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Transcript profiling during preimplantation mouse development ☆

Fanyi Zeng,[a] Don A. Baldwin,[b] and Richard M. Schultz[a,*]

[a] *Department of Biology, University of Pennsylvania, Philadelphia, PA 19104, USA*
[b] *Penn Microarray Facility, University of Pennsylvania, Philadelphia, PA 19104, USA*

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Abstract

Studies using low-resolution methods to assess gene expression during preimplantation mouse development indicate that changes in gene expression either precede or occur concomitantly with the major morphological transitions, that is, conversion of the oocyte to totipotent 2-cell blastomeres, compaction, and blastocyst formation. Using microarrays, we characterized global changes in gene expression and used Expression Analysis Systematic Explorer (EASE) to identify biological and molecular processes that accompany and likely underlie these transitions. The analysis confirmed previously described processes or events, but more important, EASE revealed new insights. Response to DNA damage and DNA repair genes are overrepresented in the oocyte compared to 1-cell through blastocyst stages and may reflect the oocyte's response to selective pressures to insure genomic integrity; fertilization results in changes in the transcript profile in the 1-cell embryo that are far greater than previously recognized; and genome activation during 2-cell stage may not be as global and promiscuous as previously proposed, but rather far more selective, with genes involved in transcription and RNA processing being preferentially expressed. These results validate this hypothesis-generating approach by identifying genes involved in critical biological processes that can be the subject of a more traditional hypothesis-driven approach.
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Introduction

Mouse preimplantation development is characterized by three major transitions, each of which entails pronounced changes in the pattern of gene expression. The first transition is the maternal-to-zygotic transition (MZT) that serves three functions. The first is to destroy oocyte-specific transcripts (e.g., *H1oo*; Tanaka et al., 2001; and *Msy2*; Yu et al., 2001). The second is to replace maternal transcripts that are common to the oocyte and early embryo, for example, actin (Davis et al., 1996) with zygotic transcripts. Although the expression of these transcripts does not result in reprogramming of gene expression in a classical sense, their expression is nonetheless essential for further development. The MZT's third function is to promote the dramatic reprogramming in the pattern of gene expression that is coupled with generating novel transcripts that are not expressed in the oocyte (Latham et al., 1991). Zygotic gene activation, as determined by expression of plasmid-borne reporter genes, transgenes, and endogenous genes (Aoki et al., 1997; Bouniol et al., 1995; Christians et al., 1995; Kigami et al., 2003; Matsumoto et al., 1994; Ram and Schultz, 1993), initiates during the 1-cell stage and is clearly evident by the 2-cell stage (Latham et al., 1991; Schultz, 1993). Superimposed on genome activation is the development of a chromatin-based transcriptionally repressive state (Majumder and DePamphilis, 1995; Schultz, 2002) and a more efficient use of TATA-less promoters (Davis and Schultz, 2000; Majumder and DePamphilis, 1994) that likely play a major role in establishing the appropriate pattern of gene expression that is required for development.

The second developmental transition is compaction, which occurs during the 8-cell stage (Fleming et al., 2001; Johnson and Maro, 1986). Compaction, which is a Ca^{2+}-dependent process that requires E-cadherin (Hyafil et al., 1981), is the first obvious morphological differentiation that occurs during preimplantation development. Up to the 8-cell stage, the blastomeres are quite distinct, but during compac-

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* Corresponding author. Department of Biology, University of Pennsylvania, 415 South University Avenue, Philadelphia, PA 19104-6018. Fax: +1-215-898-8780.
E-mail address: rschultz@sas.upenn.edu (R.M. Schultz).

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tion adhesive interactions form between individual blastomeres that are no longer clearly resolved. Accompanying this morphological transition are pronounced biochemical transitions through which the blastomeres acquire characteristics resembling somatic cells, reflected in such features as ion transport, metabolism, cellular architecture, and gene expression pattern. Development of both gap junctions (Kidder and Winterhager, 2001) and tight junctions (Fleming et al., 2001) at the late 8-cell stage results in an epithelium that is essential for continued and proper development.

Blastocyst formation is the next transition and occurs at the 32- to 64-cell stage. Following compaction, subsequent cleavage divisions allocate cells to the inside of the developing morula. These inner cells are allocated between the 8- and 16-cell stages and then again between the 16- and 32-cell stages (Pedersen, 1986). The inner cells of the morula give rise to the inner cell mass (ICM) cells that then give rise to the embryo proper, whereas the outer cells differentiate into the trophectoderm (TE), which gives rise to extraembryonic tissue. The TE is a fluid-transporting epithelium that is responsible for forming the blastocoele cavity, which is essential for continued development and differentiation of the ICM (Biggers et al., 1988; Watson and Barcroft, 2001). Blastocyst formation is the first time when overt cellular differentiation occurs and is characterized by differences in gene expression between the ICM and TE cells. For example, expression of *Oct4*, a member of the POU-domain family of transcription factors and essential to maintain totipotency of the ICM cells (Nichols et al., 1998; Pesce and Scholer, 2000), and *Fgf4* (Niswander and Martin, 1992) become restricted to the ICM; while expression of *Bex1*, the imprinted *H19* gene, and *Cdx2*, a homolog of the *Drosophila* homeotic caudal (*Cad*) gene, are restricted to the TE in the preimplantation embryo (Beck et al., 1995; Doherty et al., 2000; Williams et al., 2002).

To date, quantitative analysis of high-resolution, two-dimensional protein gels (Latham et al., 1991), mRNA differential display (Ma et al., 2001), and analysis of expressed sequence tags (EST) derived from libraries of various preimplantation stages (Ko et al., 2000; Sharov et al., 2003) have been used to assess the global changes in gene expression that occur during preimplantation development. Although these approaches have shed some light on the molecular basis underlying preimplantation development, each has major limitations. Microarray techniques provide a powerful approach to study global patterns of gene expression. The ability to amplify the small amounts of mRNA present in preimplantation mouse embryos, which can only be isolated in limited numbers, makes it feasible to generate enough material for microarray analysis.

Transcript profiling provides a measure of RNA abundance, which can be affected not only by levels of transcription but also by RNA processing and degradation. Moreover, not all transcripts are translated and RNA abundance may not correspond to protein levels, nor does RNA provide information about protein modification, activity, or location. These important processes are not the focus of this study; instead RNA expression is used as an initial indicator of the overall activities of the genome during early mouse development.

Using Affymetrix oligonucleotide arrays containing >39,000 transcripts and variants, together with a T7-based linear double amplification method, we report here changes in the global patterns of gene expression that occur during preimplantation development in the mouse by analyzing the gene expression profile in oocytes, 1-cell, 2-cell, and 8-cell embryos, and blastocysts. While this work was being prepared for publication, two studies appeared that used microarrays to assess global patterns of gene expression during preimplantation development (Hamatani et al., 2004; Wang et al., 2004).

Materials and methods

Collection of preimplantation mouse embryos

Mouse CF-1 × B6D2F1/J embryos were isolated from superovulated mice as previously described (Zeng and Schultz, 2003). Oocytes were collected 46–48 h after PMSG injection, and the following stages collected during the indicated time period after hCG administration: 1-cell (18–20 h), 2-cell (44–46 h), 8-cell (68–70 h), and blastocyst (96–98 h). Four equal pools of embryos from each stage were independently collected from separate sets of fertilized mice, and a range of 80–430 embryos per pool was collected to make the amount of total RNA recovered approximately equivalent among all stages.

cDNA preparation for microarray analysis

Embryo pools were transferred to 150 μl of Trizol (Invitrogen) containing 20 μg of glycogen and stored at −80°C. When all replicates were collected, total RNA was extracted according to the manufacturer's instructions for small sample preparation. Samples were submitted to the Penn Microarray Facility for target preparation and GeneChip hybridization. RNA mass and size distribution were determined using the Agilent Bioanalyzer with RNA 6000 Nano LabChips; total RNA yield was 82–113 ng per replicate pool. This total RNA was used for linear, two-round amplification by in vitro transcription (Affymetrix Small Sample Prep Technical Bulletin, www.affymetrix.com). cRNA yield after the first amplification was 2.0–10.8 μg, and 0.5 μg of each replicate was used as input template for the second amplification. Final yield of biotinylated cRNA was 62–119 μg, of which 15 μg per replicate was fragmented and hybridized to Affymetrix GeneChips. cRNA samples were serially hybridized to MG_U74Av2, MOE430A, and MOE430B GeneChips, then washed and stained on fluidics stations and scanned at 3 μm resolution according to the manufacturer's instructions (GeneChip Analysis Technical Manual, http://www.affymetrix.com).

