Detection of Oocyte mRNA in Starfish Polar Bodies

Oogenesis is a conserved process that requires gene transcription and storage of RNA for development until the embryonic genome is activated (Evsikov and Evsikov, 2009). Analysis of oocyte mRNA profiles detected in polar bodies may allow evaluation of gene expression in single oocytes without destroying the cell. Here we demonstrate the detection of such oocyte mRNA in its sibling polar body.

We biopsied polar bodies from eggs of the starfish, Asterina miniata, without disrupting either cell. The estimated volume of an A. miniata oocyte is $3.05 \times 10^{-3}$ pl, nearly 2,000 times greater than that of its polar body, which has an estimated volume of $1.77 \times 10^{-6}$ pl. Individual polar bodies and oocytes were isolated and transferred to a reaction buffer, heat lysed, DNAse treated, and reverse transcribed without isolation of the RNA (Protocol described in the Supplemental Material). We first tested if the polar body had detectable ribosomal RNA using 1/30th of the RT-reaction from each cell, and we consistently amplified the appropriate product from each cell (Supplemental Fig. 2). We then tested for specific mRNAs; these were detectable with greater success for transcripts that were more abundant (had lower Ct values) in the sibling oocyte (Table I). Detection of a specific mRNA transcript in the polar body decreased 60% for every unit increase in corresponding Ct value for that transcript in its sibling oocyte (odds ratio = 0.40; $P=0.01$). This result provides a baseline from which to predict transcripts that may be detectable in polar bodies if levels in the oocyte are known. Confocal imaging supports the hypothesis that representative ooplasm containing mitochondria is extruded with the first polar body (Supplemental Fig. 3).

We compared the level of mRNA for six genes in seven different sibling pairs. The $\Delta$Ct value between oocytes and polar bodies for a given gene ranged from 4.6 for eukaryotic initiation factor 2 (eif2) transcripts to 14.0 for histone2A (h2a) transcripts. These values correspond to 25- to 16,000-fold relative differences in mRNA abundance between oocytes and polar bodies. Since the variance within sample replicates is $<2\%$, variability between transcripts may reflect a difference in retention or localization between these sibling cells.

Further research is needed to correlate variation of mRNA levels in a polar body with its sibling oocyte. It will be important to examine cell-to-cell variability among developmentally critical gene transcripts in polar bodies and to test if variability between individuals may reflect biological differences between oocytes as these may reflect dynamic changes in mRNA abundance during oocyte maturation. Translating this technique to the evaluation of human oocytes is promising; although the human oocyte is smaller ($120 \mu$m diameter compared to $180 \mu$m), the polar body is comparable or larger than the starfish polar body (Vreek, 1990). Technological advances including incorporation of non-biased amplification of mRNA may permit clinical analysis of human oocyte gene expression from a single polar body (Noutsias et al., 2008). Such a method may aid with the assessment of oocyte quality and developmental competence in patients using assisted reproductive technologies.

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Received 16 December 2009; Accepted 15 January 2010

ACKNOWLEDGMENTS

We are greatly appreciative of the contributions from Linda Sousa, who assisted with microdissection and polar body biopsies, Drs. Mamiko Yajima, Julian Wong, and S. Zak Schwartz for their assistance with imaging, as well as Iris Chen for statistical analysis. This research was supported by grants from the NIH, the NSF, and the Provost of Brown University; lab and financial support was obtained from the Center for Reproduction & Infertility at Women & Infants Hospital of Rhode Island.

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