

## **Up-regulation of Transcriptional Factor E2F-1 in Mammalian Embryos: a Lesson from Gene Expression Profiles by DNA Microarray.**

**Shunping Wang<sup>1,2</sup>, Chad Cowan<sup>3</sup>, Doug Melton<sup>3</sup> and Doug Powers<sup>1,2</sup>**  
**<sup>1</sup>Boston IVF, <sup>2</sup>Embryomics, and <sup>3</sup>Harvard University**

**Objective:** In the past, the studies of gene expression in preimplantation stage embryos have been limited due to the availability of material and the sensitivity of experimental methods. Thousands of embryos needed to be collected and only a few genes could be investigated even with a labor-intensive cDNA library assay. In this study, we overcame these two limitations by using two-round PCR and microarrays to analyze expression profiles of more than 20,000 genes from oocyte to blastocyst stages of the mouse embryo. This study was designed to investigate global gene expression and regulation during early mammalian pre-implantation development.

**Materials and Methods:** Three hundred cells were collected at the following developmental stages: oocyte, 1-, 2-, 4-, 8-cell, compacting embryos, morulae, and blastocysts. RNA was prepared by PicoPure RNA Isolation kit (Arcturus), and the amplification was achieved by two-round in vitro transcription with Ribo-Amp RNA amplification kit (Arcturus). Biotin-labeled amplified probes were then hybridized to DNA microarrays (Affymetrix U74Av2). Scanned arrays were analyzed using dChip software. All experiments were performed in triplicate to ensure consistent results.

**Results:** With two-round amplification, we are able to amplify the signals from relatively small number of cells (300 each stage). The consistency of our data is suggested by the high correlation coefficients ( $> 0.9$ ) between each experiment. Gene expression profiles for more than 20,000 genes and unknown ESTs were obtained in mouse preimplantation stage embryos from oocytes to blastocysts. The gene expression profiles of some previously studied genes such as ZP-1, ZP-2, and GDF-9 all correspond to the patterns shown in our results, which demonstrates the quality of our database.

In our study, we observed a large, dynamic change of gene expression profiles between different embryonic stages. One hundred and sixteen genes were up/down regulated between oocyte to 1-cell stage, and 1227 genes between 1- to 2-cell, 340 genes between 2- to 4-cell, 524 genes between 4- 8-cell, 343 genes between compacting embryos to morulae, and 133 genes between morulae to blastocysts (Figure 1). Surprisingly, there are very few genes up/down regulated between 8-cell and compacting embryos. Dramatic changes of gene expression profiles were especially observed between the 1 and 2 cell stage. Six hundred and eighty-five genes were up-regulated, indicating that many new messages were transcribed within a very short time frame. We further analyzed and categorized these genes by molecular functions as shown in Figure 2.

Among known genes, transcription regulation (20 genes) and signal transduction (8 genes) related genes account for less than 15% of these upregulated genes. However, they are known to be critical in the regulation of development. E2F-1, which is a member of a transcription factor family consisting six E2F species, is known to be tightly regulated through cell cycles. It is hypothesized E2F-1 plays an important role in the apoptotic pathways of early embryos. Our data shows the expression level of E2F-1 starts low at egg and 1-cell stage, and significantly increases at 2-cell and 4-cell stage. The level then drops at the 8-cell stage, and remains low through blastocyst (Figure 3). The surge of this transcriptional factor at 2-cell stage may lead to a cascade of newly transcribed genes crucial for embryo development at later stages.

**Conclusion:** In this study, we establish the gene expression profiles with more than 20,000 genes for mouse preimplantation stage embryos. To our knowledge, this is the first thorough database constructed with microarray analysis and can be used as a future reference for both research and clinical applications. The outcome of this study will enable us to further understand the mechanisms involved in the regulation of preimplantation embryos and accelerate the discovery of novel genes critical for human embryo development.

