CHAPTER 4

Gene expression in oocytes during growth and maturation

Ursula Eichenlaub-Ritter

INTRODUCTION: RELEVANCE OF EXPRESSION PATTERNS STAGE-BY-STAGE

Mammalian oocytes are exceptional cells. Unlike male germ cells they are extremely long-lived and initiate the meiotic program during fetal development in the embryonic ovary. Much unlike most other cell types, they arrest for long periods at the G2-phase of the cell cycle and thus undergo a discontinuous meiosis. Their development is associated with major starts and stops in meiotic progression as well as major changes in gene expression patterns during transitional phases of oogenesis, e.g. when oocytes complete the first stages of prophase of meiosis I[1], when oocytes become meiotically arrested, or when follicles and oocytes become recruited from the resting to the growing pool[2]. Before resumption of maturation oocytes undergo extensive growth, accompanied by high transcription rates. However, fully mature oocytes subsequently become transcriptionally repressed[3,4], whereas specific stored messages are recruited by polyadenylation to be translated during maturation or early embryogenesis[5,6].

With respect to cell cycle progression, S-phase, chromatin condensation, pairing, and recombination take place during meiotic prophase I of oogenesis within the fetal ovary, followed by a long meiotic arrest phase with decondensed chromatin in the G2-phase of the cell cycle (dictate or dictyate stage), in which the oocyte is surrounded by a single layer of squamous non-proliferating granulosa cells within a primordial follicle[7]. Oocytes have an intact nucleus (germinal vesicle, GV) throughout this arrest period and remain quiescent, up to decades in humans, before growth is initiated and the transition from the resting primordial to the primary stage of folliculogenesis is initiated. However, during the arrest oocytes may be more 'active' in expression than previously anticipated[8]. Primary follicle development is followed by development from the preantral to the large antral, preovulatory stage, and finally, oocyte meiotic resumption and ovulation.

To acquire full maturational and developmental competence the oocyte has to undergo an extensive growth phase. This is associated with an accumulation of components for nuclear maturation, dramatic increases in numbers of cell organelles like mitochondria, chromatin remodeling, and rises in protein and RNA content. Most mammalian oocytes mature to metaphase II, in response to activation of maturation promoting factor (MPF)[9]. Most protein is subsequently synthesized in the mammalian oocyte[10,11] as an

04-Human Oocytes-chapter04-ppp.i49 49 14/9/06 16:19:52

essential component of the cytostatic factor (CSF) that arrests oocytes with well aligned chromosomes in metaphase II (for reviews see references 12 and 13). This is much unlike the situation in mitotic cells, that trigger anaphase once chromosomes are stably attached to spindle fibers and aligned at the spindle equator (termed: congression) (e.g. reference 14). Meiosis is completed after fertilization and inactivation of maturation promoting factor (MPF) downstream from calcium oscillations[15–17]. Subsequently, ooplasmic factors mediate chromatin remodeling and male pronuclear formation. Global changes in methylation imprinting are initiated by oocyte derived factors (e.g. references 18 and 19 and Chapter 6). Importantly, maternal and paternal imprinting marks acquired during gametogenesis have to be established and oocytes conditioned such that specific marks maintained during maturation and early embryogenesis for mono-allelic gene expression (e.g. references 20 and 21 and Chapter 6).

The oocyte provides all proteins, especially transcription factors, mRNA, tRNA, and ribosomal RNA, as well as cell organelles like mitochondria to support the first mitotic divisions and early embryogenesis. It has to provide for initiation of zygotic gene activation until full zygotic gene expression is achieved. All the information obtained by genetic models and observations on correlations between oocyte quality and developmental competence in assisted reproduction thus support the notion that embryogenesis is governed by and begins during oogenesis[22,23] and is dependent on timed expression during oogenesis. The continued development and maturation of the oocyte within the ovarian follicle in vivo facilitates the production of oocytes of the highest developmental potential. Up to now, in-vitro conditions do not fully support the process of oocyte growth and maturation as effectively. This may relate to deficiencies in the extracellular milieu, or result from the suboptimal quality of the oocyte at the beginning of and during culture, especially in primates (e.g. reference 24).

TEMPORAL AND SUSTAINED EXPRESSION OF EARLY MEIOTIC GENES FOR GENOMIC INTEGRITY

The early stages of oocyte development in the fetal ovary, prior to birth, are characterized by the expression of genes that are necessary for chromosome pairing, homologous recombination, and DNA repair. This accounts for the requirement of meiotic exchange to establish physical attachments between homologous chromosomes and the formation of chiasmata. The latter are required for reductional division, to help chromosomes orient properly on the spindle and to segregate from each other at meiosis I (for discussion see references 25–27). For instance, genes expressed in recombination encode proteins of the synaptonemal complex and chromosome cohesion like SCP1, SCP 3, REC 8, and SMC1 beta[28–30], as well as genes involved in recombination and DNA repair such as Atm, Spo11, Dmc1, Msh2, Msh 3, Msh 4, Msh 5, and Mlh 1[31–37]. Reduced expression, mutation, or knockout of such genes can have long lasting effects and severe implications for fertility in the female, causing death of oocytes[38,39] and/or affecting the ability of the oocyte to faithfully segregate chromosomes at meiosis I and ovulate metaphase II oocytes with normal chromosomal constitution (for review see references 26 and 40).

The relevance of stage-specific regulation of gene expression by transcription factors and factors in initiation of translation at this early meiotic stage can be deduced from knockout models. For instance, the gene for CPEB (cytoplasmic polyadenylation element binding protein) is a sequence-specific RNA binding protein that is involved in translation initiation during vertebrate oogenesis. Ablation of CPEB expression causes arrest at the pachytene stage in female meiosis of the mouse. Synaptonemal complexes that mediate efficient pairing and recombination do not form, presumably because expression of mRNAs with cytoplasmic polyadenylating elements (CPE), binding sites for CPEB,

04-Human Oocytes-chapter04-ppp.i50 50 14/9/06 16:19:52

that encode synaptonemal complex proteins, is reduced[41]. However, translation of CPE-containing mRNA is also required at resumption of meiosis. Regulation at pachytene appears to rely on phosphorylation of CPEB by kinase Aurora A followed by dephosphorylation when oocytes enter dictyate stage[42]. Differential phosphorylation also modulates transcription of polyadenylated mRNAs at resumption of maturation and at early development (see below).

Genetic models demonstrate how a disturbed expression of meiotic genes at early stages of oogenesis like Mlh1 affects pool size and leads to premature ovarian failure, but also causes predisposition to non-disjunction (errors in chromosome segregation) in oocytes at early maternal ages[28,30,34,43]. Interestingly, ceasing of expression of the gene for synaptonemal complex protein 1 (SCP1) is implicated in formation of primordial follicles in the mouse[1]. Most genes in meiotic recombination are no longer expressed at the end of meiotic prophase I, when nuclear maturation becomes arrested in the G2-phase. In contrast, genes that apparently remain to be expressed at low levels up to meiotic resumption are ones that mediate chromatid cohesion, like Smc1β. Usually, sister chromatids stay attached to each other until anaphase I, when chiasmata are resolved synchronously by the loss of cohesion between arms of sister chromatids (reviewed in references 44 and 45). Reduced Smc1β expression may be one of the conditions responsible for premature loss of sister chromatid cohesion and chiasma resolution that predispose to random segregation of chromosomes and aneuploidy in aged oocytes[43]. Once chromatids lose cohesion, there may be no possibility of re-attachment. Although it has been claimed that the ooplasm of fully grown GV-stage or metaphase II oocytes is capable of expressing genes that induce reductional segregation of somatic chromosomes, the lack of coordinated expression of prophase I-specific recombination genes at postnatal stages of oogenesis and the absence of a physical connection between homologous parental chromosomes render it rather unlikely that the ooplasm is able to promote normal chromosome segregation. In fact, random segregation was observed in oocyte reconstitution approaches[46–48], although meiosis-like spindles may be formed after somatic cell nuclear transfer[49]. This appears to be different for oocytes derived by in-vitro development from ES cells in animal models, which exhibit stage-specific expression of meiotic markers and develop within a follicle-like somatic compartment[50]. It will be of great interest to assess their stage-specific gene expression patterns to identify essential pathways regulating early and late meiotic events and, particularly, chromatin remodeling and chromosome separation at oogenesis. This may also be useful in improving future in-vitro maturation (IVM) approaches.

GENE EXPRESSION AT RECRUITMENT OF PRIMORDIAL FOLLICLES AND THROUGHOUT FOLLICLE AND OOCYTE GROWTH

Oocyte survival at the dictyate stage initially depends on follicle assembly, and primordial follicle formation prior to birth as in humans[51,52] or shortly after birth as in rodents (e.g. references 53 and 54), depending on species (see reference 55). At this and later stages, oocyte and follicular health or atresia rely on complex interactions of oocytes with somatic cells, e.g. through interactions via growth and survival factors and later via gap junctional communication[56,57] (see Chapter 3). For instance, failure of expression of Foxl2 encoding a transcription factor in mice results in impaired follicle formation and deregulated oocyte growth[58,59]. Oocytes in primordial follicles express Gdf9 precociously to induce folliculogenesis shortly after birth and this causes atresia and premature ovarian failure. Early stages mainly depend on locally produced intercellular signaling and regulatory feedback signaling between the germ cell compartment and the oocyte (e.g. reference 60).

04-Human Oocytes-chapter04-ppp.i51 51 14/9/06 16:19:53

Later, additional feedback signaling with the neuroregulatory axis is essential for normal follicular development and differentiation, and for oocyte growth (see Chapter 2). As an example, female mice lacking FSHβ are infertile and follicular development is arrested at the preantral stage of folliculogenesis[61].

Multiple gene products act on the oocyte or are expressed by oocytes at several distinct stages of folliculogenesis and during oocyte growth, having distinct and diverse functions. For example, Factor in the Germaline alpha (FIG-α), a basic helix-loop-helix transcription factor, is initially required for primordial follicle formation, but also regulates expression of zona proteins during oocyte growth[62,63]. Other factors expressed in oocytes at primordial follicle formation include, for example, neurotrophins and their receptors, like the tyrosine kinase B receptor (TrkB receptor)[64,65].

Cohorts of primordial follicles are continuously recruited from the resting into the growing phase throughout reproductive life. This is accompanied by differentiation of flattened granulosa cells into large cuboidal granulosa cells of the primary follicle by still largely unknown factors. Anti-Müllerian hormone (AMH), a member of the TGF (β) family that signals through a bone morphogenetic protein (BMP)-like signaling pathway is expressed in the granulosa cells of growing and preovulatory follicles and appears to play a role in inhibiting primordial follicle recruitment. It is also expressed in oocytes of preantral and preovulatory oocytes and may enhance survival and growth of follicles at these later stages of oocyte and follicle development[66–69]. Expression of AMH and levels of AMH in serum are inversely proportional to follicle pool size and can possibly be used to assess physiologic age[70]. Thus AMH may present a marker of aging and thus may be predictive of the success of IVM.

The length of the oocyte growth phase differs between species from about 20 days in small rodents like the mouse to months in larger mammals (e.g. references 71 and 72). Oocyte growth and follicular development to the preovulatory stage lasts several months in the human. The volume of the oocyte increases 100-fold during this period and oocyte diameter expands from about 35 to 120 μm[73,74]. In the mouse the diameter of the oocyte increases from about 10–15 μm in the primordial follicle to about 80 μm in the preovulatory antral follicle[75]. Accordingly, oocyte volume also increases about 150-fold between primordial and large antral stage[2]. Most proteins and RNAs are produced during the oocyte's growth phase, when rapid divisions of granulosa cells commence and the oocyte significantly increases in volume. RNA content rises by about 300% during the 2–3 weeks of oocyte growth in the mouse. The meiotically competent, fully grown mouse oocyte contains about 200 times more RNA than a typical somatic cell[76]. About 20–25% of total RNA accounts for tRNA. About 60–65% of the total RNA comprises ribosomal RNA, and 10–15% represents heterogeneous RNA. About 8% of the latter is polyadenylated. While RNA synthesis is high at the early and middle growth phase, it is already reduced during the late growth period of the oocyte. Ribosomal RNA synthesis reaches highest levels at the beginning of follicular antrum formation.

Unlike in other vertebrate species (e.g. *Xenopus*) there is no amplification of ribosomal DNA for efficient rRNA synthesis in mammalian oogenesis[77]. However, there is a steady and rapid increase in RNA due to active transcription with peak values of 0.175 pg/h and a rise in protein content due to translation of mainly polyadenylated mRNA. Over 90% of the egg's ribosomes are already present in the mouse oocyte at 14 days of oocyte growth, although the oocyte has by then only reached 60–70% of its final size[78]. The nucleolus enlarges and nuclear proteins like fibrillarin, nucleoplasmin, nucleolin, and RNA polymerase I as well as nucleolar upstream binding factor are constantly synthesized. In fact, gene expression at the transition from the primordial to primary stage particularly

04-Human Oocytes-chapter04-ppp.i52 52 14/9/06 16:19:53

involves genes in protein synthesis, ribosome biosynthesis and assembly, and translation to support oocyte growth[2]. Interestingly, Pan and co-workers also detected a significant increase in expression of mRNA of genes coding for proteins in DNA repair and chromatin conformation in mouse oocytes at this stage of folliculogenesis[2]. The latter may protect from mutation but may also preset the pattern of expression at later stages. For instance, expression of DNA damage factors like murine HR6A from maternal stores may be essential for development since oocytes of mHR6A–/– knockout mice do not develop beyond the embryonic two-cell stage[79].

There are two major transitions in the follicular and oocyte growth phase that correspond to steps in acquisition of maturational and developmental competence in oocytes, respectively, in association with characteristic changes in expression. Oocytes initially gain the competence to resume nuclear maturation although they may not be capable to support development after fertilization. At a later stage, full developmental competence is acquired. In the mouse, maturational competence is reached by the secondary to small antral follicle stage. Only afterwards do oocytes become fully competent for supporting embryogenesis to the blastocyst stage, corresponding to the transition from the small antral to the large antral stage of folliculogenesis in the mouse[80–82]. Analysis of genes that are up- or downregulated at these transition stages should help to identify critical factors in oocytes that support full cytoplasmic and developmental competence[2].

Acquisition of full meiotic competence coincides with a markedly decreased rRNA transcriptional activity. Fully mature oocytes of several species examined so far become transcriptionally quiescent when they reach full nuclear and developmental competence[3,4]. This is associated with characteristic alterations in nuclear and nucleolar morphology[83,84]. In pig oocytes it was shown that pocket protein, p130, is involved in the downregulation of rRNA transcription at the end of the oocyte growth phase through an inhibition of the activity of upstream binding factor (UBF). The latter protein is necessary for the function of RNA polymerase I (RNA Pol I), which is the actual enzyme driving rRNA gene transcription[85]. Ribosomal RNA genes are re-activated only at zygotic gene activation. Messenger RNA synthesis inevitably ceases in oocytes when they resume maturation and undergo germinal vesicle breakdown (GVBD).

At the end of the growth period, the oocyte is packed with ribosomes, mitochondria, protein, tRNA, and mRNA, which are maternally provided for the resumption of meiotic division, for fertilization, and for the rapid divisions and early stages of development of the preimplantation embryo. All of this requires substantial gene expression in a temporally and quantitatively controlled fashion at the transcriptional and translational level. Identification of key factors expressed in oocytes to initiate and promote oocyte and follicle growth and oocyte maturation to provide a high quality egg supporting embryonic development has been greatly facilitated in recent years by the analysis of transgenic mouse models[86], characterization of expression libraries, and analysis of differential expression patterns of oocyte-specific genes throughout oogenesis and at transition from early to advanced stages of folliculogenesis in several species, e.g. by transcript profiling using microarray analysis, in silico analysis, etc.[2,8,87–97].

