Transcription Profile Analysis Reveals That Zygotic Division Results in Uneven Distribution of Specific Transcripts in Apical/Basal Cells of Tobacco

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Abstract

Background: Asymmetric zygotic division in higher plants results in the formation of an apical cell and a basal cell. These two embryonic cells possess distinct morphologies and cell developmental fates. It has been proposed that unevenly distributed cell fate determinants and/or distinct cell transcript profiles may be the underlying reason for their distinct fates. However, neither of these hypotheses has convincing support due to technical limitations.

Methodology/Principal Findings: Using laser-controlled microdissection, we isolated apical and basal cells and constructed cell type-specific cDNA libraries. Transcript profile analysis revealed difference in transcript composition. PCR and qPCR analysis confirmed that transcripts of selected embryogenesis-related genes were cell-type preferentially distributed. Some of the transcripts that existed in zygotes were found distinctly existed in apical or basal cells. The cell type specific de novo transcription was also found after zygotic cell division.

Conclusions/Significance: Thus, we found that the transcript diversity occurs between apical and basal cells. Asymmetric zygotic division results in the uneven distribution of some embryogenesis related transcripts in the two-celled proembryos, suggesting that a differential distribution of some specific transcripts in the apical or basal cells may involve in guiding the two cell types to different developmental destinies.

Introduction

In some angiosperms, including Arabidopsis and tobacco, the first zygotic cell division is transverse and asymmetric, and results in a two-celled proembryo consisting of an apical cell and a basal cell, which differ in both their morphology and destiny [1–3]. The smaller apical cell develops into the embryo proper, while the larger basal cell develops into a suspensor or joins the embryonic root formation [4].

It has been a mystery for many years how apical and basal cells, which are descended from the same mother cell, show distinct divisional patterns and cell fates. To explain their distinct cell fates, it has been proposed that asymmetric divisions generate daughter cells containing different developmental determinants [5] or that the different developmental pathways of the cells occur due to different positional cues [6]. However, neither of these proposals has been demonstrated.

Cytoplasmic determinants play a predominant role in cell fate determination [7]. Researchers have identified several genes that are expressed differently in progeny after zygote division. In Arabidopsis, MERISTEM LAYER 1 (AtML1) encodes a homeobox gene, and its expression has been confirmed only in the apical cell of two-celled proembryos [8]. In Phaseolus coccineus, the transcripts of two genes, G564 and G341, accumulate shortly after fertilization and are present within the two embryonic basal cells at the four-cell stage [5]. It was recently [9] reported that the transcription factors WUSCHEL HOMEOBOX2 (WOX2) and WOX9 are expressed specifically in the apical and basal cells of the Arabidopsis two-celled proembryo. These data suggest that the two zygotic daughter cells may assume different transcriptional profiles, although no evidence has been presented. Thus, a direct apical and basal cell transcriptional profile analysis would be useful in unraveling this mystery.

Because the zygote and early embryo are deeply embedded in the ovular tissue and are therefore not easy to access, it has been difficult to identify the transcriptome and detect dynamic changes in gene expression. In the last 20 years, techniques have been established to isolate gametes and early-stage embryos from a number of flowering plant species [10], and these specific cells have become available for direct use in large-scale analyses such as cDNA library construction and microarray analysis. Using in vitro-fertilized zygote culture, apical and basal cells have been isolated from maize, and apical and basal cell-specific genes have been identified using RAPD primers [11]. Expression pattern analysis...
revealed that the genes are upregulated in the apical or basal cell in the early zygote, suggesting that the transcripts are portioned in their respective cells after zygote division, or that the transcripts are rapidly degraded in one of the daughter cells after zygotic cell division [11]. However, until now, data on the apical and basal cell transcriptome have been lacking.

