Transcript Profiling of Individual Twin Blastomeres Derived by Splitting Two-Cell Stage Murine Embryos

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Transcript Profiling of Individual Twin Blastomeres Derived by Splitting Two-Cell Stage Murine Embryos

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ABSTRACT

In invertebrates and amphibians, informational macromolecules in egg cytoplasm are organized to provide direction to the formation of embryonic lineages, but it is unclear whether vestiges of such prepatterning exist in mammals. Here we examined whether twin blastomeres from 2-cell stage mouse embryos differ in mRNA content. mRNA from 26 blastomeres derived from 13 embryos approximately mid-way through their second cell cycle was subjected to amplification. Twenty amplified samples were hybridized to arrays. Of those samples that hybridized successfully, 12 samples in six pairs were used in the final analysis. Probes displaying normalized values >0.25 (n = 4573) were examined for consistent bias in expression within blastomere pairs. Although transcript content varied between both individual embryos and twin blastomeres, no consistent asymmetries were observed for the majority of genes, with only 178 genes displaying a >1.4-fold difference in expression across all six pairs. Although class discovery clustering showed that blastomere pairs separated into two distinct groups in terms of their differentially expressed genes, when the data were tested for significance of asymmetrical expression, only 39 genes with >1.4-fold change ratios in six of six blastomere pairs passed the two-sample t-test (P < 0.05). Transcripts encoding proteins implicated in RNA processing and cytoskeletal organization were among the most abundant, differentially distributed mRNA, suggesting that a stochastically based lack of synchrony in cell cycle progression between the two cells might explain at least some and possibly all of the asymmetries in transcript composition.

developmental biology, embryo, embryonic genome activation, gene regulation, lineage, maternal effect, pregnancy, zygote

INTRODUCTION

In most if not all invertebrates and amphibians, informational macromolecules, especially RNA and proteins, deposited in the egg by the mother are not distributed uniformly but instead are organized regionally [1, 2]. Although such molecules may become reoriented as the zygote forms [3], there is little doubt that their distribution in egg cytoplasm is a major determinant of cell fate specification and body axis determination in the developing embryo, although some plasticity in developmental outcome remains. In those taxons, e.g., ascidians and amphibians, in which fertilization is immediately followed by a series of specifically orientated cell divisions, prepatterned regions of the ooplasm are allocated to particular blastomeres and guide their fate. In Drosophila and other insects, in which nuclear divisions proceed initially without cell formation, gradients of morphogens are established within the cytoplasm by localized translation of specific mRNAs and direct the formation of specific cell lineages [4].

Whether or not some vestige of prepatterning exists in mammalian embryo development remains a subject of debate [5–8]. Most mammalian oocytes are not radially symmetrical and are often ovoid in profile [9]. The female pronucleus is usually not located in the center of the egg, and there is frequently a striking unevenness in cytoplasmic texture and organelle distribution, including the location of the microtubule organizing centers [9]. Accordingly, the first cleavage division of most zygotes, whatever its plane, would be unlikely to create two progeny blastomeres that are equivalent in content. On the other hand, it is unclear whether this heterogeneous cytoplasm is organized into distinct zones containing molecules with different informational content that might be allocated preferentially and consistently to particular progeny blastomeres and direct their development [10]. If such selective distribution were to occur, it would be dependent upon how the first cleavage plane was established. If cleavage planes were relatively random rather than occurring along predetermined axes [11, 12], prepatterning would seem improbable. On the other hand, if the first division plane were to some extent predictable, there would be the opportunity for consistent, asymmetrical partitioning of informational macromolecules as the zygote divides. For example, it has been reported that the hormone leptin and the signaling molecule STAT3 are initially localized to the animal pole of oocytes close to the plasma membrane and become more concentrated in one blastomere than in the other in the 2-cell stage embryo [13]. Although the leptin (Lep) gene itself is not transcribed during early embryo development, the maternal protein survives and ultimately becomes localized to murral trophectoderm, with diminished presence in the more polar cells close to the inner cell mass (ICM). VEGF and TGFβ2 may also undergo an analogous partitioning process in both human and mouse embryos [14]. Although there has been no published confirmation of these observations and no attempt to assess their functional significance [10], the data imply that materials in the egg may not be partitioned equally between sister blastomeres when the zygote cleaves. More recently, the MOEP19/ FLOPEd RNA-binding protein, encoded by the Ooep gene

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during oocyte development, has been observed to be located in the peripheral cytoplasm of the mouse oocyte and zygote and, at the morula stage, to become partitioned into the outer blastomeres that become trophoderm [5], but there was no suggestion that the protein became asymmetrically distributed between twin blastomeres when the zygote cleaved.