Growth and survival factors and signaling pathways

Many locally produced factors promote the primordial to primary follicle transition, for instance, growth factors such as kit ligand (KL), leukemia inhibitory factor (LIF), bone morphogenetic proteins (BMPs), keratinocyte growth factor (KGF), and basic fibroblast growth factor (bFGF) (for reference see Picton and Harris, Chapter 2). Primary oocytes in primordial follicles and granulosa cells express the ligand for

04-Human Oocytes-chapter04-ppp.i53 53 14/9/06 16:19:53

the c-kit gene for a tyrosine kinase receptor, kit ligand (KL), while c-kit mRNA concentration increases during oocyte growth in the mouse[98]. FSH-stimulated oocyte growth is inhibited by Gleevec, an inhibitor of kit activity, suggesting that the correct concentration of FSH is crucial for appropriate modulation of expression of KL and also of BMP15 to support oocyte growth[99]. Autocrine and paracrine signaling by c-kit/KL may be important for oocyte survival and transition from primordial to primary stage, as is leukemia inhibitory factor[100]. Basic fibroblast growth factor increases KL expression, and together they are believed to promote primary follicle formation[101]. Keratinocyte growth factor appears to be a mesenchymal factor, produced by progenitor theca cells, that promotes the primordial to primary follicle transitions with unknown downstream targets for altered gene expression in oocytes[102].

Several recent studies addressed the characteristic changes in expression patterns during primordial follicle assembly and in oocytes progressing from primordial to primary to preantral and large antral stages in rodents using microarray analysis, analyzing expression in either follicles or isolated oocytes (e.g. references 2 and 102). Several mRNAs for steroidal enzymes as well as inhibin, Müllerian inhibiting substance (MIS, another term for AMH), and all three zona genes appear unregulated at primordial follicle formation in the rat ovary[102]. As expected, genes encoding proteins of the synaptonemal complex are downregulated in the oocytes. About 15% of all the genes upregulated at the transcriptional level at the primordial to primary stage of follicle formation comprise metabolic enzymes. Endocrine factors inhibin and AMH appear downregulated at the transition from primordial to primary stage, in contrast to their increased expression at primordial follicle formation. In the experimental approach by Pan et al. using mRNA from mouse oocytes, one third of all genes identified by gene chip analysis to be expressed in oocytes of primordial follicles changed 2-fold in expression at the transition to the primary stage[2]. About every second gene appeared to be either up- or downregulated at this stage, as may be expected by initiation of oocyte growth. At the protein level, the dramatic increase in growth is associated with transitions in transcription accompanied by major increases in RNA polymerase activity at the progression from primordial to growing oocytes[103]. The final acquisition of competence to undergo nuclear maturation and to support embryonic development is accompanied by global, characteristic changes in protein synthesis, first shown in the mouse[75].

Apart from supporting development after fertilization, the oocyte is by itself the major driving force in the development, organization, and function of the somatic cells of ovarian follicles[104–106]. Growth differentiation factor 9 (GDF-9), one of the members of the TGF-β growth factor family, is a key regulatory oocyte synthesized and secreted molecule that contributes to oocyte mediated differentiation of granulosa cells and differential expression patterns in the somatic compartment of the follicle. For instance, it is required for the survival and growth of follicle cells, suppresses apoptosis, and is involved in cumulus expansion[107–109] (see Chapter 3). Follicular development from the primary stage of growth is accordingly disturbed in homozygous mutations for GDF-9 in sheep, in specific GDF-9 RNAi-microinjected hamster oocytes, and in GDF-9–/– knockout mice[108,110]. Both GDF-9 and BMP-15 proteins are secreted proteins in follicular fluid. Type II and type I activin receptor-like kinase (ALK) receptors in granulosa cells like ALK-6 signaling appear to be downstream from the receptor-mediated BMP signaling since the ability of BMPs to inhibit differentiation of follicular cells was decreased in the presence of ALK-6 mutations. Messenger RNA for ALK-6 together with related genes for cell-surface receptors such as ALK-5 and BMP-RII mRNA is also present in oocytes at most, if not all, stages of follicular growth. In sheep carrying the ALK-6 mutation,

04-Human Oocytes-chapter04-ppp.i54 54 14/9/06 16:19:54

ovarian follicles undergo precocious maturation. Three to seven follicles are ovulated, mostly with a smaller diameter. BMP-15 and GDF-9 mutations in sheep are thought to result in reduced levels of mature protein or altered binding to cell-surface receptors. Sheep heterozygous for mutations in BMP-15 or GDF-9 or homozygous for an ALK-6 mutation have higher ovulation rates (up to 10 times higher) than wild-type animals. In sheep, GDF-9 mRNA is present in oocytes before and after follicle formation as well as throughout follicular growth, whereas BMP-15 appears expressed only from the primary stage of growth. In mouse, both orthologs, GDF-9 and BMP-15, as well as other members of the TGF-β superfamily, BMP-5 and BMP-6, are highly expressed at the primordial and primary follicle stage.

Expression is reduced at the mRNA levels from the small antral stage[2]. Although expression patterns of BMPs may differ slightly between species, possibly related to mono- or multi-ovular species, the necessity of BMP-15 for folliculogenesis in humans has been recently supported by the discovery of a BMP-15 mutation that is associated with ovarian dysgenesis (for review see reference 111). The list of oocyte-controlled signaling pathways during follicular development includes also Sonic hedgehog (Shh) signaling, signaling through epidermal growth factor (EGF), and transforming growth factor alpha (TGF-α) as well as their respective ligands that are highly expressed at primordial to primary follicle stage[2,97]. For instance, Deltex2 coding for a cytoplasmic SH3-ring finger gene that mediates Notch receptor signaling is expressed in oocytes (see reference 97). Components of Notch pathway were also identified in cDNA clones from human primordial follicles[8]. Interestingly, there was an abundance of retroviral elements and transcriptional repressor in the cDNA library from human primordial follicles, suggesting that they could contribute to the maintenance of this stage and to meiotic arrest.

With respect to essential oocyte–granulosa cell signaling, expression of connexins that form gap junctions between the oocyte and granulosa cells is necessary to allow for development of the follicle beyond the secondary follicle stage[112] (see also Chapter 3). The family of 2′,5′oligoadenylate synthasc 1 (OAS1) proteins was originally recognized by its involvement in the control of the interferon/OAS/RNase L pathway of degradation of viral double stranded RNAs (dsRNAs) in host defense against viral infection. OAS1D has recently been identified in the cytoplasm of growing and maturing oocytes and may protect from loss of oocytes in response to viral infection. OAS1D–/– mice are subfertile, revealing the significance of expression of this gene in mammalian oogenesis[113]. In conclusion, it is far beyond the scope of this review to list all regulatory components that are expressed in growing oocytes and their signaling pathways. The significance of expression of the plethora of known and novel oocyte and germ cell specific genes encoding growth and survival factors and their ligands that appear up- or downregulated at oocyte growth is awaiting further analysis.

Transcription factors in oocyte growth and developmental competence

The homeobox gene Nobox (*n*ewborn *o*vary homeo*box*-encoding gene), first identified in a cDNA library of the mouse ovary[114], is expressed in mouse oocytes from primordial follicles through antral stages and appears essential for progression from primordial to primary stage of oogenesis[115], possibly because downstream expression of genes like mos, oct-4, rfl4, fgf8, zar1, dnmtlo, gdf9, bmp15, and H1oo is regulated. The gene for oocyte-specific mammalian H1 linker histone, H1FOO, is exclusively expressed during oocyte growth and maturation. The message is alternatively spliced, and protein synthesis coincides with recruitment of resting primordial follicles into a developing primary follicular cohort. Association with perinucleolar heterochromatin suggests a role in restructuring chromatin at the transition to oocyte growth[115].

04-Human Oocytes-chapter04-ppp.i55 55 14/9/06 16:19:54

The transcription factor Oct-4 that is involved in maintaining pluripotency in stem cells and the embryo may have two additional roles in oogenesis: in the recruitment of oocytes for initiating growth and in the selection of oocytes for ovulation[116]. Messenger RNA for Oct-4 is transcribed during oocyte growth but is not present in oocytes at the primordial follicle stage. Oogenesin-1 is a recently recognized oocyte-specific transcription factor that is expressed in mouse oocytes from primordial to preovulatory follicles, while related family members of the transcription factor oogenesin 2–4 appear first expressed in primary but not in primordial follicles[117]. Thus, expression of these factors may contribute to the transition in stage-specific gene expression in oocytes. The functional significance of oocyte-specific homeobox transcription factors like obox-1, 2, 3, and 6, whose mRNAs were detected in mouse oocytes, is still unclear – they may contribute to transcriptional activation at initiation of and during oocyte growth (for discussion see reference 118). The obox-5 gene that may be central to initiation of oocyte growth, is markedly increased at primordial to primary follicle transition as well as the transcription factors Mrg1, Tcfap2e, and Gli3[2]. The latter may regulate transcription of target genes like the BMPs[119], coinciding with the increase in their expression in the growing oocyte. Mrg1 may stimulate expression of multiple other transcription factors[2]. Zfp37 codes for a KRAB-box zinc finger protein that was inferred to be expressed preferentially in growing oocytes[97]. Expression of Sox8 transcription factor was found only in the oocytes of preantral follicles and in the oocytes, cumulus cells, and mural granulosa cells of preovulatory follicles. Initially Sox8 was believed to affect expression of AMH, but this could be disproved experimentally, so that its oocyte-specific function is still unclear[67].

Major increases in expression of some genes at early stages of mouse oocyte growth are followed by substantial reductions in mRNA levels in oocytes when folliculogenesis progresses to the antral stage. Such patterns of expression are characteristic for Polr2h and j, Tafl1, Cebpb, and Cebpd genes. Downregulation at antral stages is also characteristic for genes like Sp4, Crebbp, and Tcfl2, which are implicated in reduced activity of RNA polymerase II. Sp4 mRNA is reduced by 33%, and Tcf12 decreases by 12.5% at small to large antral follicle stage in the mouse oocyte[2] concomitantly with cessation of oocyte growth and acquisition of maturational competence.

Many transcripts have to remain dormant until their products are spatially and temporally required in development. Clast-4 is a homolog of the Drosophila Cup protein, a translational repressor during female germ line development in the fruit fly. Clast-4 mRNA and protein are highly expressed within the cytoplasm of growing oocytes. The mouse Clast-4 protein is stable during this developmental window and post-translationally modified by phosphorylation upon oocyte meiotic maturation[120]. This might release translational repression in order to synthesize maturation proteins that are required for oocyte and embryo development (see below).

Oocyte growth, and chromatin structure and remodeling in control of expression

Histone mRNAs are the only metazoan mRNAs that are not polyadenylated. Instead, they end with a conserved stem-loop structure, which is recognized by the stem-loop binding protein (SLBP)[121]. Histone synthesis in immature and maturing mammalian oocytes appears governed by a translational control mechanism that is directly regulated by changes in the amount of SLBP. SLBP binds to the highly conserved sequence in the 3′-untranslated region of the non-polyadenylated histone mRNAs. Unlike in somatic cells where SLBP is expressed during S-phase, SLBP is expressed in growing oocytes of the mouse at the G2-phase of the cell cycle and further accumulates substantially during meiotic maturation. Ablation of expression of SLBP

04-Human Oocytes-chapter04-ppp.i56 56 14/9/06 16:19:55

causes a significant decrease in pronuclear size and in the amount of acetylated histone detectable on the chromatin of fertilized zygotes of SLBP-depleted mouse oocytes. This supports the concept of a tight link between oocyte quality and developmental potential. The competence to control chromatin constitution during oocyte growth impinges on expression patterns at early embryogenesis[122].

DNA methylation (for review see reference 18 and Chapter 6), histone post-translation modifications, e.g. acetylation[123], and changes in chromatin organization and nuclear structure[124,125] are all involved in global and specific changes in gene expression and represent epigenetic mechanisms in control of gene expression. Chromatin structure and imprinting involving methylation at GpG islands at promoters of developmentally expressed genes is intimately involved in programming expression, and this is particularly important during oocyte growth and after fertilization, e.g. when oocytes are matured in vitro (e.g. references 21 and 126 to 128, and Chapter 6). This review will not focus in detail on such regulatory mechanism in gene expression in oocytes but it is of interest that expression profiles of oocytes developing in vitro to metaphase II either from mouse oocytes obtained from primordial follicles or from the secondary follicle stage are quite similar. However, some genes differ in expression profile, possibly reflecting the lower developmental competence when culture was from the primordial follicle stage. Of note, these may be relevant for epigenetic modulation of gene expression. It is remarkable that Ctcf, coding for a protein that blocks methylation of specific DNA sequences[129], as well as Dnmt-3a, coding for an active de novo DNA methyltransferase required for development[130], appear upregulated in the metaphase II mouse oocytes that have a reduced developmental potential[2]. Increased repression by methylated DNA due to increased expression of these genes has therefore been discussed in respect to critical changes in the growing oocytes that could contribute to reduced size characteristics for in-vitro grown and matured oocytes[131,132]. It could also critically affect expression patterns at embryogenesis. Alterations in expression of genes in chromatin conformation and imprinting, e.g. in association with IVM or adverse exposures, might therefore be of high relevance for developmental potential as well as for genetic disease in the offspring (discussed in Chapter 6).

Screening ovarian libraries by in-silico subtraction approaches as well as by differential display identified new oocyte-specific genes that are expressed in a temporally controlled fashion. Interestingly, there is increasing evidence that some of the oocyte-specific genes are either up- or downregulated in a coordinate fashion[2] as they are transcribed from specific chromosome domains in clusters[133]. For instance, the clusters on mouse chromosome 9 comprise the oogenesin genes, those on chromosome 7 the Obox genes, and another cluster on chromosome 7 the Nalp family of genes, related to Mater, a maternal effect gene that is essential for embryogenesis. Tcl1 genes are in a cluster on mouse chromosome 9, shown to have human orthologs expressed in oocytes. The F-box genes (FBO12) on mouse chromosome 9 belong to a family of proteins that serve as link between target proteins and ubiquitin-ligases such as early mitotic inhibitor 1 (Emi-1), an inhibitor of anaphase promoting complex that prevents MPF inactivation (see below)[134]. Other genes may code for proteins that interact with the cytoskeleton (e.g. actin/dynein) and other proteins that may help to reorganize the chromatin[133]. Finally, some genes on mouse chromosome 12 encode known oocyte-specific genes (e.g. *oo*cyte *s*ecreted *p*rotein 1, Oosp-1) with zona protein-like domains that may be involved in zona formation. It has been suggested that the clustering of expressed genes reflects coordinated gene regulation in oocytes by large-scale genome organization and oocyte-specific regulation of chromatin constitution[2,133]. Pan et al. suggested that local differences in histone modifications are involved[2].

04-Human Oocytes-chapter04-ppp.i57 57 14/9/06 16:19:55

Spatial organization of telomeres and chromatin domains within the nucleus during oogenesis may also play a role[133]. This suggests that spatial-temporal changes in the cytoskeleton, nucleus, and cellular organization may affect efficiency and control of expression as well as the quality of the oocyte.

A major change in chromatin/DNA configuration in the GV is characteristic for the acquisition of meiotic competence[135]. While DNA is diffusely distributed within the nucleus of meiotically incompetent oocytes, termed non-surrounded nucleolus (NSN), condensation of DNA in a rim-like fashion is characteristic for fully mature oocytes (termed surrounded nucleolus, NS)[81,136] that are transcriptionally suppressed[31,83]. In NSN oocytes ribosomal transcripts are accumulated within the nuclear matrix with nucleolus organizing regions (NORs) within the nucleolus. In contrast, NORs are in the nucleolar periphery in the mature SN oocytes[136]. The expression of histone deacetylase 4 (HDAC4) mRNA appears low in growing oocytes, but to be characteristic for fully grown oocytes. The HDAC4 protein has been implicated in transcriptional repression but is also associated with the chromosomes at resumption of maturation. Message decreases sharply after fertilization[137]. Currently it is unclear in which way chromatin reorganization contributes to transcriptional silencing in mature GV stage oocytes because mouse oocytes deficient in nucleoplasmin 2 (Npm2) do not develop to the SN stage but are still transcriptionally repressed. Also, chromatin hyperacetylation due to Trichostatin A (a deacetylase inhibitor) exposure does not release from the transcriptionally repressed state, although it induces chromatin decondensation[4]. In contrast, core histone deacetylation is associated with the modification of chromatin and termination of RNA synthesis from blastomere nuclei when they are exposed to cytoplasm of mature, fully grown oocytes[138]. ATRX, a member of the SNF2 family of helicase/ATPases, that is associated with chromosomes, may also critically affect chromatin constitution. Expression appears to be required for chromosome alignment and meiotic spindle organization in metaphase II stage mouse oocytes[4]. The regulatory mechanisms for transcriptional silencing and spatio-temporal regulation of chromatin conformation have still to be uncovered and the functional significance of the silencing is still an enigma in oogenesis research.