Analysis of the microarrays

Microarray Analysis Suite 5.0 (MAS, Affymetrix) was used to quantify microarray signals with default analysis parameters and global scaling to target mean = 150. Quality control parameters for all samples were within the following ranges: scale factor 0.7–3.5 and background 44–83 for U74Av2 and MOE430A; percent genes detected 31–49% on U74Av2 or MOE430A and 16–23% on MOE430B; actin 3′ /5′ signal ratio 0.5–6.0, and GAPDH 3′ /5′ signal ratio 0.8–8.7 for all GeneChips. GeneChip tabular data are available at the Gene Expression Omnibus repository, www.ncbi.nlm.nih.gov/geo. The MAS metrics output was loaded into GeneSpring v6 (Silicon Genetics) with per-chip normalization to the 50th percentile and per-gene normalization to the median. A filtered list was created of all genes detected (MAS "P" call) in at least three of four replicates of at least one embryo stage. K-means hierarchical clustering using this gene list correctly grouped all replicates as most related by embryo stage, so the filtered list was used to search for transcripts showing abundance differences. (Note: all analyses described below were performed using Affymetrix probe set lists, some of which were converted to Unigene accession numbers as noted in the text and figure legends to illustrate gene expression with reduced redundancy.)

Three independent analyses were then applied to identify genes with statistically significant differences in any of the stages tested. The GeneSpring pairwise comparison (Welch *t* test with ANOVA, $P = 0.05$, Benjamini and Hochberg multiple testing correction) was conducted between all possible stage pairs. The GeneSpring multiclass analysis (Welch *t* test with Welch ANOVA, $P = 0.05$, Benjamini and Hochberg multiple testing correction) was applied to the entire sample using the filtered gene list described above. A one-way ANOVA for microarrays (lgsun.grc.nia.nih.gov/ANOVA/index.html, default parameters except FDR < 0.05) was also conducted. A nonredundant list was compiled containing candidate genes called significantly different in at least one analysis. Expression profiles for all genes on the candidate list were plotted in GeneSpring across all stages to conduct pattern searches by the following strategies.

The GeneSpring "filter on fold change" and "draw gene" tools were applied to the nonredundant differentially expressed gene list (Affymetrix probe sets) to locate genes with expression profiles closely resembling various simple, hypothetical patterns such as sustained increase or decrease. Annotation-directed searches were performed to query genes previously known to be involved in early developmental processes. Transient change filters were applied to identify genes increased by at least 1.4-fold in a single stage compared to expression in all other stages. Similar fold-change filters were used to find increases or decreases spanning more than two consecutive stages. The pattern searches based on fold-change filters generated subset lists of candidate genes, which were then imported to EASE (version 2.0) to test for overrepresentation of annotation classes (Hosack et al., 2003). EASE analyses with Bonferroni multiplicity correction tested each subset list against the population of all genes detected, and an EASE score was calculated for likelihood of overrepresentation in the Gene Ontology Consortium annotation categories GO biological process, GO cell component, and GO molecular function, as well as KEGG pathway and SwissProt keyword. GeneSpring "genomes" were built for each of these annotation categories so that EASE scores from all subset lists could be visualized in parallel across all embryo stages. EASE analysis was also conducted for genes expressed at each developmental stage.

Real-time RT-PCR analysis

In vivo-developed 1- and 2-cell embryos were collected as described above and total RNA was isolated from 100 embryos at each stage. Two embryo equivalents of template RNA were used for each real-time RT-PCR assay according to manufacturer's protocol using ABI Prism Sequence Detection System 7000. To confirm the ability of this microarray analysis to resolve small differences in expression level, six genes that showed a statistically significant 40–100% increase in expression between the 1- and 2-cell stages were selected. The corresponding ABI TaqMan Assay-on-Demand probe/primer sets used were Mm00520817_m1 (*LOC217716*), Mm00485509_m1 (*Rad51ap1*), Mm00457046_m1 (*Tbpl1*), Mm00494300_m1 (*Zfp103*), Mm00432498_m1 (*Chkl*), Mm00442360_m1 (*Ceacam1*), and Mm00501974_s1 (*Hist2h2aa1*). Three replicates were used for each real-time PCR reaction; a minus RT and a minus template served as controls. Quantification was normalized to the endogenous histone H2A within the log-linear phase of the amplification curve obtained for each probe/primer using the comparative CT method (ABI PRISM 7700 Sequence Detection System, user bulletin #2).

Results and discussion

Hierarchical cluster analysis

Oocytes and embryos from four preimplantation stages (1-cell, 2-cell, 8-cell, and blastocyst) were used for expression profiling to encompass the three aforementioned major transitions that govern preimplantation development, that is, maternal-to-zygotic transition (MZT), compaction, and blastocyst formation. We elected to analyze embryos that developed in vivo, rather than in vitro, to avoid the effect of embryo culture on gene expression (Doherty et al., 2000; Ho et al., 1994).

An unsupervised hierarchical clustering using either all the genes on MOE430 or U74Av2 chips, or the nonredundant list of genes that are expressed in the preimplantation mouse embryo (Table 1), independently clustered correctly all replicates by their appropriate developmental stage (Fig.

Table 1
Distribution of genes detected in the five preimplantation stages

Stage	Genes detected	Genes differentially expressed
Oocyte	9909	9414
1-Cell	8793	8413
2-Cell	9951	9514
8-Cell	8312	7957
Blastocyst	10,242	9776
Total (nonredundant)	14,119	13,378

The number of Unigene accessions derived from genes detected (Affymetrix "P" call in at least 3 of 4 replicates) on the MOE430A and B GeneChips in each stage, as well as the total number of non-redundant genes detected from all five stages, are listed in the left column. One-way ANOVA and GeneSpring ANOVA/Welch *t*-test (false discovery rate = 0.05) were then used to identify genes showing a statistically significant expression difference when compared to any other stage, and the number of non-redundant genes resulting from this analysis is listed in the right column.

1) with the branch tree distances minimal between replicates. Furthermore, with the exception of a few genes, most of the genes behaved similarly across the four replicates for each developmental stage. The wider range of expression among replicates for some genes may reflect the asynchrony of embryo development in vivo.

The clustering dendogram (Fig. 1) reveals that the oocyte and 1-cell, and 8-cell and blastocyst, are subgrouped together. In contrast, the 2-cell is separated from the other stages, forming an intermediate node between the oocyte/1-cell and 8-cell/blastocyst. The separation of the 2-cell embryo from the other stages is consistent with previous results indicating that the major reprogramming of gene expression occurs during the 2-cell stage and is linked with the major period of genome activation (Latham et al., 1991). This clearly separates the 2-cell embryo from the 1-cell. The other burst of reprogramming that occurs during the 4- to 8-cell stages (Van Blerkom and Brockway, 1975) likely further distances the 8-cell embryo from the 2-cell embryo. The dramatic reprogramming in gene expression during the 2-cell stage, as detected by hierarchical clustering analysis, was also reported in the two recent microarray studies (Hamatani et al., 2004; Wang et al., 2004). The changes in gene expression that precede compaction, and recognized some 30 years ago (Van Blerkom and Brockway, 1975), were likewise observed in those studies (Hamatani et al., 2004; Wang et al., 2004).

The clustering of oocyte and 1-cell embryo transcript profiles was anticipated. Although many changes in the pattern of protein synthesis are observed following fertilization, the majority of these are due to recruitment of maternal mRNAs or posttranslational modifications of existing proteins (Van Blerkom, 1981). In addition, few newly synthesized transcripts have been identified in the 1-cell embryo (Kigami et al., 2003) that is now recognized as being transcriptionally active (Aoki et al., 1997; Bouniol et al., 1995). Last, although degradation of maternal mRNA initiates during oocyte maturation, destruction of these mRNAs is typically not complete until the late 2-cell stage. Thus, most of the transcripts present in the 1-cell embryo are maternally derived. Following compaction, the patterns of protein synthesis of 8-cell embryos and blastocysts are very similar as assessed by 1D (Van Blerkom and Brockway, 1975) and high-resolution 2D gels (Latham, personnel communication). The clustering of the 8-cell and blastocyst stages was anticipated because the 8-cell embryos used in our analyses were typically approximately 60% compacted and 40% uncompacted. In toto, the concordance of the hierarchical cluster analysis with previous results collected by totally independent methods lends confidence to the reliability of conclusions drawn from our transcript profiling of preimplantation mouse embryos.

Derivation of nonredundant gene expression lists

All genes detected (Affymetrix "P" call) on MOE430A and B GeneChips that were present in at least three of four replicates of each stage were used to construct a nonredundant list of genes expressed in the oocyte and preimplantation stages (Table 1). The resulting list of 18,108 Affymetrix probe sets, corresponding to 14,119 Unigene annotations, was used for three independent ANOVA analyses to identify genes with statistically significant differences in RNA expression in any of the stages tested as described under Material and methods. A second nonredundant list was compiled containing all of the candidate genes from all the ANOVA tests and identified genes that are significantly different in at least one stage. This list contains 13,378 (17,193 probe sets) of the 14,119 detected genes. As the ANOVA analyses will eliminate genes that are not statistically different in any of the pairwise comparisons or across all stages, as well as genes with poor quality signals from the GeneChip hybridization and MAS procedures, we find that approximately 95% of the detected genes have altered expression levels in one or more stages during preimplantation development. In particular, as shown in Table 1, the majority of the genes that are present in each stage are also statistically significantly different. The decrease in the number of genes in the 1-cell embryo relative to the oocyte and the subsequent increase by the 2-cell stage reflects degradation of maternal mRNAs that initiates during oocyte maturation and the expression of zygotic transcripts concomitant with genome activation during the 2-cell stage as described above. Similar overall results were obtained using the U74Av2 microarray (data not shown).