There also continues to be disagreement as to whether the first cleavage division of the murine embryo segregates blastomeres with different developmental potential, as might be anticipated if each blastomere received different information from the oocyte. For example, using lineage tracing experiments, some investigators have reported that the murine zygote generally cleaves at approximately 90° relative to the pole demarcated by the position of the second polar body, thereby dividing the cytoplasm somewhat predictably into two halves [8, 15]. Of the two blastomeres that result, the one that divides first is more likely to contribute to the embryonic pole of the blastocyst and hence inner cell mass and polar trophoderm, while the blastomere that divides later is usually the precursor of murral trophoderm. If such data are correct, and some disagree that it is, the main axes of the embryo are already established at the first cleavage division of the embryo. Other researchers, however, have made contrasting observations, considering both blastomeres to possess equivalent potential, and they have forcefully argued that prepatterning of the zygote does not occur in the mouse and, by analogy, in other mammals [7, 16, 17]. In fact, when the twins are separated from each other, 2-cell stage murine blastomeres are equally capable of advancing to blastocyst stage [18–20], and each can be a separate source of embryonic stem cells [21], although for reasons that are unclear, the production of monozygotic twins from such murine demi-embryos occurs very rarely [22–26]. The goal of this work was to test the hypothesis that sister blastomeres derived by embryo splitting at the 2-cell stage differ in their relative content of gene transcripts. Consistent differences in mRNA composition occurring between blastomere pairs would be an indication that the two cells were apportioned different quantities of key genetic information from the mother and might differ in developmental potential.

**MATERIALS AND METHODS**

**Embryo Collection and Culture**

Embryos were derived from CF1 female mice by natural breeding. CF1 stud males were introduced to the female mice (Harlan Laboratories) at 17.00 h, and females were selected for retrieval of 2-cell stage embryos between 10.00 and 11.00 h on the day following observation of the copulatory plug, i.e., ~36 h postcoitus [27]. Oviducts were flushed in CZB-Hepes buffer [28] lacking glucose (mCZB-Hepes) to collect embryos. All experiments with mice were conducted in accordance with National Research Council publication Guide for Care and Use of Laboratory Research Involving Animals protocols (1935 and 4073), approved by the University of Missouri’s animal care and use committee.

**Isolation of Blastomeres from 2-Cell-Stage Embryos**

Micromanipulation of embryos was performed with an inverted microscope (Nikon DIAPHOT-300 model; Spectra Services, Ontario, NY) equipped with a standard micromanipulation system (model M; Leitz, Bannockburn, IL). Two-cell stage embryos were transferred to 2-μl microdrops containing Ca2+/Mg2+-free phosphate-buffered saline supplemented with 0.1% polyvinyl alcohol and covered with mineral oil. An embryo was secured by using a holding pipette through a slit made in the zona pellucida (ZP), close to the side of one blastomere, by means of a sharp glass needle. The blunt tip of a glass rod was then used to put pressure on the ZP-enclosed embryo, thereby allowing the blastomere closest to the slit to be squeezed out, generally without apparent damage [20]. The second blastomere was recovered in a similar manner. Although the two blastomeres were physically indistinguishable, they were arbitrarily assigned a letter, e.g., “L,” designating the blastomere pair and, also arbitrarily, either the number 1 or 2. Two collections were performed approximately 3 months apart, yielding 26 blastomeres for RNA amplification (see below). Twenty amplified samples were hybridized to arrays. Of those that hybridized successfully, 12 samples of six pairs were used in the final analysis. To distinguish samples from the two collections, those from Collection 2 are designated with asterisks throughout.

**RNA Processing**

Total RNA was isolated by using RNAqueous-Micro Technology (AM1931; Applied BioSystems, Carlsbad, CA) as described by the manufacturer. The procedure consisted of an initial extraction in guanidinium thiocyanate solution, dilution in ethanol solution, and purification with an RNA-binding glass fiber filter. Concentrated RNA was eluted from the column and assessed for quantity and quality with a Bioanalyzer using an RNA6000 Pico Lab chip (Agilent Technologies; Santa Clara, CA). Labeled cRNA was prepared from each RNA sample. Briefly, the poly(A)+ RNA population within total RNA was linearly amplified through two rounds of in vitro transcription by using an Agilent RiboAmp HS reagents (Molecular Devices, Sunnyvale, CA) as described previously [29, 30]. After the second round of reverse transcription and second-strand cDNA synthesis, the double-stranded cDNA was purified and in vitro-transcribed by T7 RNA polymerase with Cy3-CTP included in the reaction mixture to fluorolabel the resulting cRNA. Labeled cRNA was assayed qualitatively with a Bioanalyzer (Agilent Technologies) before being subjected to microarray analysis.