There may be also a link between gene expression in oocytes, the presence of maternal mRNA and protein at early embryogenesis, and changes in chromatin organization that facilitate zygotic gene expression and nuclear reprogramming. Mouse nucleoplasmin (Npm2) protein accumulates in oocyte nuclei and persists in preimplantation embryos. Npm2–/– knockout females have fertility defects owing to failed preimplantation embryo development[139]. Experimental data showed that pretreatment of mouse somatic nuclei with Npm2 facilitated activation of oocyte-specific genes from somatic cell nuclei injected into *Xenopus laevis* oocytes[140]. Zygote arrest 1 (Zar1 and ZAR1 in mouse and human, respectively), the first identified oocyte-specific maternal-effect gene, is also transcribed during oocyte growth. Maternal and paternal genomes remain separate in Zar1–/– mutants and there is a marked reduction in the synthesis of the transcription-requiring complex Zar1, suggesting that the protein is required for zygotic gene activation[141]. The role of MATER (maternal antigen that embryos require) and other members of the NACHT NTP family of proteins that are expressed during oocyte growth and required for early embryogenesis[142,143] is still under investigation. MATER protein was detected in mitochondria and nucleoli, suggesting that it may participate in both cytoplasmic and nuclear events during early development[143].

Notably, among approximately 11 000 genes whose transcripts were detected in mouse oocytes by microarray, about 5% showed statistically significant expression changes with advanced maternal age, including members of the NALP gene family and genes involved in

04-Human Oocytes-chapter04-ppp.i58 58 14/9/06 16:19:55

chromatin structure, DNA methylation, genome stability, and RNA helicases[144]. It remains to be determined whether optimizing oocyte growth and maturation in vivo or in vitro may reduce or prevent age-associated changes in expression of such genes, in particular, when they are involved in nuclear remodeling and zygotic gene activation at embryogenesis in order to obtain healthy oocytes with high developmental potential. Polycomb group (Pc-G) gene products that are expressed in growing and mature oocytes are essential to maintain stable repression of homeotic (HOM C) genes during development. Messenger RNA of members of the Pc-G like Yin Yang 1 (YY1), Enhancer of Zeste (EZH2), and Early Ectoderm Development (EED) are involved in X-chromosome inactivation[145]. Their deregulation in ART or, possibly, in IVM could contribute to disturbances in epigenetic regulation with adverse effects on embryo health (see reference 145 and Chapter 6).

DIFFERENTIAL EXPRESSION OF GENES REQUIRED FOR OOCYTE MATURATION AND EARLY EMBRYOGENESIS

The fate of mRNAs can differ depending on the need to provide proteins for metabolic activities associated with growth and acquisition of meiotic competence of the oocyte, or processes essential for meiotic progression or early embryogenesis[146]. For instance, housekeeping genes like β-actin are actively transcribed and translated during murine oocyte growth, accounting for 1% of the total protein synthesized. The store of mRNA rises to 2×10^4 molecules, then decreases by 50% at metaphase II and becomes further reduced to 10% of the initial amount by the two-cell stage. The zona proteins ZP-1, ZP-2, and ZP-3 are coordinately expressed, predominantly during oocyte growth under the control of Fig-α transcription factor[62,147]. At mid growth phase they represent a large fraction, 1.5% of the total polyadenylated RNA. At ovulation mRNA is substantially lower (5% of peak values) and is undetectable in the embryo. Other mRNAs like that encoding lactate dehydrogenase (LDH-β) are more stable in mature oocytes and embryos. The synthesis of LDH protein reaches high levels, comprising up to 2–5% of the totally synthesized protein or 200–400 pg in murine oocytes[148]. However, it only recently became obvious that the relative rate of expression of genes, for instance as in metabolism, is influenced by oocyte–granulosa cell cooperativity, which may have a major impact on egg quality (for discussion, see reference 106 and Chapter 2).

Regulation of translation by polyadenylation

Generally, most mRNAs are immediately polyadenylated within the nucleus and then transcribed in the cytoplasm of the growing oocyte (Figure 4.1). Abundant mRNAs for typical housekeeping genes with long poly(A) tails actively transcribed during oocyte growth include actin and globin mRNAs. However, others are temporarily stored and expressed at distinct times during maturation or in early development in a temporally coordinated fashion. Stored mRNAs especially abundant in fully grown mouse oocytes are, for instance, those encoding c-kit, zona protein ZP-3, lactate dehydrogenase, and the product of the c-mos proto-oncogene[76,149]. Control of expression was first extensively studied for genes like tissue plasminogen activator, tPA, and hypoxanthine phosphoribosyltransferase (HPRT) in the mouse[148], and mRNA for the spindle associated protein spindlin[150], and notably, cell cycle regulatory components like cyclins A1 and B2[151–153], Mos[154,155], and Cdc25 [156] in several species. Maturation dependent polyadenylation associated with translational initiation and expression was earlier also described for mRNAs of antioxidant enzymes, glutathione peroxidase (GPX), and Mn-superoxide dismutase (Mn-SOD)[157].

04-Human Oocytes-chapter04-ppp.i59 59 14/9/06 16:19:56

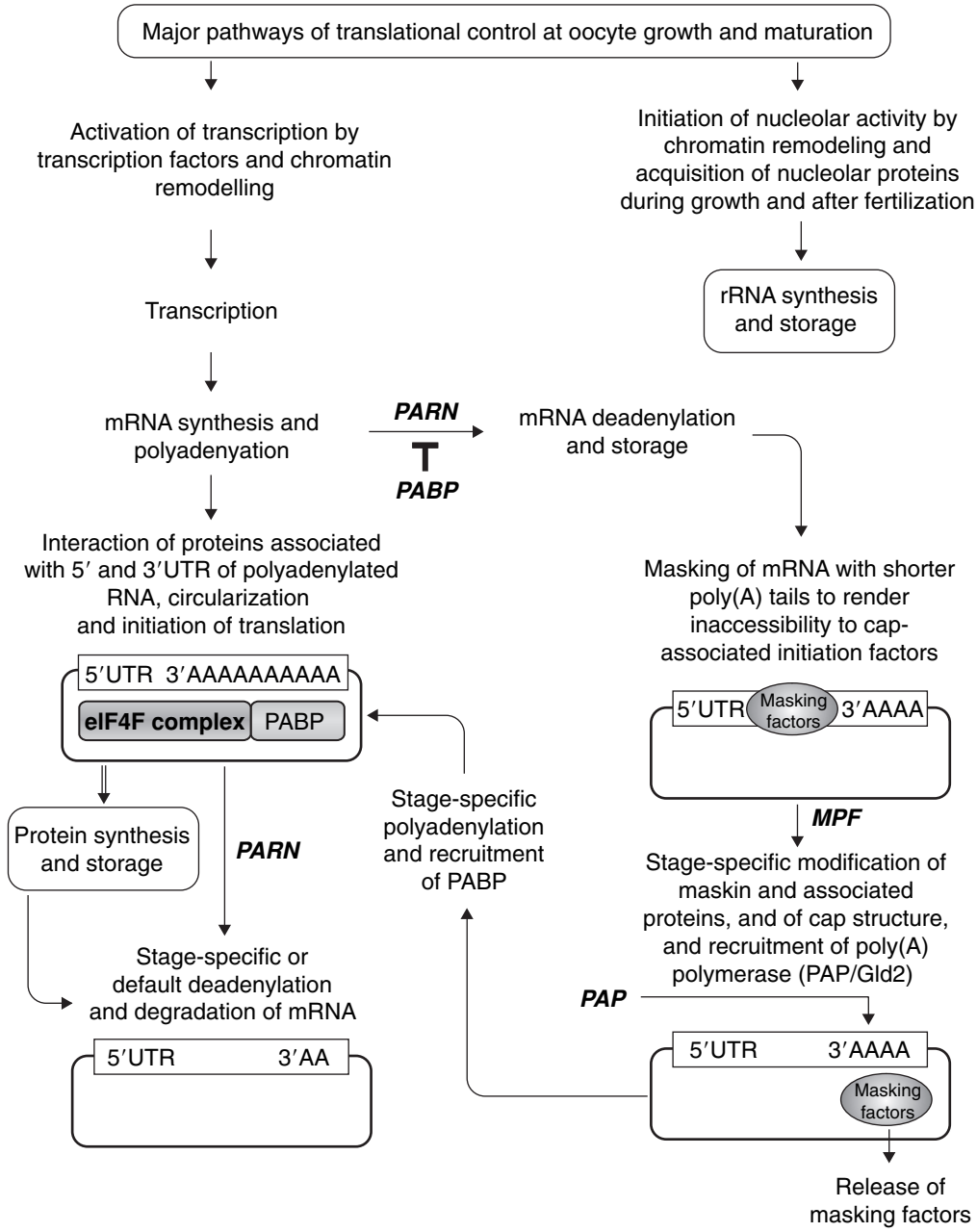


Figure 4.1 Overview of storage, recruitment, and translation initiation of mRNAs in growing and maturing oocytes. PAP: poly(A) polymerase; PARN: poly(A) RNase; PABP: cytoplasmic poly(A) binding protein; MPF: maturation promoting factor. For further explanation see text. (Modified from reference 179)

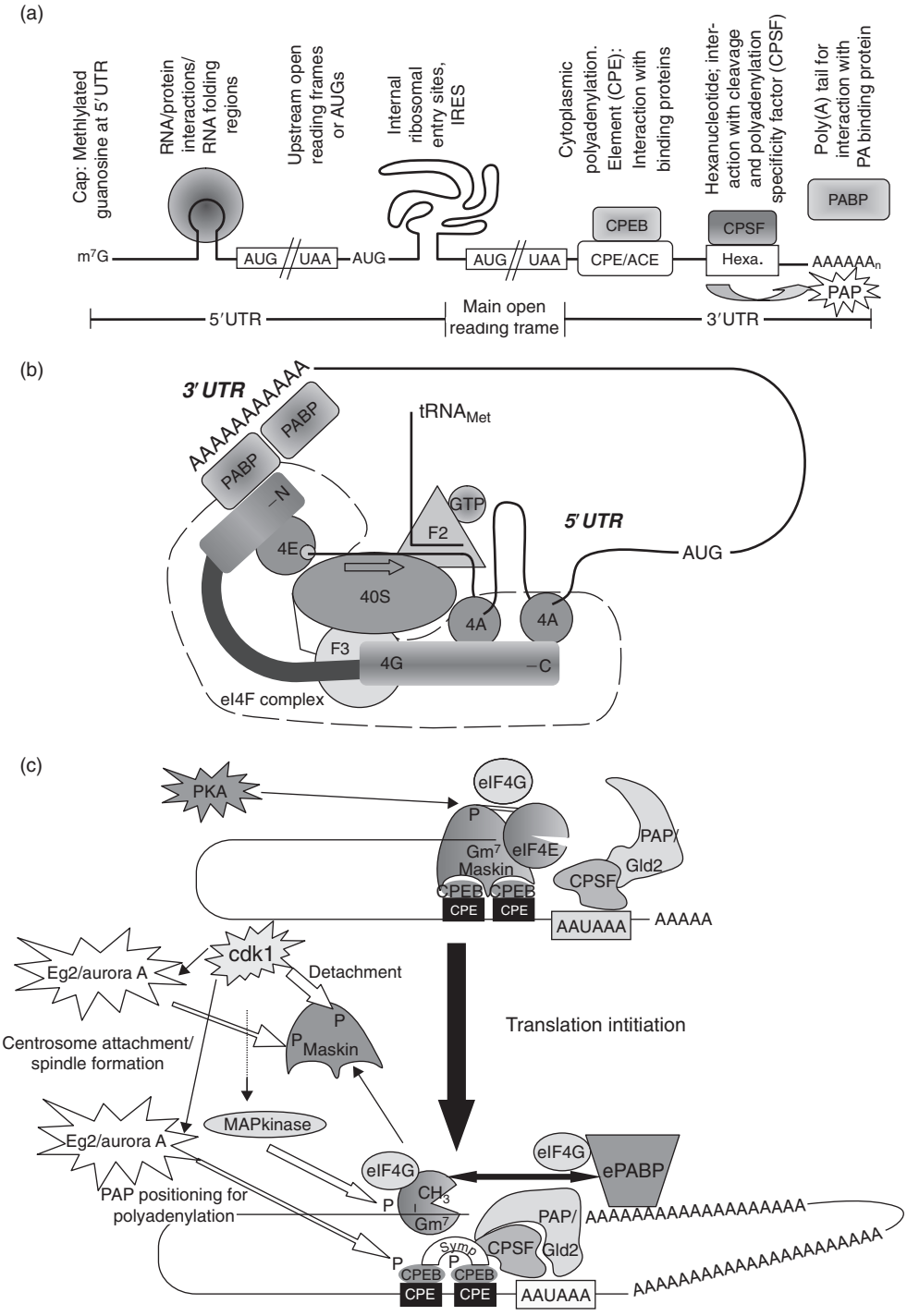
04-Human Oocytes-chapter04-ppp.i60 60 14/9/06 16:19:56

Prior to maturation, repression of a large number of these mRNA species is controlled by deadenylation of the 3′ end of the untranslated region (3′UTR) of the mRNA, usually to about 20 nucleotides[146]. Messages with a short poly(A) tail are much less susceptible to degradation[150]. For translation, polyadenylation by poly(A) polymerases (PAPs) is required (see Figure 4.1). The core factors that control adenylation and translational initiation in vertebrate oocytes and embryos include key components like the cytoplasmic polyadenylation element binding protein (CPEB), an RNA binding protein with recognition motif and zinc finger, associated with the cytoplasmic polyadenylation element on the mRNA (CPE) that specifies which mRNAs undergo polyadenylation[158,159] (Figure 4.2a). Furthermore, cleavage and polyadenylation specificity factor (CPSF) is a multi-factor complex that interacts with a conserved, ubiquitous hexanucleotide AAUAAA of the 3′UTR of the mRNA[160] (Figure 4.2a). In addition, symplectin, believed to be a scaffolding protein that anchors the CPEB and CPSF proteins, may help to position the poly(A) polymerase to prolong the poly(A) tail[161,162] (Figure 4.2c). In contrast, Maskin, a CPEB and initiation factor (eIF4E) binding protein, prevents polyadenylation[163,164] and inhibits the association of the mRNA with ribosomes (Figures 4.1, 4.2b, and 4.2c). The masking inhibits not only polyadenylation but also the recruitment of the elF4F elongation initiation complex to the 3′UTR of the mRNA by preventing ring formation of the mRNA required for interactions between the 3′ and 5′ UTR of the mRNA. Ring formation for initiation of translation is achieved by association of the elongation initiation complex at the 5′UTR cap structure with the poly(A) binding protein (PABP) that is itself associated with the 3′UTR of polyadenylated mRNAs (Figure 4.1). This requires a long poly(A) tail for PABPs to interact with the mRNA (about 100–150 nucleotides)[165]. Therefore, it is mainly the regions at the 3′ untranslated end of the mRNA (Figure 4.2a) and the presence/release of maskin or related proteins allowing or preventing polyadenylation (Figure 4.2c) that direct storage, masking, and recruitment of mRNAs[6,161,166,167].

Deadenylation is catalyzed by a poly(A)-specific ribonuclease (PARN; Figure 4.1). When oocytes re-enter the meiotic cycle, removal of masking factors occurs downstream from inactivation of protein kinase A[164] and activation of MPF[166,167] (Figure 4.2c), by displacement of masking factors and recruitment and activation of poly(A) polymerases (PAP or Gld2) by CPEB/CPSF. Stage-specific post-translational modifications of proteins like maskin and CPEB (Figure 4.2c) then contribute together with the poly(A) binding protein (PABP) to recruit the eIF4F initiation complex to the 5′UTR and cap structure of the mRNA and to initiate translation (Figures 4.1 and 4.2b).

At resumption of meiosis and at fertilization there are major switches for control of polyadenylation of mRNAs that greatly affect translational initiation and/or mRNA stability[168]. The timing of adenylation/deadenylation depends on the specific cis sequences in the 3′UTP of the message and their relative distance on the mRNA for deadenylation and polyadenylation at the appropriate time (Figure 4.2a) (e.g. reference 155). During oocyte maturation it is predominantly the cytoplasmic polyadenylation elements on the 3′UTP (CPEs or adenylation control element, ACE), and the canonical AAUAAA hexamer of the premRNA in the 3′UTR that associate with proteins like CPEB, and CPSF, respectively (Figure 4.2a). They direct polyadenylation by influencing activity of RNA PAP (or rather Gld2 PAP in oocytes), or poly(A) deadenylases (PARN), respectively (Figure 4.2c). Screening by microarray analysis showed that in *Xenopus* oocytes more than 500 mRNAs from 3000 in an array were regulated at the post-transcriptional level during oocyte maturation and early embryogenesis[169].

