (Charpentier *et al.* 2000, Finlin *et al.* 2001, Bouras *et al.* 2002, Inoue *et al.* 2002, Lobenhofer *et al.* 2002,

Hodges *et al.* 2003) and from microarray expression data from estrogen-treated ZR-75.1 and MCF-7 cells available from our laboratories (A. Weisz, unpublished data). This led to the identification of 95 hormone-regulated genes whose distribution within chromosome 17 is reported, at 4 Mbs resolution, in Table 9. When compared with the local gene density (R/T ratios in Table 9), distribution of these hormone-responsive transcription units along the chromosome does not appear uniform. The average density of such genes within the centromeric region between cytogenetic loci 17p11.2 and 17q21.2–q21.3 (12 ~ 40 Mbs interval from pter), for example, is much lower (2.74%) than that of the more telomeric regions of either 17p or 17q (6.61% and 9.16% respectively). Furthermore, we observed some distribution outliers, in particular within 17p13.1–p13.3, 17q21.33, 17q22–q23 and 17q25.2–q25.3, where the hormone-responsive gene density is much higher than the average. All these data suggest the possible existence of estrogen-responsive domains in chromosome 17, an interesting novelty concerning the relationships between gene regulation by steroid hormones and

genome architecture and functional organization. Indeed, in several instances hormone-regulated genes appear to cluster together on this chromosome (Table 10). For example, ten hormone-responsive genes are concentrated within ~1.71 Mbs of 17p13, including *ITGAE*, *ATP2A3* and *KIAA0399* which cluster in a <400 Kbs subregion, and *CXCL16*, *PSMB6*, *PLD2*, *GP1BA* and *ENO3*, which are closely spaced within ~200 Kbs. Interestingly, in the same cytogenetic band *NUP88* and *C1 QBP* genes from cluster 7 (Fig. 2b) are only 13 Kbs apart from each other on the same DNA strand, while five other genes which also cluster together in the same cytogenetic location (*EIF4A1*, *SHBG*, *TP53*, *VAMP2* and *STK12*) are all activated by estrogen, suggesting two possible examples of colinear gene regulation. Several other examples of physical clustering of co-regulated genes can be found also in 17q, including the three keratin genes *KRT13*, *KRT15* and *KRT17*, *TUBG1*, *PSME3*, *BRCA1* and *NBR2* or *NPEPPS* and *KPNB1* at 17q21, *TOB1*, *NME1* and *NME2*, *SFRS1*, *MPO* and *RAD51C* or *RPS6 KB1*, *NY-REN-60* and *PPM1D* at 17q23, *KPNA2*, *LOC51321* and *SLC16A6*, *LLGL2*, *ITGB4* and *GALK1* or *TK1*, *BIRC5* and *SSI3/SOCS3* at 17q25 (Table 10). These observations, which arise from a zoom-in on the estrogen-responsive transcriptome from chromosome 17 and are likely to apply also to other cytogenetic locations (see Tables 7 and 8), while awaiting further confirmation by whole genome studies of the estrogen-responsive transcriptomes from different cell types, already suggest some intriguing possibilities. Clustering of estrogen-responsive genes, for example, could provide a structural basis to allow them to respond in a coordinated fashion during development, cell differentiation or cell growth, as recently suggested for other gene expression ‘neighborhoods’ (Oliver *et al.* 2002). In this case, an epigenetic control of estrogen-responsive gene clusters could involve locus-specific gene regulation domains, where the chromatin can shift between ‘closed’ and ‘open’ conformations allowing coordinated silencing/activation of many genes located in proximity to each other. In this respect, long-ranging chromatin unfolding by ER- α has recently been demonstrated experimentally (Nye *et al.* 2002), while the involvement of chromatin remodeling factors and histone modifying enzymes in nuclear receptor – including ER – actions on target genes is well known. It would be