The Penn Microarray Facility has processed a wide variety of mouse tissues and cell types on MOE430 GeneChips. The number of genes detected generally ranges from 30% to 50% of all genes on the MOE430A or U74Av2, and 10–15% on the MOE430B. The proportion detected (see Materials and methods) in this study is within the normal range for the MOE430A GeneChip (or for U74Av2) and somewhat higher than usual for the MOE430B GeneChip. The MOE430B probe sets assay genes that tend to be expressed at lower levels and are represented by EST sequences only or do not occur frequently in sequence

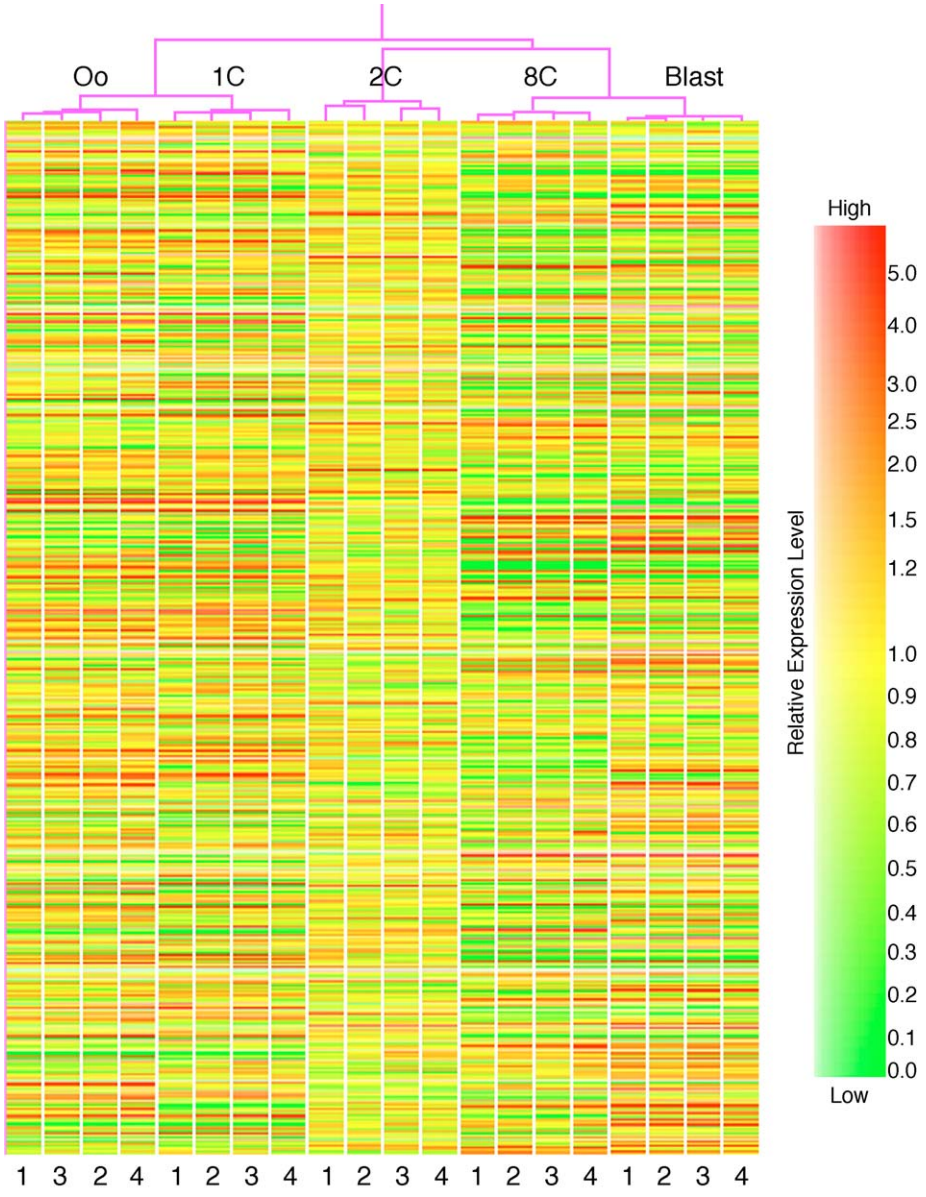


Fig. 1. Hierarchical clustering analysis of all samples from different developmental stages. Unsupervised clustering in GeneSpring was used to analyze similarities among replicate samples across all stages tested. Replicate sample numbers are indicated at the bottom of the figure. Stage abbreviations in the tree diagram are as indicated under Materials and methods. Colors correspond to relative RNA abundance for the detected genes (Affymetrix "Present" call in at least one embryo stage, 18,108 probe sets), each of which is represented by one horizontal bar.

databases. Our results suggest that the preimplantation embryo stages tested may express a number of these rarer and less well-characterized genes.

Our analysis strategy focused first on consistently detected transcripts, then measured the likelihood of difference given observed levels of variance, and finally considered the magnitude of change. Furthermore, the use of four biological replicates provided sufficient statistical power and confidence levels to detect a 40% change of RNA abundance for a significant portion of the detected genes (Table 2, and see below). That such modest changes in the expression of a gene can result in profound differences is gaining more appreciation. For example, the transcription factor OCT4 is critical in maintaining pluripotency of stem cells (Pesce and Scholer, 2000). *Oct4* is expressed initially in all blastomeres, but in the late blastocyst it is expressed in the inner cell mass (ICM) cells, down-regulated in trophectoderm, but up-regulated in primitive endoderm (Palmieri et al., 1994). Eventually, *Oct4* expression is confined exclusively to the developing germ cells (Yeom et al., 1996). Experimentally, manipulating levels of *Oct4* expression by approximately 50% implicated *Oct4* as a developmental switch in regulating the fate of ES cells, for example, higher levels lead to differentiation into primitive endoderm and mesoderm, intermediate levels lead to pluripotent stem cells, and reduced levels result in trophectoderm (Niwa et al.,

Table 2
Number of genes with altered expression levels by stage

Stage	Fold change		
	>1.4×	>2×	>5×
Oocyte	1370	572	46
1-Cell	1877	313	3
2-Cell	911	427	91
8-Cell	1131	368	22
Blastocyst	2739	1401	306

The table displays the number of Affymetrix probe sets on MOE430 arrays showing a higher RNA abundance in the indicated stage compared to any other stage. Genes are categorized by magnitude of difference between expression levels in the indicated stage and the next highest level among all other stages. These data were generated from the nonredundant list of significantly different genes throughout preimplantation development.

2000). Thus, small changes in expression of a critical transcription factor or a gene that acts in the early steps of a signal transduction pathway could function as a molecular switch that would lead to widespread and dramatic changes in gene expression.

Validation of expression patterns

Before analyzing the transcript profiles, we first validated expression patterns observed using the GeneChips with those of several genes for which the temporal pattern of expression has been previously determined by other methods, for example, RT-PCR. For all the genes queried against the data set, temporal patterns of expression were observed to be similar to those reported. Some representative genes that show interesting patterns of expression are shown in Fig. 2. For example, the maternally expressed *Mos* proto-oncogene showed a dramatic decrease in expression from the oocyte to blastocyst stage; the murine endogenous retrovirus-like gene (*MuERV-L*) (Wang et al., 2001) displayed a transient increase in expression during the 2-cell stage; the Na^+/K^+-ATPase, β1 subunit (*Atp1b1*) (Gardiner et al., 1990) and the imprinted *H19* gene (Doherty et al., 2000) increased between the 8-cell and blastocyst stage; maternal G protein alpha inhibiting 3 (*Gnai3*) (Williams et al., 1996) was degraded and then replaced by zygotic transcripts; and expression of the inactive X specific transcript (*Xist*) was first detected in 2-cell embryos and then accumulated and reached a plateau by the morula stage (Hartshorn et al., 2002).

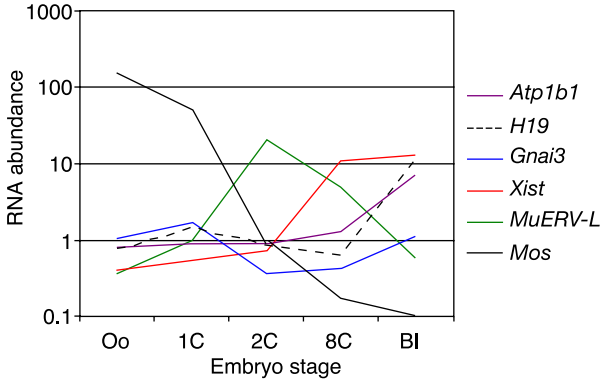
To confirm that the microarray analysis could detect a small-fold change in expression, six genes that displayed such a small and statistically significant increase between the 1- and 2-cell stages were arbitrarily selected for real-time PCR analysis and in each case an increase was observed between these two developmental stages (Fig. 3). These two independent confirmations shown in Figs. 2 and 3 strongly suggest that the expression profiles obtained from the GeneChip analysis accurately reflect qualitative changes in gene expression that occur during preimplantation development.

