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Oogenesis as a foundation for embryogenesis

R.G. Gosden *

Department of Obstetrics and Gynecology, Division of Reproductive Biology, Royal Victoria Hospital, McGill University, Women's Pavilion (F3.38), 687 Pine Avenue West, Montreal, Quebec, Canada H3A 1A1

Abstract

The majority of oocytes in postnatal ovaries are small, non-growing and reside in primordial follicles. They have to undergo a prolonged phase of growth and differentiation before nuclear and cytoplasmic maturation enables them to resume meiosis and undergo fertilization. A better knowledge of this phase of oogenesis is essential for understanding causes of oocyte pathology and optimizing methods for growing oocytes in vitro and for cryopreservation. There could also be spin-off discoveries for contraceptive strategies and pharmacologically controlling oocyte maturation. During oocyte growth, a molecular programme for development is assembled for the timely expression of mRNAs, some of which are expressed throughout oogenesis while others are 'masked' until or after meiotic maturation. Masking and stability in storage are largely due to a truncated poly(A) tail, controlled by regulatory sequences on the 3′-untranslated region (UTR) of the mRNA. Most maternal RNAs are degraded early in cleavage, there being a narrow overlap between persisting maternal mRNAs and activation of the embryonic genome. Accumulation of RNAs and proteins are not, however, the only major changes taking place during oogenesis. Cytoplasmic organelles multiply and redistribute, and there are epigenetic modifications of DNA for monoallelic expression of imprinted genes. The granulosa cells are obligatory for they provide physical support, nutrients and mediate the regulatory influences of gonadotrophic hormones. On the other hand, the oocyte actively influences the growth and differentiation of its granulosa cells. Thus, healthy embryos reflect the quality of both the oocyte and the granulosa cells. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

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1. Oocyte quality is a primary determinant of embryo quality

As the contributor of the bulk of the cytoplasm to the zygote and half of its nuclear DNA, the key importance of the oocyte is unquestionable. The principle, that embryogenesis is rooted in oogenesis, has been understood for nearly a century, and is now becoming better understood at the molecular level.

Starting at the primordial follicle stage, the oocyte takes weeks or months to complete its growth phase and increase its total volume more than 100-fold (Gougeon, 1996). Its growth is not a simple process of increasing mass, but involves qualitative and quantitative changes in key molecules for metabolism, structure and information. There is also an increase in cytoplasmic organelles, with the Golgi apparatus and mitochondria also changing distribution (Gosden and Bownes, 1995). Very little is known about the transcriptional activity of small oocytes in non-growing follicles, except that it is never completely silent. Once a follicle has become recruited for growth, however, there is a burst of transcription and translation in its oocyte, and this is sustained until the cell reaches full size. At this stage, mRNA synthesis virtually ceases and does not resume until 1–3 days after the egg has been fertilized. Some of these RNAs are long lived (half-lives are days or longer, according to species) and their expression must be finely controlled temporally (and perhaps spatially) so that they are expressed and degraded at the appropriate time. Degradation of maternal mRNAs is accelerated after meiosis and fertilization, such that 70% of the original payload of mRNA is lost by the 2-cell stage (Bachvarova, 1985).

In the most intensively studied species, the mouse, RNA synthesis increases 300-fo1d to 15 fg min^{-1} during peak oocyte growth (Piko and Clegg, 1982). Mature

* Tel.: +1-514-842-1231x4735/4747; fax: +1-514-843-1662.
E-mail address: gosdenrg@eums.edu (R.G. Gosden).

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oocytes contain 0.3–0.5 ng RNA (cf. perhaps 1 ng in humans), including 60–65% rRNA, 20–25% 5S and tRNA and 10–15% heterogeneous RNA. Eight percent of hnRNA is translatable poly(A), which is rather more than in somatic cells. There are pronounced differences in the time when the transcripts are translated, which is timed according to physiological need. Some genes, such as *zp3* responsible for part of the zona pellucida, are translated along with cellular housekeeping genes throughout oogenesis. Others, such as *mos* and tissue-type plasminogen activator (tPA) involved in the cell cycle or at ovulation, do not express their mature proteins until later. And finally there are some, like stem loop binding protein (SLBP), which is not required until after fertilization when the creation of new chromatin during cleavage causes a need for histone protein synthesis.

Control of the respective RNA populations in oocytes has been the subject of intensive investigation, though more often in oviparous species than in mammals. What do we know about the process(es) responsible for masking stored RNAs in mammals so that they can be expressed in a timely manner?