Pured cRNA (1 μg) was fragmented to uniform size and applied to Agilent 4x44K Whole Mouse Genome microarrays (design ID 14868; Agilent Technologies) in hybridization buffer. All fragmented samples were visualized with a Bioanalyzer to verify complete fragmentation to ~0.1-kb size before the sample was applied to the array. Arrays were hybridized at 65°C for 17 h on a rotating incubator and washed at 37°C for 1 min. Rinsed and dried arrays were scanned with a G2565 Microarray Scanner (Agilent Technologies) at 5-μm resolution. Agilent Feature Extraction software was used to process the scanned images from arrays (gridding and feature intensity extraction), and data generated for each probe on the array were analyzed with GeneSpring GX version 7.3.1 software (Agilent Technologies).

**Data Analysis**

To compare individual expression values across arrays, raw intensity data from each gene probe were normalized to the 75th percentile intensity of probes above background level on each array and further scaled assuming maximum correlation for all genes within and across pairs. Genes with expression values above background level and with normalized values of >0.25 in at least one sample for six pairs were used for further analysis. Genes were further filtered for ≥1.4-fold differential expression in at least 5 of 6 pairs and 6 of 6 pairs. The fold-change values shown in figures and tables are the ratios of normalized values. The expression values for the genes that qualified for differential expression by the above-described criteria were normalized to the median expression within each blastomere pair and used in hierarchical clustering analysis (Spearman correlation with complete linkage) to segregate blastomere pairs into two cluster groups [31]. To find differentially expressed genes that displayed a consistent asymmetry in expression across blastomere pairs, a two-sample t-test assuming unequal variance was conducted between cluster groups, using the expression values normalized within each blastomere pair. The Welch t-test was used because it is an established method for comparing the difference between two means when there is possibly unequal variance.

All data have been deposited in GEO, a publicly accessible data bank, under accession number GSE21688.

**RESULTS**

**Recovery of RNA from Individual Twin Blastomeres**

Two experiments were performed for embryo splitting, with each yielding several blastomere pairs whose RNA was extracted. The cRNA amplified from the original extract from each blastomere was assessed by electrophoresis, thereby allowing pairs of samples to be selected for microarray analysis. Figure 1 illustrates the high quality of the cRNA samples generated from the first round of collections. RNA that had not been amplified successfully, e.g., H1, H2, K1, and J1, or had been amplified poorly, e.g., K2, were discarded. At this stage, biotinylated target cRNA from successful amplifications...
was fragmented to a uniform size for hybridization on Agilent arrays.

Due to the challenging nature of amplifying RNA from single cells, it was necessary to be as stringent as possible in filtering out genes that were expressed just above background level and that provided the most variability to the data set. Thus, we removed genes expressed at intensities below background level and below a specified normalized value of 0.25 but preserved the ability to distinguish relative transcript concentrations extended over 3 orders of magnitude, i.e., approximately three logs of data (0.25 to \(250\)), as indicated in the scatter plots shown in Figure 2.

**Transcriptome Profiles of Individual Blastomeres**

About one quarter (n = 10,526) of the gene probes provided a signal above background level in at least one blastomere from the six pairs of embryos (GEO accession number, GSE21688). Normalized signals ranged from 0.01 to >100 for the most strongly expressed genes. Of these expressed genes, 4573 had normalized intensity values \(>0.25\), the cut-off value for our analyses (see Supplemental Table S1, available online at www.bioreprod.org). As experimental variability might have been introduced during RNA extraction, copying, and amplification, as well as during microarray processing, especially as the blastomeres had been isolated and processed as two separate lots, we were concerned that the data would be inconsistent. However, an inspection of data submitted to GEO, which provided values for all 41,174 probes (Supplemental Table S1), indicated that confounding variability arising from experimental procedures had probably not occurred. For example, the *mt-Co2*, *Oog1*, and *Fithl* genes, which were the three most strongly expressed genes in the embryos, provided comparably high expression in all 12 blastomeres analyzed. Such consistency between Experiments 1 and 2 is also evident in a comparison of 75th-percentile normalized intensity values for genes encoding ribosomal proteins (Supplemental Table S2). Although values varied, they were generally not markedly different either between blastomere pairs or across all blastomeres analyzed. Such coherent data provided confidence that the microarray analyses could be used to explore differences among sister pairs.

