How to make an egg: transcriptional regulation in oocytes

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Abstract The oocyte is a highly differentiated cell. It makes organelles specialized to its unique functions and progresses through a series of developmental stages to acquire a fertilization competent phenotype. This review will integrate the biology of the oocyte with what is known about oocyte-specific gene regulation and transcription factors involved in oocyte development. We propose that oogenesis is reliant on a dynamic gene regulatory network that includes oocyte-specific transcriptional regulators.

Key words oogenesis · primordial germ cells · oocytes · gonadal development · transcriptional regulation

Introduction

An oocyte must undergo several developmental transitions during which it acquires a specialized extracellular matrix and synthesizes a unique set of proteins in order to become a fertilizable egg. The making of an egg is dictated by networks of oocyte-specific gene expression patterns that are at least in part regulated by transcriptional mechanisms discussed in this review. Little is known about the transcriptional profile within an oocyte and how oocyte-specific genes are regulated throughout the lifetime of an egg.

Development of the egg begins with the formation of primordial germ cells (PGCs) in the embryo and is followed by oogonial proliferation by mitosis, and initiation of meiosis I as primary oocytes. Oocytes may be arrested in the first prophase of meiosis from 12–50 years in humans, up to 3 years in frogs, and up to a year for most echinoderms. In response to various signals from gonadal somatic cells, the primary oocytes then grow, differentiate, and meiotically mature. Throughout the development of the oocyte, or oogenesis, the oocyte must make all the necessary maternal factors in preparation for fertilization. A hallmark of the oocyte across many species is its high level of transcription, reflecting the importance of maternal mRNAs and proteins that are crucial for supporting not only the growth of the oocyte, but the newly fertilized zygote as well (Smith and Richter, 1985; Davidson, 1986; Wassarman and Kinloch, 1992). The complexity and careful regulation of the transcriptional activity of the oocyte dictate its ultimate acquisition of developmental competence. This review will combine data and models from diverse oocyte types to emphasize our current knowledge of oocyte transcriptional regulation and transcription factors that play a role in the regulation of oocyte development.

Biology of the oocyte

An oocyte is a specialized cell whose differentiated phenotype supports fertilization and early development. In addition to having vast numbers of organelles typical to a eukaryotic cell such as the endoplasmic reticulum, mitochondria, and the Golgi apparatus, it also possesses organelles unique to the oocyte functions. These include annulate lamellae, cortical granules, and yolk granules (Lash and Whittaker, 1974; Wessel et al., 2001). Each of these structures is made largely by the oocyte and relies on oocyte-specific gene activity at high levels. In addition, all animal eggs are surrounded by one or more extracellular coats made specifically by the oocyte (Dumont and Brummett, 1985).

The extracellular matrix of the oocyte plays a crucial role in both fertilization and early development. Many eggs of both vertebrate and invertebrate animals, such
as frogs and sea urchins, are surrounded by an outer jelly coat and an inner vitelline envelope (Fig. 1) (Wessel et al., 2001). In contrast, flies and fish are surrounded by a tough chorion layer, whereas all mammalian eggs are surrounded by a thick coat, called the zona pellucida (ZP). The molecular composition of each of these extracellular coats appears distinct from each other; yet, they are functionally analogous. In mammals and fish, an extensive oocyte extracellular matrix is synthesized during oogenesis prior to fertilization, and the contents of the cortical granule, membrane-bound, stimulus-dependent secretory vesicles, further modify the extracellular matrix at fertilization (Fig. 1) (Wessel et al., 2001). At the other extreme are frog and sea urchin oocytes that have a minimal extracellular matrix but massive secretions of cortical contents transform the extracellular matrix following fertilization.

The extracellular matrix of the egg contains specialized components that ensure proper binding and activation of the sperm. In sea urchins, a protective, physical barrier for the developing oocyte is composed of the egg jelly synthesized by somatic cells and contains sperm chemoattractants and peptides (such as speract and resact) that activate the sperm (Gilbert, 2000). Approached to the plasma membrane of the egg is the vitelline layer, which consists of a network of glycoproteins (Dumont and Brummett, 1985). The main function of the vitelline layer prior to fertilization is for sperm attachment but is sufficiently porous to allow the transfer of macromolecules into the growing oocyte. The vitelline...
layer also serves as a scaffold for the attachment of proteins released by the cortical granules at fertilization in the formation of the fertilization envelope (reviewed in Wessel et al., 2001). Molecules in the cortical granules are specific to the oocyte and serve their function only during fertilization (Fig. 2). So far, of the 12 cortical granule proteins characterized in the sea urchin, all except two are the result of genes whose activities are unique to the oocytes (Wessel et al., 2001; Wong and Wessel, 2004).

The analogous extracellular structure in mammals, the ZP, is formed during oogenesis and increases in thickness as the oocyte grows. During fertilization, sperm must bind to and penetrate the ZP in order to fuse with the egg plasma membrane (reviewed in Wassarman et al., 2004). Following fertilization, the cortical granule contents released by the oocyte modify the ZP to block additional sperm penetration. In addition, the ZP prevents the developing mammalian blastocyst from adhering to the oviduct wall as it travels to the uterus. The blastocyst embryo hatches from the ZP by using a trypsin-like protease, trypsinin, thus enabling implantation (Perona and Wassarman, 1986).