Since it is still very difficult to isolate zygote and two-celled proembryos from Arabidopsis and we have well established relevant techniques in tobacco [3,12], in this report, we present a comparison of the transcript profiles between the two zygotic daughter cells of tobacco, and we compare the two cell types with the zygote. We also address two questions: 1) Do apical and basal cell possess distinct transcript profiles that may be responsible for their distinct cell fates? and 2) Can zygotic transcripts be portioned into the different daughter cells, which may involve in cell fate regulation. Our data suggest that the transcript diversity occurs between apical and basal cells. Asymmetric zygotic division result in uneven distribution of some specific transcripts in two daughter cells, thereby triggering their distinct developmental pathways.

Results

Isolation of apical and basal cells

Because the two-celled embryos of tobacco are deeply embedded in the ovules and are difficult to approach, viable two-celled proembryos must be isolated from ovules by enzymatic maceration combined with grinding [13]. We tested two different techniques to isolate apical and basal cells. When isolated two-celled proembryos were placed in an enzymatic solution for an extended duration, the apical and basal cells could be completely separated and became protoplasts (Figure 1A–C). We also used a laser microdissection device (LMD) to quickly ablate the apical and basal cells, respectively, and, after a brief washing procedure, individual apical or basal cells were collected (Figure 1D–F). As long-term enzymatic treatment may weaken cell viability and promote stress-induced gene expression, which might greatly alter the expression profile of the cells, we finally chose LMD to isolate the cells. During enzymatic treatment of the ovules to isolate two-celled proembryos, two transcriptional inhibitors, actinomycin D (50 mg/L) and cordycepin (100 mg/L) were added to all solutions [12,14] to suppress stress-induced gene expression. After laser ablation, individual apical or basal cells were collected manually and immediately washed to avoid possible contamination from the broken cells. A pure population of apical or basal cells was collected efficiently using this method and all the cells were viable (Figure S1).

Library construction and expressed sequence tag (EST) cluster analysis

Seventy-two apical cells and 88 basal cells were used to construct two cell type-specific cDNA libraries. The titers of the unamplified apical and basal cell libraries were 2.5 × 10⁶ and 2 × 10⁶ pfu·ml⁻¹, respectively. More than 99% of the clones had an insert, indicating that the libraries were of high quality. The insert size of the cDNA clones in the apical cell cDNA library ranged from 0.3 to 1.7 kb (centered around 0.6 kb), compared to 0.3 to 1.7 kb in the basal cell cDNA library (centered around 0.5 kb).

EST sequencing was performed from the 5’-end of randomly picked clones from the two primary libraries. A total of 2,772 ESTs from the apical cell cDNA library were obtained and assembled into 2,072 non-redundant clusters containing 1,750 singletons; 2,776 ESTs were assembled into 1,950 non-redundant clusters containing 1,532 singletons for the basal cell cDNA library. In total, 45.42% of the clusters from the apical cells and 43.85% of the clusters from the basal cells could be assigned putative functions. Some of the clusters (25.63% for the apical cells and 28.92% for the basal cells) showed no significant homology with National Center for Biotechnology Information (NCBI) databases. The remaining clusters (28.96% for the apical cells and 27.23% for the basal cells) displayed similarities without specific annotations.

Our results showed that 16.0% of the apical cell EST clusters were present in basal cells and that 16.2% of the basal cell EST clusters were present in apical cells (Figure 2). Since this is EST-based and sampling analysis, the diversity of the transcript profiles between the two cell types needs to be further confirmed.
Apical and basal cells share the majority of their most abundant transcripts

We further compared the transcript component between the two cell types. The most abundant clusters in apical and basal cells are shown in Tables 1 and 2, respectively. Several clusters containing ESTs related to ribosomal proteins, chaperones, ubiquitin, calmodulin, and histones appeared in both the apical and basal cells as abundant clusters, suggesting that apical and basal cells share the majority of their most abundant transcripts.

