Cortically localized maternal messenger RNAs in sea urchin unfertilized eggs, a transcriptomic approach

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Abstract

Maternally accumulated mRNAs and proteins play different roles in early development of animals, cell cycle progression, cell architecture maintenance, metabolism and patterning. In some animals, mRNAs required for early development, especially for embryo patterning, are may be localized in oocyte particular regions. Later on, these localized mRNAs participate in specification of particular regions in embryos. In sea urchin eggs, vegetal region is determinated by maternal factors, from which Dishevelled protein has only been known to be localized in vegetal pole of unfertilized eggs. We suggest that determination of vegetal region of sea urchin eggs and specification of other embryonic regions should require some or many localized maternal factors both mRNAs and proteins. The goal of this study is analysis of localized maternal mRNAs that are potentially necessary for early sea urchin development. To detect localized transcripts, we performed transcriptomic analysis of unfertilized eggs and their isolated cortical layers. We detected a pool of cortically-enriched transcripts. Using gene ontology analysis, we found 62 gene probes corresponded to 27 unique genes. Further analysis showed that among 17 found terms the most prevalent 'Cellular components' category is nucleic acid binding (20 genes). The most prevalent 'Biological processes' categories are DNA metabolic process (7 genes) and nucleic acid metabolic process (9 genes). We suppose that found cortically-associated transcripts coding nucleic acid binding proteins may be necessary for cell specification in early sea urchin development.

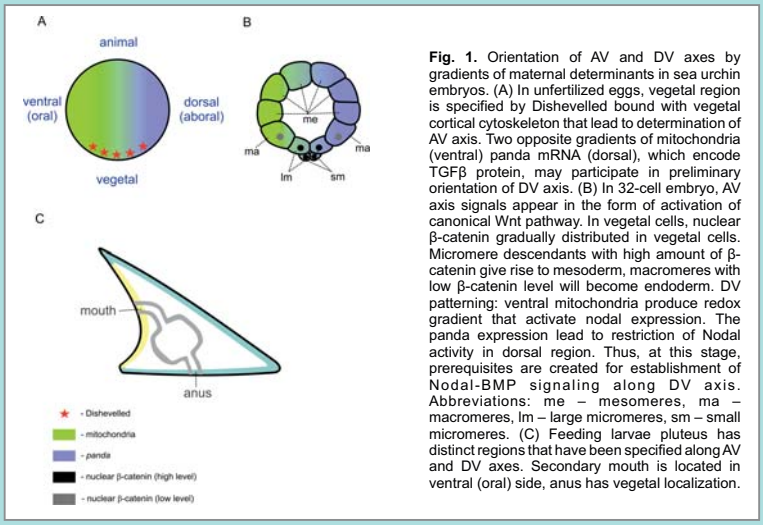

Fig. 1. Orientation of AV and DV axes by gradients of maternal determinants in sea urchin embryos. (A) In unfertilized eggs, vegetal region is specified by Dishevelled bound with vegetal cortical cytoskeleton that lead to determination of AV axis. Two opposite gradients of mitochondria (ventral) panda mRNA (dorsal), which encode TGFβ protein, may participate in preliminary orientation of DV axis. (B) In 32-cell embryo, AV axis signals appear in the form of activation of canonical Wnt pathway. In vegetal cells, nuclear β-catenin gradually distributed in vegetal cells. Micromere descendants with high amount of β-catenin give rise to mesoderm, macromeres with low β-catenin level will become endoderm. DV patterning: ventral mitochondria produce redox gradient that activate nodal expression. The panda expression lead to restriction of Nodal activity in dorsal region. Thus, at this stage, prerequisites are created for establishment of Nodal-BMP signaling along DV axis. Abbreviations: me – mesomeres, ma – macromeres, lm – large micromeres, sm – small micromeres. (C) Feeding larvae pluteus has distinct regions that have been specified along AV and DV axes. Secondary mouth is located in ventral (oral) side, anus has vegetal localization.