Another specialized family of proteins whose presence is dependent upon oocyte-specific gene activity is the proteins associated with yolk acquisition and storage. Yolk proteins are the nutritional source for all non-placental embryos and depending on the animal and the protein, they can either be synthesized in various organs of the adult and imported into the oocyte, or be synthesized by the oocyte directly (reviewed in Brooks and Wessel, 2003). In egglaying species, oocytes contain low-density lipoprotein receptors on their surface that bind to yolk components such as vitellogenin and other ligands and selectively transport them into oocytes via receptor-mediated endocytosis (Schneider, 1996). Vitellogenin receptors have been identified in a wide range of animals including insects, frogs, fish, and birds (Schneider, 1996; Sappington and Raikhel, 1998). In sea urchin oocytes, in addition to the proteins responsible for the uptake of exogenously synthesized MYP (Major Yolk Protein), the oocyte also synthesizes YP30 (Yolk Platelet Protein of 30 KDa) (Wessel et al., 2000b; Brooks and Wessel, 2003). YP30 is synthesized exclusively by sea urchin oocytes, is packaged selectively into yolk platelets, and is hypothesized to function in the packaging and storing of other yolk proteins during oogenesis. In other animals, including mollusks, polychaetes, and crustaceans, ultrastructural analyses suggest that yolk is also synthesized by the oocyte (Eckelberger, 1979; Zerib, 1980; Kress, 1982; reviewed in Brooks and Wessel, 2003). In order for the oocyte to carry out its specialized functions, it likely requires a unique repertoire of transcriptional strategies.

Following the transition from an oogonia, the developing oocyte generally increases its transcriptional activity, resulting in a stockpile of mRNA and proteins that can be utilized quickly following fertilization and embryogenesis (Smith and Richter, 1985; Davidson, 1986; Wassarman and Kinloch, 1992). The accumulation
of mRNAs by oocytes during its growth phase is crucial to later development, and oocytes that have not completed their growth phase fail to develop properly as embryos (Fair et al., 2004; reviewed in Fair, 2003). Some gene products are only synthesized and utilized during the life of the oocyte. For example, the RNA transcripts of the ZP genes, which encode gene products that make up the mammalian egg coat, increase markedly during mouse oogenesis and then decrease to undetectable levels prior to ovulation (Roller et al., 1989; Epifano et al., 1995). Similarly, in sea urchins, oocyte-specific genes involved in modification of the extracellular matrix analogous to the ZP, such as Soft Fertilization Envelope genes, SFE1, SFE9, and Ovoperoxidase (OPO), also increase in abundance during oogenesis until oocyte maturation (Laidlaw and Wessel, 1994; LaFleur et al., 1998; Wessel et al., 2000a). Each of these transcripts is then selectively degraded, and their expression is repressed, so that these transcripts are not detectable following oocyte maturation. Ovoperoxidase is an enzyme that catalyzes the covalent tyrosine cross-linking of the structural proteins of the envelope, SFE1 and SFE9. These structural proteins modify the extracellular environment of the oocyte to prevent polyspermy and are constructed promptly after fertilization (LaFleur et al., 1998; Wessel et al., 2000a). The transcript dynamics of YP30 is similar to that of the SFE and OPO in that its mRNA is most abundant in developing oocytes and is no longer detectable upon oocyte maturation (Wessel et al., 2000b).

What makes the oocyte unique begins with the proper transcription of oocyte-specific genes. Numerous studies have focused on the general biology of oocyte development and meiotic regulation, but little is known of the transcriptional regulatory mechanisms of oocytes. Is the transcriptional profile within the oocyte uniform throughout oogenesis or do transitions occur indicative of a progressive transcriptional network? Are oocyte-specific genes regulated by unique sets of regulatory proteins throughout oogenesis or do the cells use master gene regulators?

Gametes develop from PGCs

Gametes develop from PGCs that are established during early embryogenesis. In the nematode Caenorhabditis elegans, the fruit fly Drosophila melanogaster, and the frog Xenopus laevis, PGCs originate from a morphologically distinct region of the zygote during early embryonic divisions (Fig. 3) (Ikenishi, 1998). In sea urchins and mammals, the PGCs appear to be induced de novo from other cells in the early gastrulating embryo (Lawson and Hage, 1994; Ransick et al., 1996; McLaren, 2003). PGCs have an extragonadal origin and navigate through various tissues to reach the somatic gonad. Overall, the mechanisms of PGC migration are highly conserved in divergent animal classes and involve intrinsic and somatic cues, attraction and repulsion, and amoeboid motility (Matova and Cooley, 2001; McLaren, 2003; Raz, 2003).

Several molecules involved in the molecular mechanisms of germline establishment have been localized to PGCs in different animal species. Some of these molecules are conserved germline determinants, such as vasa, tudor, pandilla, nanos, germ cell less, and mago nashi, while others appear species specific, such as oskar in flies and pgl-1 in worms (reviewed in Matova and Cooley, 2001; Extavour and Akam, 2003). Most of these germline factors are involved in translational regulation. Oct-4, however, is a maternally inherited transcription factor that is essential for the maintenance of the mammalian germline (Pesce and Scholer, 2000; Fuhrmann et al., 2001) (discussed below). Oct-4 is postulated to function as a transcriptional activator of genes required in maintaining an undifferentiated totipotent state and may repress the transcription of lineage-specific regulatory genes. Once in the gonad, germ cells begin to actively divide mitotically and become either oogonia or spermatogonia. In many invertebrate and vertebrates, oogonia divide to form clusters of interconnected cells (Pepling et al., 1999; Matova and Cooley, 2001). As the meiotic process is initiated, oogonial germ cells are referred to as primary oocytes.