Of the 4573 probes that had normalized values greater than 0.25 in at least one blastomere of each pair, we next examined those probes that showed a consistent bias in expression within blastomere pairs, using a cut-off value of \(>1.4\)-fold difference. A total of 769 genes were differentially expressed in at least five of the six pairs of blastomeres and 178 in all six pairs. We then used class discovery clustering to confirm that the blastomere pairs separated into two distinct groups (Fig. 3, Cluster Group A and Cluster Group B) in terms of their differentially expressed genes. For this analysis, we used both the more stringent list of 178 genes (Fig. 3A) and the less stringent list of 769 genes (Fig. 3B). A similar clustering pattern, which resulted in an identical separation of samples, was achieved with both gene lists. These data suggest that the transcriptome profiles of each blastomere in a 2-cell-stage embryo are subtly different.

**Genes Differentially Expressed Across Blastomere Pairs**

Once blastomere pairs were separated into Cluster Groups A and B, a statistical comparison was conducted. We used intensity values normalized to the median expression within each pair to conduct a two-sample *t*-test, assuming unequal variance between Cluster Group A versus B (Fig. 3). The
resulting $P$ values represented the level of confidence that a gene is differentially expressed, i.e., the significance of the mean ratio of expression values between A and B across six pairs.

The 163 genes that were differentially expressed in Cluster Group A versus B, i.e., across blastomere pairs, were then ranked by $P$ value (Supplemental Table S3), and their relative expression levels were compared between blastomeres of each embryo pair analyzed. Importantly, only 39 genes with $>1.4$-fold higher ratios in six of six blastomere pairs passed the two-sample $t$-test ($P < 0.05$).

We then examined those differentially expressed genes whose transcripts were abundant, as well as those that demonstrated the most significant differences between blastomeres. Table 1 lists the 13 transcripts with a normalized expression $>10$ that showed a consistent, asymmetric skewing between twin blastomeres ($P < 0.05$). The highly expressed ataxin2-like ($Atxn2l$) gene is probably involved in RNA destruction [32]. At least three other plentiful, asymmetrically expressed transcripts, those for the $Hnrnpu$, $Oas1f$, and $Ybx2$ genes, have been implicated in RNA metabolism, while some others encode proteins associated with dynamics of the cytoskeleton (e.g., $Mast2$, $Myo9b$, and $Cfl1$). The $Mfap5$ gene (sometimes named $Magp2$) encodes a glycoprotein with an integrin binding motif that is normally part of a fibrillin-based, extracellular microfibrillar complex [33]. Transcripts for 12 of these 13 genes (the exception being the $Rsp13a$ gene), were higher in the Group A blastomeres of Embryos D, D*, G*, and H*. In Embryo I, 10 of the same genes were Group A-biased, while in Embryo L, 9 of the 13 genes showed such skewing. The exceptions ($Cfl1$ 9 for Embryo I, and $Hnrpau$ and $Mast2$ for Embryo L, respectively) showed almost identical expression in both blastomeres. In contrast to the other genes in the list, $Rsp13a$ (formerly $Rsh2a$) transcripts were overrepresented in the B group blastomeres. The data in Table 1, therefore, reinforce the concept that there are a few genes in 2-cell stage mouse embryos whose transcripts are differentially expressed across blastomeres.

Table 2 lists the 15 genes whose transcripts demonstrated the most significant ($P < 0.0003$) asymmetric distribution between twin blastomeres. Transcripts for two transcription factor ($Runx1t1$ and $Irf3$) genes fell into this group, but no common themes appeared to be represented among the genes. Of these 15 genes, 12 genes were entirely Group A-biased, while three ($Ndfip1$, in 6/6 pairs; $Ccnb1$, in 6/6 pairs; and $Irf3$, in 5/6 pairs), like $Rsp13a$ in Table 1, were overrepresented in Group B blastomeres.

