From egg to embryo: a peripatetic journey

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Abstract

The recent surge of interest in oocyte development has been spurred in large part by the increasing implementation of assisted reproductive technologies (ART) to treat human infertility. What is becoming apparent is that ‘egg quality’ is a primary factor in the success of ART (Sauer 1998), and yet we know virtually nothing about the molecular signature of a ‘high quality’ oocyte, i.e., an oocyte that is capable of maturing, being fertilized and supporting development to term. We are gaining marked insights, however, into how sperm activate eggs and the changes in gene expression that accompany preimplantation development. Nevertheless, embryo culture is known to effect gene expression (Rinaudo & Schultz 2004), the long-term consequences of which are only recently being unmasked. This review will briefly highlight these topics that were presented during the Biennial Joint Meeting of the UK Fertility Societies at Warwick University in April 2005.

Gene expression during oocyte development

Oocyte development is characterized by initiation of growth during the primordial to primary follicle transition and the subsequent sequential acquisition of meiotic and developmental competence, i.e., the ability of oocyte to resume and complete meiosis, and the ability of the metaphase II-arrested egg to be fertilized and support development to term, respectively. We used Affymetrix microarrays to characterize global changes in gene expression that occur in oocytes obtained from primordial to large antral follicles and used Expression Analysis Systematic Explorer (EASE) to identify biological and molecular processes that accompany these transitions and likely underpin acquisition of meiotic and developmental competence (Hosack et al. 2003).

Most striking is that greatest degree of change is observed during primordial to primary follicle transition, in which approximately one third of the transcripts exhibit at least a two-fold change in relative abundance (of those transcripts which change, about a half increase and another half decrease; Pan et al. 2005). Changes that occur during subsequent transitions, e.g., primary to secondary follicle, are about ten-fold less. Of particular note is the marked up-regulation of several transcription factors, including Gli3, which is critically involved in the hedgehog signaling pathway that appears ubiquitously involved in cell-cell interactions (Cohen 2003). But also of note was the totally unanticipated finding that accompanying the primordial to primary follicle transition is an increase or decrease in transcriptional activity of specific chromosomes – such changes are not observed at later stages of oocyte development – and that these changes are largely confined to specific regions of these specific chromosomes. It will be interesting to learn if these regional changes in gene expression correlate with local differences in the state of histone modifications that are linked to either transcriptionally permissive or repressive chromatin (Jenuwein & Allis 2001, Fischle et al. 2003).

Oocyte development in vitro results in eggs of reduced developmental competence (Eppig & Schroeder 1989). Remarkably the global pattern of gene expression in oocytes that developed from either the primordial or secondary follicles stage differed from that of oocytes that developed in vivo by only 4% and 2%, respectively. EASE analysis revealed that there is an over-representation of genes involved in transcription of the commonly mis-expressed genes (1%). For example, the increased expression of Ctcf and Dnmt3a, genes involved in establishing the correct pattern of maternal DNA methylation, could result in reduced developmental competence of oocytes (or preimplantation embryos due to maternally-inherited CTCF and DNMT3A).

Egg activation

Activation of mammalian eggs is characterized by a series of calcium oscillations that initiate shortly after sperm--egg...
fusion and terminate with pronucleus formation (Ducibella et al. 2002). The events of egg activation include exit from metaphase II-arrest and entry into interphase, cortical granule exocytosis, and recruitment of maternal mRNAs. Until recently it has not been clear if these oscillations are simply an outcome of the calcium releasing and sequestration machinery or whether information encoded in these oscillations is ‘decoded’ by the egg to generate specific responses of egg activation.

Recently, it has become possible to experimentally manipulate calcium oscillations in mammalian eggs, i.e., one can modulate their frequency, amplitude and duration (Ducibella et al. 2002). By doing so, it has become apparent that the events of egg activation are controlled by the number of calcium oscillations (the eggs in fact seem to be summing the total amount of calcium that is released, (Ozil et al. 2005) such that early events (e.g., cortical granule exocytosis) require only a few oscillations, whereas later events (e.g., cell cycle resumption and recruitment of maternal mRNAs) require many more. This may serve as a mechanism to insure that the events of egg activation occur in a specific temporal fashion that is necessary for successful development. For example, a block to polyspermy needs to be established early and this would occur because cortical granule exocytosis is triggered by only a few calcium oscillations.

The basis for calcium oscillations is IP$_3$-mediated release from intracellular stores (Miyazaki et al. 1992), presumably by a phospholipase C (PLC) hydrolyzing inositol bisphosphate (PIP$_2$). How sperm generate IP$_3$, however, has undergone recently a paradigm shift from a ligand-receptor model to a model in which a sperm-specific PLC, PLC$_ζ$, diffuses into the egg following sperm–egg membrane fusion to initiate calcium oscillations in the egg (Swann et al. 2004). Consistent with this model is that expression of PLC$_ζ$ mRNA in mammalian eggs mimics sperm-induced calcium oscillations and activates eggs, and sperm extracts immunodepleted of PLC$_ζ$ are no longer capable of initiating calcium oscillations when injected into eggs (Saunders et al. 2002). The best evidence to date that PLC$_ζ$ is the sperm-derived factor is that a transgenic RNAi approach to target PLC$_ζ$ mRNA and thereby reduce the amount of PLC$_ζ$ protein reveals that sperm derived from these transgenic mice trigger patterns of Ca$^{2+}$ oscillations following fertilization in vitro that terminate prematurely (Knott et al. 2005). But the most compelling observation implicating PLC$_ζ$ is that no transgenic offspring are found when females are mated to transgenic founder males.