EASE analysis of gene expression

The mouse genome is estimated to contain approximately 30,000 protein-coding genes, and their biological process classification by GO annotation indicates a distribution of functional classes very similar to that found in the human genome. The 10 most common processes, in descending order by percentage of all genes, were reported as follows: protein metabolism, other metabolic processes, RNA metabolism, DNA metabolism, transport, cell cycle or proliferation, cell organization or biogenesis, signal transduction, cell adhesion, and developmental processes (Waterston et al., 2002). This distribution is mirrored in the GO annotations of genes assayed on the Affymetrix mouse GeneChip microarrays used in this study, as well as in the distribution of all genes detected in at least one embryo stage. Listing detected genes by individual stage, however, suggests that a number of biological processes are overrepresented compared to the overall distribution described above. These lists were analyzed to describe biological process themes that may be of functional importance in individual embryo stages, using Expression Analysis Systematic Explorer (EASE) (Hosack et al., 2003).

EASE is a recently developed program that facilitates the biological interpretation of gene lists derived from results of a microarray analysis. It provides statistical methods (reported as an EASE score) for discovering biological themes within gene lists using previously published annotation databases to generate gene annotation tables. In particular, the overrepre-

A



B

Gene	Oo- 1C	1C - 2C	2C - 8C	8C - B1
Atp1b1				*
H19	*	*		*
Gnai3	*	*		*
Xist			*	
MuERV-L	*	*	*	*
Mos	*	*		

Fig. 2. Validation of expression profiles of previously characterized genes of interest. (A) Shown are average microarray expression levels of selected genes. These profiles match previously determined individual patterns. (B) Statistically significant differences between adjacent stages are indicated by an asterisk.

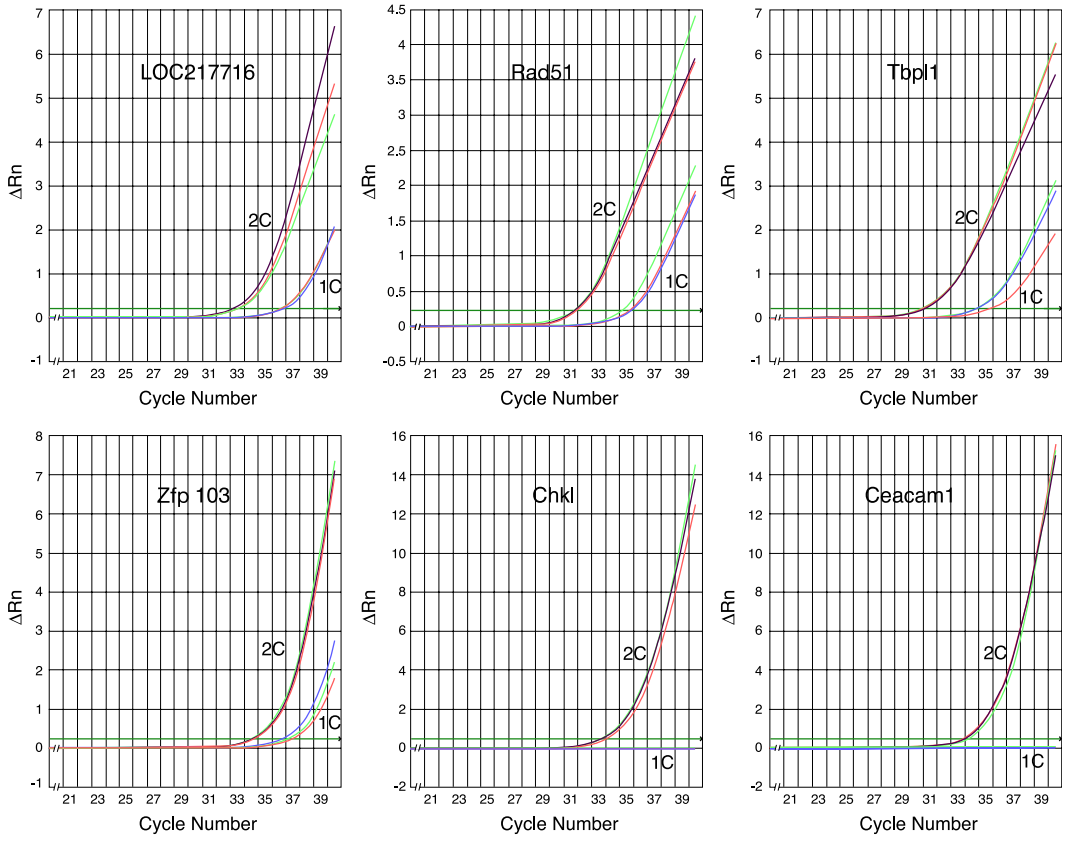


Fig. 3. Real-time RT-PCR confirmation of selected genes. Shown are real-time RT-PCR results of six arbitrarily selected genes that display a 1.4- to 2-fold increase in expression between the 1- and 2-cell stages according to microarray analysis; equal numbers of 1- and 2-cell embryos were used. Three replicates were used for each real-time PCR reaction from each stage. In each case, an increased expression was observed in 2-cell embryos compared to 1-cell embryos after normalizing to histone H2A, which served as an internal control (data not shown).

sentation analysis of functional gene categories is a unique way of identifying and converting the results of functional genomics studies from "genes to themes" (Hosack et al., 2003). Overrepresentation does not refer to abundance of gene expression but rather describes a class of genes that have similar functions, for example, transcription factors, regardless of their expression level, that appear more often in a list of interest than would normally be predicted by their distribution among all genes assayed. The method incorporates jackknife iterative resampling of Fisher exact probabilities, with Bonferroni multiple testing correction, to generate an EASE score that indicates increasing confidence in overrepresentation as the score decreases toward zero. The overrepresented set thus describes a "biological theme" of categories enriched in a particular condition or stage compared to a background population.

We applied EASE analysis to find gene categories that are overrepresented in a particular stage compared to the population of all genes that are expressed in preimplantation mouse embryos from the MOE430 chips, using GO biological process and other annotation categories (see Material and methods). Listed in Table 3 are the overrepresented GO biological processes that have EASE scores of 0.05 or lower in any stage and the corresponding EASE scores across the five stages. (See Supplemental Table S1 for a complete list of GO biological processes that passed EASE <0.05 filter).

The robustness of the EASE analysis is highlighted by it identifying overrepresented GO biological process categories that are consistent with previous findings (Table 3). For example, the absolute rate of protein synthesis increases during preimplantation development from the oocyte to 8-cell stage (Schultz et al., 1979), and EASE analysis identified overrepresentation of genes in protein synthesis. Moreover, overrepresentation of genes involved in ribosome biogenesis and assembly processes (LaMarca and Wassarman, 1979), such as rRNA processing, was identified by EASE analysis. Lastly, the assembly of a functional nucleolus (Zatsepina et al., 2003) following fertilization in the preimplantation embryo is consistent with the overrepresentation of genes related to transcription from Pol I promoters. These changes in genes implicated in rRNA synthesis and ribosome biogenesis may underlie the global increase in the rate of protein synthesis.

The increase in GO biological processes classified as tricarboxylic acid cycle and oxidative phosphorylation at the 8-cell stage and carbohydrate metabolism at the blastocyst stage are consistent with a metabolic switch that occurs around the 8-cell stage. Before compaction, pyruvate is the main energy source (Gardner, 1998), mitochondrial cristae

Table 3
Selected biological processes overrepresented in each developmental stage

GO biological process	Overrepresentation (EASE score)				
	Oo	1C	2C	8C	BL
Protein biosynthesis	6.3E−06	2.4E−04	6.0E−04	5.4E−12	1.2E−14
Ribosome biogenesis and assembly	1.8E−04	3.4E−02	9.1E−04	1.6E−10	1.3E−06
Transcription from Pol I promoter	4.0E−02	*	2.4E−02	2.7E−04	*
Carbohydrate metabolism	*	*	*	*	2.6E−02
Tricarboxylic acid cycle	*	*	*	4.8E−03	4.2E−02
Oxidative phosphorylation	3.2E−02	*	*	2.0E−02	4.2E−02
Chromatin assembly or disassembly	*	1.7E−02	1.3E−02	*	*
DNA repair	5.9E−03	*	*	*	*
Response to DNA damage stimulus	9.8E−04	*	*	*	*

The EASE analysis tool was used to calculate likelihood of overrepresentation for annotation categories associated with each group of genes detected in at least three of four replicates of a stage. Lists of genes detected in each stage were compared to the population of all genes detected in any stage (the lists of genes detected from Table 1). Biological process categories with an EASE score <0.05 were grouped into functional classes. A complete list of GO biological processes from each stage can be found in Supplemental Table S1.
* Functional classes with an EASE score >0.05 in that particular stage.

are condensed (Calarco and Brown, 1969), ATP/ADP ratios are high (Ginsberg and Hillman, 1975), and oxygen consumption is low and largely cyanide insensitive (30%) (Trimarchi et al., 2000), which taken together indicate a low level of oxidative phosphorylation. By the blastocyst stage, there is an increase in oxidative phosphorylation as evidenced by glucose being the main energy source (Gardner, 1998), mitochondrial cristae are of normal morphology, that is, traverse (Calarco and Brown, 1969), expression of genes critical for oxidative phosphorylation is increased (Pikó and Taylor, 1987; Taylor and Piko, 1995), ATP/ADP ratios are markedly reduced (Ginsberg and Hillman, 1975), and oxygen consumption is dramatically increased and largely cyanide sensitive (70%) (Trimarchi et al., 2000). The increase in ATP production is likely critical for supporting increased anabolic pathways such as protein and RNA biosynthesis and the ion-transporting activities required for blastocyst formation.