The principle factor determining masking-storage versus translation-degradation is selective, cytoplasmic polyadenylation of mRNAs. Truncation of the poly(A) tail masks the mRNA and produces translational silencing, whereas extension produces activation. RNAs contain a highly conserved and specific sequence in the 3′-untranslated region (UTR) which signals polyadenylation (AAUAAA). Masked transcripts, such as tPA, contain an additional U-rich conserved sequence 5′ to the polyadenylation signal (UUUUUAU), which is called the cytoplasmic polyadenylation element (CPE; Fox et al., 1989). The CPE is an important regulator of the stored-masked RNAs. The significance of the tail length can be illustrated by tPA, which normally has ~ 250 poly(A)s (Meric et al., 1996), but is stored with tail lengths reduced to ~ 40 residues until oocyte maturation. Translational silencing is probably due to a *trans*-acting translational repressor protein which, if displaced by a competitor transcript, leads to rapid elongation of the tail (Stutz et al., 1998; Richter, 1999). There may also be physical masking by merging mRNAs in ribonucleoprotein (RNP) particles (Matsumoto et al., 1998), or perhaps in association with cytoskeletal elements to produce large RNP particles combined with translation factors (Jansen, 1999). Packaged RNA excludes ribosomes from gaining access to regulatory elements, and the synthesis and translation of RNA into protein becomes uncoupled. Thus, more than one mechanism may account for the long RNA half-lives before oocyte maturity and the rapid degradation that happens afterwards when the maternal mRNAs are becoming progressively redundant.

Histone RNAs form an important exception to the general rule about polyadenylation control. Uniquely, they lack poly(A) tails and end in a conserved stem-loop, which serves as its equivalent. A specific RNA-binding protein, the SLBP, is essential for translation and associates with the mRNA as a component of the polyribosomal histone mRNP. Whilst the role of SLBP is still being clarified in mammalian oocytes, it appears that in *Xenopus* there is exchanged binding between two SLBPs, which may contribute to translational activation when histone demands rise (Wang et al., 1999).

In addition to the creation of a molecular program for development, the nuclear genomes of oocytes and sperm undergo epigenetic modifications. These changes, called genomic imprints, are allele-specific. They affect either the paternal or maternal allele, but only a minority of genes is known to be imprinted (< 100). Primordial germ cells (PGCs) are almost globally demethylated but subsequently during gametogenesis they acquire specific DNA methylation patterns responsible for suppressing expression of the affected allele. For example, in the case of *H19* the paternally-derived gene is methylated and silenced whereas the maternal copy is expressed (Fig. 1). The major enzyme responsible is DNA (cytosine-5)-methyltransferase, and mutations of Dnmt1 in mice cause prenatal death associated with severe abnormalities in both genomic imprinting and X-chromosome inactivation (Li et al., 1993). Interestingly, sex-specific alternatively-spliced forms of Dnmt1 are generated in mature oocytes and preimplantation embryos, and levels are very high (Howlett and Reick, 1991). It is sequestered in the cytoplasm except during oocyte growth, which is when the nuclei are losing their gynogenetic potential and gaining biparental potential (Kono et al., 1996; Mertineit et al., 1998). Until more data are available it is not possible to state the proportion of reproductive wastage that can be attributed to imprinting errors.

2. Granulosa cells influence the growth and quality of the oocyte

Oocytes extracted from small follicles must maintain contact with granulosa cells in order to grow, even though they remain alive in a nutrient culture medium for several days. The two cell types are metabolically coupled to each other—this union becoming much more intimate after follicle growth is initiated. Granulosa cells are also coupled to each other via gap junctions, presumably to guarantee coordinated development of the clonal descendents of the pregranulosa cells (Boland and Gosden, 1994). The character of heterologous and homologous gap junctions differs. Between the oocyte and granulosa cells, the channels are characterized by connexin 37, whereas homologous

junctions between granulosa cells contain connexin 43 (Juneja et al., 1999). The importance of these junctions for fertility has been proven by targeted mutagenesis and, in either case, fertility is impaired with arrested follicle development and inability of oocytes to resume meiosis (Simon et al., 1997). Pores in conventional gap junctions permit molecular exchange by diffusion of substances <1000 Da, including sugars, amino acids, lipid precursors and nucleotides. This is, therefore, a major route of entry to the oocyte. Cytoplasmic continuity is not, however, the only pathway. Oocytes are active in endocytosis, and this may be a route for transferrin-bound iron (Briggs et al., 1999). Also, granulosa cells produce a variety of growth factors and cytokines that have a demonstrated or potential impact on the fate of the oocyte. One of the best-known is kit-ligand (KL), which binds to a tyrosine kinase receptor, c-*kit*, on the oocyte surface. Spontaneous mutations of the respective genes (steel, *W*) have corresponding effects—germ cell deficiency and arrested development of follicular oocytes, depending on the product of the mutation. Confirmation of a role in oocyte growth has also been obtained using KL in culture experiments (Packer et al., 1994).

The gonadotrophins influence the oocyte indirectly since the cell does not express these receptors. FSH is constitutively expressed by granulosa cells, at least after the primordial follicle stage (Oktay et al., 1997) and, when the FSH receptor is deleted, follicles arrest at preantral stages (Abel et al., 2000). LH receptors are induced in the Graafian follicle, though predominantly in the mural granulosa cells. Certainly, these hormones have a profound effect on the fate of oocytes insofar that they determine the number of follicles selected for ovulation versus atresia by apoptosis (Gosden and Spears, 1997; Hsu and Hsueh, 1998). Oocytes appear to remain viable until atresia is well-advanced, perhaps because cumulus cells become apoptotic at a late stage. Whether the impact on the oocyte is all-or-none or if follicular rescue from atresia by gonadotrophins affects the quality of oocytes is not known. Some evidence that embryo quality is more heterogeneous in superovulated animals than during natural ovulation supports this suspicion.