**Gene expression during preimplantation development**

Affymetrix microarrays have also been used to characterize global patterns of gene expression that accompany development of preimplantation mouse embryos (Zeng et al. 2004, Zeng & Schultz 2005). The analysis confirmed previously described processes/events. For example, the expression profiles of oocytes and 1-cell embryos are very similar, presumably because the mRNA complement of the 1-cell embryo is inherited from the oocyte. A major reprogramming of gene expression occurs concomitant with zygotic genome activation during the 2-cell stage and the expression profile of the 2-cell embryo differs markedly from that of oocytes/1-cell embryos and 8-cell/blastocysts. EASE analysis, however, provided several new and unanticipated insights, one being that genome activation, in which 17% of the genes expressed during the 2-cell stage are α-amanitin sensitive, is not as promiscuous as previously proposed with genes involved in RNA metabolism and transcription, ribosome biogenesis and assembly, and protein synthesis being preferentially over-represented. Of particular note is that Ingenuity Pathway Analysis (IPA), which identifies biological pathways and the interrelationships between network genes in the subsets of candidate genes with particularly interesting patterns, revealed a network of focus genes centered around Myc. MYC can regulate expression of many genes including genes involved in ribosome biogenesis, and its ability to stimulate its own expression makes it an ideal candidate to play a central role in genome activation and the associated reprogramming of gene expression that is essential for continued development.

Superimposed on genome activation is the development of a chromatin-mediated transcriptionally repressive state (Schultz 2002). For example, inhibiting histone deacetylases (HDAC) relieves this repression. Many Hdac’s are expressed in the preimplantation mouse embryo, but IPA analysis identified Hdac1 as a critical component in a network centered on repression of gene expression. Thus, we have identified specific candidate genes that may be critical for genome activation.

**Long-term effects of embryo culture**

The number of children conceived by ART is estimated to be between 1–3% in developed countries (Maher 2005). The problem of multiple gestation pregnancies is a clearly perceived and recognized problem by practitioners of ART, who are confronted with the problem of identifying the best embryos for transfer, which is usually performed after 2–3 days of culture. The morphological criteria for identifying such embryos, however, are weak at best. Culturing embryos to the blastocyst stage, which requires 5–6 days of culture, has been proposed as a solution to identify embryos of higher developmental competence within the developing cohort (Gardner et al. 2000). Nevertheless, embryo culture is known to perturb gene expression (Rinaudo & Schultz 2004), especially genes involved in protein synthesis and amino acid and water transport. In addition, embryo culture can perturb the expression imprinted genes such as H19, such that biallelic expression is observed and coupled with loss of DNA
methylation of the paternal allele in a region that is essential for repression of the paternal allele (Doherty et al. 2000, Mann et al. 2004). Moreover, biallelic expression of H19 and other imprinted genes persists in extraembryonic tissue following embryo transfer and subtly compromise placental function that would ultimately impact the developing embryo (Mann et al. 2004). It should be noted that loss-of-imprinting is associated with Angelman and Beckwith–Weidemann syndromes, of which a significantly higher incidence is observed in ART-conceived children, and which in most instances this is due to loss of DNA methylation in critical regulatory sequences (see Maher 2005), i.e., the syndrome is due to an epigenetic change.

The Barker hypothesis or Fetal Origins of Adult Disease (FOAD) proposes that fetal adaptations in utero to maternal undernutrition or malnutrition can lead to specific diseases in the adult, including coronary heart disease, high blood pressure, and type II diabetes (Hales & Barker 2001). FOAD has been extrapolated back to preimplantation development using a rat model in which a low protein diet restricted to preimplantation stage led to changes in birthweight, postnatal growth rate, hypertension and organ/body-weight ratios in either male or female offspring (Kwong et al. 2000). The long-term effects of embryo culture on behavior, however, have not been assessed.

Using a mouse model we found that adults derived from cultured embryos did not exhibit any differences in development to term or early developmental events (e.g., righting response, day of eyelid opening, motor coordination) but did exhibit specific behavioral alterations in anxiety (elevated zero maze) and spatial memory (Morris hidden water maze) (Ecker et al. 2004). Male mice exhibited reduced anxiety, and both males and females displayed reduced retention of spatial information. We also found that although embryo culture had long-term effects on behavior in the offspring it had no effect on longevity (Somovilla et al. 2005).

These results suggest that special effort should be made to minimize the effect of culture on the human preimplantation embryo, especially in light of the recent reports that detected an increased incidence of Angelman and Beckwith–Wiedemann syndromes. They also provide a powerful justification for the careful monitoring of ART-conceived children, in particular with follow-up studies assessing development and behavior over their lifetime. When these suggestions were first offered four years ago they evoked the ire of certain members of the ART community and were viewed as draconian. It is reassuring that these voices are now silent and that the ART community is both sensitive and receptive to these suggestions.

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