Primary oocytes undergo extreme growth, from 10 μm up to several centimeters, depending on the animal, and this growth involves bidirectional communication between the germ cells and the gonadal somatic cells (De La Fuente and Eppig, 2001; Matzuk et al., 2002). The somatic cells provide nutrients and secrete signals important for the growth of the oocyte. In Drosophila and C. elegans, the oocyte develops in a syncytium (Gilbert et al., 1984; reviewed in Matova and Cooley, 2001). In different vertebrates, including zebrafish, Xenopus, chicken, and most mammals, clusters of oogonial cells undergo a growth phase within cysts (Ukeshima and Fujimoto, 1991; Matova and Cooley, 2001; Raz, 2003). The surface membranes of oocytes and their surrounding granulosa cells in mice are connected by gap junctions, which facilitate the transfer of glucose metabolites, amino acids, and nucleotides from the follicular cells to the growing oocytes (Eppig, 1991; Wright et al., 2001).

Oocyte-secreted factors play key roles in the development and differentiation of ovarian follicles. Oocyte-specific factors that influence follicular development include proteins of the transforming growth factor β superfamily members, growth differentiation factor (GDF-9), and bone morphogenic protein (BMP-15) (Dong et al., 1996; Yan et al., 2001). Mice lacking GDF-9 are infertile because of a lack of follicular development, and mice lacking BMP-15 have decreased ovulation and fertilization rates (Dong et al., 1996; Yan et al., 2001). The bidirectional communication between
the oocyte and follicle cells involves autocrine, paracrine, and endocrine regulations and ensures their proper differentiation and development.

**Discovery of transcriptional activity in the oocyte—a historical perspective**

The study of transcriptional regulation has a deep history in oocytes. Even before the central dogma was posed, structures within the oocyte nucleus were intensively investigated that led to our understanding of transcriptional units. For example, the oocytes of many animals contain lambrush chromosomes, readily observed in the growing oocytes of organisms that produce large eggs. These chromatin loops of transcriptional activity were first observed by Flemming (1882) in urodele oocytes and examined in detail by Ruckert (1892) in shark oocytes. In the 1960s, autoradiographic studies carried out with the light microscope showed that intense RNA synthesis occurs throughout the lateral loops of the lambrush chromosomes (Gall and Callan, 1962; Hill and Macgregor, 1980; Davidson, 1986). In large oocytes that contain excessive pools of maternal RNA, lambrush chromosomes reflect a maximal and continuous flow of transcription products...
throughout oogenesis (Smith and Richter, 1985; Davidson, 1986). Lampbrush chromosomes are not a pre-requisite for the synthesis and accumulation of maternal message though, because not all oocytes contain these structures (Smith and Richter, 1985; Davidson, 1986).

Maternal RNAs were demonstrated in the 1930s and are defined as RNAs synthesized by the oocyte prior to fertilization but utilized in early embryogenesis (reviewed in Davidson, 1986). In many species examined (including echinoderms and amphibians), maternal RNA has high complexity and is sufficient to support all the needed protein biosynthesis required during early development (reviewed in Davidson, 1986). The timing of activation of zygotic genes is species dependent and is highly variable. For example, zygotic gene activation occurs within minutes in sea urchins, at the two-cell stage in mice, four- to eight-cell stage in cows and humans, eight- to 16-cell stage in sheep and rabbits, and not until the mid-blastula transition in amphibians (Schultz and Heyner, 1992). Prior to zygotic gene activation, the zygote is supported by maternal mRNAs transcribed and translated during oogenesis.

First example of oocyte-specific gene regulation: oocyte-type rRNA

In the early 1960s the bulk of the maternal RNA stored in eggs was known to be rRNA (Davidson, 1986). The store of maternal ribosomes inherited in the egg cytoplasm is sufficient to support protein synthesis immediately following fertilization and in the first few stages of embryogenesis. The most thoroughly examined gene regulation in the oocyte is the transcription of 5S RNA genes (reviewed in Smith and Richter, 1985; Wolffe, 1994). Two types of 5S RNA genes exist in the *Xenopus* oocyte: an “oocyte type” present in about 20,000 copies and a “somatic type” present in about 400 copies per haploid genome (Brown and Sugimoto, 1973; Ford and Southern, 1973; Peterson et al., 1980). The regulation of 5S RNA was initially examined by a cell-free transcription system in which purified somatic 5S RNA gene was observed to be transcribed in extracts from both oocyte and unfertilized eggs, whereas oocyte 5S RNA gene was only transcribed from the oocyte extract (reviewed in Wormington and Brown, 1983; Smith and Richter, 1985).

The transcription of 5S RNA genes requires the general transcription factors TFIIIA, B, and C, and the 5S RNA gene promoter lies within the gene itself (internal control region (ICR)) (Fig. 4) (Wolffe, 1994). The oocyte and somatic 5S RNA genes have a total of 5 bp difference within the ICR, of which 3 bp are located within the TFIIIC-binding site. This sequence variation contributes to the preferential association of TFIIIC to the somatic 5S RNA gene (Fig. 4) (Wolffe, 1988, 1994; Keller et al., 1990, 1992). TFIIIA forms a specific but unstable complex with 5S RNA and recruits TFIIIB, which is then recognized by RNA polymerase III (Bieker et al., 1985; Setzer and Brown, 1985; Kassavetis et al., 1990). During oogenesis, the somatic and oocyte 5S RNA genes are transcribed with nearly the same efficiency when TFIIIA is in excess (Wolffe, 1994). So where is the transcriptional switch that distinguishes somatic and oocyte 5S gene activities? Recent evidence suggests that phosphorylation of *Xenopus* TFIIIA by casein kinase II allows the factor to act as an activator of the somatic 5S RNA and as a repressor of the oocyte-type 5S RNA upon oocyte maturation (Ghose et al.,
Phosphorylated TFIIIA binds to the promoters of both the somatic and oocyte-type 5S RNA genes, but it represses the transcription of the oocyte-type 5S RNA selectively, possibly by interfering with protein–protein interactions necessary for oocyte-type transcription (Ghose et al., 2004).