interesting to verify now whether these co-regulated chromatin domains contain hormone-sensitive LCR elements. Furthermore, epigenetic control of the activity of genes and gene clusters is also the basis of genetic imprinting (Reik & Walter 2001), which is suggested as one of the mechanisms responsible for genetic predisposition to cancer, including BC (Schofield *et al.* 2001). Chromosomal clustering of hormone-responsive genes can also be envisioned to provide a functional link with certain genomic imbalances frequently observed in tumor cells. BC cells, including ZR-75.1 and MCF-7 cells, show numerous karyotypic aberrations, in particular amplification and deletion of large chromosomal regions (Forozan *et al.* 2000). Interestingly, in the case of 17q23, amplification results in overexpression of many genes included in the amplified segment (Barlund *et al.* 2000, Monni *et al.* 2001). Thus, it is possible to envisage that the non-random distribution of estrogen-regulated genes observed in this study might reflect chromosomal imbalances in ZR-75.1 cells (Forozan *et al.* 2000). Amplification, for example, could lead to ‘gene dosage’ effects causing differences in the number of genes detectable by expression analysis between diploid and aneuploid chromosomal segments. In turn, this suggests also how clustering of responsive genes in defined genomic loci might play a role in hormone-responsive breast carcinogenesis and tumor progression. In fact, significant modifications of cell sensitivity to estrogens could result from amplification and deletion of chromosomal segments rich in hormone-responsive genes.

All these exciting hypotheses can now be effectively addressed, as the list of genes identified here and in other similar investigations aimed at mapping the hormone-regulated transcriptomes of responsive cell types can be exploited *in silico* to pinpoint potential chromosomal domains with strong evidence of colinear gene regulation, which may then be studied experimentally in model systems to identify, for example, primary and secondary gene responses to ERs within each cluster, as well as potential functional hierarchies among co-regulated genes and their contribution towards defined phenotypic modifications occurring in hormone-stimulated cells.

In conclusion, the gene expression changes described here represent a comprehensive view of the gene expression program activated by estrogen

Table 10 Physical location of estrogen responsive genes from chromosome 17

Symbol	Description	UniGene ^b	Response to E2 ^c	Ensembl gene ID ^d	Strand ^d	Coordinates from pter (kbp) ^d		Transcription unit (bp) ^d
						5'	3'	
Cytogenetic^a	17p13.3	MYO1C						
	17p13.3	SKIP	Hs.286226 Hs.178347	-	-	1318-9 1348-7	1345-8 1370-8	26,833 22,167
	17p13.3	SREC / SCARF1	Hs.57735	-	-	1488-0	1449-9	11,893
	17p13.3	KIAA0397	Hs.273138	-	-	2191-6	2235-2	43,539
	17p13.2	ITGAE	Hs.389133	+	-	3568-7	3655-3	86,615
	17p13.2	ATP2A3	Hs.5541	-	-	3778-0	3818-6	40,567
	17p13.2	KIAA0399	Hs.172179	+	-	3858-6	3978-0	119,490
	17p13.2	CXCL16	Hs.82407	+	-	4587-6	4594-0	6,391
	17p13.2	PSMB6	Hs.77060	+	+	4605-4	4652-8	2,333
	17p13.1	PLD2	Hs.104519	-	+	4661-4	4677-8	16,327
	17pter-p12	GP1BA	Hs.1472	+	+	4780-4	4782-7	2,227
	17pter-p11	ENO3	Hs.224171	-	+	4797-3	4805-2	7,925
	17p13.2	NUP88	Hs.172108	+	-	5234-0	5267-8	33,826
	17p13.3	C1QBP	Hs.78614	+	-	5280-7	5287-3	6,537
	17p13.2	HSXIAPAF1	Hs.441975	-	+	6603-6	6622-2	18,660
	17p13.2	EIF4A1	Hs.129673	+	+	7419-0	7424-8	5,813
	17p13.1	SHBG	Hs.46319	+	+	7474-2	7479-5	5,326
	17p13.1	TP53	Hs.426890	+	-	7514-6	7533-7	19,178
	17p13.1	VAMP2	Hs.25348	+	-	8005-0	8009-4	4,400
	17p13.1	STK12	Hs.442658	+	-	8050-6	8056-5	5,888
	17p13.1	MDS006	Hs.47668	+	+	10 544-0	10 558-0	13,938
	17p11.2	MAP2K4	Hs.134106	+	+	11 867-2	11 990-1	122,915
	17p12	PMP22	Hs.372031	-	-	15 076-2	15 111-7	35,548
	17p11.2	TRIM16	Hs.241305	+	-	15 474-3	15 530-7	56,339
	17p12	COPS3	Hs.6076	+	-	17 093-2	17 127-6	34,458
	17p11.2	ALDH3A1	Hs.575	-	-	19 545-8	19 556-3	10,450
	17q11.2	MAC30	Hs.199695	+	+	26 498-0	26 507-1	9,089
	17q13.2	SLC13A2	Hs.102307	-	+	26 652-5	26 676-7	24,132
	17q11.2	BLMH	Hs.387183	+	-	28 427-1	28 470-9	43,824
	17q11.2	CENTA2	Hs.415471	+	+	29 100-6	29 138-1	37,517
	17q12	PSMD11	Hs.443379	+	+	30 620-2	30 658-1	37,892
	17q11.2							
	17q11.2							
	17q11.2							