04-Human Oocytes-chapter04-ppp.i61 61 14/9/06 16:19:57



04-Human Oocytes-chapter04-ppp.i62 62 14/9/06 16:19:59

Translational inactivation of mRNA

Messenger RNAs in growing mouse oocytes that contain a short poly(A) tail (<90 residues) are much more stable compared to those with longer poly(A) tails. Those with about 150 residues are immediately translated[150]. Messenger RNA can be stored in the mouse oocyte for long periods during the growth phase with half-life times of approximately 28 days[170]. In *Xenopus* some messages without poly(A) tails are stable up to the mid-blastula transition[171,172]. Microinjection of RNAs containing long poly(A) tails (100–200 As) into mouse oocytes has shown that PARN reduces the length of the poly(A) tail of injected, foreign cytoplasmic mRNAs efficiently to about 20 to 50 adenosines at the 3′UTR of the mRNA during oocyte growth. A message with a short poly(A) tail is not only more stable, but initiation of translation is repressed by the deadenylation[173], as shown above. Thus, poly(A) tail removal is the initial and rate-limiting step in mRNA turnover that controls storage as well as decay. Deadenylation is the main mechanism responsible for translational silencing of maternal mRNAs during oocyte maturation and early development to cause translational repression or save them for their timed recruitment at a specific stage of maturation or development, depending on the 3′UTRs[172]. Most mRNAs, especially those of housekeeping genes like actin, appear deadenylated at GVBD as shown in the mouse[174], and this requires the activity of poly(A)-specific ribonuclease (PARN, or deadenylating nuclease, DAN)[175]. PARN is a member of the RNaseD family of RNA deadenylating nucleases. Interestingly, disturbances in default deadenylation and enhanced, untimely expression appear characteristic for bovine oocytes with low developmental potential[176]. Thus it

Figure 4.2 Messenger RNA sequences and RNA-binding proteins in regulation of circularization and initiation of translation of mRNAs during oocyte maturation and early development (modified from references 197, 224, and 246). (a): Modulation of expression of mRNAs by elements in RNA and binding of conserved proteins. CPEB and CPSF influence targeting of PAP to elongate the poly(A) tail for binding of PAPB. (b) Model of circularization of the mRNA. The N-terminal domain of elF4G adapter protein (hook-shaped gray structure, 4G) of the el4F complex (indicated by strippled line) is associated with the elFE cap binding protein (4E) to mediate binding to the PABP while the C-teminal domain of the elF4G protein of the elF4F complex is associated with the helicases elF4A (4A) and recruits the 40S ribosomal subunit with elF3 (F3) and the methyl tRNA with elF2 (triangle F2) to initiate RNA translation. (c) Upper part: masking of mRNAs with short poly(A) tail by binding to Maskin or related proteins to the CPEB, thus preventing tight interaction between CPEB and CPSF to position PAP/Gld2 poly(A) polymerase to elongate the poly(A) tail. Maskin phosphorylated by PKA when bound to elF4E excludes the elF4G protein from the complex and prevents ring formation by attachment to PABP. Lower part: upon resumption of maturation and activation of MPF/cdk1, Maskin is phosphorylated by cdk1 and this releases its interaction with elF4G (and possibly CPEB). Further phosphorylation by Eg2/aurora A kinase may facilitate centrosome attachment and regulation of microtubule length by maskin. Downstream from synthesis of Mos and activation of MAP kinases, phosphorylation of elF4E by kinases facilitates binding to the methylated cap mRNA at the 5′UTR and interaction with ePABP in circularization. Phosphorylation of CPEB by Eg2/aurora A kinase may induce conformational changes that promote tight interaction between CPEB, CPSF, and symplekin to position the PAP/Gld2 poly(A) polymerase at the 3′UTR to induce polyadenylation. In turn, this provides sites for attachment of embryonic PABP (ePAB) for ring formation and association of the ePAB with the elF4G adapter protein of the el4F initiation complex. For further explanation and references, see text

04-Human Oocytes-chapter04-ppp.i63 63 14/9/06 16:19:59

is feasible that overexpression of genes due to insufficient deadenylation in not fully developmentally competent oocytes such as derived from a suboptimal follicular environment might contribute to reduced quality.

Microinjection of PARN antibodies into *Xenopus* oocytes resulted in untimely overexpression of housekeeping genes[175]. Overexpression of PABP also prevented translational silencing[177] (Figure 4.1), suggesting that the relative abundance of PARN, PAP/Gld2, and PABP affects temporal control of expression at oogenesis. Alizadeh et al. showed, by subtractive cDNA analysis of oocytes and one-cell embryos, that H1oo, c-mos, tPA (tissue type plasminogen activator gene), and Gdf-9 transcripts underwent rapid degradation after fertilization of mouse oocytes[178]. This appeared associated with the presence of CPEs near a poly(A) signal in the 3′UTR of all of these mRNA species, suggesting that polyadenylation, translation, and subsequent degradation of these stage-specifically expressed genes may have occurred.

Cell cycle control and translation of genes for maturation

Before discussing cytoplasmic polyadenylation it is necessary to understand the salient features of cell cycle regulation in mammalian oocytes. Resumption of maturation is species-specifically regulated. In vertebrates like *Xenopus*, the signaling by progesterone initially induces translation of the Mos protein, which then triggers increases in expression of the catalytic subunit of maturation promoting factor (MPF) and MPF activation (for review see references 146, 172, and 179). In mammals like the mouse, protein synthesis is not required for initial resumption of maturation. The G-protein coupled receptors rendering adenylate cyclase active[180–182] and the cAMP transmitted by gap junctions from the cumulus to the oocyte retain high cAMP and, accordingly, high activity of the cAMP-dependent protein kinase A (PKA) in meiotically arrested oocytes (for review see reference 183). The latter inhibits activation of preMPF, the complex of inactive cdc2/cdk1 (cyclin dependent kinase 1) and cyclin B, that is already present in sufficient concentrations to induce resumption of maturation in meiotically competent oocytes, until inactivation of PKA has occurred (Figure 4.3). PKA mediates meiotic arrest by phosphorylation and inactivation of the cdc25 phosphatase[184–186], concomitantly with the phosphorylation and activation of the Wee1 kinase (Figure 4.3). The latter renders MPF inactive by phosphorylation of the catalytic subunit of MPF, the cdc2 kinase (also termed cyclin dependent kinase 1, cdk1) on a regulatory site in the meiotically blocked, maturation competent oocyte[187]. Resumption of maturation is mediated by activation of phosphodiesterase 3 (PDE3), causing reduction in cAMP levels[188,189] (Figure 4.3). Moreover, downstream from the LH surge, Leydig insulin-like 3 protein (INSL3) appears to activate inhibitory G proteins, thereby decreasing cAMP production and initiating resumption of meiosis[190]. Inactivation of PKA changes the balance between activity of the phosphatase Cdc25 and the kinase Wee1. Eventually, removal of inhibitory phosphorylation on residues of the cdk1 kinase will activate MPF (Figure 4.3). In species like the mouse, no new protein synthesis is required for meiotic resumption, e.g. after removal of cumulus, whereas oocytes of other species like the pig still require synthesis of Cdc25C phosphatase from stored mRNA for GVBD[176]. Accordingly, in mouse and most other oocytes the key factors in cell cycle control that mediate initial resumption of meiosis are already present in fully grown oocytes. However, progression to metaphase II and meiosis II arrest depend on synthesis of several additional key proteins in cell cycle control and maturation (Figure 4.3), and this appears to be conserved between vertebrate oocytes. For instance, new protein synthesis of the regulatory subunit of MPF, cyclin B, is required after GVBD throughout oocyte maturation. Accordingly, the relative rate of cyclin B synthesis may influence the kinetics of meiotic progression[191].

04-Human Oocytes-chapter04-ppp.i64 64 14/9/06 16:19:59

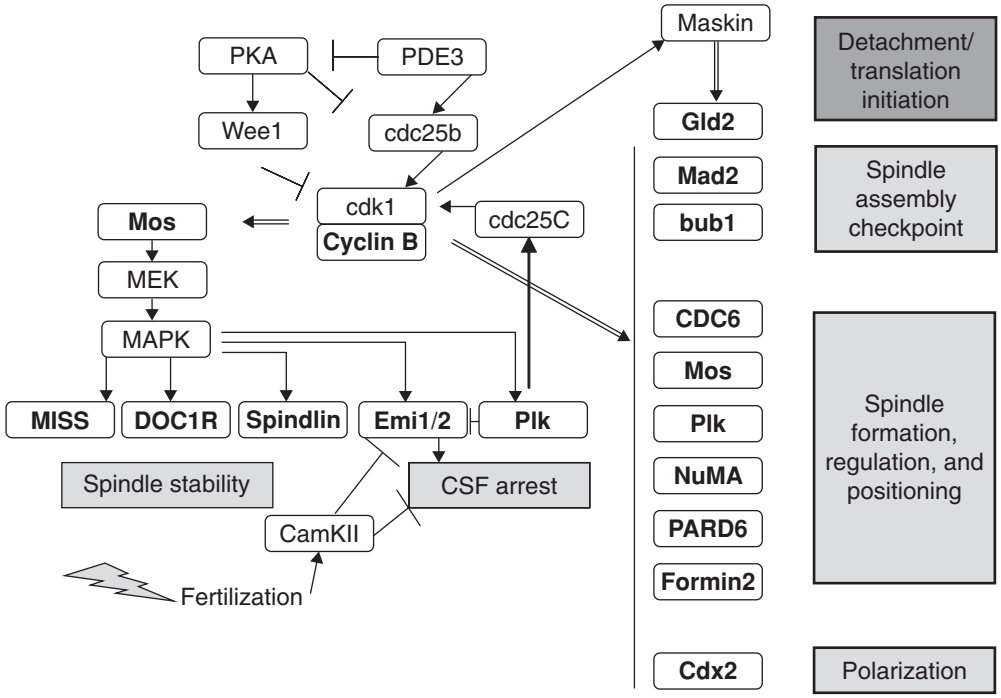


Figure 4.3 Major pathways in cell cycle control and regulation of maturation and early development by post-translational mechanisms involving newly synthesized proteins. Gene products that are synthesized at maturation are printed in bold letters; boxes indicate function of newly synthesized proteins at maturation or in the preimplantation embryo. Arrows and T lines indicate activation or inactivation, respectively, by post-translational modification; double arrows symbolize induction of synthesis as a consequence of activation of kinases, e.g. MPF (cdk1/CyclinB). For further explanation, see text

Acquisition of meiotic competence in the mouse is accompanied by characteristic changes in expression of genes engaged in regulation of maturation. Proteins like the catalytic and regulatory subunits of maturation promoting factor, CDC2/CDK1, and cyclin B, as well as their activating/inactivation phosphatase/kinase Cdc25 and Wee1, respectively, change in subcellular distribution and expression[192]. Accordingly, mouse cyclin cdcnb1 mRNA and phosphatase cdc25b mRNA are already upregulated at the primordial to primary stage of folliculogenesis, while cdc2a mRNA encoding the catalytic subunit of MPF increases at the small antral to large antral stage. Wee1 is downregulated at the transcriptional level concomitantly, presumably in preparation for decreased activities at resumption of maturation[2]. Acquisition of meiotic competence in the mouse coincides with an increase in cdc25c transcription, probably supporting the enhanced synthesis of this protein that induces activation of preMPF at resumption of maturation in an autocatalytic loop by dephosphorylating preMPF at the regulatory site of cdk1 (Figure 4.3). Active MPF in turn is required for inducing polyadenylation of c-mos mRNA, thus initiating translation of Mos protein and the downstream activation of MAP kinases in mammalian oocytes

04-Human Oocytes-chapter04-ppp.i65 65 14/9/06 16:20:01

(e.g. reference 193). Selective ablation of cyclin B expression by RNAi in GV stage mouse oocytes thus results in low MPF activity and prevents polyadenylation of c-mos mRNA.

After oocytes have undergone GVBD, formed a spindle, and aligned all chromosomes at the equator (termed chromosome congression), entry into anaphase I is initiated by activation of APC/C, the anaphase promoting complex[45]. This complex is present and induces degradation of the regulatory subunit of MPF, cyclin B, as well as the protein securin. Securin is associated with separin, a protease, up to metaphase, thus rendering it inactive. Upon release of securin, separase is free to proteolytically cleave proteins of the meiotic cohesin complex that hold sister chromatid arms attached to each other before anaphase, thus preventing chiasma resolution (for review, see references 179 and 194). Upon APC/C activation cohesins are cleaved by separase and cohesion is lost between the arms of sister chromatids, so that homologs separate. MPF is also transiently inactivated by degradation of cyclin B[45]. The sustained synthesis of cyclin B from mRNA stores and recruitment of protein from the cytoplasm restores MPF activity and is also required for sustained cytostatic arrest at metaphase II, in the presence of aligned chromosomes[195,196]. Timed translation of cyclin B has been studied extensively in oocytes, revealing regulatory regions in the 3′ untranslated part of the mRNAs that are responsible for regulation (see below). Ret finger protein-like 4 (Rfpl4) was discovered during an in silico search for germ cell specific genes. RFPL4 mRNA accumulates in all growing oocytes and rapidly disappears at early embryogenesis. Downstream from recognition and initiation of polyubiquinylation by APC/C it appears to target proteins like cyclin B for degradation at anaphase of meiotic divisions as a component of the ubiquitin–degradation pathway[197].

Another key factor that is essential for meiotic cell cycle regulation in mammalian oocytes is Mos kinase. Mos mRNA is translated at resumption of maturation. It is an essential component of cytostatic factor (CSF) (for discussion, see references 13, 198, and 199). The kinase phosphorylates MEK and MAP kinases in a phosphorylation cascade together with several other substrates, which contribute to meiotic arrest at metaphase II (Figure 4.3). Mos protein can also bind to tubulin and regulates spindle formation and positioning of the spindle for unequal division at first polar body formation[13,200]. In effect, Mos translation is required for normal cell cycle regulation and CSF arrest in oocytes, although the pivotal factor in CSF is probably the Emi2 meiotic inhibitor of APC/C (see below).

At the transition from meta- to anaphase the so-called spindle attachment checkpoint (SAC) is expressed in oocytes (see references 201–204). Initially believed to be a component of CSF, it is now clear that SAC senses attachment of spindle fibers to chromosomes and tension on centromeres by bipolar attachment of chromosomes at mitosis and meiosis I and II[202]. Several essential proteins involved in checkpoint control are synthesized from mRNA after resumption of maturation as suggested by RNAi approaches. For instance, knockdown of the checkpoint component Mad2 in oocytes[45,203,204] by injection of specific small interfering RNAs may speed up meiotic resumption and ablate checkpoint control at meiosis, showing the significance of timed synthesis for ordered chromosome segregation in oocytes and for maturation kinetics (Figure 4.3). Indeed, checkpoint control may be essential for prevention of aneuploidy when there are disturbances in spindle formation[45,203,204]. Emi1, another essential component of cell cycle control in oogenesis, is expressed throughout meiosis, albeit the protein is rather unstable (Figure 4.3). Emi1 is an inhibitor of the APC/C. Ablation by RNAi in mouse oocytes results in spontaneous activation, suggesting that it is a pivotal CSF component[205]. Possibly, Emi2 represents the major and most important component of CSF as an inhibitor of the APC at meiosis II[17]. Emi2 is more stable compared to Emi1 and was the first

04-Human Oocytes-chapter04-ppp.i66 66 14/9/06 16:20:01

component identified to be a direct target of the calcium calmodulin kinase II (CamKII) (Figure 4.3). This calcium activated kinase is induced by fertilization and mediates CSF inactivation[206–208]. Emi2 is also a target of polo-like kinase 1[207] that marks it for destruction by phosphorylation. As long as Emi2 concentration is high, oocytes remain arrested at metaphase II by inhibition of APC/C. It was earlier shown that levels of polo-like kinase protein increase at GVBD (plk in Figure 4.3) and the kinase associates initially with the spindle poles, depending on phosphorylation by MAP kinases[209,210], downstream from Mos kinase. Polo-like kinase 1 (plk1) has multiple tasks. It appears involved in an autocatalytic loop between CDC25C phosphatase and M-phase promoting factor (MPF), supporting the conversion of pre-MPF to active MPF by dephosphorylation of cdk1 (Figure 4.3). Plk1 is activated before MPF in maturing porcine oocytes[211]. Destruction of Emi2 is triggered in coordination with fertilization events. Activation of CamKII leads initially to phosphorylation of Emi2 at a specific motif (Figure 4.3). This then enables Plk1 to strongly interact with Emi2, and, in turn, phosphorylate and target it for destruction[207] (as indicated in Figure 4.3). Comparable to fertilization, ablation of Emi2 by RNAi causes exit from CSF-mediated metaphase II arrest[17]. The experimental data thus suggest that key components of cell cycle regulation, including Emi2, need to be synthesized from a pre-existing message at maturation to mediate normal meiotic progression as well as arrest at meiosis II (Figure 4.3).