An important mediator of 5S RNA gene transcription also involves changes in chromatin structure (reviewed in Panetta et al., 1998; Crane-Robinson, 1999). The expression of linker histone protein (H1) is developmentally regulated; in *Xenopus* it is translated during early embryogenesis but not in oocytes (Wolffe, 1994; Panetta et al., 1998; Crane-Robinson, 1999). In the presence of the histone H1, a stable nucleosome is positioned on the oocyte 5S RNA gene that prevents binding of TFIIIA, whereas on the somatic 5S RNA gene, essential promoter elements remain accessible to TFIIIA (Bouvet et al., 1994; Panetta et al., 1998; Sera and Wolffe, 1998). Thus, this oocyte-specific transcriptional mechanism does not rely on oocyte-specific factors. Rather, differential regulation of shared or common factors results in this oocytestatic activity.

**Oct-4: one of the early master gene regulators?**

One of the few transcription factors known to be involved in the self-renewal of embryonic stem (ES) cells is Oct-4. This factor belongs to the Pit-Oct-Unc (POU) family of transcription factors containing both a homeobox sequence and a POU-specific domain (Verrijzer et al., 1992). It binds to an octamer motif and interacts with DNA through a helix–turn–helix domain. Orthologs of Oct-4 in mice, bovines, and humans share highly conserved genomic organization and regulatory regions (Nordhoff et al., 2001; Kuroyama et al., 2004).

*Oct-4* is selectively expressed in totipotent embryonic and germ cells (Pesce et al., 1998b; Pesce and Scholer, 2000). At the blastocyst stage in mammals, the outer layer of the blastocyst is the trophectoderm and the cells inside are the inner cell mass (ICM). The ICM is composed of pluripotent stem cells that give rise to all cell types of the embryo, including the germ cell lineage. *Oct-4* mRNA is present uniformly throughout the morula stage and becomes restricted to the ICM of the blastocyst (Fig. 5A). After embryo implantation in the mouse, *Oct-4* transcription is restricted to the epiblast and ES cells (Yeom et al., 1996; Pesce et al., 1998a). *Oct-4* transcription is then progressively down-regulated during gastrulation and eventually confined to the PGCs (reviewed in Pesce and Scholer, 2000). This function is presumably involved in retention of germline stem cell fates.

The candidate target genes that are up-regulated by *Oct-4* during mouse implantation include *Fibroblast Growth Factor-4 (FGF-4)* and *Osteopontin (OPN)* (Guo et al., 2002). *FGF-4* is thought to stimulate ICM growth or maintenance and is involved in the establishment of the primitive endoderm *in vivo* (Wildier et al., 1997), and *OPN* is an endodermal-specific, extracellular phosphoprotein that mediates adhesion by interacting with integrins (Guo et al., 2002). In contrast, both α and β forms of human chorionic gonadotropin are downregulated by *Oct-4* (Liu et al., 1997). Chorionic gonadotropin prevents the regression of the corpus luteum during early pregnancy, and is expressed by trophodermal cells. Therefore, *Oct-4* functions as both a transcriptional activator and repressor of its different target genes in ensuring the end result of potency in ES cells.

Given its critical role in maintaining pluripotency of the germ cells, *Oct-4* is of special interest in our understanding of the continuity of the germline. The *Oct-4* gene is driven by a TATA-less minimal promoter and at least two enhancer elements (Okazawa et al., 1991; Yeom et al., 1996). The minimal promoter contains a GC box with putative-binding sites for Sp1 and Sp3 transcription factors and a hormone-responsive element (HRE) (Sylvester and Scholer, 1994). The HRE contains binding sites for the steroid–thyroid receptor family and orphan nuclear receptor superfamily, specifically *COUP-TFI, ARP-1, EAR-2, and GCNF*, which downregulate *Oct-4* transcription (Okazawa et al., 1991; Pikarsky et al., 1994; Sylvester and Scholer, 1994; Ben-Shushan et al., 1995; Schoorlemmer et al., 1995; Fuhrmann et al., 2001). The distal enhancer is necessary for *Oct-4* transcription in the morula, ICM, and PGCs, while the proximal enhancer (PE) activates *Oct-4* in the epiblast (Yeom et al., 1996). The PE element is not only a transcriptional enhancer but is also a *cis*-demodification element that demethylates *Oct-4* during specific stages of embryogenesis, spermatogenesis, and oogenesis, when other genes undergo de novo methylation (Ben-Shushan et al., 1993; Gidekel and Bergman, 2002; Pan et al., 2002).