Table 10 Continued

Symbol	Description	UniGene ^b	Response to E2 ^c	Ensembl gene ID ^d	Strand ^d	Coordinates from pter		Transcription unit (bp) ^d
						5'	3'	
Cytogenetic^a Cont								
17q11.2-q12	AP2B1	Hs.370123	+	ENSG000000006125	+	33 763-0	33 902-1	139,135
17q12	ERBB2	Hs.446352	-	ENSG00000141736	+	37 766-7	37 795-7	29,048
17q12	RPL23	Hs.406300	+	ENSG00000125691	-	36 914-5	36 920-4	5,934
17q12-q21.1	IGFBP4	Hs.1516	+	ENSG00000141753	+	38 508-5	38 522-7	14,269
17q21.2	SMARCE1	Hs.437546	+	ENSG00000073584	-	38 692-7	38 713-9	21,154
17q21.2		Hs.2785	-	ENSG00000126337	-	39 541-7	39 689-9	148,153
17q12-q21	KRT17	Hs.433871	-	ENSG00000171401	-	39 566-0	39 570-6	4,632
17q21.2	KRT13	Hs.80342	-	ENSG00000171346	-	39 578-8	39 586-8	8,028
17q21.2	KRT15	Hs.309517	+	ENSG00000171345	-	39 588-6	39 593-3	4,687
17q21.2	KRT19	Hs.2340	-	ENSG00000173801	-	39 819-6	39 851-8	32,087
17q21.2	JUP	Hs.387567	+	ENSG00000131473	-	39 931-9	39 984-1	51,112
17q12-q21	ACLY	Hs.176901	-	ENSG00000108786	+	40 612-7	40 616-0	3,247
17q21.2	HSD17B1	Hs.21635	+	ENSG00000131462	+	40 670-5	40 676-0	5,566
17q21	TUBG1	Hs.152978	+	ENSG00000131467	+	40 885-2	40 904-3	19,080
17q21	PSME3	Hs.194143	+	ENSG000000012048	-	41 105-1	41 186-2	81,090
17q21.31	BRCA1	Hs.500268	+	ENSG00000182076	+	41 186-3	41 214-5	28,109
17q21.31	NBR2	Hs.180577	-	ENSG000000030582	+	42 433-3	42 441-2	7,981
17q21.31	GRN	Hs.431279	+	ENSG00000073969	+	45 050-1	45 177-1	126,942
17q21	NSF	Hs.224667	-	ENSG00000108379	-	45 183-9	45 238-2	54,232
17q21.31	WNT3	Hs.293007	+	ENSG00000141279	+	45 950-6	46 042-7	92,153
17q21.31	NPEPPS	Hs.439683	+	ENSG00000108424	+	46 069-3	46 103-1	33,751
17q21.32	KPNB1	Hs.9028	-	ENSG00000108840	-	46 164-9	42 211-8	46,890
17q21.32	HDAC5	Hs.77254	+	ENSG00000108468	-	46 489-5	46 520-9	31,431
17q 17q21.32	CBX1	Hs.129951	+	ENSG00000121067	-	48 019-4	48 097-5	78,043
17q21.33	SPOP	Hs.265829	-	ENSG00000005884	+	48 475-5	48 509-9	34,421
17q21.33	ITGA3	Hs.370927	+	ENSG00000108829	-	48 800-7	48 817-0	16,316
17q23.33	PRO1855	Hs.90786	-	ENSG00000108846	+	49 054-3	49 111-1	56,853
17q22	ABCC3	Hs.178137	+	ENSG00000141232	-	49 281-7	49 285-8	4,121
17q21.33	TOB1							