Mouse meiotic mutants as well as ablation of expression of genes by degradation and inactivation of translation by introduction of anti-sense RNA, double-stranded RNA, or RNAi into oocytes have revealed that several other genes involved in regulation of the cell cycle, spindle formation, and early development need to be synthesized from mRNAs during maturation (Figure 4.3). For instance, DOC1R (deleted oral cancer 1 related), initially described as a tumor proto-oncogene, has been identified as a MAP kinase substrate that is synthesized during oocyte maturation and that, when knocked down, will result in spindle deregulation and random microtubule aster formation in mouse oocytes[212]. DOC1R protein increases during maturation whereas MISS protein (MAP kinase-interacting and spindle stabilizing protein), another MAP kinase substate, is unstable in meiosis I, and only stably associates with the meiosis II spindle[213] (Figure 4.3). PARD6A is a protein that is apparently synthesized at resumption of meiosis, concentrated on the spindle half that will attach to the cell cortex, and is essential for unequal division at meiotic anaphase in oocytes[214]. Formin 2 encodes an actin-polymerizing protein involved in spindle migration to the oolemma that needs to be synthesized at oocyte maturation[215]. Furthermore, ablation of expression of CDC6, a checkpoint component in S-phase in mitosis, showed that translation of mRNA is essential for spindle formation at meiosis I and thus constitutes another essential oocyte factor for maturation that is synthesized stage-specifically[211]. Finally, several mRNAs translated after resumption of maturation or in early embryogenesis are essential maternal components for meiosis and/or early development. For instance, it appears that NuMA (nuclear mitotic apparatus antigen) needs to be synthesized during maturation[179]. This protein is required for spindle formation and function in meiosis. In addition, it is one of the maternal products that are transiently enriched at the meiotic metaphase II spindle, subsequently transits to the female and male pronucleus after fertilization, and is required for normal development[216]. Spindlin (Spin) is another maternal effect gene expressed from maternal mRNA stores at maturation. It is associated with the spindle at metaphase II when it is phosphorylated by MAP kinase[217,218]. The message for Spin encoding the 30-kDa protein is highly expressed in mature oocytes (Figure 4.3).

Differential polyadenylation and translation occurs at two critical points: upon oocyte maturation and after fertilization. While the message

04-Human Oocytes-chapter04-ppp.i67 67 14/9/06 16:20:01

is diminished in meiosis II, the protein level is high in the zygote and may play an important role in zygotic gene activation (ZGA)[219]. Depletion of oocytes of maternally expressed spindle-associated proteins like NuMA and spindlin by removal of chromosomes and the spindle before somatic cell nuclear transfer in cloning may be one etiologic factor in the failure to obtain viable embryos[179,220]. The apolar localization and recruitment of proteins by the spindle may also facilitate their targeting to specific sites in the embryo (e.g. pronuclei or mitotic spindles) such that disturbances due to oocyte freezing or suboptimal conditions at maturation might adversely affect development. Several studies support the notion that not only control of timely expression but also spatial distribution of gene products influence development (e.g. references 221 and 222). For instance, Cdx2 mRNA coding for a transcription factor is localized toward the vegetal pole in the mammalian oocyte. The message reorients after fertilization and mRNA and protein become concentrated in the late-dividing, two-cell-stage blastomere that defines the lineage to trophectoderm[223]. In conclusion, many proteins that are cell cycle dependently synthesized during maturation are pivotal in the control of cytoskeletal, chromatin, and embryonal regulation in mammalian oocytes and in polarization of the embryo (Figure 4.3).

Translation of polyadenylated mRNA

Initiation of translation is a complex multi-step process that requires a large number of protein factors and multi-protein complexes, in addition to ribosomes[165] (Figure 4.2b). Cap-dependent translational initiation in eukaryotes requires a methylated guanosine residue at the 5′untranslated region of the mRNA (m7GpppN) (Figure 4.2a and b). The 5′cap structure attracts the eukaryotic initiation factor eIF4F complex (indicated by strippled lines in Figure 4.2b) to recruit it to the mRNA. The eIF4F complex is heterotrimeric, consisting of a cap-binding protein, eIF4E (4E in Figure 4.2b), an RNA-dependent helicase, eIF4A (4A in Figure 4.2b), and a large protein serving as an adapter in the complex, eIF4G (hook-like structure with N- and –C-terminal domains termed 4G in Figure 4.2b)[168,221]. Concomitantly, a ternary complex with GTP and the initiator Met-tRNA forms that associates with the 40S ribosomal subunit and several initiation factors, such as eIF1, eIF1A, and eIF3 (triangle termed F2 in Figure 4.2b). The 43S preinitiation complex binds to the eIF4F complex to form the 48S preinitiation complex. After completion of initiation, the initiation complex scans the mRNA for the translation start codon AUG, and GTP hydrolysis occurs. Finally, the translational elongation by the 80S ribosome starts.

Synergistically with the cap structure circularization of the mRNA by protein–protein interactions between proteins attached to the 3′ and 5′ ends of the mRNAs acts as a rate-limiting step in initiation to promote the initiation process (Figure 4.2b). For this, the eIF4G adapter protein in the initiation complex associates with its N-terminal domain with the poly(A) tail of the 3′UTP of the mRNA by binding to PABP (gray boxes in Figure 4.2b). Concomitantly, eIF4G recruits eIF3 (termed F3 in Figure 4.2b) with its C-terminal domain to the 40S ribosomal complex. In combination with eIF5 it may serve as an adapter for proteins associated with the heterotrimeric eIF2 complex and the Met-t-RNA[224]. When all the factors for translational initiation have assembled they promote initiation of translation. In mouse, expression of embryonal poly(A) binding protein (ePAB), an ortholog of *Xenopus* embryonal PABP, appears to be particularly relevant. It is present during maturation and at metaphase II, as well as in the one-cell and two-cell embryo[225]. The protein becomes undetectable from the four-cell stage. The expression up to major zygotic gene activation argues for a role in translational activation of maternally derived mRNAs during mammalian oocyte and early preimplantation development[226].

04-Human Oocytes-chapter04-ppp.i68 68 14/9/06 16:20:02

According to the need for circularization, the presence of a poly(A) tail and interaction of PABP with the elF4F complex on mRNAs decide on whether and when mRNAs can be translated. In effect, it is also the timing of polyadenylation and modifications of molecules in the protein–protein interactions that influence translation. For instance, modification of the cap structure in maturing oocytes contributes to efficient translational initiation. The cap of certain mRNAs may be modified by methylation of riboses in the trinucleotide at the 5′UTR[227]. Such ribose methylation occurs concomitantly with recruitment and translational activation of mRNAs like Mos in *Xenopus* oocytes[227]. The cap-binding factor also becomes phosphorylated at resumption of maturation, probably by the MAP kinase pathway (Figure 4.2c[228]). This enhances the affinity of the protein for the mRNA 5′ cap in the initiation event[222]. The hypermethylated cap within the eIF4F complex may ensure that certain molecules become very efficiently associated with polyribosomes and translated, right after polyadenylation. When eIF4G, which mediates the binding of the 5′ cap to PABP, is enzymatically cleaved, the recruitment of stage-specifically expressed mRNAs coding for c-mos, cyclin B1, and other proteins to polyribosomes is blocked[179,229].

Post-translational mechanisms in mRNA recruitment

According to their function, most cellular mRNAs encoding housekeeping proteins that are efficiently translated during oocyte growth attain the poly(A) tail already in the nucleus (about 450 residues) and, after nuclear export, can directly associate with ribosomes. Initiation of translation can proceed according to the mechanisms shown in Figures 4.1 and 4.2b. As demonstrated above, especially during gametogenesis/oogenesis, several types of mRNAs appear to become deadenylated and masked by proteins associating with the mRNA, preventing their expression. Translational repression may involve masking factors, which associate with RNA in the cytoplasm but also already in the nucleus. For instance, association of a masking protein FRGY2, a DEAD-box RNA-helicase, with mRNA species starts within the nucleus of the growing *Xenopus* oocyte[230]. The catalytic subunits of casein kinase II (CkII) and two other proteins, FRGY2a and FRGY2b, were found to be involved in the masking of mRNAs. FRGY2 is phosphorylated by CkII, which appears to be important for the masking/unmasking reaction[231]. MSY2, the mouse ortholog of *Xenopus* FRY2 and of human contrin[232], functions as a co-activator of transcription in male germ cells and plays an important role in the translational repression and storage of both paternal and maternal mRNAs in spermatocytes, spermatids, and oocytes (see reference 233). In the mouse, MSY2 is one of the most abundant proteins (2% of total protein) in fully grown oocytes. Deletion in transgenic msy2–/– mice causes infertility in both sexes, and early loss of oocytes, and defects in ovulation in females[234]. The reduction of MSY2 in GV-arrested oocytes by using Zp3-promoter-based transgenic RNAi methodology for knockdown of expression also leads to subfertility or infertility. This is consistent with the proposed function of MSY2 to stabilize mRNAs in oocytes and thereby facilitate mRNA accumulation during oocyte growth. Specificity of binding appears to be linked to transcripts derived from genes with a Y-box-containing promoter. MSY2 is inactivated by degradation at resumption of oocyte maturation, which may contribute to the recruitment of maternal mRNAs at this transition.

The recruitment of stored maternal mRNAs for polyadenylation in the cytoplasm is a complex process that mainly relies on post-translational mechanisms and sequential synthesis and activation of stimulatory kinases. The processes have been studied most extensively in *Xenopus* oocytes, but appear largely conserved. For instance, as in *Xenopus*, masking and inhibition of polyadenylation by exposure to polyadenylation inhibitor 3′-deoxyadenosine (3′-dA)

04-Human Oocytes-chapter04-ppp.i69 69 14/9/06 16:20:02

up to metaphase I prevents meiosis progression in in-vitro maturing bovine oocytes, possibly by inhibiting the constant lengthening of cyclin B1 mRNA[151]. Such observations in experimental models underline the significance of timed polyadenylation and expression of meiotic genes from stored, timely recruited mRNAs. Release from repression of masked, dormant RNAs requires the CPE (also termed adenylation control element, ACE) and a hexanucleotide downstream in the 3′UTR of the mRNA or a related consensus sequence. In masked mRNA the CPE is associated with the CPEB protein that, in turn, appears bound to the Maskin protein in stored deadenylated mRNAs (Figure 4.2c). This prevents the elongation initation complex efficiently docking to the 3′UTR of the mRNA because the message is rendered with a short poly(A) tail and has no site for binding of PABP. As described above, PABP is involved in ring formation. CPSF associated with the hexamer in the 3′UTP is also prevented from tight interaction with CPEB by the presence of Maskin and therefore cannot position the PAP on the 3′UTR for polyadenylation (upper part of Figure 4.2C). The two proteins CPEB and CPSF are in contact with the protein symplekin[161]. The latter helps to attract and activate the meiotic poly(A) polymerase Gld2. Once maskin is released and CPEB phosphorylated this may induce conformational changes so that Gld2 can elongate the poly(A) tail (lower part of Figure 4.2c). Maskin also binds translation initiation factor 4E (eIF4E), an interaction that excludes eIF4G and in this way prevents formation of the eIF4F initiation complex (Figure 4.2c).

However, upon resumption of maturation and activation of MPF/cdk1, maskin is phosphorylated by MPF and this releases it from CPEB and from eIF4F[164] (detachment in Figure 4.2c). Mutation of residues for MPF phosphorylation alleviates the cdk1-induced dissociation of maskin from eIF4E and translation initiation. Downstream from cdk1 activation another kinase, Eg2/aurora A kinase, a member of the aurora family of mitotic serine/threonine kinases, is activated during maturation, which, in turn, can phosphorylate CPEB[235]. The quantity of aurora A/Eg2 protein is already high in the GV of mouse oocytes and remains stable during maturation up to metaphase II[236]. Phosphorylation of CPEB by aurora A participates in the control of sequential protein synthesis by enhancing the affinity of binding between CPEB and CPSF, promoted by attachment to symplekin[161]. CPSF in turn may recruit/position PAP. CPEB/CPSF proteins complexed by symplekin can target poly(A) polymerase GLD2 to catalyze polyadenylation of the 3′UTR of the message[161]. The mechanisms are conserved since mouse GLD-2 (mGld2), a recently identified cytoplasmic PAP, is also expressed in the oocytes exclusively after GVBD at meiosis I and II and appears essential for the progression from metaphase I to metaphase II during oocyte maturation[237]. Upon elongation of the poly(A) tail, PABP can bind and recruit eIF4G of the elF4E complex for ring formation and stimulation of expression. Maskin may have several functions as it can be phosphorylated by different kinases and at different sites. Before maturation, Maskin is already phosphorylated by protein kinase A. This does not influence initiation of translation but appears critical for the protein to localize on the spindle of somatic cells[161]. After resumption of maturation, phosphorylation of Maskin by E2/aurora A kinase (Figure 4.2c) may help to promote microtubule growth from asters and contribute to the determination of microtubule steady-state length[238].

Apart from these mechanisms of translational initiation involving differential phosphorylation of Maskin, the early cytoplasmic polyadenylation and translational activation of multiple maternal mRNAs can also occur in a CPE- and CPEB-independent manner. The sequential action of distinct 3′-UTR-directed translational control mechanisms and related RNA-binding proteins can possibly coordinate the complex temporal patterns and extent of protein synthesis during vertebrate meiotic cell cycle progression in such

04-Human Oocytes-chapter04-ppp.i70 70 14/9/06 16:20:03

cases[155,172,239]. Generally, it appears to be the context, for instance, the number and distance between the CPEs and the hexanucleotide, the presence of RNA-binding and interacting proteins, and the activity of kinases, which controls the timing and extent of polyadenylation[155,240]. As an example, DAZL proteins like human DAZ and BOULE stimulate translation by promoting initiation. Collier et al. showed that DAZL proteins interact with PABPs and thus may contribute to the activation of specific translationally silent mRNAs during germ cell development[241]. DAZL protein is abundantly expressed in mature human oocytes and embryos, and expression in the embryo may relate to quality and developmental capacity[242].

CONCLUSIONS AND PERSPECTIVES

Currently we are at a time when qualitative analysis of gene expression in mammalian oocytes of different species is progressing towards quantitative assessments, in order to proceed from genomics and proteomics to metabolomics and systems biology. We are still far from understanding the complex regulatory processes that govern regulation of gene expression in oocyte growth and maturation, in para- and autocrine signaling cascades during folliculogenesis, and in ovulation and early development. Even less so, we do not comprehend the influences of environment, in-vitro conditions, and aging on gene expression. However, several recent studies analyzing global gene expression or expression of particular genes in unstimulated versus stimulated cycles provide evidence that the endocrine environment has a profound influence and may greatly impinge on gene expression, and oocyte quality and developmental competence (e.g. references 2, 21, 116, and 243). For instance, the expression of the transcription factor Oct-4 is increased in primordial follicles of mice primed with PMSG, and following the LH surge in preovulatory antral oocytes[116]. This suggests that Oct-4 may not only have a role in regulation of gene expression to initiate growth, but also in the selection of oocytes for ovulation in response to endocrine signaling. Expression patterns of preovulatory oocytes of the mouse were shown to differ between oocytes obtained from unstimulated cycles and stimulated cycles, and this was associated with a better developmental potential of oocytes from the stimulated compared to the unstimulated cycle[2]. Such approaches may reveal pivotal components that are needed for normal development. Currently, there is a hunt for predictive indicators of oocyte health, in particular with respect to optimize in-vitro maturation. For instance, the expression of insulin-like growth factor II (IGF-II) was not found in metaphase II in-vitro grown oocytes from preantral follicle culture compared to in-vivo controls, and this was associated with reduced developmental potential after fertilization[113]. Differential expression related to maternal age and/or depletion of the follicular reserve is also of high relevance, especially in ART, when patients of advanced age ask for help in conception. Thus, there are reports suggesting that aging may affect expression of components of the spindle assembly checkpoint[244] and chromosome cohesion[43] that may contribute to increase risks for meiotic non-disjunction (errors in chromosome segregation). Other genes that appear repressed with advanced maternal age are candidate maternal effect genes required for early embryogenesis[144]. The large increase in numbers of libraries of genes expressed at distinct stages of oogenesis, coupled with improvement in methodology for assessing gene expression in silico as well as through experimental approaches, the availability of animal models, and the improvement in culture methods, now greatly facilitates comparative/subtractive and quantitative approaches to identify key factors in regulation and test for their influence on developmental potential (e.g. reference 245). The present review just provides a glimpse at the complexity of differential expression and its temporal, stage-specific regulation in oocytes. Apart from assessing

04-Human Oocytes-chapter04-ppp.i71 71 14/9/06 16:20:03

expression at the translational and protein level, it should however be kept in mind that the spatio-temporal regulation is crucial in the context of oocyte quality, the polar distribution of cellular components, and the functional integrity at the level of the cytoskeleton and the chromatin configuration. Therefore, it is essential to analyze gene expression in a global fashion as well as by functional approaches to improve IVM and oocyte quality in ART.