During gonadal development, transcription of *Oct-4* in mouse and human germ cells remains high until the onset of meiosis (Pesce and Scholer, 2000; Kuroyama et al., 2004; Rajpert-De Meyts et al., 2004). *Oct-4* transcription in oogonia and spermatogonia is down-regulated at specific stages of oogenesis and spermatogenesis (Pesce et al., 1998a; Kuroyama et al., 2004; Rajpert-De Meyts et al., 2004). In the spermatocyte lineage of the adult mouse, *Oct-4* protein is expressed in undifferentiated A spermatogonia and is down-regulated as the germ cells begin spermatogenic maturation (Pesce et al., 1998a). *Oct-4* expression in human testes is similar to findings in mice, except that *Oct-4* protein is undetectable from adult spermatogonia (Rajpert-De Meyts et al., 2004). In mouse oocytes, *Oct-4* mRNA and...
protein are down-regulated when the oocyte enters prophase of the first meiotic division and are re-expressed near oocyte maturation (Fig. 5B) (Pesce et al., 1998a, 1998b). Similarly, in fetal human ovaries, Oct-4 is present in primordial oogonia during fetal development and is down-regulated in oocytes that entered the first meiotic prophase; some Oct-4 molecules might be transported to the cytoplasm at the onset of meiosis, but the biological significance of this phenomenon is unknown (Rajpert-De Meyts et al., 2004). Oct-4 may play a role in the growth or acquisition of meiotic competence of oocytes, and/or it may be involved in transcriptional repression of oocyte-specific genes, since Oct-4 is transcribed minimally during a time when the oocyte is undergoing an overall increase in transcriptional activity, and Oct-4 is transcribed abundantly at oocyte maturation when transcriptional activity in the oocyte decreases dramatically (Pesce et al., 1998b). Recently, the expression of Oct-4 has been examined in human testes and ovaries during fetal development, and its aberrant expression may contribute to disorders in sex differentiation and malignant gonadal tumors in humans, highlighting its critical role in gonadal development (Looijenga et al., 2003; Rajpert-De Meyts et al., 2004).

**The germ cell-specific transcription factor FIGα regulates oocyte-specific genes**

FIGα is a germ cell-specific transcription factor that regulates the coordinated transcription of the three ZP glycoproteins, ZP1, ZP2, and ZP3, and may regulate additional pathways critical for ovarian development (Fig. 6) (Liang et al., 1997; Dean, 2002). The ZP proteins form an extracellular matrix that surrounds the growing mammalian oocyte and are critical for sperm activation and for the block to polyspermy. Mouse ZP3 has been shown to contain the primary species-specific sperm receptor of the oocyte, binding sperm via O-linked oligosaccharide chains and inducing the sperm

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**Fig. 5** (A) Oct-4 expression in preimplantation and early postimplantation development in mouse. Present as a maternal transcript in the zygote, Oct-4 is expressed at low levels until the eight-cell stage, when the zygotic Oct-4 gene is turned on. Until the compaction of the morula, Oct-4 is expressed equally in all blastomeres to be eventually confined in the inner cell mass, and after implantation, in the epiblast. From Pesce et al. (1998a). (B) In female germ cells, Oct-4 expression is down-regulated coincident with the entry into the prophase of the first meiotic division. At 15.5 dpc, corresponding to the zygotene/pachytene stage of meiotic prophase I, Oct-4 is absent in oocytes. It is not re-expressed until birth, when diplotene-arrested oocytes start expressing it de novo. Modified from Pesce et al. (1998a).
acrosome reaction (Bleil and Wassarman, 1990; Cheng et al., 1994, 1998; reviewed in Wassarman, 1988; Dean, 2002, 2004; Wassarman et al., 2004). Mouse ZP2 acts as a secondary sperm receptor and plays a role in the prevention of polyspermy, while ZP1 cross-links the other two ZP proteins (Wassarman et al., 2004). Targeted mutagenesis in mice demonstrated that either ZP2 or ZP3 homozygous nulls result in infertility in females but not in males (Rankin et al., 2001). Mice lacking ZP1 have abnormal zona matrices that are more porous than normal and result in early embryonic loss (Rankin et al., 1999). In most vertebrates analyzed, including mammals, *Xenopus*, and several fish, ZP genes are present, active, and contribute to the egg’s extracellular matrix, even though the extracellular matrix may vary structurally. The transcription of ZP glycoproteins is temporally and spatially restricted to oocytes in most vertebrates (Wassarman, 1990; Epifano et al., 1995; Kubo et al., 1997; Wang and Gong, 1999; Zeng and Gong, 2002). However, in some organisms the ZP proteins are present, active, and contribute to the egg’s extracellular matrix, even though the extracellular matrix may vary structurally. The transcription of ZP glycoproteins is temporally and spatially restricted to oocytes in most vertebrates (Wassarman, 1990; Epifano et al., 1995; Kubo et al., 1997; Wang and Gong, 1999; Zeng and Gong, 2002). However, in some organisms the ZP proteins are synthesized elsewhere. For example, the chick ZP3 is synthesized by follicle cells surrounding the embryo and the ZP1 homolog is transcribed in the liver (Waclawek et al., 1998; Bausek et al., 2000). In some teleost fish species also, ZP components are transcribed in the liver (Del Giacco et al., 1998).

Transcription of ZP genes in some organisms provides a paradigm for examining mechanisms and evolution of oocyte-specific gene transcription. In the mouse, transcription of ZP proteins is coordinately regulated by FIGα (Liang et al., 1997). FIGα heterodimerizes with a ubiquitous β-helix–loop–helix (bHLH) E12 protein and regulates the mouse ZP genes by binding to a canonical E-Box (CANNTG) approximately 200 bp upstream of the transcription start sites of ZP genes (Millar et al., 1991; Epifano et al., 1995; Liang et al., 1997). Functional homologs of FIGα have been identified in humans, zebrafish, and medaka (Liang et al., 1997; Kanamori, 2000; Huntriss et al., 2002; Onichtchouk et al., 2003; Bayne et al., 2004), and FIGα homologs bind interchangeably to the zebrafish ZPC and human ZP2 promoters, respectively (Onichtchouk et al., 2003; Bayne et al., 2004). FIGα is expressed in both the testis and ovary, but is expressed most abundantly in the ovary of mouse (Liang et al., 1997). FIGα is first detected in oocytes at E13.5 in mice and its transcript persists in oocytes into adulthood (Liang et al., 1997). Mice lacking FIGα are unable to express ZP genes or form primordial follicles, resulting in massive depletion of oocytes and sterility (Liang et al., 1997). However, cotransfection of FIGα and E12 in 10T1/2 embryonic fibroblasts is not sufficient to activate endogenous zona gene transcription, indicating that FIGα plays an important regulatory role in conjunction with other, yet unknown factors (Liang et al., 1997). Mice lacking FIGα have been found to heterodimerize with E12 protein and bind to the E-Box of the human ZP2 promoter, suggesting a conserved mechanism in controlling ZP genes and primordial follicle formation in the human as in the mouse ovary (Bayne et al., 2004).