3. Oocytes influence the granulosa cells

At one time, there was a widespread assumption that the oocyte is passive in the follicle and completely subject to control by its granulosa cells. There were, however, even in the older literature, indications from ovectomy experiments that the oocyte is actively influencing follicular development and controlling its environmental conditions. There is now much more supporting evidence for this view (Gosden et al., 1997). The discovery of GDF-9, a member of the TGF-β family which is specifically expressed in the oocytes of mice (though in some other cell types in humans and sheep) has revealed an oocyte protein that controls the granulosa cells. Targeted deletion to produce $gdf9^{-/-}$ mice causes an infertile phenotype in which follicles halt at the one-layer stage with enlarged oocytes (Dong et al., 1996). GDF-9 is needed for granulosa cells to proliferate, and at the Graafian stage it causes the cumulus cells to mucify prior to ovulation.

4. Potential benefits and applications

Among the major milestones in reproductive science in the last quarter century none is more dramatic than

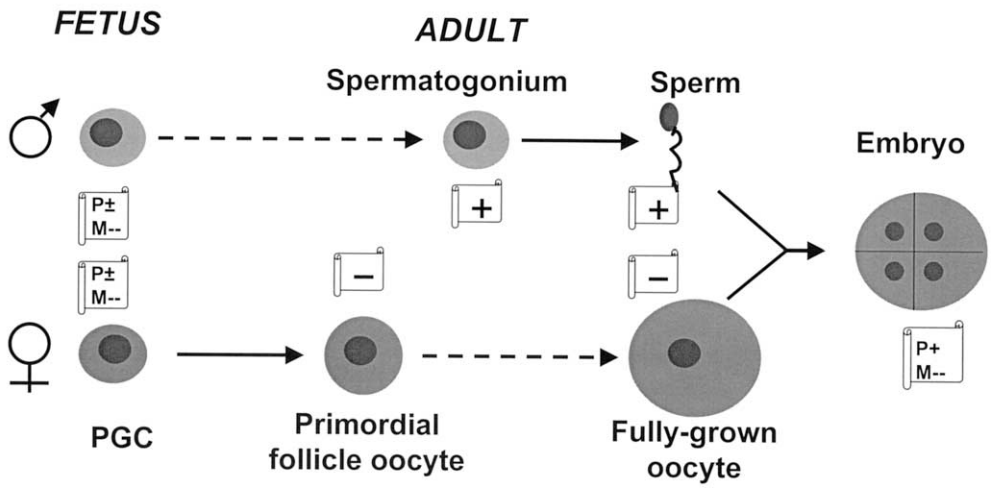


Fig. 1. Diagrammatic illustration of methylation patterns of *H19* on the maternally-derived chromosome in oogenesis and spermatogenesis, which creates complementarity in the zygote. *H19* is unusual in being incompletely demethylated in the PGCs.

assisted reproductive technology (ART) and the ongoing conquest of infertility. Yet, there is still a dearth of knowledge about the underlying causes of many types of infertility, and some technologies require fuller scientific validation or refining for improved efficiency and acceptability. It is widely believed that success rates with IVF are mainly limited by poor egg quality or our limited criteria for identifying the best embryos for transfer. Embryos often fail to thrive, and frequently one or more blastomere fragments or undergoes apoptosis. Not surprisingly, many of these embryos are aneuploid, mosaic or have chaotic karyotypes (Harper et al., 1995). On top of these problems, clinical egg donation has revealed that oocyte quality deteriorates markedly with age. Whilst preimplantation screening for aneuploidy may be useful in screening for the best quality embryos for transfer, hope also rests on a better understanding of the key determinants of egg quality—genetic, environmental and iatrogenic—and perhaps identifying molecular markers of cellular health. The introduction of PCR-SAGE is a significant stride in this direction (Neilson et al., 2000), and DNA microarrays should carry forward this progress. The capacity for high-throughput screening of candidate RNAs is exciting, although current applications are limited by the requirement for relatively large amounts of RNA from scarce cells and problems of uneven preamplification.

ART is not the only strong driving force for research on oocytes. In the future, it may be possible to use the oocyte as a target for contraception, and indeed there have already been considerable efforts in that direction (e.g. zp3, PDE3 inhibitors). Furthermore, it is hoped that a 'front-end' technology to conventional IVF can be refined for freeze-banking oocytes and subsequently maturing them in vitro (Newton et al., 1999). Recent results showing that culture conditions can alter the monoallelic expression of *H19* in embryos should be taken as a warning to be cautious, since methylation patterns are laid down during oogenesis (Doherty et al., 2000). Whilst attention tends to rest mainly on germ cells, it is clear that we shall not fully attain any of these goals unless research also engages the role of the granulosa cells in the health of the oocyte and, indirectly, of the embryo.

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