Functional category analysis indicates strong similarity between apical and basal cells

The clusters and ESTs matching-characterized proteins or proteins with putative functions were grouped according to functional categories (Figure 3). Similar to the results of our analysis of the most common clusters in apical and basal cells, the functional categories showed a high degree of similarity between the cell types. No distinct functional group of transcripts was found in either cell type. Because apical and basal cells undergo cell division to generate the embryo and suspensor, it is reasonable that most of the annotated clusters and ESTs were related to protein synthesis, metabolism, and DNA processing in both cells. The similar functional categories in apical and basal cells suggest that the cells inherit parallel groups of transcripts from zygote.

Existence confirmation of clusters in apical and basal cells by RT-PCR

To further confirm the existence of the clusters from the two cDNA libraries, 34 clusters from the apical cell cDNA library and 37 clusters from the basal cell cDNA library were selected for RT-PCR. The clusters used for the analysis were involved in several

Table 1. Most abundant clusters in tobacco apical cells.

| Cluster id | EST No. | AGI      | Putative identity                  | e-value   |
|------------|---------|----------|------------------------------------|-----------|
| NtAc 1     | 21      | AT1G14980| chaperone                          | 2.00E-30  |
| NtAc 2     | 21      | No hit   |                                     |           |
| NtAc 3     | 18      | ATMG00030| NitaMp027                          | 9.00E-36  |
| NtAc 4     | 14      | AT5G65360| histone H3                         | 5.00E-71  |
| NtAc 5     | 10      | AT4G33865| 40S ribosomal protein S29          | 3.00E-28  |
| NtAc 6     | 9       | AT3G46030| HTB11; DNA binding                 | 1.00E-65  |
| NtAc 7     | 9       | AT3G12410| 3’-5’ exonuclease/ nucleic acid binding | 4.00E-58 |
| NtAc 8     | 9       | AT5G65360| histone H3                         | 1.00E-70  |
| NtAc 9     | 9       | AT3G48810| calmodulin                         | 9.00E-78  |
| NtAc 10    | 8       | AT5G02560| HTA12; DNA binding                 | 2.00E-64  |
| NtAc 11    | 8       | AT2G09990| 405 ribosomal protein S16          | 3.00E-70  |
| NtAc 12    | 8       | AT5G57290| 60S acidic ribosomal protein P3    | 1.00E-41  |
| NtAc 13    | 7       | AT3G49010| structural constituent of ribosome | 3.00E-93  |
| NtAc 14    | 7       | AT5G59910| HTB4; DNA binding                  | 2.00E-64  |
| NtAc 15    | 7       | AT3G04400| structural constituent of ribosome | 6.00E-76  |
| NtAc 16    | 7       | AT5G27670| H2A histone                        | 5.00E-62  |
| NtAc 17    | 7       | AT5G27670| H2A histone                        | 1.00E-61  |
| NtAc 18    | 7       | AT5G59910| ubiquitin extension protein         | 2.00E-69  |
| NtAc 19    | 7       | AT5G57290| 60S acidic ribosomal protein P3    | 1.00E-40  |
| NtAc 20    | 6       | AT1G73230| BTF3 [Nicotiana benthamiana]        | 9.00E-65  |

Table 2. Most abundant clusters in tobacco basal cells.