**NoBox regulates oocyte-specific gene transcription**

The homeobox gene, *Nobox* (newborn ovary homeobox-encoding gene), was first identified by *in silico* subtraction of a murine newborn ovary cDNA library.
against the entire collection of mouse expression sequence tags (Suzumori et al., 2002). Nobox transcripts are detected in both mouse male and female gonads by reverse transcription-polymerase chain reaction (RT-PCR), and in situ hybridization results indicated that Nobox transcripts are present in murine oocytes from primordial through antral follicles but not in somatically derived granulosa cells, theca cells, and corpora lutea (Suzumori et al., 2002). NoBox protein localizes to the nuclei of germ cells and primordial follicles in the mouse ovary (Rajkovic et al., 2004). Disruption of 90% of the Nobox coding region including the homeodomain in the mouse resulted in infertile female mice, while males were unaffected (Rajkovic et al., 2004). Histomorphometric analyses indicated that the relative numbers of oocytes, germ cells, and primordial follicles were similar in Nobox+/− and Nobox−/− newborn ovaries (Rajkovic et al., 2004). However, by day 14 the Nobox−/− oocytes revealed a great loss of oocytes, indicating that the majority of oocyte and follicle growth beyond the primordial follicle stage was disrupted by the absence of Nobox (Rajkovic et al., 2004). Further examination indicated that in Nobox−/− oocytes, transcripts of genes that are preferentially transcribed in the oocyte, including Mos, Oct-4, Rfpl4, Fgf8, Zaf1, Dnm1lo, Gdf9, Bmp15, and H100, were downregulated (Rajkovic et al., 2004). The transcription of Nobox precedes the transcription of these genes, suggesting that Nobox may directly or indirectly regulate these genes that are important for oocyte and early embryo development (Rajkovic et al., 2004). A portion of the human Nobox homolog was also identified, but its function has not been examined (Suzumori et al., 2002).

Germ cell-specific general transcription factor ALF

The differentiation and restoration of totipotency of germ cells involve tightly controlled gene expression patterns. A set of general transcription factors are necessary for an accurate initiation of transcription. ALF (TFIIAα) is a counterpart of the large α/β subunit of the general transcription factor TFIIA. ALF interacts with the small TFIIA subunit to form a heterodimeric complex that stabilizes binding of TBP (TATA-binding protein) to core promoter DNA. ALF is transcribed specifically in testis and ovary of Xenopus and mice (Han et al., 2001). It was first identified in male germ cells and is expressed during the pachytene stage of meiotic prophase I in males (Han et al., 2001). In immature Xenopus oocytes, the maternal TFIIAα/β mRNAs are translationally repressed through a conserved 3’ UTR, and ALF compensates for the maternal storage and inactivation of TFIIAα/β mRNAs (Han et al., 2003). When oocytes commit to meiosis, a transition from TFIIAα/β to ALF occurs, and during maturation, fertilization, and early embryogenesis, ALF is inactivated and replaced by somatic TFIIAα/β (Fig. 7) (Han et al., 2003).

The transcriptional regulation of ALF in male germ cells is associated with reduced methylation at promoter-proximal CpG dinucleotides, whereas silencing in somatic tissues is associated with increased methylation (Xie et al., 2002). The promoters of mouse and human ALFs contain a short GC-rich region that is active when transfected into COS-7 and 293 cells (Xie et al., 2002). Transgenic mice containing this 133 bp ALF promoter linked to β-galactosidase and GFP reporter genes indicated that this promoter fragment was sufficient to direct specific transcription of ALF in both male and female germ cells (Han et al., 2004). Factors that regulate the differentiated transcription of ALF, however, have not been identified. The results from Xenopus and mice suggest an evolutionary conserved role for ALF in the germ cell differentiation programs.

ALF is one of a few germ cell-specific general transcription factors identified to date. Similar critical regulators of the gene transcription program directing male gametogenesis in the fruit fly are the Drosophila testis-specific TAF homologs: the cannonball (homolog of dTAF5), nht (homolog of dTAF4), mia (homolog of dTAF6), sa (homolog of dTAF8), and rye (homolog of dTAF12) (Lin et al., 1996; Hiller et al., 2001, 2004). Mutations in can, nht, sa, and mia all block spermatic differentiation. ALF may regulate germ cell-specific gene expressions during gametogenesis, since RNA polymerase II does not initiate transcription efficiently without general transcription factors, including TFIIA. Examination of the transcriptional regulation of ALF indicates that epigenetic modification such as

**Fig. 7** Multiple targets for FIGα. FIGα heterodimerizes with a ubiquitous bHLH protein, E12, and binds upstream of one or more genes, the expression of which is required for primordial follicle formation. In addition, FIGα is required for the expression of the three zona pellucida genes (Zp1, Zp2, and Zp3), without which the zona matrix is not formed. The persistence of FIGα in oocytes from embryonic day 13 until the oocytes are fully grown suggests that FIGα may regulate additional genes that are important for normal oogenesis and early development. From Soyal et al. (2000).
methylation may be an important regulatory mechanism of transcription. Identification of germ cell-specific transcription factor ALF and other tissue-specific TAF homologs suggest that the germ cell genome has evolved specialized transcription machinery to ensure the proper activation of genes that are required for germ cell development.