The enrichment of genes functioning in chromatin assembly or disassembly in the 1- and 2-cell stages is consistent with the temporally regulated changes in chromatin structure that commence in the 1-cell embryo and progress through the 2-cell stage. Remodeling of the paternal genome entails a protamine-histone exchange, active DNA demethylation (Oswald et al., 2000; Santos et al., 2002), and preferential accumulation of hyperacetylated histones (Adenot et al., 1997). Collectively, these changes may provide a window of opportunity for transcription factors to gain access to their *cis*-cognate DNA binding sequences and account for the higher level of transcription that occurs in the male pronucleus when compared to the female pronucleus (Aoki et al., 1997; Bouniol et al., 1995). During the 2-cell stage, a chromatin-mediated transcriptionally repressive state develops (Majumder and DePamphilis, 1995) that can be relieved by inducing histone acetylation or inhibiting the second round of DNA replication (Davis et al., 1996; Ma et al., 2001; Majumder and DePamphilis, 1995), which could provide another opportunity to remodel the zygotic genome.

For the majority of these processes, the EASE values are highest at the 8-cell stage, when the first epithelium forms and the blastomeres assume more somatic cell-like properties. These changes may highlight this transition and serve to prepare the embryo for differentiation into trophectodermal and ICM cells.

EASE analysis revealed certain processes that were not suggested from previous studies. For example, response to DNA damage and DNA repair genes is overrepresented in the oocyte when compared to 1-cell through blastocyst stages. This overrepresentation may reflect the oocyte's response to selective pressures to insure genomic integrity. Following birth, the female inherits her full complement of oocytes, which are nondividing cells. The oocytes remain arrested in the first meiotic prophase before their recruitment into a growing follicle, and this period before oocyte growth and development can last for years, for example, >40 years in humans. Thus, genes critical to maintain genomic integrity of the female germline, that is, genes that compose the DNA repair machinery and involved in responding to DNA damage, may become overrepresented. Two examples are *Rad51*, a component of the postreplication, homologous DNA mismatch repair machinery (Sung et al., 2003), and *Xrcc5* (*Ku80*), which is critical for the nonhomologous end joining DNA repair pathway (Featherstone and Jackson, 1999).

EASE analysis of genes with interesting expression profiles

To examine further possible roles of genes whose expression profile is linked with specific developmental transitions (see Introduction), the nonredundant, differentially expressed gene list (Table 1) was used to group genes according to their temporal expression patterns. These gene lists were then subjected to EASE analysis to identify functional categories that are overrepresented for a particular expression pattern. Our discussion focuses on six expression profiles, namely, maternal, maternal-to-zygotic, 1-cell transient, 2-cell transient, 8-cell transient, and blastocyst

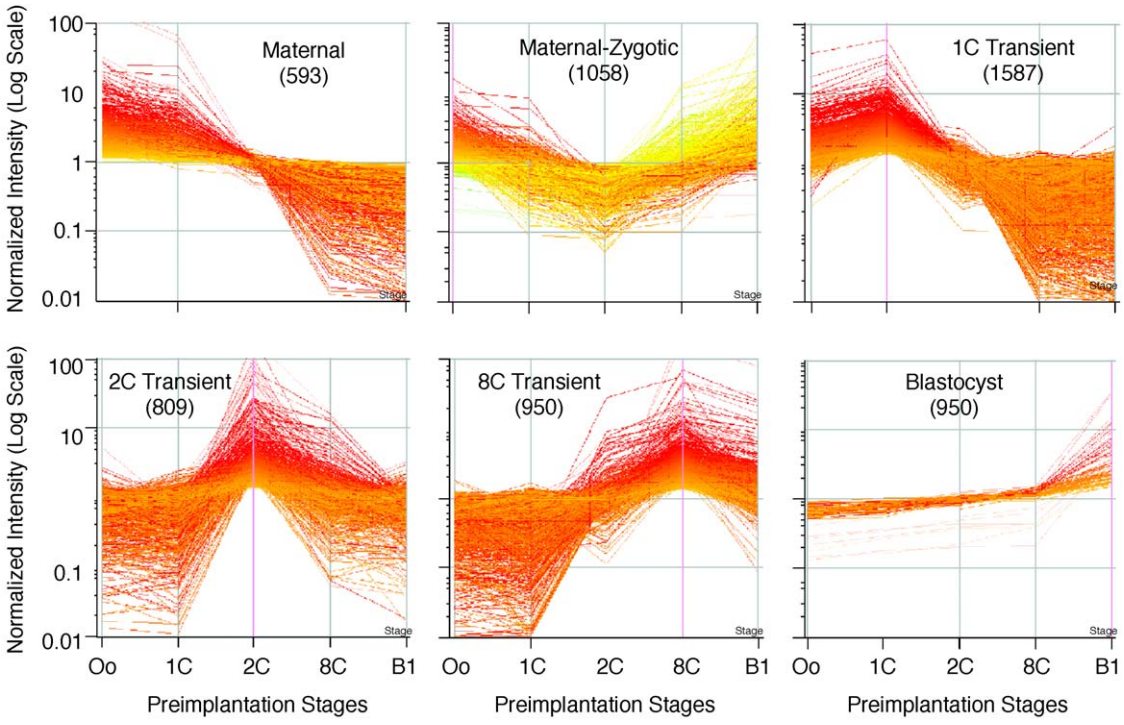


Fig. 4. Expression profiles of six major expression patterns during preimplantation development. The number of Unigenes presented in these patterns (designated maternal, maternal-zygotic, 1C transient, 2C transient, 8C transient, and blastocyst) is indicated in parentheses. Colors correspond to relative RNA abundance (as in Fig. 1) at the stage most characteristic of that particular pattern. For example, expression levels at the oocyte stage set each gene's profile color used for comparison to other stages in the "maternal" expression pattern; the 1C stage was similarly used as the relative abundance reference for the "1C transient" pattern.

(Fig. 4 and Table 4). (See Supplemental Table S2 for a complete list of genes that show each expression profile, Supplemental Table S3 for lists of genes that are represented in each functional category in Table 4, and Supplemental Table S4 for complete lists of GO categories, including biological process and molecular function, as well as the Swissprot keyword and KEGG pathway search that passed EASE score <0.05 filter.)

Oocyte maturation initiates the destruction of many maternal RNAs and this destruction continues following fertilization and through the 2-cell stage, resulting in a >90% decrease in mass of these transcripts (Schultz, 2002). One class of transcripts continues to be degraded following genome activation at the 2-cell stage (i.e., maternal) while another class is replaced by zygotic transcripts (i.e., maternal-zygotic). Because the "maternal-zygotic" pattern represents replacing maternal transcripts with zygotic transcripts that are common to the oocyte and early embryo, for example, actin, it is not surprising that gene categories overrepresented in this group consist largely of basic cellular functions. In particular, out of the 632 genes in this group that have GO biological process annotation, 63% function in metabolism, such as electron transport and oxidative phosphorylation, coenzyme metabolism, protein metabolism, lipid metabolism, or nucleotide metabolism including ATP synthesis.

Genes with "maternal" patterns include oocyte-specific genes such as *Mos* and *Zp3* but also include many non-oocyte-specific transcripts. Of interest is that the most prominent overrepresented category (approximately 27% of the 297 genes with GO annotation for biological process) is involved in cell communication (including the subcategory of signal transduction, half of which are related to cell surface receptor-linked signal transduction and cell adhesion). The oocyte and companion follicle cells communicate bidirectionally during folliculogenesis by both gap junction-mediated and paracrine mechanisms (Matzuk et al., 2002). The outcome is a preovulatory antral follicle that harbors a highly differentiated oocyte; oocytes, which are the only cells in the female that can undergo meiosis, express oocyte-specific genes, and only oocyte cytoplasm can reprogram a nucleus to support development to term. Following fertilization, however, oocytes are transformed to blastomeres that remain totipotent until the 8-cell stage (Handyside, 1978). The embryo develops in an environment rich in extracellular signals and the decrease in representation of genes involved in cell communication may reflect a requirement to insulate the embryo from these signals so that the blastomeres may remain totipotent.