| Cluster id | EST No. | AGI      | Putative identity                  | e-value   |
|------------|---------|----------|------------------------------------|-----------|
| NtBc 1     | 34      | No hit   |                                     |           |
| NtBc 2     | 34      | ATMG00030| NitaMp027                          | 8.00E-30  |
| NtBc 3     | 19      | AT4G33865| 405 ribosomal protein S29          | 6.00E-27  |
| NtBc 4     | 17      | AT3G43810| calmodulin                         | 2.00E-78  |
| NtBc 5     | 12      | AT1G55020| lipoygenase                        | 2.00E-57  |
| NtBc 6     | 12      | AT1G14980| chaperonin                         | 9.00E-41  |
| NtBc 7     | 11      | AT5G65360| histone H3                         | 4.00E-71  |
| NtBc 8     | 10      | AT4G39340| unknown protein                    | 4.00E-28  |
| NtBc 9     | 9       | AT5G59850| 405 ribosomal protein S15A         | 2.00E-70  |
| NtBc 10    | 9       | No hit   |                                     |           |
| NtBc 11    | 8       | AT3G04400| 605 ribosomal protein L17          | 6.00E-76  |
| NtBc 12    | 8       | AT1G55020| lipoygenase                        | 8.00E-64  |
| NtBc 13    | 7       | AT3G52590| ubiquitin extension protein         | 4.00E-69  |
| NtBc 14    | 7       | AT3G46030| HTB11; DNA binding                 | 2.00E-65  |
| NtBc 15    | 7       | No hit   |                                     |           |
| NtBc 16    | 7       | AT3G59540| 605 ribosomal protein L38          | 1.00E-31  |
| NtBc 17    | 7       | AT4G33865| 405 ribosomal protein S29          | 3.00E-28  |
| NtBc 18    | 6       | AT3G46030| HTB11; DNA binding                 | 2E-65     |
| NtBc 19    | 6       | AT1G26880| 605 ribosomal protein L34          | 3.00E-59  |
| NtBc 20    | 6       | AT5G59970| histone H4                         | 1E-53     |

doi:10.1371/journal.pone.0015971.t001
biological processes (Table S1). These chosen clusters were estimated as numerous in one cell type; thus, the results will help evaluate whether particular clusters in a given cell type could be cell type-specific. The PCR parameters were optimized to identify ESTs that differed dramatically between apical and basal cells: 25 cycles for house-keeping genes and 35 cycles for candidate genes.

Among the 34 clusters in the apical cell cDNA library (Figure 4), five showed slight reduction in basal cells, and none were reduced in apical cells. Among the 37 clusters from the basal cell cDNA library, two in the basal cell cDNA library existed in basal cells and not in apical cells. Although the tested samples were limited, large-scale distinct transcript portioning could not be confirmed in the apical or basal cells during asymmetric zygotic division. But, some of transcripts were indeed specifically localized in one of the cell types.

To trace the fate of the transcripts identified from apical or basal cells in the subsequent development stages, hundreds of transcripts from the two cDNA libraries were examined in global-stage proembryos and heart-stage embryos. Among 126 transcripts detected (49 from apical cells, 77 from basal cells), seven were not found in global-stage embryos (two from apical cells, five from basal cells). Among 166 ESTs detected (49 from apical cells, 117 from basal cells), fourteen were not detected in heart-stage embryos (four from apical cells, ten from basal cells). The transcripts tested are listed in Table S1. These data suggest that most of the transcripts identified in the two-celled proembryos still existed at the subsequent developmental stages, indicating their persist role in embryo development.

Real-time RT-PCR reveals preferential transcript accumulation in apical or basal cells

Our RT-PCR results indicated that most of the transcripts identified existed in both apical and basal cells. To detect the relative transcript level in the two cell types, 42 ESTs were examined by real-time RT-PCR. Among them, 19 ESTs showed a significant expression difference between apical and basal cells. Two ESTs (tc0001 and nta1105) were expressed exclusively in apical cells (Figure 5A, B) and other 13 ESTs (tc0003, tc0005, tc0007, nta0281, nta0833, nta1389, nta1473, nta1524, nta1527,
Most of the differentially accumulated transcripts in apical or basal cell were found in zygote

To estimate that the unevenly distributed transcripts are inherited from zygote or from de novo transcription in apical or basal cell, we also checked the 42 ESTs in zygote by qPCR. The result indicates that most of these transcripts were found in zygote and only three of them are not expressed in zygote (Figure 6; Table S2). This suggests most of the transcripts that preferentially distributed in apical or basal cell were inherited from zygote. Interestingly, among the three transcripts that not expressed in zygote, two (tc0001 and nta1105) specifically expressed in apical cell and not in basal cell (Figure 5), indicating cell-type specific de novo transcription occurred after zygotic division.