**Tissue-specific transcription factors important for somatic contribution to oogenesis**

Ovarian development involves combinatorial factors present in both the oocytes as well as in the somatic ovarian tissues. Transcription factors expressed by the follicular cells in the ovarian tissue are also now known to be important for oogenesis, such as the mouse TAFII105 and FOXL2 factors. TAFII105 consists of TBP-associated factors that are part of the TFIID general transcription initiation complex. The TFIID complex binds to the core promoters and directs RNA polymerase II to begin transcription. TAFII105 is highly expressed in granulosa cells that surround the maturing mouse oocytes and in the testis (Freiman et al., 2001). Female mice lacking TAFII105 are viable but are sterile, whereas male mice lacking TAFII105 are both viable and fertile (Freiman et al., 2001). TAFII105 controls gene transcription during female gametogenesis as a tissue-specific TFIID subunit. Microarray analysis of heterozygous and knock-out TAFII105 ovaries indicated down-regulation of ovarian-specific genes, including inhibin, aromatase, follistatin, cyclin D2, and 17-β-hydroxysteroid dehydrogenase type I (Freiman et al., 2001). These findings suggest that tissue-specific TFIID-related factors are important in the developmental regulation of gene transcription in gonadal development.

Another transcription factor important for ovarian development and function is FOXL2, a winged helix/forkhead transcription factor that is highly conserved in sequence and pattern of transcription in the early development of the vertebrate female gonad (Cocquet et al., 2003). Mutations in FOXL2 cause the blepharophimosis–ptosis–epicanthus inversus syndrome (BPES), characterized by eyelid malformations and premature ovarian failure in humans (Cocquet et al., 2002, 2003). FOXL2 is expressed in mammalian ovaries early in development, even before the onset of folliculogenesis, and persists until adulthood (Cocquet et al., 2003). FOXL2 mRNA is detected in both granulosa cells and some oocytes of the fetal and adult mouse ovaries (Loffler et al., 2003), although the FOXL2 protein is present only in follicular granulosa cells (Cocquet et al., 2002; Schmidt et al., 2004). Female mice lacking FOXL2 have a depleted pool of primordial follicles because of granulosa cell differentiation failure that leads to oocyte atresia (Schmidt et al., 2004). Therefore, proper gene expressions in ovarian somatic cells, as exemplified by TAFII105 and FOXL2, affect the growing oocyte and contribute to the proper overall ovarian development.

**Potential oocyte-specific regulatory factor—Oogenesin**

Most of the known gonadal transcription factors are present in both the testis and ovary, or the so-called “germline-specific” factors. These include FIGz, No-Box, and ALF transcription factors. Recently, two groups have independently identified an oocyte-specific superfamily of leucine-rich proteins, oogenesin-1–4, by two different methods. Dade et al. (2003) used in silico subtraction of cDNA libraries generated from mouse unfertilized eggs and two-cell zygote to identify oogenesin-1–4. Using differential display comparing RNA transcription of mouse oocytes and preimplantation embryos, Minami et al. (2003) identified oogenesin-1, which is transcribed in oocytes from 15.5 dpc fetus, coinciding with the start of oogenesis. Oogenesin-1 mRNA and protein are localized in oocytes in primordial, primary, secondary, and antral follicles in the mouse ovary, but in contrast, oogenesin-2–4 mRNAs are detected in oocytes from primary to preovulatory follicles but are not detectable in the primordial follicles (Dade et al., 2003). Low levels of oogenesin-4 transcripts are detected, however, in the testis. The exact roles of oogenesin proteins are not known. Since oogenesin-1 is expressed in the oocytes coincident with the start of primordial follicle formation, oogenesin-1 may be involved in oocyte–somatic cell interactions that lead to folliculogenesis (Minami et al., 2003).

**Potential regulatory role of Sox in ovarian development**

The Sox proteins belong to the SRY-type HMG (high mobility group) box superfamily of DNA-binding proteins. They are involved in the regulation of diverse developmental processes such as germ layer formation, organ development, sex determination, and cell type specification and are found in all metazoan species (reviewed in Wegner, 1999; Wilson and Koopman, 2002). Sox3 is homologous to the SRY gene (Sex-determining Region of the Y chromosome) and is expressed in the mouse brain and both gonads of the mouse (Collignon et al., 1996; Weiss et al., 2003). In the mouse, Sox3 was determined to be required for gonadal function but not in sex determination in both males and females (Weiss et al., 2003). The mouse Sox3 protein was localized in Sertoli cells of the testis and not in the ovary, although...
transcripts of mouse Sox3 are detected in oocytes and cumulus cells in the ovary (Weiss et al., 2003). The Sox3 knock-out mice exhibited abnormal growth, tooth development, and altered function of the somatic and germ cells in both sexes (Weiss et al., 2003). Therefore, Sox3 may play a role in gonadal development; however, its target genes have yet to be identified.