We next examined three transient expression patterns designated 1-cell, 2-cell, and 8-cell; transiently expressed genes are defined as genes whose expression is at least 40% higher in one stage compared to the other four stages. The existence of transient expression during the 2-cell stage is well documented from previous studies using quantitative

Table 4
Selected biological processes overrepresented in major expression patterns

Gene pattern	Overrepresented categories of biological processes	EASE score	No. of genes	Supplemental Table S3
Maternal	Cell communication	4.69E−05	81	A1
	A. Signal transduction	4.07E−03	61	A2
	Cell surface receptor linked signal transduction	3.71E−03	30	A3
	B. Cell adhesion	6.91E−03	19	A4
Maternal-zygotic	Metabolism	1.14E−03	400	B1
	A. Electron transport or oxidative phosphorylation	3.09E−04	48	B2
	B. Coenzyme metabolism	1.12E−05	25	B3
	C. Nucleotide metabolism—nucleoside triphosphate metabolism	7.89E−03	10	B4
	D. Lipid metabolism	3.56E−03	16	B5
	E. Protein metabolism	1.64E−02	167	B6
Transient 1-Cell	Protein metabolism—protein modification	3.17E−05	114	C1
	Protein amino acid phosphorylation	2.91E−03	60	C2
	Cell proliferation—cell cycle	9.56E−04	78	C3
	Mitotic cell cycle	3.40E−02	36	C4
	Transcription—regulation of transcription	2.32E−02	131	C5
	DNA metabolism	4.23E−02	52	C6
Transient 2-Cell	Transcription	2.54E−04	86	D1
	Regulation of transcription, DNA dependent	9.46E−03	70	D2
	RNA metabolism—RNA processing	2.22E−03	23	D3
	mRNA processing	7.79E−03	13	D4
	Metabolism	2.66E−03	264	D5
	Phosphate metabolism—protein amino acid dephosphorylation	3.40E−02	10	D6
	Cell cycle—regulation of cell cycle	2.37E−02	23	D7
Transient 8-Cell	Metabolism			
	A. Biosynthesis	7.63E−04	70	E1
	1. Protein biosynthesis	1.18E−02	40	E2
	2. Phospholipid biosynthesis	1.67E−02	6	E3
	B. Amine metabolism or amino acid metabolism	8.37E−03	18	E4
	Ribosome biogenesis	6.05E−03	12	E5
	Transport—protein targeting	2.63E−02	19	E6
Blastocyst	Metabolism	1.23E−02	32	F

Gene patterns listed in the table are as shown in Fig. 4. After EASE analysis, characteristic biological processes are listed for annotated genes in that particular pattern with an EASE score less than 0.05. The number of genes in each pattern or category is shown next to the EASE score. See Supplemental Table S2 for a complete list of genes for each gene expression pattern. Details for genes listed in column 4 can be found in Supplemental Table S3 (A1–F). See Supplemental Table S4 for a complete list of functional categories that are overrepresented.

analysis of high-resolution, 2D protein gels (Latham et al., 1991), mRNA differential display (Ma et al., 2001), and RT-PCR (e.g., Davis et al., 1996; Latham et al., 1995), but the number of genes identified was quite limited. We identified 1587 (1879), 809 (911), and 950 (1132) Unigenes (Affymetrix probe sets) transiently expressed in 1-cell, 2-cell, and 8-cell stages, respectively (Fig. 4 and Supplemental Table S2 for a complete list of genes).

The large number of transcripts displaying a transient increase during the 1-cell stage was unanticipated. This subset includes 18% of all genes (8793 Unigenes) expressed in the 1-cell embryo (Table 1). Note that one highly overrepresented gene category is "regulation of transcription" that consists of 15.5% of all genes annotated (131 of 848) in the 1-cell transient list. Even though the 1-cell embryo is transcriptionally competent (Aoki et al., 1997; Bouniol et al., 1995), the majority of these transcripts are likely maternal mRNAs recruited following egg activation—mRNA recruitment entails polyadenylation (e.g., Oh et al., 2000) that would lead to more efficient oligo-dT priming and hence an apparent increase in transcript abundance—because α-amanitin inhibited the up-regulation of only 1 out of 47 up-regulated 1-cell transcripts (Hamatani et al., 2004). Recruitment of maternal mRNAs appears coupled to genome activation because inhibiting polyadenylation inhibits transcription in 1-cell embryos (Aoki et al., 2003). The other gene categories that are overrepresented include protein modification (13.4%), cell cycle (9.2%), and DNA metabolism (6.1%). Many of the changes in the pattern of protein synthesis that occur following egg activation are due to protein phosphorylation, and in particular, protein phosphorylation catalyzed by the cAMP-dependent protein kinase, PKA, appears necessary for genome activation (Latham et al., 1992; Poueymirou and Schultz, 1989). These previous findings agree with the EASE analysis that revealed a significant number of genes function in protein modification, half of which are involved in protein phosphorylation, including PKA. In addition, after fertilization, the newly formed 1-cell embryo must initiate DNA replication for the first time following a long hiatus; the last round of DNA replication occurred when the oogonia underwent DNA replication and entered the

first meiotic prophase during fetal development. Thus, it is not surprising that genes functioning in cell cycle (9.2%), especially in the mitotic cell cycle (50% of this group), as well as DNA metabolism (6.1%) would be overrepresented (e.g., DNA replication genes *Recc1*, *Blm*, *Nfib*, *Pcna*, and *Orc6l*, and DNA methylation genes *Dnmt2* and *Dnmt3a*).

Although the 1-cell embryo is transcriptionally active, the major phase of genome activation occurs during the 2-cell stage (Schultz, 2002) and likely underlies the transformation of the differentiated oocyte into the totipotent blastomeres; this reprogramming was evidenced from the hierarchical cluster analysis described above. The class of genes transiently expressed during genome activation has been proposed to indicate that genome activation is global and relatively promiscuous, and that a function of the transcriptionally repressive state is to dictate the appropriate profile of gene expression that is compatible with further development (Ma et al., 2001). Thus, although genes with strong promoters and/or enhancers would be preferentially expressed, many other genes may be inappropriately, that is, opportunistically expressed (especially at basal levels of transcription) during this transition. An example is the transient expression of *MuERV-L* during the 2-cell stage (Ma et al., 2001; Wang et al., 2001). The formation of a transcriptionally repressive state could preferentially decrease the expression of these genes but permit the continued expression of genes that are regulated by strong promoters or enhancers. EASE analysis suggests that this view is an oversimplification.

EASE analysis of the 411 GO biological process annotated genes in the 2-cell transient list showed that 86 genes (20.9%) are related to transcription. Also note that EASE analysis at each developmental stage indicated an overrepresentation of genes involved in chromatin remodeling during genome activation (Table 3). The majority of these genes regulate DNA-dependent transcription (17.0%), 20% of which are for transcription from Pol II promoters (examples include *Gabpa*, *Dr1*, *Idb4*, *Crsp2*, *Klf9*, *Chd1*, *Zfp148*, and *Taf9*). Similarly, RNA metabolism in the form of RNA processing (5.6%) is overrepresented. More than half of these genes function in mRNA processing, including *ASF*, *Rbmx*, *Nssr*, and *Rngtt*. These results suggest that a specific subset of genes, that is, genes involved in transcription and RNA metabolism, is transiently expressed and may be critical to "kick-start" genome activation.

A smaller class of 23 genes (5.6%) functioning in regulation of cell cycle is overrepresented in the 2-cell transient as compared to the 1-cell transient group (9.2%). Although similar in functional annotation, the individual cell cycle genes from the 1- and 2-cell transient groups are different. Six of the 23 cell cycle genes in the 2-cell transient list function in the G1/S transition of mitotic cell cycle. This may reflect the relatively short G1 phase in the 2-cell embryo (Bolton et al., 1984), which contrasts to the lengthy G1 phase (approximately 10 h) in the 1-cell embryo (Aoki and Schultz, 1999). Another overrepresented gene category includes 10 genes implicated in protein dephosphorylation in contrast to the overrepresentation of genes involved in protein phosphorylation detected in the 1-cell transient group.

Genes overrepresented in the transient 8-cell groups (a total of 457 genes annotated for GO biological process) were involved in the biological processes of biosynthesis (15.3%, 57.1% of which are for protein biosynthesis), ribosome biogenesis (2.6%), and protein targeting (4.2%). These changes may underlie the dramatic increase in protein synthesis that follows compaction (Epstein and Smith, 1973; Van Blerkom and Brockway, 1975) and the transition from totipotent blastomeres to blastomeres of restricted potential (Handyside, 1978) and more somatic cell-like. Consistent with an increased demand for plasma membrane biogenesis—there is an increase in surface area due to cell division in the absence of net growth—is the overrepresentation of genes in phospholipid biosynthesis. Last, a relatively small set of genes (80) characterized the "blastocyst" expression profile, that is, genes first turned on at the blastocyst stage or after the 8-cell stage. Only one biological process, the generalized "metabolism" category (76.2% out of the 42 annotated in this group), passed the EASE score filter (≤0.05) and could be considered as overrepresented.

As mentioned in the Introduction, there are two very recent published studies that used microarrays to assess global patterns of gene expression during preimplantation development (Hamatani et al., 2004; Wang et al., 2004). The combination of these studies and the results described here offer a comprehensive data set covering nearly all mouse genes throughout embryogenesis. Wang et al. (2004) utilized the Affymetrix U74Av2 GeneChip to profile many developmental stages whereas Hamatani et al. (2004) used the NIA 22K microarray, a printed oligomer array of genes identified from mouse embryo cDNA libraries and thought to be embryo specific or enriched. RNA profiles reported here were initially assayed on the U74Av2 GeneChip, then expanded to MOE430A and B GeneChips, with good concordance between the two versions of Affymetrix arrays. The MOE430 set provides greater gene coverage than the U74Av2 array (2.8×) and benefits from whole-genome sequencing to reduce the number of redundant probe sets and improve probe sequence quality. Preliminary mapping of the overlap between the MOE430 set and the NIA 22K platforms indicates that approximately 60% of the NIA array is covered in the MOE430 set, and another approximately 8100 transcripts are assayed only on the NIA array. In addition, approximately 26,500 transcripts are assayed only on the MOE430 chips, approximately 8400 of which are detected in the preimplantation mouse embryo.