ACKNOWLEDGMENTS

I thank Rudolf Eichenlaub (University of Bielefeld) for critical reading of the manuscript and Helen Picton (University of Leeds) for helpful information. I apologize to all whose work could not be cited due to limited space.

REFERENCES

1. Paredes A, Garcia-Rudaz C, Kerr B et al. Loss of synaptonemal complex protein–1, a synaptonemal complex protein, contributes to the initiation of follicular assembly in the developing rat ovary. Endocrinology 2005; 146: 5267–77.
2. Pan H, O'Brien MJ, Wigglesworth K et al. Transcript profiling during mouse oocyte development and the effect of gonadotropin priming and development in vitro. Devel Biol 2005; 286: 493–506.
3. De La Fuente R, Eppig JJ. Transcriptional activity of the mouse oocyte genome: companion granulosa cells modulate transcription and chromatin remodeling. Devel Biol 2001; 229: 224–36.
4. De La Fuente R, Viveiros MM, Burns KH et al. Major chromatin remodeling in the germinal vesicle (GV) of mammalian oocytes is dispensable for global transcriptional silencing but required for centromeric heterochromatin function. Devel Biol 2004; 275: 447–58.
5. Cao Q, Richter JD. Dissolution of the maskin-eIF4E complex by cytoplasmic poly adenylation and poly (A)-binding protein controls cyclin B1 mRNA translation and oocyte maturation. EMBO J 2002; 21: 3852–62.
6. Wilkie GS, Dickson KS, Gray NK. Regulation of mRNA translation by 5′- and 3′-UTR-binding factors. Trends Biochem Sci 2003; 28: 182–8.
7. Amleh A, Dean J. Mouse genetics provides insight into folliculogenesis, fertilization and early embryonic development. Hum Reprod Update 2002; 8: 395–403.
8. Serafica MD, Goto T, Trounson AO. Transcripts from a human primordial follicle cDNA library. Hum Reprod 2005; 20: 2074–91.
9. Choi T, Aoki F, Mori M et al. Activation of p34cdc2 protein kinase activity in meiotic and mitotic cell cycles in mouse oocytes and embryos. Development 1991; 113: 789–95.
10. Sagata, N. What does Mos do in oocytes and somatic cells? Bioessays 1997; 19: 13–21.
11. Araki K, Naito K, Haraguchi S et al. Meiotic abnormalities of c-mos knockout mouse oocytes: activation after first meiosis or entrance into third meiotic metaphase. Biol Reprod 1996; 55: 1315–24.
12. Tunquist BJ, Maller JL. Under arrest: cytostatic factor (CSF)-mediated metaphase arrest in vertebrate eggs. Genes Dev 2003; 17: 683–710.
13. Brunet S, Maro B. Cytoskeleton and cell cycle control during meiotic maturation of the mouse oocyte: integrating time and space. Reproduction 2005; 130: 801–11.
14. Rieder CL, Schultz A, Cole R et al. Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitors sister kinetochore attachment to the spindle. J Cell Biol 1994; 127: 1301–10.
15. Marangos P, Carroll J. Fertilization and InsP3-induced Ca2+ release stimulate a persistent increase in the rate of degradation of cyclin B1 specifically in mature mouse oocytes. Devel Biol 2004; 272: 26–38.
16. Tung JJ, Hansen DV, Ban KH et al. A role for the anaphase-promoting complex inhibitor Emi2/XErp1, a homolog of early mitotic inhibitor 1, in cytostatic factor arrest of *Xenopus* eggs. Proc Natl Acad Sci USA 2005; 102: 4318–23.

04-Human Oocytes-chapter04-ppp.i72 72 14/9/06 16:20:03

17. Shoji S, Yoshida N, Amanai M et al. Mammalian Emi2 mediates cytostatic arrest and transduces the signal for meiotic exit via Cdc20. EMBO J 2006; 25: 834–45.

18. Morgan HD, Santos F, Green K et al. Epigenetic reprogramming in mammals. Hum Mol Genet 2005; 14(Spec No 1): R47–58.

19. Xu Y, Zhang JJ, Grifo JA et al. DNA methylation patterns in human tripronucleate zygotes. Mol Hum Reprod 2005; 11: 167–71.

20. Lucifero D, Mann MR, Bartolomei MS et al. Gene-specific timing and epigenetic memory in oocyte imprinting. Hum Mol Genet 2004; 13: 839–49.

21. Borghol N, Lornage J, Blachere T et al. Epigenetic status of the H19 locus in human oocytes following *in vitro* maturation. Genomics 2006; 87: 417–26.

22. Davidson EH. Gene Activity in Early Development. Academic Press, New York, 1986.

23. Gosden RG. Oogenesis as a foundation for embryogenesis. Mol Cell Endocrinol 2002; 186: 149–53.

24. Zheng P, Patel B, McMenamin M et al. Effects of follicle size and oocyte maturation conditions on maternal messenger RNA regulation and gene expression in rhesus monkey oocytes and embryos. Biol Reprod 2005; 72: 890–7.

25. Watanabe Y. Sister chromatid cohesion along arms and at centromeres. Trends Genet 2005; 21: 405–12.

26. Eichenlaub-Ritter U. Mouse genetic models for aneuploidy induction in germ cells. Cytogenet Genome Res 2005; 111: 392–400.

27. Revenkova E, Jessberger R. Keeping sister chromatids together: cohesins in meiosis. Reproduction 2005; 130: 783–90.

28. Yuan L, Liu JG, Hoja MR et al. Female germ cell aneuploidy and embryo death in mice lacking the meiosis-specific protein SCP3. Science 2002; 296: 1115–18.

29. Bannister LA, Reinholdt LG, Munroe RJ et al. Positional cloning and characterization of mouse mei8, a disrupted allelle of the meiotic cohesin, Rec8. Genesis 2004; 40: 184–94.

30. Revenkova E, Eijpe M, Heyting C et al. Cohesin SMC1 beta is required for meiotic chromosome dynamics, sister chromatid cohesion and DNA recombination. Nat Cell Biol 2004; 6: 555–62.

31. Xu Y, Ashley T, Brainerd EE et al. Targeted disruption of ATM leads to growth retardation, chromosomal fragmentation during meiosis, immune defects, and thymic lymphoma. Genes Dev 1996; 10: 2411–22.

32. Pittman DL, Cobb J, Schimenti KJ et al. Meiotic prophase arrest with failure of chromosome synapsis in mice deficient for Dmc1, a germline-specific RecA homolog. Mol Cell 1998; 1: 697–705.

33. de Vries SS, Baart EB, Dekker M et al. Mouse MutS-like protein Msh5 is required for proper chromosome synapsis in male and female meiosis. Genes Dev 1999; 13: 523–31.

34. Woods LM, Hodges CA, Baart E et al. Chromosomal influence on meiotic spindle assembly: abnormal meiosis I in female Mlh1 mutant mice. J Cell Biol 1999; 145: 1395–406.

35. Kneitz B, Cohen PE, Avdievich E et al. MutS homolog 4 localization to meiotic chromosomes is required for chromosome pairing during meiosis in male and female mice. Genes Dev 2000; 14: 1085–97.

36. Baudat F, Manova K, Yuen JP et al. Chromosome synapsis defects and sexually dimorphic meiotic progression in mice lacking spo11. Mol Cell 2000; 6: 989–98.

37. Kolas NK, Svetlanov A, Lenzi ML et al. Localization of MMR proteins on meiotic chromosomes in mice indicates distinct functions during prophase I. J Cell Biol 2005; 171: 447–58.

38. Bhalla N, Dernburg AF. A conserved checkpoint monitors meiotic chromosome synapsis in *Caenorhabditis elegans*. Science 2005; 310: 1683–6.

39. Guillon H, Baudat F, Grey C et al. Crossover and noncrossover pathways in mouse meiosis. Mol Cell 2005; 20: 563–73.

40. Hunt PA, Hassold TJ. Sex matters in meiosis. Science 2002; 296: 2181–3.

41. Tay J, Richter JD. Germ cell differentiation and synaptonemal complex formation are disrupted

04-Human Oocytes-chapter04-ppp.i73 73 14/9/06 16:20:04

in CPEB knockout mice. Devel Cell 2001; 1: 201–13.

42. Tay J, Hodgman R, Sarkissian M et al. Regulated CPEB phosphorylation during meiotic progression suggests a mechanism for temporal control of maternal mRNA translation. Genes Dev 2003; 17: 1457–62.

43. Hodges CA, Revenkova E, Jessberger R et al. SMC1beta-deficient female mice provide evidence that cohesins are a missing link in age-related nondisjunction. Nat Genet 2005; 37: 1351–5.

44. Watanabe Y. Sister chromatid cohesion along arms and at centromeres. Trends Genet 2005; 21: 405–12.

45. Herbert M, Levasseur M, Homer H et al. Homologue disjunction in mouse oocytes requires proteolysis of securin and cyclin B1. Nat Cell Biol 2003; 5: 1023–5.

46. Tateno H, Akutsu H, Kamiguchi Y et al. Inability of mature oocytes to create functional haploid genomes from somatic cell nuclei. Fertil Steril 2003; 79: 216–18.

47. Heindryckx B, Lierman S, Van der Elst J et al. Chromosome number and development of artificial mouse oocytes and zygotes. Hum Reprod 2004; 19: 1189–94.

48. Galat V, Ozen S, Rechitsky S et al. Cytogenetic analysis of human somatic cell haploidization. Reprod Biomed Online 2005; 10: 199–204.

49. Chen SU, Chang CY, Lu CC et al. Microtubular spindle dynamics and chromosome complements from somatic cell nuclei haploidization in mature mouse oocytes and developmental potential of the derived embryos. Hum Reprod 2004; 19: 1181–8.

50. Hubner K, Fuhrmann G, Christenson LK et al. Derivation of oocytes from mouse embryonic stem cells. Science 2003; 300: 1251–6.

51. Fortune JE, Cushman RA, Wahl CM et al. The primordial to primary follicle transition. Mol Cell Endocrinol 2000; 163: 53–60.

52. Skinner MK. Regulation of primordial follicle assembly and development. Hum Reprod Update 2005; 11: 461–71.

53. Peters H. The development of the mouse ovary from birth to maturity. Acta Endocrinol (Copenh) 1969; 62: 98–116.

54. Juneja SC, Barr KJ, Enders GC et al. Defects in the germ line and gonads of mice lacking connexin43. Biol Reprod 1999; 60: 1263–70.

55. Kezele PR, Ague JM, Nilsson E et al. Alterations in the ovarian transcriptome during primordial follicle assembly and development. Biol Reprod 2005; 72: 241–55.

56. Carabatsos MJ, Elvin J, Matzuk MM et al. Characterization of oocyte and follicle development in growth differentiation factor-9-deficient mice. Devel Biol 1998; 204: 373–84.

57. Gittens JE, Kidder GM. Differential contributions of connexin37 and connexin43 to oogenesis revealed in chimeric reaggregated mouse ovaries. J Cell Sci 2005; 118(Pt 21): 5071–8.

58. Uda M, Ottolenghi C, Crisponi L et al. Foxl2 disruption causes mouse ovarian failure by pervasive blockage of follicle development. Hum Mol Genet 2004; 13: 1171–81.

59. Schmidt D, Ovitt CE, Anlag K et al. The murine winged-helix transcription factor Foxl2 is required for granulosa cell differentiation and ovary maintenance. Development 2004; 131: 933–42.

60. Kol S, Adashi EY. Intraovarian factors regulating ovarian function. Curr Opin Obstet Gynecol 1995; 7: 209–13.

61. Kumar TR, Wang Y, Lu N et al. Follicle stimulating hormone is required for ovarian follicle maturation but not male fertility. Nat Genet 1997; 15: 201–4.

62. Soyal SM, Amleh A, Dean J. FIGalpha, a germ cell-specific transcription factor required for ovarian follicle formation. Development 2000; 127: 4645–54.

63. Bayne RA, Martins da Silva SJ, Anderson RA. Increased expression of the FIGLA transcription factor is associated with primordial follicle formation in the human fetal ovary. Mol Hum Reprod 2004; 10: 373–81.

64. Spears N, Molinek MD, Robinson LL et al. The role of neurotrophin receptors in female germ-

04-Human Oocytes-chapter04-ppp.i74 74 14/9/06 16:20:04

cell survival in mouse and human. Development 2003; 130: 5481–91.

65. Paredes A, Romero C, Dissen GA et al. TrkB receptors are required for follicular growth and oocyte survival in the mammalian ovary. Devel Biol 2004; 267: 430–49.

66. Durlinger AL, Gruijters MJ, Kramer P et al. Anti-Mullerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. Endocrinology 2002; 143: 1076–84.

67. Salmon NA, Handyside AH, Joyce IM. Expression of Sox8, Sf1, Gata4, Wt1, Dax1, and Fog2 in the mouse ovarian follicle: implications for the regulation of Amh expression. Mol Reprod Dev 2005; 70: 271–7.

68. Schmidt KL, Kryger-Baggesen N, Byskov AG et al. Anti-Mullerian hormone initiates growth of human primordial follicles in vitro. Mol Cell Endocrinol 2005; 234: 87–93.

69. Visser JA, Themmen AP. Anti-Mullerian hormone and folliculogenesis. Mol Cell Endocrinol 2005; 234: 81–6.

70. Ficicioglu C, Kutlu T, Baglam E et al. Early follicular antimullerian hormone as an indicator of ovarian reserve. Fertil Steril 2006; 85: 592–6.

71. Gandolfi TA, Gandolfi F. The maternal legacy to the embryo: cytoplasmic components and their effects on early development. Theriogenology 2001; 55: 1255–76.

72. Sirard MA. Resumption of meiosis: mechanism involved in meiotic progression and its relation with developmental competence. Theriogenology 2001; 55: 1241–54.

73. Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. Endocr Rev 1996; 17: 121–55.

74. Gougeon A. Ovarian follicular growth in humans: ovarian ageing and population of growing follicles. Maturitas 1998; 30: 137–42.

75. Schultz RM, LaMarca MJ, Wassarman PM. Absolute rates of protein synthesis during meiotic maturation of mammalian oocytes in vitro. Proc Natl Acad Sci USA 1978; 75: 4160–4.

76. Wassarman PM, Kinloch RA. Gene expression during oogenesis in mice. Mutat Res 1992; 296: 3–15.

77. Tian Q, Kopf GS, Brown RS et al. Function of basonuclin in increasing transcription of the ribosomal RNA genes during mouse oogenesis. Development 2001; 128: 407–16.

78. Kaplan G, Abreu SL, Bachvarova R. rRNA accumulation and protein synthetic patterns in growing mouse oocytes. J Exp Zool 1982; 220: 361–70.

79. Roest HP, Baarends WM, de Wit J et al. The ubiquitin-conjugating DNA repair enzyme HR6A is a maternal factor essential for early embryonic development in mice. Mol Cell Biol 2004; 24: 5485–95.