Discussion

Transcript diversity occurs between apical and basal cells after asymmetric zygote division

After zygotic cell division, the resulting apical and basal cells differ in terms of their morphology and developmental fate. Proper basal and apical cell formation is critical not only for embryo and suspensor differentiation, but also for the apical-basal axis establishment [15–17]. It has been unclear how two cells derived from the same mother cell can differentiate into distinct structures and how their fate is determined. In recent decades, different hypotheses, which are not mutually exclusive, were proposed to explain the distinct cell fates of this pair of sister cells. For example, cell fate could be determined by the position of the cells in the embryo sac, as the cell that is directly attached to the maternal tissue usually forms the suspensor [18]; cell fate could also be decided by an interaction between two attached or adjacent cells [19–23]. Another attractive hypothesis is that cytoplasmic determinants may play a predominant role in cell fate determination [7]. Asymmetric cell division generates daughter cells containing different developmental determinants [5]. In fact, some genes that may be involved in cell fate determination have been reported in Arabidopsis; for example, WOX2 expression is confined to apical daughter cells of the zygote, while WOX9 expression is initiated in the basal daughter cell of the zygote [9]. Our data confirm that the diversity of transcript profiles indeed occurs between apical and basal cells in tobacco. RT-PCR and qPCR further confirm the difference of transcript composition between the two cell types, suggesting that the diversity of transcript profiles in the cells is at least initiated at two-celled proembryo stage. However, it is difficult to calculate the percentage of differentially expressed transcripts in the whole transcript composition of apical or basal cells due to the limitation of transcript profile analysis (Table S3, S4). Further transcriptome analysis based on novel sequencing technique will enable a more complete survey of the diversity or similarity between apical and basal cells at the transcriptional level and a more accurate answer to this question.

Preferential distribution of zygote transcripts and cell type specific de novo transcription may be involved in apical/basal cell fate determination

To explain the mechanism of basal cell and suspensor specification, scientists ever proposed that basal cell-specifying morphogenetic factors are distributed asymmetrically in the cytoplasm of the egg cell or zygote. Upon asymmetric division, these factors are inherited by the basal cell and trigger the
Figure 5. Transcript levels of selected genes in apical/basal cells as shown by real-time RT-PCR. (A, B): The expressed sequence tags (ESTs) showed specific distribution in apical cell. (C–O): ESTs showed a significantly higher level in apical vs. basal cells (≥2-fold difference). (P–S): ESTs showed a significantly higher level in basal vs. apical cells (≥2-fold difference). Ap, apical cell; Ba, basal cell; Zy, zygote. Expression level in apical cell was set to 1. 

doi:10.1371/journal.pone.0015971.g005
transcription of basal region–specific genes and, eventually, the specification of suspensor [5].

In our experiments, we confirmed that among 42 ESTs examined by real-time RT-PCR, 19 showed significant expression level differences between apical and basal cells (Figure 5 and 6; Table S2). We further confirmed that the most of these transcripts were inherited from zygote. Thus, it is likely that these zygotic transcripts are preferentially portioned into the different daughter cells during asymmetric cell division. This finding offer direct evidence for Weterings’ proposal.

Among thirteen transcripts expressed at significantly higher levels in apical cells than in basal cells, Tc0003 encodes part of the open reading frame, and it possess 20 N-terminal amino acids of the basic helix-loop-helix (bHLH) domain [24,25]. BIM1, a bHLH protein involved in brassinosteroid signaling, contributes to embryo patterning via its interaction with AP2-type transcription factors [26]. Nta1473 is a P0-related acidic ribosomal protein. Zmp1p0 is relatively abundantly expressed in unfertilized egg cells but down regulated in zygotes by 18 hour after in-vitro fertilization [27]. Nta1826 is similar to the Arabidopsis transcription factor NFYB8 (NUCLEAR FACTOR Y, SUBUNIT B8). Of the ten Arabidopsis HAP3 subunits, NF-YB8 is a non-LEC1-type protein, while LEC1-type HAP3 subunits LEC1 and L1L define a class of regulators essential for embryo development [28]. Among four transcripts expressed at significantly higher levels in basal cells than in apical cells, tc0002 is similar to a Leu zipper domain containing transcription factor. A reported such transcription factor in Arabidopsis, GLUTAMINE-RICH PROTEIN23 (GRP23), is essential for early embryogenesis [29]. The grp23 showed abnormal division in both apical and basal cell development, nta1115 and ntb1853 are quite similar to WOX9. Interestingly, nta1527 is quite similar to WOX2 and was expressed more highly in apical cells than in basal cells. In Arabidopsis, WOX2 expression is confined to apical cells, whereas WOX9 expression has been reported in basal cells [9]. These genes play critical role in early embryogenesis and embryo pattern formation.