In summary, our large-scale microarray analysis for transcript profiling during preimplantation mouse embryo development appears reliable and has a high degree of statistical confidence. The analysis confirmed previous

conclusions drawn from analyses of a limited number of genes, for example, a dramatic reprogramming of gene expression occurs during the 2-cell, but also revealed unanticipated insights that could not be deduced previously from analysis of a limited number of genes. In addition, these results validate this hypothesis-generating approach by identifying genes involved in critical biological processes that will be the subject of the more traditional hypothesis-driven approach.

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References

Adenot, P.G., Mercier, Y., Renard, J.-P., Thompson, E.M., 1997. Differential H4 acetylation of paternal and maternal chromatin precedes DNA replication and differential transcriptional activity in pronuclei of 1-cell mouse embryos. Development 124, 4615–4625.

Aoki, E., Schultz, R.M., 1999. DNA replication in the 1-cell mouse embryo: stimulatory effect of histone acetylation. Zygote 7, 165–172.

Aoki, F., Worrad, D.M., Schultz, R.M., 1997. Regulation of transcriptional activity during the first and second cell cycles in the preimplantation mouse embryo. Dev. Biol. 181, 296–307.

Aoki, F., Hara, K.T., Schultz, R.M., 2003. Acquisition of transcriptional competence in the 1-cell mouse embryo: requirement for recruitment of maternal mRNAs. Mol. Reprod. Dev. 64, 270–274.

Beck, F., Erler, T., Russell, A., James, R., 1995. Expression of Cdx-2 in the mouse embryo and placenta: possible role in patterning of the extra-embryonic membranes. Dev. Dyn. 204, 219–227.

Biggers, J.D., Bell, J.E., Benos, D.J., 1988. Mammalian blastocyst: transport functions in a developing epithelium. Am. J. Physiol. 255, C419–C432.

Bolton, V.N., Oades, P.J., Johnson, M.H., 1984. The relationship between cleavage, DNA replication, and gene expression in the mouse 2-cell embryo. J. Embryol. Exp. Morphol. 79, 139–163.

Bouniol, C., Nguyen, E., Debey, P., 1995. Endogenous transcription occurs at the 1-cell stage in the mouse embryo. Exp. Cell Res. 218, 57–62.

Calarco, P.G., Brown, E.H., 1969. An ultrastructural and cytological study of preimplantation development of the mouse. J. Exp. Zool. 171, 253–283.

Christians, E., Campion, E., Thompson, E.M., Renard, J.-P., 1995. Expression of the HSP 70.1 gene, a landmark of early zygotic gene activity in the mouse embryo, is restricted to the first burst of transcription. Development 121, 113–122.

Davis Jr., W., Schultz, R.M., 2000. Developmental change in TATA-box utilization during preimplantation mouse development. Dev. Biol. 218, 275–283.

Davis Jr., W., DeSousa, P.D., Schultz, R.M., 1996. Transient expression of translation initiation factor eIF-4C during the 2-cell stage of the preimplantation mouse embryo: identification by mRNA differential display and the role of DNA replication. Dev. Biol. 181, 296–307.

Doherty, A.S., Mann, M.R., Tremblay, K.D., Bartolomei, M.S., Schultz, R.M., 2000. Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo. Biol. Reprod. 62, 1526–1535.

Epstein, C.J., Smith, S.A., 1973. Amino acid uptake and protein synthesis in preimplantation mouse embryos. Dev. Biol. 33, 171–184.

Featherstone, C., Jackson, S.P., 1999. Ku, a DNA repair protein with multiple cellular functions? Mutat. Res. 434, 3–15.

Fleming, T.P., Sheth, B., Fesenko, I., 2001. Cell adhesion in the preimplantation mammalian embryo and its role in trophectoderm differentiation and blastocyst morphogenesis. Front. Biosci. 6, D1000–D1007.

Gardiner, C.S., Williams, J.S., Menino Jr., A.R., 1990. Sodium/potassium adenosine triphosphatase α- and β-subunit and α-subunit mRNA levels during mouse development in vitro. Biol. Reprod. 43, 788–794.

Gardner, D.K., 1998. Changes in requirements and utilization of nutrients during mammalian preimplantation embryo development and their significance in embryo culture. Theriogenology 49, 83–102.

Ginsberg, L., Hillman, N., 1975. Shifts in ATP synthesis during preimplantation stages of mouse embryos. J. Reprod. Fertil. 43, 83–90.

Hamatani, T., Carter, M.G., Sharov, A.A., Ko, M.S., 2004. Dynamics of global gene expression changes during mouse preimplantation development. Dev. Cell 6, 117–131.

Handyside, A.H., 1978. Time of commitment of inside cells isolated from preimplantation mouse embryos. J. Embryol. Exp. Morphol. 45, 37–53.

Hartshorn, C., Rice, J.E., Wangh, L.J., 2002. Developmentally-regulated changes of Xist RNA levels in single preimplantation mouse embryos, as revealed by quantitative real-time PCR. Mol. Reprod. Dev. 61, 425–436.

Ho, Y., Doherty, A.S., Schultz, R.M., 1994. Mouse preimplantation embryo development in vitro: effect of sodium concentration in culture media on RNA synthesis and accumulation and gene expression. Mol. Reprod. Dev. 38, 131–141.

Hosack, D.A., Dennis Jr., G., Sherman, B.T., Lane, H.C., Lempicki, R.A., 2003. Identifying biological themes within lists of genes with EASE. Genome Biol. 4, R70.

Hyafil, F., Babinet, C., Jacob, F., 1981. Cell–cell interactions in early embryogenesis: a molecular approach to the role of calcium. Cell 26, 447–454.

Johnson, M.H., Maro, B., 1986. Time and space in the mouse early embryo: a cell biological approach to cell diversification. In: Rossant, J., Pedersen, R.A. (Eds.), Experimental Approaches to Mammalian Embryonic Development. Cambridge Univ. Press, Cambridge, pp. 35–65.

Kidder, G.M., Winterhager, E., 2001. Intercellular communication in preimplantation development: the role of gap junctions. Front. Biosci. 6, D731–D736.

Kigami, D., Minami, N., Takayama, H., Imai, H., 2003. MuERV-L is one of the earliest transcribed genes in mouse one-cell embryos. Biol. Reprod. 68, 651–654.

Ko, M.S., Kitchen, J.R., Wang, X., Threat, T.A., Hasegawa, A., Sun, T., Grahovac, M.J., Kargul, G.J., Lim, M.K., Cui, Y., Sano, Y., Tanaka, T., Liang, Y., Mason, S., Paonessa, P.D., Sauls, A.D., DePalma, G.E., Sharara, R., Rowe, L.B., Eppig, J., Morrell, C., Doi, H., 2000. Large-scale cDNA analysis reveals phased gene expression patterns during preimplantation mouse development. Development 127, 1737–1749.

LaMarca, M.J., Wassarman, P.M., 1979. Program of early development in the mammal: changes in the absolute rates of synthesis of ribosomal proteins during oogenesis and early embryogenesis in the mouse. Dev. Biol. 73, 103–119.

Latham, K.E., Garrels, J.I., Chang, C., Solter, D., 1991. Quantitative analysis of protein synthesis in mouse embryos: I. Extensive reprogramming at the one- and two-cell stages. Development 112, 921–932.

Latham, K.E., Solter, D., Schultz, R.M., 1992. Acquisition of a transcriptionally permissive state during the 1-cell stage of mouse embryogenesis. Dev. Biol. 149, 457–462.

Latham, K.E., Rambhatla, L., Hayashizaki, Y., Chapman, V.M., 1995. Stage-specific induction and regulation by genomic imprinting of the