80. Sorensen RA, Wassarman PM. Relationship between growth and meiotic maturation of the mouse oocyte. Devel Biol 1976; 50: 531–6.

81. Wickramasinghe D, Ebert KM, Albertini DF. Meiotic competence acquisition is associated with the appearance of M-phase characteristics in growing mouse oocytes. Devel Biol 1991; 143: 162–72.

82. Eppig JJ, Schroeder AC. Capacity of mouse oocytes from preantral follicles to undergo embryogenesis and development to live young after growth, maturation, and fertilization in vitro. Biol Reprod 1989; 41: 268–76.

83. Bouniol-Baly C, Hamraoui L, Guibert J et al. Differential transcriptional activity associated with chromatin configuration in fully grown mouse germinal vesicle oocytes. Biol Reprod 1999; 60: 580–7.

84. Zuccotti M, Boiani M, Garagna S et al. Analysis of aneuploidy rate in antral and ovulated mouse oocytes during female aging. Mol Reprod Dev 1998; 50: 305–12.

85. Bjerregaard B, Maddox-Hyttel P. Regulation of ribosomal RNA gene expression in porcine oocytes. Anim Reprod Sci 2004; 82–83: 605–16.

86. Rajkovic A, Matzuk MM. Functional analysis of oocyte-expressed genes using transgenic models. Mol Cell Endocrinol 2002; 187: 5–9.

87. Neilson L, Andalibi A, Kang D et al. Molecular phenotype of the human oocyte by PCR-SAGE. Genomics 2000; 63: 13–24.

88. Robert C, Barnes FL, Hue I et al. Subtractive hybridization used to identify mRNA associ-

04-Human Oocytes-chapter04-ppp.i75 75 14/9/06 16:20:04

ated with the maturation of bovine oocytes. Mol Reprod Dev 2000; 57: 167–75.

89. Tanaka M, Hennebold JD, Miyakoshi K et al. The generation and characterization of an ovary-selective cDNA library. Mol Cell Endocrinol 2003; 202: 67–9.

90. Rajkovic A, Yan MSC, Klysik M et al. Discovery of germ cell-specific transcripts by expressed sequence tag database analysis. Fertil Steril 2001; 76: 550–4.

91. Stanton JL, Bascand M, Fisher L et al. Gene expression profiling of human GV oocytes: an analysis of a profile obtained by Serial Analysis of Gene Expression (SAGE). J Reprod Immunol 2002; 53: 193–201.

92. Goto T, Jones GM, Lolatgis N et al. Identification and characterisation of known and novel transcripts expressed during the final stages of human oocyte maturation. Mol Reprod Dev 2002; 62: 13–28.

93. Vallee M, Gravel C, Palin MF et al. Identification of novel and known oocyte-specific genes using complementary DNA subtraction and microarray analysis in three different species. Biol Reprod 2005; 73: 63–71.

94. Arraztoa JA, Zhou J, Marcu D et al. Identification of genes expressed in primate primordial oocytes. Hum Reprod 2005; 20: 476–83.

95. Pennetier S, Uzbekova S, Guyader-Joly C et al. Genes preferentially expressed in bovine oocytes revealed by subtractive and suppressive hybridization. Biol Reprod 2005; 73: 713–20.

96. Bermudez MG, Wells D, Malter H et al. Expression profiles of individual human oocytes using microarray technology. Reprod Biomed Online 2004; 8: 325–37.

97. Herrera L, Ottolenghi C, Garcia-Ortiz JE et al. Mouse ovary developmental RNA and protein markers from gene expression profiling. Devel Biol 2005; 279: 271–90.

98. Doneda L, Klinger FG, Larizza L et al. KL/KIT co-expression in mouse fetal oocytes. Int J Devel Biol 2002; 46: 1015–21.

99. Thomas FH, Ethier JF, Shimasaki S et al. Follicle-stimulating hormone regulates oocyte growth by modulation of expression of oocyte and granulosa cell factors. Endocrinology 2005; 146: 941–9.

100. Nilsson EE, Kezele P, Skinner MK. Leukemia inhibitory factor (LIF) promotes the primordial to primary follicle transition in rat ovaries. Mol Cell Endocrinol 2002; 188: 65–73.

101. Nilsson E, Parrott JA, Skinner MK. Basic fibroblast growth factor induces primordial follicle development and initiates folliculogenesis. Mol Cell Endocrinol 2001; 175: 123–30.

102. Kezele P, Nilsson EE, Skinner MK. Keratinocyte growth factor acts as a mesenchymal factor that promotes ovarian primordial to primary follicle transition. Biol Reprod 2005; 73: 967–73.

103. Moore GP, Lintern-Moore S. Transcription of the mouse oocyte genome. Biol Reprod 1978; 18: 865–70.

104. Eppig JJ, Wigglesworth K, Pendola FL. The mammalian oocyte orchestrates the rate of ovarian follicular development. Proc Natl Acad Sci USA 2002; 99: 2890–4.

105. Matzuk MM, Burns KH, Viveiros MM et al. Intercellular communication in the mammalian ovary: oocytes carry the conversation. Science 2002; 296: 2178–80.

106. Eppig JJ, Pendola FL, Wigglesworth K et al. Mouse oocytes regulate metabolic cooperativity between granulosa cells and oocytes: amino acid transport. Biol Reprod 2005; 73: 351–7.

107. Pangas SA, Matzuk MM. The art and artifact of GDF9 activity: cumulus expansion and the cumulus expansion-enabling factor. Biol Reprod 2005; 73: 582–5.

108. Juengel JL, McNatty KP. The role of proteins of the transforming growth factor-beta superfamily in the intraovarian regulation of follicular development. Hum Reprod Update 2005; 11: 143–60.

109. Hussein TS, Froiland DA, Amato F et al. Oocytes prevent cumulus cell apoptosis by maintaining a morphogenic paracrine gradient of bone morphogenetic proteins. J Cell Sci 2005; 118(Pt 22): 5257–68.

110. Dong J, Albertini DF, Nishimori K et al. Growth differentiation factor–9 is required during early ovarian folliculogenesis. Nature 1996; 383: 531–5.

04-Human Oocytes-chapter04-ppp.i76 76 14/9/06 16:20:05

111. Moore RK, Shimasaki S. Molecular biology and physiological role of the oocyte factor, BMP-15. Mol Cell Endocrinol 2005; 234: 67–73.

112. Simon AM, Goodenough DA, Li E et al. Female infertility in mice lacking connexin 37. Nature 1997; 385: 525–9.

113. Yan W, Ma L, Stein P et al. Mice deficient in oocyte-specific oligoadenylate synthetase-like protein OAS1D display reduced fertility. Mol Cell Biol 2005; 25: 4615–24.

114. Rajkovic A, Pangas SA, Ballow D et al. NOBOX deficiency disrupts early folliculogenesis and oocyte-specific gene expression. Science 2004; 305: 1157–9.

115. Becker M, Becker A, Miyara F et al. Differential in vivo binding dynamics of somatic and oocyte-specific linker histones in oocytes and during ES cell nuclear transfer. Mol Biol Cell 2005; 16: 3887–95.

116. Monti M, Garagna S, Redi C et al. Gonadotropins affect Oct–4 gene expression during mouse oocyte growth. Mol Reprod Dev 2006; 73: 685–91.

117. Dade S, Callebaut I, Paillisson A et al. *In silico* identification and structural features of six new genes similar to MATER specifically expressed in the oocyte. Biochem Biophys Res Commun 2004; 324: 547–53.

118. Song JL, Wessel GM. How to make an egg: transcriptional regulation in oocytes. Differentiation 2005; 73: 1–17.

119. Cohen MM Jr. The hedgehog signaling network. Am J Med Genet 2003; 123: 5–28.

120. Villaescusa JC, Allard P, Carminati E et al. Clast4, the murine homologue of human eIF4E-Transporter, is highly expressed in developing oocytes and post-translationally modified at meiotic maturation. Gene 2006; 367: 101–9.

121. Kaygun H, Marzluff WF. Translation termination is involved in histone mRNA degradation when DNA replication is inhibited. Mol Cell Biol 2005; 25: 6879–88.

122. Allard P, Yang Q, Marzluff WF et al. The stem-loop binding protein regulates translation of histone mRNA during mammalian oogenesis. Devel Biol 2005; 286: 195–206.

123. Cheung P, Lau P. Epigenetic regulation by histone methylation and histone variants. Mol Endocrinol 2005; 19: 563–73.

124. Vignon X, Zhou Q, Renard JP. Chromatin as a regulative architecture of the early developmental functions of mammalian embryos after fertilization or nuclear transfer. Cloning Stem Cells 2002; 4: 363–77.

125. Marshall TW, Link KA, Petre-Draviam CE et al. Differential requirement of SWI/SNF for androgen receptor activity. J Biol Chem 2003; 278: 30605–13.

126. Kono T. Influence of epigenetic changes during oocyte growth on nuclear reprogramming after nuclear transfer. Reprod Fertil Dev 1998; 10: 593–8.

127. Obata Y, Kaneko-Ishino T, Koide T et al. Disruption of primary imprinting during oocyte growth leads to the modified expression of imprinted genes during embryogenesis. Development 1998; 125: 1553–60.

128. Gioia L, Barboni B, Turriani M et al. The capability of reprogramming the male chromatin after fertilization is dependent on the quality of oocyte maturation. Reproduction 2005; 130: 29–39.

129. Fedoriw AM, Stein P, Svoboda P et al. Transgenic RNAi reveals essential function for CTCF in H19 gene imprinting. Science 2004; 303: 238–40.

130. Okano M, Bell DW, Haber DA et al. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 1999; 99: 247–57.

131. Kim DH, Ko DS, Lee HC et al. Comparison of maturation, fertilization, development, and gene expression of mouse oocytes grown *in vitro* and *in vivo*. J Assist Reprod Genet 2004; 21: 233–40.

132. Hu Y, Betzendahl I, Cortvrindt R et al. Effects of low O_2 and ageing on spindles and chromosomes in mouse oocytes from pre-antral follicle culture. Hum Reprod 2001; 16: 737–48.

133. Paillisson A, Dade S, Callebaut I et al. Identification, characterization and metagenome analysis of oocyte-specific genes organized in clusters in the mouse genome. BMC Genomics 2005; 6: 76.

134. Tung JJ, Jackson PK. Emi1 class of proteins regulates entry into meiosis and the meiosis I to mei-

04-Human Oocytes-chapter04-ppp.i77 77 14/9/06 16:20:05

osis II transition in *Xenopus* oocytes. Cell Cycle 2005; 4: 478–82.

135. Parfenov V, Potchukalina G, Dudina L et al. Human antral follicles: oocyte nucleus and the karyosphere formation (electron microscopic and autoradiographic data). Gamete Res 1989; 22: 219–31.

136. Zuccotti M, Garagna S, Merico V et al. Chromatin organisation and nuclear architecture in growing mouse oocytes. Mol Cell Endocrinol 2005; 234: 11–17.

137. Kageyama S, Liu H, Nagata M et al. Stage specific expression of histone deacetylase 4 (HDAC4) during oogenesis and early preimplantation development in mice. J Reprod Dev 2006; 52: 99–106.

138. Borsuk E, Milik E. Fully grown mouse oocyte contains transcription inhibiting activity which acts through histone deacetylation. Mol Reprod Dev 2005; 71: 509–15.

139. Burns KH, Viveiros MM, Ren Y et al. Roles of NPM2 in chromatin and nucleolar organization in oocytes and embryos. Science 2003; 300: 633–6.

140. Tamada H, Van Thuan N, Reed P et al. Chromatin decondensation and nuclear reprogramming by nucleoplasmin. Mol Cell Biol 2006; 26: 1259–71.

141. Wu X, Viveiros MM, Eppig JJ et al. Zygote arrest 1 (Zar1) is a novel maternal-effect gene critical for the oocyte-to-embryo transition. Nat Genet 2003; 33: 1871–91.

142. Tong ZB, Gold L, Pfeifer KE et al. Mater, a maternal effect gene required for early embryonic development in mice. Nat Genet 2000; 26: 267–8.

143. Tong ZB, Gold L, De Pol A et al. Developmental expression and subcellular localization of mouse MATER, an oocyte-specific protein essential for early development. Endocrinology 2004; 145: 1427–34.

144. Hamatani T, Falco G, Carter MG et al. Age-associated alteration of gene expression patterns in mouse oocytes. Hum Mol Genet 2004; 13: 2263–78.

145. Hinkins M, Huntriss J, Miller D et al. Expression of Polycomb-group genes in human ovarian follicles, oocytes and preimplantation embryos. Reproduction 2005; 130: 883–8.

146. Richter JD. Cytoplasmic polyadenylation in development and beyond. Microbiol Mol Biol Rev 1999; 63: 446–56.

147. Liang L, Soyal SM, Amleh A, Dean J. FIGalpha, a germ cell-specific transcription factor involved in the coordinate expression of the zona pellucida genes. Development 2000; 124: 4939–47.

148. Picton H, Briggs D, Gosden R. The molecular basis of oocyte growth and development. Mol Cell Endocrinol 1998; 145: 27–37.

149. Roller RJ, Kinloch RA, Hiraoka BY et al. Gene expression during mammalian oogenesis and early embryogenesis: quantification of three messenger RNAs abundant in fully grown mouse oocytes. Development 1989; 106: 251–61.

150. Bachvarova RF. A maternal tail of poly (A): the long and the short of it. Cell 1992; 69: 895–7.

151. Tay J, Hodgman R, Richter JD. The control of cyclin B1 mRNA translation during mouse oocyte maturation. Devel Biol 2000; 221: 1–9.

152. Fuchimoto D, Mizukoshi A, Schultz RM et al. Posttranscriptional regulation of cyclin A1 and cyclin A2 during mouse oocyte meiotic maturation and preimplantation development. Biol Reprod 2001; 65: 986–93.

153. Traverso JM, Donnay I, Lequarre AS. Effects of polyadenylation inhibition on meiosis progression in relation to the polyadenylation status of cyclins A2 and B1 during in vitro maturation of bovine oocytes. Mol Reprod Dev 2005; 71: 107–14.

154. Mendez R, Hake LE, Andresson T et al. Phosphorylation of CPE binding factor by Eg2 regulates translation of c-mos mRNA. Nature 2000; 404: 302–7.

155. Dai Y, Newman B, Moor R. Translational regulation of MOS messenger RNA in pig oocytes. Biol Reprod 2005; 73: 997–1003.

156. Dai Y, Lee C, Hutchings A et al. Selective requirement for Cdc25C protein synthesis during meiotic progression in porcine oocytes. Biol Reprod 2000; 62: 519–32.

157. El Mouatassim S, Guerin P, Menezo Y. Expression of genes encoding antioxidant

04-Human Oocytes-chapter04-ppp.i78 78 14/9/06 16:20:05

enzymes in human and mouse oocytes during the final stages of maturation. Mol Hum Reprod 1999; 5: 720–5.

158. Stebbins-Boaz B, Hake LE, Richter JD. CPEB controls the cytoplasmic polyadenylation of cyclin, Cdk2 and c-mos mRNAs and is necessary for oocyte maturation in *Xenopus*. EMBO J 1996; 15: 2582–92.

159. Hodgman R, Tay J, Mendez R et al. CPEB phosphorylation and cytoplasmic polyadenylation are catalyzed by the kinase IAK1/Eg2 in maturing mouse oocytes. Development 2001; 128: 2815–22.

160. Dickson KS, Bilger A, Ballantyne S et al. The cleavage and polyadenylation specificity factor in *Xenopus laevis* oocytes is a cytoplasmic factor involved in regulated polyadenylation. Mol Cell Biol 1999; 19: 5707–17.

161. Barnard DC, Ryan K, Manley JL et al. Symplekin and xGLD–2 are required for CPEB-mediated cytoplasmic polyadenylation. Cell 2004; 119: 641–51.

162. Rouhana L, Wang L, Buter N et al. Vertebrate GLD2 poly(A) polymerases in the germline and the brain. RNA 2005; 11: 1117–30.

163. Stebbins-Boaz B, Cao Q, de Moor CH et al. Maskin is a CPEB-associated factor that transiently interacts with elF–4E. Mol Cell 1999; 4: 1017–27.

164. Barnard DC, Cao Q, Richter JD. Differential phosphorylation controls Maskin association with eukaryotic translation initiation factor 4E and localization on the mitotic apparatus. Mol Cell Biol 2005; 25: 7605–15.