Table 10 Continued

Symbol	Description	UniGene ^b	Response to E2 ^c	Ensembl gene ID ^d	Strand ^d	Coordinates from pter	Transcription unit (bp) ^d
						5' 3'	
Cytogenetic^a Cont							
17q21.3	NME1	Hs.118638	+	ENSG00000011052	+	49 573-0 49 581-6	8,601
17q21.33							
17q21.33	Non-metastatic cells 1, protein (NIM23A) expressed in						
17q21.3	NME2	Hs.433416	+	ENSG000000121054	+	49 585-0 49 591-2	6,213
17q21.33							
17q23.1	ZNF147	Hs.1579	+	ENSG000000121060	-	55 310-6 55 333-5	22,841
17q23.2							
17q21-q23	A kinase (PRKA) anchor protein 1	Hs.78921	+	ENSG000000121057	+	55 504-7 55 540-7	36,010
17q23.2							
17q21.3-q22	SFRS1	Hs.68714	+	ENSG000000136450	-	56 408-5 56 426-7	18,222
17q23.2	Splicing factor, arginine/serine-rich 1 (splicing factor 2, alternate splicing factor)						
17q23.1							
17q23.2	MPO	Hs.458272	+	ENSG000000005381	-	56 689-3 56 700-4	11,094
17q23.2							
17q22-q23	RAD51 homolog C (S. cerevisiae)	Hs.421587	+	ENSG000000108384	+	57 112-0 57 153-8	41,768
17q23.2							
17q23.2	RPS6KB1	Hs.86858	+	ENSG000000108443	+	58 312-3 58 366-7	54,440
17q23.3							
17q23.3	NY-REN-60	Hs.436133	+	ENSG000000170832	-	58 596-4 58 811-3	214,924
17q23.2							
17q23.3	PPM1D	Hs.286073	+	ENSG000000170836	+	59 019-4 59 083-9	64,482
17q23.2							
17q23.2	TLK2	Hs.445078	+	ENSG000000146872	+	60 897-3 61 031-7	134,367
17q23.3							
17q23.3	ACE	Hs.298469	+	ENSG000000159640	+	61 895-2 61 940-0	44,769
17q23.3							
17q24.2	RNAHP / DDX42	Hs.8765	+	ENSG000000087187	+	62 192-4 62 237-5	45,110
17q23.3							
17q21	DDX5	Hs.279806	+	ENSG000000108654	-	62 913-3 62 919-9	6,673
17q24.1							
17q24.3	HELZ	Hs.3085	+	ENSG000000141331	-	65 491-6 65 659-6	168,038
17q24.2							
17q23.1-q23.3	KPNA2	Hs.159557	+	ENSG000000182481	+	66 449-3 66 460-5	11,134
17q24.2							
17q24.3	LOC51321	Hs.268122	+	ENSG000000108934	+	66 666-6 66 717-8	51,251
17q24.2							
17q24.3	SLC16A6	Hs.42645	+	ENSG000000108932	-	66 729-3 66 751-8	22,494
17q24.2							
17q25.2	SLC9A3R1	Hs.396783	+	ENSG000000109062	+	73 208-8 73 229-7	20,803
17q25.1							
17q24-q25	LLGL2	Hs.3123	-	ENSG000000073350	+	73 985-9 74 035-4	49,482
17q25.1							
17q11-qter	ITGB4	Hs.85266	-	ENSG000000132470	+	74 181-6 74 218-0	36,383
17q25.1							
17q24	GALK1	Hs.407966	-	ENSG000000108479	-	74 218-1 74 225-9	7,774
17q25.1							