Other transcription factors expressed in the gonads

An increasing number of transcription factors are found to be expressed in the gonads, but their roles in gonadal development are not known. Northern blot analyses and RT-PCR from adult mouse tissues identified six Obox (oocyte-specific homeobox) family transcripts that are preferentially expressed in the gonads and that are distinct from the NoBox transcription factors (Rajkovic et al., 2002; Yeh et al., 2002). Obox-1, 2, 3, and 6 mRNA were detected in oocytes, and Obox-4 transcripts were detected in the testes (Rajkovic et al., 2002; Yeh et al., 2002). The protein localization of Obox has not been determined, but they may function either in transcriptional activation of the oocyte genome within the growing follicle, and/or they may be maternal factors that play a role in early embryogenesis.

Several groups have examined the transcription and function of homeotic genes in mouse, human, and bovine oocytes and preimplantation stage embryos (Verlinsky et al., 1995; Kuliev et al., 1996; Adjaye and Monk, 2000; Ponsuksili et al., 2001; Villaescusa et al., 2004). In vertebrates, HOX homeobox-containing genes are arranged on four separate chromosomal clusters, A, B, C, and D (reviewed in Gehring et al., 1994). Previous studies using RT-PCR detected HOXA4, HOXA7, HOXB4, HOXB5, HOXD8, and HOXD1 in human oocytes and cleaving embryos (Verlinsky et al., 1995; Kuliev et al., 1996). In addition, the ubiquitously expressed POU family member Oct1, and HEX (Homeotic gene Expressed in the Hematopoiesis) were detected in cDNA libraries derived from human unfertilized oocytes and early embryos (Adjaye and Monk, 2000). In bovine oocytes, HOXA3 and HOXD1 mRNA were detected, with HOXD1 also present in the four-cell stage bovine embryo (Ponsuksili et al., 2001). A similar study was conducted to identify HOX genes expressed in the mouse ovary resulting in the identification of HOXA5, HOXA9, HOXB6, HOXB7, HOXC6, and HOXC8 and HOX co-factors PREP and PBX (Villaescusa et al., 2004). The role of HOX transcription factors in mammalian oocytes and early embryonic stages remains to be elucidated.

Summary

The diverse families of transcription factors discussed in this review include regulatory proteins that are expressed predominantly in oocytes, proteins that are enriched in the gonads, and proteins that are present in oocytes, gonads, and early embryos (Table 1). A survey of existing studies on transcription factors that

<table>
<thead>
<tr>
<th>RNA expression</th>
<th>Protein localization</th>
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<tbody>
<tr>
<td>Oocytes</td>
<td>Sperm</td>
</tr>
<tr>
<td>Oct-4</td>
<td>+++</td>
</tr>
<tr>
<td>FIGz</td>
<td>+++</td>
</tr>
<tr>
<td>NoBox</td>
<td>+++</td>
</tr>
<tr>
<td>ALF</td>
<td>+++</td>
</tr>
<tr>
<td>TAF</td>
<td>105</td>
</tr>
<tr>
<td>FOXL2</td>
<td>+</td>
</tr>
<tr>
<td>Oogenesin-1–3</td>
<td>+++</td>
</tr>
<tr>
<td>Oogenesin-4</td>
<td>+++</td>
</tr>
<tr>
<td>mSox3</td>
<td>–</td>
</tr>
<tr>
<td>Obox 1, 2, 3, 6</td>
<td>–</td>
</tr>
<tr>
<td>Obox 4</td>
<td>–</td>
</tr>
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1Not distinguished between sperm and somatic testis.
2 Oogenesin 1 only.

–, not detected; N/D, not determined.
have a putative role in gonadal development revealed that the majority of regulatory proteins are not restricted to oocytes, but rather, are present elsewhere in the animal. Therefore, the majority of transcriptional mechanisms used in the oocyte will likely center on differences in the regulation of populations of transcription factors that overlap somatic cells of the gonad, embryo, or adult. Studies on gene regulation of 5S RNA, Oct-4, and ALF suggest that chromatin remodeling events such as histone acetylation and DNA methylation are at least part of the molecular mechanism that regulates transcription of these genes. It is likely that the interaction between the chromatin structure and transcription factors together determine the transcriptional activity of specific sets of genes during gametogenesis. The transcriptional regulatory machinery in specifying oocyte development may involve a unique combination of only a few master transcription factors, such as FIGz in regulating oocyte-specific ZP genes in the mouse, utilization of tissue-specific general transcription machinery, interactions with chromatin remodeling and modification complexes, and hormonal signals.

Concluding remarks and future perspectives

Oocytes go through many transitions in their life history. These begin with the specification of the PGCs in the embryo, the entry of the germ cells into the gonadal rudiment, the transition of oogonial stem cell to a primary oocyte, and their development into a mature, fertilizable egg. These developmental stages must entail a dynamic network of gene expressions. One model to explain these changes is that this single cell undergoes progressive transitions in transcription factor profiles and activities (Fig. 8). While currently we are in a phase of identifying what factors might be present, with dramatic new knowledge in genomics even in oocyte biology, we will quickly confront the problem of how the accumulated factors identified in oocytes interact to propel the cell forward in a developmental scheme. One would invoke a network of interacting trans-factors with the cis-regulatory elements of the promoters of select transcription factors to regulate the specific expression of these factors. Once a certain level of such a target factor has been achieved, feedback—either directly, or indirectly through additional transcription factors—would up- or down-regulate transcription of the first factor.

In addition, because of the prolonged periods required for oocyte development, homeostasis of transcription-stable periods would require feedback in the form of a network of positive and negative interactions. While some of these processes are shared with other cells, understanding the oocyte transcriptome would be of great utility to our understanding of germ cells specifically, or stem cells in general. Thus, for both changes in oocyte development and for homeostasis, feedback regulation in the transcriptional profile of oocytes is a necessary process that needs further attention.

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References