We also confirmed that two ESTs (tc0001 and nta1105) existed exclusively in apical cells and not in zygotes, indicating that cell type specific de novo transcription occurs after zygotic cell division. Tc0001 showed no similarity to any sequence in the NCBI or TAIR database. Nta1105 is similar to the Arabidopsis transcription factor HSFB2B (class B heat shock factor). HsfB2b is involved in the regulation of Pdf1.2 defensin gene expression and pathogen resistance in Arabidopsis [30]. The function of such de novo transcripts remain to be elucidated. Their occurrence only in apical cell may suggest their potential role in embryogenesis.

In summary, we conclude that zygotic division results in a divergence in apical and basal cell transcript profiles. The asymmetric zygotic division indeed brings unequal or uneven distribution of embryogenesis-related transcripts in apical and basal cell. We have further confirmed that these transcripts previously exist in zygote. The cell-type specific de novo transcription in these two cell types also occurs. Thus, both the preferential distribution of zygotic transcripts and the cell-type specific de novo
transcription may contribute to the different composition of transcripts in apical or basal cells.

Materials and Methods

Apical and basal cell isolation from tobacco

Tobacco (Nicotiana tabacum cv SR1) plants were grown in a greenhouse at 25°C with a light period of 16 h. Two-celled proembryos were isolated by enzymatic maceration combined with brief manual grinding [13]. During the isolation process, two transcription inhibitors, actinomycin D (50 mg/L) and cordycepin (100 mg/L), were added to all solutions to inhibit potential stress-induced gene expression [12,14].

An LMD (Leica, Bonn, Germany) was used to destroy one of the cells in two-celled proembryos to obtain the apical or basal cell, respectively. The ablation solution was 13% mannitol. To destroy the apical cell, the “move” mode was chosen to operate laser spot since two-celled proembryos was not fixed and might move on the film. A tailor-made slide with PET film at the bottom was used as a cell container during the ablation. A 5μl microdroplet of mannitol solution was first transferred onto the film and then two-celled proembryos were transferred into the droplet and finally the slide was covered with a coverslip to avoid evaporation. The energy of laser spot was modulated (30–60% of the full power, 200W) frequently to realize ablation. The parameter of “specimen balance” was set at 2.

Isolated apical or basal cells were gently washed twice with 13% mannitol and transferred to lysis/binding buffer (Dynal Biotech, Oslo, Norway) for mRNA isolation. The viability of the isolated cells was confirmed by FDA staining (Figure S1).

cDNA library construction and sequencing

A Dynabeads mRNA DIRECT Micro Kit (Dynal Biotech) was used for mRNA isolation. A SMART cDNA Library Construction Kit (Clontech Laboratories, Mountain View, CA, USA) was used for cDNA library construction, following the manufacturer’s instructions. Ligation reactions were packaged with Gigapack III Gold packaging reagents (Stratagene, La Jolla, CA, USA). Individual cloned cDNAs were obtained by in vitro mass excision, randomly picked, and sequenced using a DNA capillary sequencer (ABI 3730XL, Applied Biosystems, Foster City, CA, USA).