165. Gray NK; Wickens M. Control of translation initiation in animals. Annu Rev Cell Dev Biol 1998; 14: 399–458.

166. Mendez R, Richter JD. Translational control by CPEB: a means to the end. Nat Rev Mol Cell Biol 2001; 2: 521–9.

167. Richter JD, Sonenberg N. Regulation of cap-dependent translation by eIF4E inhibitory proteins. Nature 2005; 433: 477–80.

168. de Moor CH, Meijer H, Lissenden S. Mechanisms of translational control by the 3′ UTR in development and differentiation. Semin Cell Dev Biol 2005; 16: 49–58.

169. Graindorge A, Thuret R, Pollet N et al. Identification of post-transcriptionally regulated *Xenopus tropicalis* maternal mRNAs by microarray. Nucleic Acids Res 2006; 34: 986–95.

170. Wassarman PM, Liu C, Litscher ES. Constructing the mammalian zona pellucida: some new pieces of an old puzzle. J Cell Sci 1996; 109(Pt 8): 2001–4.

171. Audic Y, Omilli F, Osborne HB. Postfertilization deadenylation of mRNAs in *Xenopus laevis* embryos is sufficient to cause their degradation at the blastula stage. Mol Cell Biol 1997; 17: 209–18.

172. Charlesworth A, Cox LL, MacNicol AM. Cytoplasmic polyadenylation element (CPE)- and CPE-binding protein (CPEB)-independent mechanisms regulate early class maternal mRNA translational activation in *Xenopus* oocytes. J Biol Chem 2004; 279: 17650–9.

173. Huarte J, Stutz A, O'Connell ML et al. Transient translational silencing by reversible mRNA deadenylation. Cell 1992; 69: 1021–30.

174. Paynton BV. RNA-binding proteins in mouse oocytes and embryos: expression of genes encoding Ybox, DEAD box RNA helicase, and polyA binding proteins. Devel Genet 1998; 23: 285–98.

175. Korner CG, Wormington M, Muckenthaler M et al. The deadenylating nuclease (DAN) is involved in poly(A) tail removal during the meiotic maturation of *Xenopus* oocytes. EMBO J 1998; 17: 5427–37.

176. Brevini-Gandolfi TA, Favetta LA, Mauri L et al. Changes in poly(A) tail length of maternal transcripts during *in vitro* maturation of bovine oocytes and their relation with developmental competence. Mol Reprod Dev 1999; 52: 427–33.

177. Wormington M, Searfoss AM, Hurney CA. Overexpression of poly(A) binding protein prevents maturation-specific deadenylation and translational inactivation in *Xenopus* oocytes. EMBO J 1996; 15: 900–9.

178. Alizadeh Z, Kageyama S, Aoki F. Degradation of maternal mRNA in mouse embryos: selective degradation of specific mRNAs after fertilization. Mol Reprod Dev 2005; 72: 281–290.

179. Eichenlaub-Ritter U, Peschke M. Expression in in-vivo and in-vitro growing and maturing

04-Human Oocytes-chapter04-ppp.i79 79 14/9/06 16:20:06

oocytes: focus on regulation of expression at the translational level. Hum Reprod Update 2002; 8: 21–41.

180. Mehlmann LM. Oocyte-specific expression of Gpr3 is required for the maintenance of meiotic arrest in mouse oocytes. Devel Biol 2005; 288: 397–404.

181. Freudzon L, Norris RP, Hand AR et al. Regulation of meiotic prophase arrest in mouse oocytes by GPR3, a constitutive activator of the Gs G protein. J Cell Biol 2005; 171: 255–65.

182. Hinckley M, Vaccari S, Horner K et al. The G-protein-coupled receptors GPR3 and GPR12 are involved in cAMP signaling and maintenance of meiotic arrest in rodent oocytes. Devel Biol 2005; 287: 249–61.

183. Dekel N. Cellular, biochemical and molecular mechanisms regulating oocyte maturation. Mol Cell Endocrinol 2005; 234: 19–25.

184. Lincoln AJ, Wickramasinghe D, Stein P et al. Cdc25b phosphatase is required for resumption of meiosis during oocyte maturation. Nat Genet 2002; 30: 446–9.

185. Duckworth BC, Weaver JS, Ruderman JV. G2 arrest in *Xenopus* oocytes depends on phosphorylation of cdc25 by protein kinase A. Proc Natl Acad Sci USA 2002; 99: 16794–9.

186. Perdiguero E, Nebreda AR. Regulation of Cdc25C activity during the meiotic G2/M transition. Cell Cycle 2004; 3: 733–7.

187. Han SJ, Conti M. New pathways from PKA to the Cdc2/cyclin B complex in oocytes: Wee1B as a potential PKA substrate. Cell Cycle 2006; 5: 227–31.

188. Masciarelli S, Horner K, Liu C et al. Cyclic nucleotide phosphodiesterase 3A-deficient mice as a model of female infertility. J Clin Invest 2004; 114: 196–205.

189. Richard FJ, Tsafriri A, Conti M. Role of phosphodiesterase type 3A in rat oocyte maturation. Biol Reprod 2001; 65: 1444–51.

190. Kawamura K, Kumagai J, Sudo S et al. Paracrine regulation of mammalian oocyte maturation and male germ cell survival. Proc Natl Acad Sci USA 2004; 101: 7323–8.

191. Polanski Z, Ledan E, Brunet S et al. Cyclin synthesis controls the progression of meiotic maturation in mouse oocytes. Development 1998; 125: 4989–97.

192. Mitra J, Schultz RM. Regulation of the acquisition of meiotic competence in the mouse: changes in the subcellular localization of cdc2, cyclin B1, cdc25C and wee1, and in the concentration of these proteins and their transcripts. J Cell Sci 1996; 109(Pt 9): 2407–15.

193. Lazar S, Gershon E, Dekel N. Selective degradation of cyclin B1 mRNA in rat oocytes by RNA interference (RNAi). J Mol Endocrinol 2004; 33: 73–85.

194. Revenkova E, Jessberger R. Keeping sister chromatids together: cohesins in meiosis. Reproduction 2005; 130: 783–90.

195. Kubiak JZ, Weber M, de Pennart H et al. The metaphase II arrest in mouse oocytes is controlled through microtubule-dependent destruction of cyclin B in the presence of CSF. EMBO J 1993; 12: 3773–8.

196. Heikinheimo O, Lanzendorf SE, Baka SG et al. Cell cycle genes c-mos and cyclin-B1 are expressed in a specific pattern in human oocytes and preimplantation embryos. Hum Reprod 1995; 10: 699–707.

197. Suzumori N, Burns KH, Yan W et al. RFPL4 interacts with oocyte proteins of the ubiquitin-proteasome degradation pathway. Proc Natl Acad Sci USA 2003; 100: 550–5.

198. Gebauer F, Richter JD. Synthesis and function of Mos: the control switch of vertebrate oocyte meiosis. Bioessays 1997; 19: 23–8.

199. Sagata N. What does Mos do in oocytes and somatic cells? Bioessays 1997; 19: 13–21.

200. Araki K, Naito K, Haraguchi S et al. Meiotic abnormalities of c-mos knockout mouse oocytes: activation after first meiosis or entrance into third meiotic metaphase. Biol Reprod 1996; 55: 1315–24.

201. Irniger S. Preventing fatal destruction: inhibitors of the anaphase-promoting complex in meiosis. Cell Cycle 2006; 5: 405–15.

202. Pinsky BA, Biggins S. The spindle checkpoint: tension versus attachment. Trends Cell Biol 2005; 15: 486–93.

04-Human Oocytes-chapter04-ppp.i80 80 14/9/06 16:20:06

203. Homer HA, McDougall A, Levasseur M et al. Mad2 is required for inhibiting securin and cyclin B degradation following spindle depolymerisation in meiosis I mouse oocytes. Reproduction 2005; 130: 829–43.

204. Vogt E, Betzendahl I, Eichenlaub-Ritter U. Model for aging: knockdown of Mad2 expression predisposes to non-disjunction in mammalian oocytes possessing aberrant spindles. Hum Reprod 2005, 20(Suppl 1):i70.

205. Paronetto MP, Giorda E, Carsetti R et al. Functional interaction between p90Rsk2 and Emi1 contributes to the metaphase arrest of mouse oocytes. EMBO J 2004; 23: 4649–59.

206. Winston NJ, Maro B. Calmodulin-dependent protein kinase II is activated transiently in ethanol-stimulated mouse oocytes. Devel Biol 1995; 170: 350–2.

207. Hansen DV, Tung JJ, Jackson PK. CaMKII and polo-like kinase 1 sequentially phosphorylate the cytostatic factor Emi2/XErp1 to trigger its destruction and meiotic exit. Proc Natl Acad Sci USA 2006; 103: 608–13.

208. Liu J, Maller JL. Calcium elevation at fertilization coordinates phosphorylation of XErp1/Emi2 by Plx1 and CaMK II to release metaphase arrest by cytostatic factor. Curr Biol 2005; 15: 1458–68.

209. Wianny F, Tavares A, Evans MJ et al. Mouse polo-like kinase 1 associates with the acentriolar spindle poles, meiotic chromosomes and spindle midzone during oocyte maturation. Chromosoma 1998; 107: 430–9.

210. Pahlavan G, Polanski Z, Kalab P et al. Characterization of polo-like kinase 1 during meiotic maturation of the mouse oocyte. Devel Biol 2000; 220: 392–400.

211. Anger M, Klima J, Kubelka M et al. Timing of Plk1 and MPF activation during porcine oocyte maturation. Mol Reprod Dev 2004; 69: 11–16.

212. Terret ME, Lefebvre C, Djiane A et al. DOC1R: a MAP kinase substrate that control microtubule organization of metaphase II mouse oocytes. Development 2003; 130: 5169–77.

213. Lefebvre C, Terret ME, Djiane A et al. Meiotic spindle stability depends on MAPK-interacting and spindle-stabilizing protein (MISS), a new MAPK substrate. J Cell Biol 2002; 157: 603–13.

214. Vinot S, Le T, Maro B et al. Two PAR6 proteins become asymmetrically localized during establishment of polarity in mouse oocytes. Curr Biol 2004; 14: 520–5.

215. Leader B, Lim H, Carabatsos MJ et al. Formin-2, polyploidy, hypofertility and positioning of the meiotic spindle in mouse oocytes. Nat Cell Biol 2002; 4: 921–8.

216. Hewitson L, Dominko T, Takahashi D et al. Unique checkpoints during the first cell cycle of fertilization after intracytoplasmic sperm injection in rhesus monkeys. Nat Med 1999; 5: 431–3.

217. Oh B, Hwang SY, Solter D et al. Spindlin, a major maternal transcript expressed in the mouse during the transition from oocyte to embryo. Development 1997; 124: 493–503.

218. Oh B, Hwang S, McLaughlin J et al. Timely translation during the mouse oocyte-to-embryo transition. Development 2000; 127: 3795–803.

219. Yao YQ, Xu JS, Lee WM et al. Identification of mRNAs that are up-regulated after fertilization in the murine zygote by suppression subtractive hybridization. Biochem Biophys Res Commun 2003; 304: 60–6.

220. Simerly C, Dominko T, Navara C et al. Molecular correlates of primate nuclear transfer failures. Science 2003; 300: 297.

221. Antczak M, Van Blerkom J. Temporal and spatial aspects of fragmentation in early human embryos: possible effects on developmental competence and association with the differential elimination of regulatory proteins from polarized domains. Hum Reprod 1999; 14: 429–47.

222. Edwards RG. Genetics of polarity in mammalian embryos. Reprod Biomed Online 2005; 11: 104–14.

223. Deb K, Sivaguru M, Yong HY et al. Cdx2 gene expression and trophectoderm lineage specification in mouse embryos. Science 2006; 311: 992–6.

224. Preiss T, Hentze MW. Starting the protein synthesis machine: eukaryotic translation initiation. Bioessays 2003; 25: 1201–11.

225. Wilkie GS, Gautier P, Lawson D et al. Embryonic poly(A)-binding protein stimulates translation in germ cells. Mol Cell Biol 2005; 25: 2060–71.

04-Human Oocytes-chapter04-ppp.i81 81 14/9/06 16:20:07

226. Seli E, Lalioti MD, Flaherty SM et al. An embryonic poly(A)-binding protein (ePAB) is expressed in mouse oocytes and early preimplantation embryos. Proc Natl Acad Sci USA 2005; 102: 367–72.

227. Kuge H, Brownlee GG, Gershon PD et al. Cap ribose methylation of c-mos mRNA stimulates translation and oocyte maturation in *Xenopus laevis*. Nucleic Acids Res 1998; 26: 3208–14.

228. Pyronnet S, Imataka H, Gingras AC et al. Human eukaryotic translation initiation factor 4G (eIF4G) recruits mnk1 to phosphorylate eIF4E. EMBO J 1999; 18: 270–9.

229. Keiper BD, Rhoads RE. Translational recruitment of *Xenopus* maternal mRNAs in response to poly(A) elongation requires initiation factor eIF4G–1. Devel Biol 1999; 206: 1–14.

230. Sommerville J, Ladomery M. Transcription and masking of mRNA in germ cells: involvement of Y-box proteins. Chromosoma 1996; 10: 435–43.

231. Braddock M, Muckenthaler M, White MR et al. Intron-less RNA injected into the nucleus of *Xenopus* oocytes accesses a regulated translation control pathway. Nucleic Acids Res 1994; 22: 5255–64.

232. Tekur S, Pawlak A, Guellaen G et al. Contrin, the human homologue of a germ-cell Y-box-binding protein: cloning, expression, and chromosomal localization. J Androl 1999; 20: 135–44.

233. Yang J, Medvedev S, Reddi PP et al. The DNA/RNA-binding protein MSY2 marks specific transcripts for cytoplasmic storage in mouse male germ cells. Proc Natl Acad Sci USA 2005; 102: 1513–18.

234. Yang J, Medvedev S, Yu J et al. Absence of the DNA-/RNA-binding protein MSY2 results in male and female infertility. Proc Natl Acad Sci USA 2005; 102: 5755–60.

235. Sasayama T, Marumoto T, Kunitoku N et al. Over-expression of Aurora-A targets cytoplasmic polyadenylation element binding protein and promotes mRNA polyadenylation of Cdk1 and cyclin B1. Genes Cells 2005; 10: 627–38.

236. Yao LJ, Zhong ZS, Zhang LS et al. Aurora-A is a critical regulator of microtubule assembly and nuclear activity in mouse oocytes, fertilized eggs, and early embryos. Biol Reprod 2004; 70: 1392–9.

237. Nakanishi T, Kubota H, Ishibashi N et al. Possible role of mouse poly(A) polymerase mGLD-2 during oocyte maturation. Devel Biol 2006; 289: 115–26.

238. Peset I, Seiler J, Sardon T et al. Function and regulation of Maskin, a TACC family protein, in microtubule growth during mitosis. J Cell Biol 2005; 170: 1057–66.

239. Sakurai T, Sato M, Kimura M. Diverse patterns of poly(A) tail elongation and shortening of murine maternal mRNAs from fully grown oocyte to 2-cell embryo stages. Biochem Biophys Res Commun 2005; 336: 1181–9.

240. Stebbins-Boaz B, Richter JD. Translational control during early development. Crit Rev Eukaryot Gene Expr 1997; 7: 73–94.

241. Collier B, Gorgoni B, Loveridge C et al. The DAZL family proteins are PABP-binding proteins that regulate translation in germ cells. EMBO J 2005; 24: 2656–66.

242. Cauffman G, Van de Velde H, Liebaers I et al. DAZL expression in human oocytes, preimplantation embryos and embryonic stem cells. Mol Hum Reprod 2005; 11: 405–11.

243. Walters KA, Binnie JP, Campbell BK et al. The effects of IGF-I on bovine follicle development and IGFBP-2 expression are dose and stage dependent. Reproduction 2006; 131: 515–23.

244. Steuerwald N, Cohen J, Herrera RJ et al. Association between spindle assembly checkpoint expression and maternal age in human oocytes. Mol Hum Reprod 2001; 7: 49–55.

245. Stein P, Svoboda P, Schultz RM.Transgenic RNAi in mouse oocytes: a simple and fast approach to study gene function. Devel Biol 2003; 256: 187–93.

246. De Moor CH, Richter JD. Translational control in vertebrate development. Int Rev Cytol 2001; 203: 567–608.

04-Human Oocytes-chapter04-ppp.i82 82 14/9/06 16:20:07