Methods

RNA samples of unfertilized eggs and their isolated cortices were sequences by Illumina paired end approach (2×100 bp). Transcriptomes were assembled de novo using Trinity in web-based Galaxy platform (www.usegalaxy.org). Quantitative gene expression was estimated using the RSEM program (computational resources provided by the Shared Facility Center 'Data Center of FEB RAS' (Khabarovsk, Russia)). Functional annotation, Gene ontology enrichment analysis (was performed using the GOseq method).

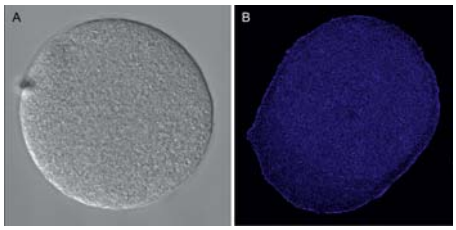

Fig. 2. Unfertilized eggs and isolated egg cortices. (A) DIC image of unfertilized egg. (B) Isolated egg cortex. Specimens were fixed (3% PFA and 0.1% glutaraldehyde) and stained wish phalloidin-Alexa Fluor 633. Actin is visible as small dots (colored by blue).

Results

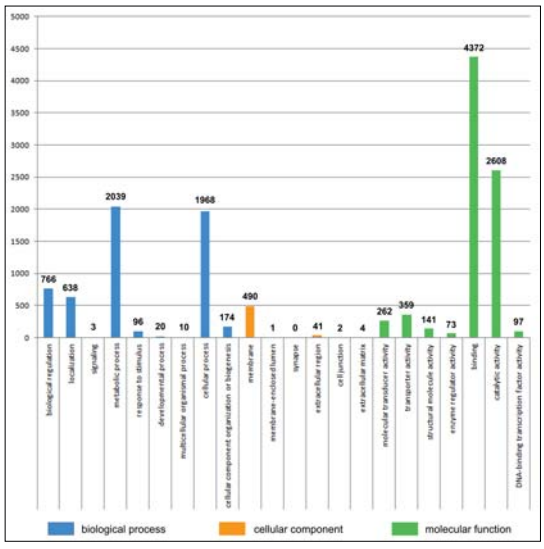

Fig. 3. Functional annotation of assembled transcripts for unfertilized eggs based on Gene Ontology (GO) categorization. GO analysis was performed at the second level for three main categories (Biological Process, Cellular component and Molecular function).

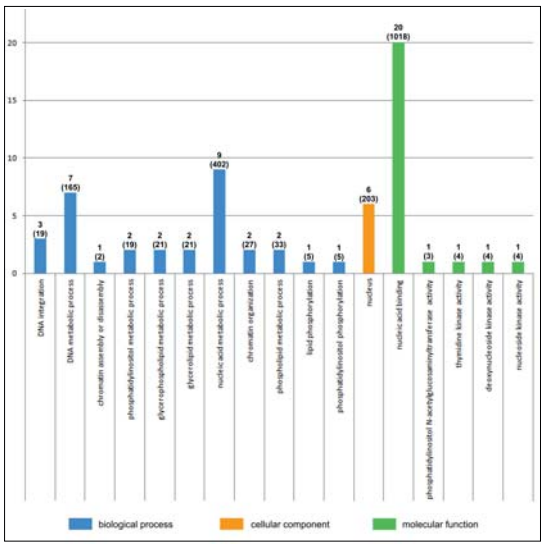

Fig. 4. Distribution of enriched GO terms for isolated egg cortices. The X-axis indicates the enriched GO terms, and the Y-axis indicates the number of upexpressed transcripts for each GO term. For comparison, numbers of annotated gene probes for each category for unfertilized eggs are given in

Conclusions

1. The de novo transcriptome assembly generated by Trinity resulted in 144271 transcripts (or 108419 unigenes). The number of annotated unigenes under the GO term 3 categories was estimated to reach 7%.

2. In transcriptome of isolated egg cortices, GO analysis showed 62 gene probes corresponded to 27 unique genes.

3. Gene ontology enrichment analysis shows that prevalent category in isolated cortices is “Nucleic acid binding”, which includes several transcription factors. Some cortically-enriched transcripts of other categories encode proteins, which are components of plasma membrane.

5. In sea urchin eggs, pool of cortically-enriched transcripts are necessary for both embryonic patterning and functioning of plasma membrane complex.