Bioinformatics

ESTs from the two individual libraries were combined for the following processes. PHRED was used for base calling and trimming of low-quality sequences. Cloning vectors and linkers were masked with the CROSS-MATCH program. The cleaned sequences were then compared against the NCBI database using BLAST searches (http://www.ncbi.nlm.nih.gov). The e-values were 10−3 for both BLASTN and BLASTX searches. For comparative analysis of ESTs derived from different cDNA libraries (apical cell, basal cell, zygote, two-celled proembryo), all these ESTs were clustered using Blastclust software. All the ESTs were submitted to NCBI database. The accession numbers for apical cell ESTs are HS808285–HS808305, and for basal cell ESTs are HS804060–HS804083, and for two-celled proembryo ESTs (tc0001–tc0007) are HO844849, HO844415, HO845026, HO843422, HO844377, HO844521, and HO844358.

RT-PCR expression analysis

mRNA was isolated from apical cells, basal cells, global-stage embryos, and heart-shaped embryos. First-strand cDNA was synthesized using Oligo (dT)15 (Sigma, Hamburg, Germany) and Superscript III Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA); a Super SMART™ cDNA PCR Synthesis Kit (Clontech Laboratories) was used for cDNA amplification. The tobacco glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (GenBank accession no. AJ133422, GAP1: 5’TCCACTCTCA-CAGGACACA-3’, GAP2s: 5’–AGACTCTTCAGGACACGC-3’) was used as a control. RT-PCR was done as described previously [12].

Real-time RT-PCR analysis

The quantitative expression of apical/basal cell-derived transcripts was estimated by real-time RT-PCR using single-stranded cDNA from specific cells preamplified using a Super SMART™ cDNA PCR Synthesis Kit. cDNAs from apical/basal cells were used as the template for real-time PCR, with gene-specific primers in 20-μl reactions containing 1 × FastStart Universal SYBR Green Master (Roche Diagnostics, Mannheim, Germany), and 300 nM each primer. Real-time PCR was performed over 45 cycles (95°C for 15 s and 60°C for 1 min) with a Roter-Gene 6000 system (Corbett Research, Mortlake, Australia). The data were analyzed using LinRegPCR [31]. In pre-experiments, expression level of the six reference genes were tested in apical/basal cell and zygote: GAPDH(AJ133422), Actin(GQ281246), Polyubiquitin(GQ281244), L25 ribosomal protein(L18908), Elongation factor 1α (EF-1α; AF120993) and Ubiquitin-conjugating enzyme E2 (Nubec2; AB026056). Stability of the reference genes was examined by geNORM v3.5 [32]. Finally, GAPDH/Polyubiquitin/Nubec2 were chosen for the calculation of normalization factor. Relative expression level was then calculated based on the N0 in LinRegPCR and normalization factor in geNORM. Thus, for each examined gene, the expression levels of the examined gene in the apical/basal cell samples were normalized to the expression levels of reference genes, GAPDH/Polyubiquitin/Nubec2. Primer pairs for reference genes are: qGAPs: 5’–AGGCTGGAGAAA-GAAGCTACCTA–3’, qGAPs: 5’–AGACTCTTCAGGACACCGA–3’, qUBCs: 5’–GGGCTGGTATGCAGATTTTC–3’, qUBCs: 5’–AGCTGCAAAGATCAGCCT–3’, qUBCs: 5’–AGCTTGCAAAGATCAGCT–3’, qUBCs: 5’–CAGGATATA TTTGCTGTAACAGATTA–3’.

Supporting Information

Figure S1 Viability of two-celled proembryo, isolated apical and basal cell. (TIF)

Figure S2 Transcript levels of selected transcripts. (TIF)

Table S1 Result of RT-PCR and detailed information of ESTs examined. (XLS)

Table S2 Result of qPCR and detailed information of ESTs examined. (XLS)

Table S3 Detailed information on apical cell ESTs. (XLS)
Table S4  Detailed information on basal cell ESTs.  
(XLS)

Acknowledgments

We thank Dr. Jingzhe Guo from Wuhan University for his help in preparation of figures.

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