mouse U2afbp-rs gene during preimplantation development. Dev. Biol. 168, 670–676.
Ma, J., Svoboda, P., Schultz, R.M., Stein, P., 2001. Regulation of zygotic gene activation in the preimplantation mouse embryo: global activation and repression of gene expression. Biol. Reprod. 64, 1713–1721.
Majumder, S., DePamphilis, M.L., 1994. TATA-dependent enhancer stimulation of promoter activity in mice is developmentally acquired. Mol. Cell. Biol. 14, 4258–4268.
Majumder, S., DePamphilis, M.L., 1995. A unique role for enhancers is revealed during early mouse development. BioEssays 17, 879–889.
Matsumoto, K., Anzai, M., Nakagata, N., Takahashi, A., Takahashi, Y., Miyata, K., 1994. Onset of paternal gene activation in early mouse embryos fertilized with transgenic mouse sperm. Mol. Reprod. Dev. 39, 136–140.
Matzuk, M.M., Burns, K.H., Viveiros, M.M., Eppig, J.J., 2002. Intercellular communication in the mammalian ovary: oocytes carry the conversation. Science 296, 2178–2180.
Nichols, J., Zevnik, B., Anastassiadis, K., Niwa, H., Klewe-Nebenius, D., Chambers, I., Scholer, H., Smith, A., 1998. Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct 4. Cell 95, 379–391.
Niswander, L., Martin, G.R., 1992. Fgf-4 expression during gastrulation, myogenesis, limb and tooth development in the mouse. Development 114, 755–768.
Niwa, H., Miyazaki, J., Smith, A.G., 2000. Quantitative expression of oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nat. Genet. 24, 372–376.
Oh, B., Hwang, S., McLaughlin, J., Solter, D., Knowles, B.B., 2000. Timely translation during the mouse oocyte-to-embryo transition. Development 127, 3795–3803.
Oswald, J., Engemann, S., Lane, N., Mayer, W., Olek, A., Fundele, R., Dean, W., Reik, W., Walter, J., 2000. Active demethylation of the paternal genome in the mouse zygote. Curr. Biol. 10, 475–478.
Palmieri, S.L., Peter, W., Hess, H., Schöler, H.R., 1994. Oct-4 transcription factor is differentially expressed in the mouse embryo during the establishment of the first two extraembryonic cell lineages involved in implantation. Dev. Biol. 166, 259–267.
Pedersen, R.A., 1986. Potency, lineage, and allocation in preimplantation mouse embryos. In: Rossant, J., Pedersen, R.A. (Eds.), Experimental Approaches to Mammalian Embryonic Development. Cambridge Univ. Press, Cambridge, pp. 143–156.
Pesce, M., Scholer, H.R., 2000. Oct-4: control of totipotency and germline determination. Mol. Reprod. Dev. 55, 452–457.
Pikó, L., Taylor, K.D., 1987. Amounts of mitochondrial DNA and abundance of some mitochondrial gene transcripts in early embryos. Dev. Biol. 123, 364–374.
Poueymirou, W.T., Schultz, R.M., 1989. Regulation of mouse preimplantation development: inhibition of synthesis of proteins in the two-cell embryo that require transcription by inhibitors of cAMP-dependent protein kinase. Dev. Biol. 133, 588–599.
Ram, P.T., Schultz, R.M., 1993. Reporter gene expression in G2 of the 1-cell mouse embryo. Dev. Biol. 156, 552–556.
Santos, F., Hendrich, B., Reik, W., Dean, W., 2002. Dynamic reprogramming of DNA methylation in the early mouse embryo. Dev. Biol. 241, 172–182.
Schultz, R.M., 1993. Regulation of zygotic gene activation in the mouse. BioEssays 15, 531–538.
Schultz, R.M., 2002. The molecular foundations of the maternal to zygotic transition in the preimplantation embryo. Hum. Reprod. Updat. 8, 323–331.
Schultz, R.M., Letourneau, G.E., Wassarman, P.M., 1979. Program of early development in the mammal: changes in patterns and absolute rates of tubulin and total protein synthesis during oogenesis and early embryogenesis in the mouse. Dev. Biol. 68, 341–359.
Sharov, A.A., Piao, Y., Matoba, R., Dudekula, D.B., Qian, Y., VanBuren, V., Falco, G., Martin, P.R., Stagg, C.A., Bassey, U.C., Wang, Y., Carter, M.G., Hamatani, T., Aiba, K., Akutsu, H., Sharova, L., Tanaka, T.S., Kimber, W.L., Yoshikawa, T., Jaradat, S.A., Pantano, S., Nagaraja, R., Boheler, K.R., Taub, D., Hodes, R.J., Longo, D.L., Schlessinger, D., Keller, J., Klotz, E., Kelsoe, G., Umezawa, A., Vescovi, A.L., Rossant, J., Kunath, T., Hogan, B.L., Curci, A., D'Urso, M., Kelso, J., Hide, W., Ko, M.S., 2003. Transcriptome analysis of mouse stem cells and early embryos. PLoS Biol. 1, 410–419.
Sung, P., Krejci, L., Van Komen, S., Sehorn, M.G., 2003. Rad51 recombinase and recombination mediators. J. Biol. Chem. 278, 42729–42732.
Tanaka, M., Hennebold, J.D., Macfarlane, J., Adashi, E.Y., 2001. A mammalian oocyte-specific linker histone gene H1oo: homology with the genes for the oocyte-specific cleavage stage histone (cs-H1) of sea urchin and the B4/H1M histone of the frog. Development 128, 655–664.
Taylor, K.D., Piko, L., 1995. Mitochondrial biogenesis in early mouse embryos: expression of the mRNAs for subunits IV, Vb, and VIIc of cytochrome *c* oxidase and subunit 9 (P1) of H(+)-ATP synthase. Mol. Reprod. Dev. 40, 29–35.
Trimarchi, J.R., Liu, L., Porterfield, D.M., Smith, P.J., Keefe, D.L., 2000. Oxidative phosphorylation-dependent and-independent oxygen consumption by individual preimplantation mouse embryos. Biol. Reprod. 62, 1866–1874.
Van Blerkom, J., 1981. Structural relationship and posttranslational modification of stage-specific proteins synthesized during early preimplantation development in the mouse. Proc. Natl. Acad. Sci. U.S.A. 78, 7629–7633.
van Blerkom, J., Brockway, G.O., 1975. Qualitative patterns of protein synthesis in the preimplantation mouse embryo:x I. Normal pregnancy. Dev. Biol. 44, 148–157.
Wang, Q., Chung, Y.G., deVries, W.N., Struwe, M., Latham, K.E., 2001. Role of protein synthesis in the development of a transcriptionally permissive state in one-cell stage mouse embryos. Biol. Reprod. 65, 748–754.
Wang, Q.T., Piotrowska, K., Ciemerych, M.A., Milenkovic, L., Scott, M.P., Davis, R.W., Zernicka-Goetz, M., 2004. A genome-wide study of gene activity reveals developmental signaling pathways in the preimplantation mouse embryo. Dev. Cell 6, 133–144.
Waterston, R.H., Lindblad-Toh, K., Birney, E., Rogers, J., Abril, J.F., Agarwal, P., Agarwala, R., Ainscough, R., Alexandersson, M., An, P., Antonarakis, S.E., Attwood, J., Baertsch, R., Bailey, J., Barlow, K., Beck, S., Berry, E., Birren, B., Bloom, T., Bork, P., Botcherby, M., Bray, N., Brent, M.R., Brown, D.G., Brown, S.D., Bult, C., Burton, J., Butler, J., Campbell, R.D., Carninci, P., Cawley, S., Chiaromonte, F., Chinwalla, A.T., Church, D.M., Clamp, M., Clee, C., Collins, F.S., Cook, L.L., Copley, R.R., Coulson, A., Couronne, O., Cuff, J., Curwen, V., Cutts, T., Daly, M., David, R., Davies, J., Delehaunty, K.D., Deri, J., Dermitzakis, E.T., Dewey, C., Dickens, N.J., Diekhans, M., Dodge, S., Dubchak, I., Dunn, D.M., Eddy, S.R., Elnitski, L., Emes, R.D., Eswara P., Eyras, E., Felsenfeld, A., Fewell, G.A., Flicek, P., Foley K., Frankel, W.N., Fulton, L.A., Fulton, R.S., Furey, T.S., Gage, D., Gibbs, R.A., Glusman, G., Gnerre, S., Goldman, N., Goodstadt, L., Grafham, D., Graves, T.A., Green, E.D., Gregory, S., Guigo, R., Guyer, M., Hardison R.C., Haussler, D., Hayashizaki, Y., Hillier, L.W., Hinrichs, A., Hlavina W., Holzer, T., Hsu, F., Hua, A., Hubbard, T., Hunt, A., Jackson, I., Jaffe, D.B., Johnson, L.S., Jones, M., Jones, T.A., Joy, A., Kamal, M., Karlsson, E.K., et al., 2002. Initial sequencing and comparative analysis of the mouse genome. Nature 420, 520–562.
Watson, A.J., Barcroft, L.C., 2001. Regulation of blastocyst formation. Front. Biosci. 6, D708–D730.
Williams, C.J., Schultz, R.M., Kopf, G.S., 1996. G protein gene expression during mouse oocyte growth and maturation, and preimplantation embryo development. Mol. Reprod. Dev. 44, 315–323.
Williams, J.W., Hawes, S.M., Patel, B., Latham, K.E., 2002. Trophectoderm-specific expression of the X-linked Bex1/Rex3 gene in preimplantation stage mouse embryos. Mol. Reprod. Dev. 61, 281–287.
Yeom, Y.I., Fuhrmann, G., Ovitt, C.E., Brehm, A., Ohbo, K., Gross, M.,

Schultz, R.M., Schöler, H.R., 1996. Germline regulatory element of Oct-4 specific for the totipotent cycle of embryonal cells. Development 122, 881–894.

Yu, J., Hecht, N.B., Schultz, R.M., 2001. Expression of MSY2 in mouse oocytes and preimplantation embryos. Biol. Reprod. 65, 1260–1270.

Zatsepina, O., Baly, C., Chebrout, M., Debey, P., 2003. The step-wise assembly of a functional nucleolus in preimplantation mouse embryos involves the Cajal (coiled) body. Dev. Biol. 253, 66–83.

Zeng, F., Schultz, R.M., 2003. Gene expression in mouse oocytes and preimplantation embryos: use of suppression subtractive hybridization to identify oocyte- and embryo-specific genes. Biol. Reprod. 68, 31–39.