Expression of Genes Associated with Trophoblast Emergence

Low expression and lack of bias were generally features noted for a set of genes that had been implicated in some manner with the specification of trophoblast (Table 3). There were some exceptions. The $Tead4$ gene, for example, had relatively high expression levels in both blastomeres of Embryo D* but provided a signal barely above background level in the other five embryos. The $Tphpa$ gene showed a pattern similar to $Tead4$. A few genes appeared to be upregulated in one blastomere relative to the other, e.g., $Cdx2$ and $Eomes$ in embryo L, but this bias was not consistent across other embryos.

DISCUSSION

Sister blastomeres separated at the 2-cell stage of development are not visibly distinct [20]. Therefore, an analysis to determine whether the twin embryos can be distinguished in terms of their relative mRNA content depends upon whether...
gene expression differences emerge in such a manner that one member of each pair consistently clusters with just one member of each of the other pairs. For the majority of the gene probes examined, such clustering was not observed, even though considerable microheterogeneity [34] was evident when we compared individual blastomeres within pairs and individual embryos (Supplemental Tables S1 and S2). This variability in expression, yet lack of consistent clustering, is well illustrated for the genes encoding ribosomal proteins (Supplemental Table S2) and trophectoderm emergence (Table 3), which are known to be transcribed at the initiation of embryonic genome activation and not inherited from the egg ooplasm [35], and for almost all other highly expressed genes (Supplemental Table S1). Even among genes that were expressed less strongly, there was little evidence that for the majority, the ratios of normalized expression values for sister blastomeres were systemically biased toward expression in only one blastomere of a blastomere pair, relative to that of other genes. The most obvious and arguably the most important interpretation of these data is that, although the content of individual transcripts can differ considerably between twin blastomeres derived from the two-cell stage murine embryo, for most genes, the differences are not consistent when several embryo pairs are compared.

Clearly, a partial explanation for this acquisition of apparently random differences between individual blastomeres derived from the same 2-cell-stage conceptus is that each blastomere arbitrarily receives a heterogeneous complement of maternal transcripts when the zygote cleaves. The lack of uniform distribution of maternal mRNA across the zygote cytoplasm, particularly if combined with an unpredictable plane of cytokinesis, could clearly contribute to such uneven partitioning. Although this process may be unbiased, it establishes an asymmetric state, and this microheterogeneity, although established by chance, could drive further diversification [34].

Unfortunately, the test of whether sister blastomeres already differ in composition when the zygote divides requires that the blastomeres be separated immediately after cytokinesis, a procedure that is technically challenging, as it usually leads to lysis of one or both cells (Katayama M., unpublished observations). Instead, we chose to conduct splitting at about half way through the second cell cycle, when blastomeres separate relatively easily [20]. Most blastomeres show no signs of damage and are fully competent to advance to blastocyst, with an efficiency and speed comparable to that of control, nonmanipulated embryos [20]. This delay in splitting has a major disadvantage, however. Not only is the second cell cycle of the mouse embryo much longer than the ones that follow [36], it is a highly dynamic process in terms of mRNA metabolism [37]. While maternal mRNAs are being selectively degraded, the embryonic genome is becoming progressively activated [35, 38, 39]. Hence, the transcripts detected are likely to be a mixture of surviving maternal mRNAs derived from the oocyte and newly synthesized embryonic mRNAs transcribed early in the process of embryonic genome activation. Accordingly, neither set is likely to be in a steady state. The situation is further complicated by the fact that mouse embryos do not progress in development at identical speeds so that individuals within any randomly chosen group will likely exhibit some heterogeneity in RNA composition because activation of the embryonic genome is not occurring
### Table 1

<table>
<thead>
<tr>
<th>Gene</th>
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<th>Experiment 1b</th>
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</table>

**Note:**
- Asterisk (*) designates blastomeres from Experiment 2, here and in the text.
- Four genes listed in Table 1 (Ataxin 2-like, Heterogeneous nuclear ribonucleoprotein U, Y box protein 2, Slc6a9) are not included in the present Table.
- Terms of Use: https://bioone.org/terms-of-use
synchronously. A further complication is that the two-component blastomeres of a single 2-cell stage embryo themselves are not in perfect synchrony, with one usually dividing before the other [8, 13, 15]. These variables are likely to influence the extent to which maternal mRNAs have been destroyed and embryonic mRNAs have accumulated and the extent to which blastomeres differ in composition at the time of analysis.