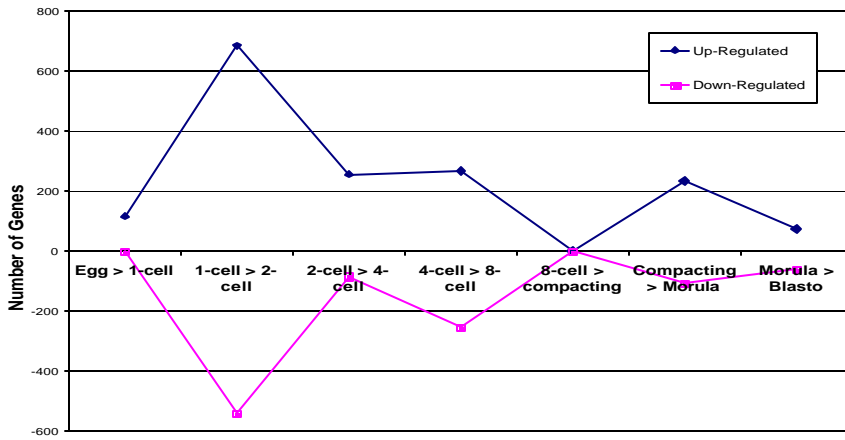


Figure 1. Number of genes up/down regulated in preimplantation stage embryos.

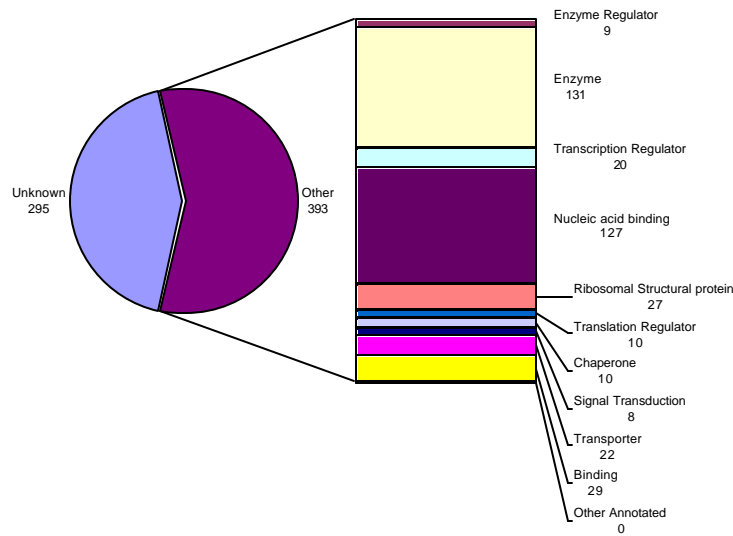


Figure 2. Functional analysis of 685 genes upregulated between 1- and 2-cell embryos

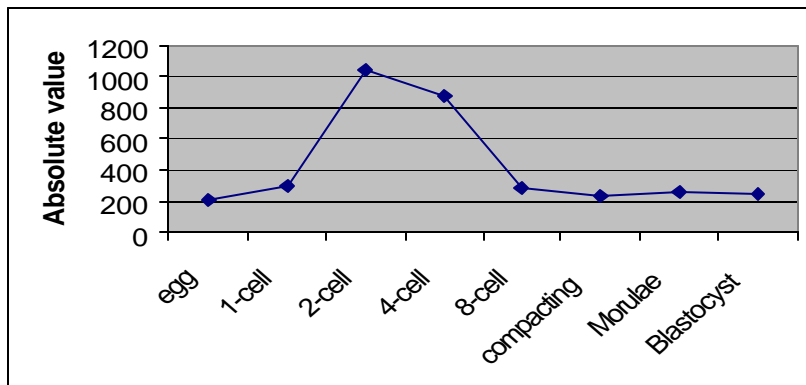


Figure 3. Gene expression pattern of E2F-1 in preimplantation stage embryos