Table 10 Continued

Symbol	Description	UniGene ^b	Response to E2 ^c	Ensembl gene ID ^d	Strand ^d	Coordinates from pter (kbp) ^d	Transcription unit (bp) ^d
						5' 3'	
Cytogenetic^a Cont							
17q25	H3F3B	Hs.451419	+	ENSG00000132475	-	74 237-7 74 240-1	2,462
17q25.1	EVER1	Hs.16165	-	ENSG00000141524	-	76 567-6 76 589-6	21,944
17q25.3	SYNGR2	Hs.433753	-	ENSG00000108639	+	76 625-7 76 630-1	4,363
17q25.3	TK1	Hs.105097	+	ENSG00000167900	-	76 631-2 76 644-2	12,985
17q23.2-q25.3							
17q25.3	BIRC5	Hs.1578	+	ENSG00000008985	+	76 671-3 76 681-9	10,515
17q25							
17q25.3							
17q25.3	SSI-3 / SOCS3	Hs.436943	+	ENSG00000184557	-	76 815-5 76 816-4	849
17q25.2-q25.3	GAA	Hs.1437	-	ENSG00000171298	+	78 637-5 78 655-9	18,396
17q25.3							
17q25	ACTG1	Hs.14376	+	ENSG00000184009	+	80 051-7 80 056-8	5,090
17q25.3	HGS	Hs.416959	-	ENSG00000185359	+	80 211-0 80 229-1	18,125
17q25.3							
17q25.3	ARHGDI	Hs.159161	-	ENSG00000141522	-	80 361-4 80 365-1	3,642
17q25.3	ALY	Hs.241520	+	ENSG00000141592		80 381-5 80 385-3	3,734
17q25.3	PCYT2	Hs.226377	-	ENSG00000185813	-	80 397-9 80 405-1	7,202
17q25.3	DCXR	Hs.9857	-	ENSG00000169738	-	80 529-6 80 531-4	1,806

^aCytogenetic location according to LocusLink and/or Ensembl databases; ^bUniGene cluster number; ^cresponse to estrogen in breast cancer cells, assessed directly by microarray analysis (see text) and/or (*italic*) reported in the literature; ^dfrom Ensembl and/or UCSC Golden Path.

in a hormone-responsive BC cell line *in vitro*, which can be used to investigate various aspects of estrogen involvement in breast carcinogenesis and tumor progression and to further our understanding of the molecular and genetic basis of the responsiveness of BC cells to hormones and antihormones. A detailed *in silico* categorization and functional analysis of this estrogen-regulated gene set will provide a logical framework to pinpoint potential novel aspects of estrogen's actions in BC cells, which may then be tested experimentally and lead in this way to the identification of new biological properties of hormone-responsive cells. At the same time, the pattern of expression of dominant estrogen-regulated genes may characterize defined, and clinically useful, hormone-responsive BC subtypes. It is reasonable to assume that a sizeable fraction of the gene expression profiles measured in hormone-stimulated BC cells *in vitro* reflects distinctive properties of these cells which, ultimately, may be exploited to type them *ex vivo*. Indeed, we have already shown that the expression pattern of some of the genes identified in this study clearly discriminates estrogen-responsive from -non-responsive BC cells *in vitro* (Weisz *et al.* 2003) and ER- α -positive and -negative breast tumor samples (Sorbelli *et al.* 2003).

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