Despite these complications to the analysis, sister blastomeres showed very few consistent differences in mRNA composition. Importantly, no such differences were observed for the genes associated with trophoderm emergence (Table 3), and there was no evidence that the two cells might already be biased toward different fates at this early stage of development. The few differences in transcript concentration that were evident between embryos were probably due to developmental asynchrony and specifically to the relative timing of embryonic genome activation. Pair D* blastomeres showed developmental asynchrony and specifically to the relative timing of embryonic genome activation. Pair D* blastomeres from Experiment 2, for example, overexpressed the gene that was more advanced earlier than the other and bias that cell's progeny to diverge further below, could explain the asymmetries in Cdx2 and Eomes expression in Pair L.

A total of 769 genes exhibited a minimum of 1.4-fold difference across five of six pairs and 178 genes across six of six pairs. These values exceed the binomial probabilities for such asymmetric expression, namely 0.09375, i.e., 429 of the 4573 qualified gene probes, and 0.0156, i.e., 71 genes, respectively. However, when the differentially expressed genes were subjected to a two-sample t-test to determine the significance of asymmetrical expression, only 39 genes with >1.4-fold ratios in six of six blastomere pairs and 163 genes with >1.4-fold ratios in five of six pairs passed the test (P < 0.05) (Supplemental Table S3). Although, there appear to be no clear themes that interconnect the genes whose transcript concentrations consistently differed between twin blastomeres, there is again the suggestion, touched upon above, that the asymmetries can be explained by variability in cell cycle stage. One illustrative example relates to the Ybx2 gene (also known in humans as MSY2), which encodes an abundant mRNA-binding protein specific to germ cells and is known to be passed from the oocyte to the embryo [40]. The life of Ybx2 transcripts in the mouse embryo is very short; they are completely destroyed by the end of the 2-cell stage of development. In our analyses, the Ybx2 transcript concentrations varied considerably between individual pairs of blastomeres examined (Table 1). For example, Pairs D and L showed >5-fold higher Ybx2 expression than Pair D*, suggesting that the former had not progressed as far through their second cell cycles as the latter had. In addition, one blastomere in each of the six pairs had a consistently higher Ybx2 expression than its twin partner. We hypothesize that the blastomere furthest along its cycle and most likely to divide first, i.e., B-group blastomeres, had degraded more Ybx2 mRNA than the lagging twin. Hence, the differences that distinguish twin blastomeres may be an indication that the two cells are not in precise phase with each other, rather than being a reflection of differing developmental potential. The asymmetric expression of the Ybx2 gene in mouse embryos is very short; they are completely degraded by the end of the second cell cycle [42]. Curiously, Rsp3a transcripts, which are most concentrated in the B group blastomeres and which are predicted to divide first, are also known not to survive past the end of the 2-cell stage [42]. We have no explanation for this anomaly.

The opposite situation occurs for the Ccnb1 gene, which encodes cyclin B1, a protein product associated with entry into the S phase of the mitotic cell cycle, when it relocates from the cytoplasm to the nucleus [43]. Although there are no specific data available for Ccnb1 mRNA, its protein product is absent in early 2-cell stage embryos and increases in amount with the onset of the S phase of the cycle, reaching maximum concentrations in late G2, just before the blastomeres cleave [44]. If these changes in protein are preceded by Ccnb1 transcription, the higher concentration of Ccnb1 mRNA in B blastomeres (Table 2) again suggests that Group B blastomeres are the ones likely to divide first in a 2-cell stage mouse embryo.

The ability to measure global gene expression profiles from single blastomeres of a mammalian embryo, as demonstrated here, potentially provides a means of linking the activities of individual genes and gene networks to the emergence of particular lineages during early embryogenesis. We found no evidence that such lineage specification events had been initiated midway through the second cell cycle. On the contrary, the majority of transcripts appeared to be rather randomly distributed across blastomere pairs, which is inconsistent with prepatternning. That is not to say that both blastomeres are identical in potential, as heterogeneity in mRNA composition undoubtedly occurs by chance when the zygote first divides. These differences may be self-reinforcing, triggering a chain of events that cause one blastomere to divide earlier than the other and bias that cell’s progeny to diverge further from that of its twin as embryogenesis proceeds.

### TABLE 3. Relative expression of genes associated with trophoblast specification in six pairs of blastomeres.

<table>
<thead>
<tr>
<th>Gene</th>
<th>D1A</th>
<th>D2B</th>
<th>I1B</th>
<th>I2A</th>
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<td>Gial cells missing homolog 1 (Drosophila)</td>
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</table>

a Note that none of these genes fell into the group of 163 that were differentially expressed in cluster group A versus B.

b Asterisk (*) designates blastomeres from Experiment 2, here and in the text.
ACKNOWLEDGMENTS

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