Re-starting life: Fertilization and the transition from meiosis to mitosis

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Abstract

Fertilization triggers a complex cellular programme that transforms two highly specialized meiotic germ cells, the oocyte and the sperm, into a totipotent mitotic embryo: linkages between sister chromatids are remodelled to support the switch from reductional meiotic to equational mitotic divisions; the centrosome, which is absent from the egg, needs to be reintroduced; the axis of cell division is shifted from extremely asymmetric to symmetric; genomic imprinting is selectively erased and re-established; and protein expression shifts from translational control back to transcriptional control. Recent work has started to reveal how this remarkable transition from meiosis to mitosis is achieved.

During fertilization of an egg with a sperm, the haploid genomes of each parent are unified to form the diploid genome of a new and unique individual. Yet the road to reproduction begins a long time before the fusion of male and female gametes. In females, egg precursor cells, termed oocytes, are stored in the ovary from before birth. It is generally thought that oocytes are not replenished after birth, but this dogma of developmental biology has recently been challenged[12]. The oocytes have already undergone meiotic DNA replication and recombination that ensures the genetic diversity of potential offspring (FIG. 1b). The stored oocytes are arrested in meiotic prophase and surrounded by somatic cells in a functional unit termed primordial follicle. Periodically some primordial follicles initiate a prolonged growth phase. The somatic cells that surround the oocyte divide and feed the oocyte with precursors of macromolecules through gap junctions[3]. The oocyte increases in size and accumulates all the storage material necessary to support the development of the early embryo. Once every menstrual cycle a surge of gonadotropins induces the meiotic maturation of these fully grown oocytes into fertilizable eggs. Still in the follicle, the oocyte nucleus breaks down and a microtubule spindle assembles around the chromosomes. The spindle then migrates to the oocyte surface and segregates half the homologous chromosomes into a small cell termed polar body. The remaining chromosomes are captured by a second meiotic spindle, and the egg remains arrested at this stage awaiting fertilization by a sperm (FIG. 1b). While the oocyte matures, which takes 12-14 hours in mice and more than 24 hours in humans, a mucified matrix develops between the somatic cells of the follicle. The matrix expands and ruptures the surface of the follicle so that the egg can be released into the oviduct.

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In the oviduct, the sperm binds to the zona pellucida, a glycoprotein matrix that surrounds the egg, and the gametes fuse to form the zygote. The egg resumes meiosis and segregates half of the remaining sister chromatids into a second polar body (FIG. 1b). The male and female haploid pronuclei form and migrate towards each other before the first mitotic spindle assembles around the now diploid zygotic genome. A series of mitotic cell divisions then produce smaller embryonic cells, termed blastomeres. The blastomeres start to adhere to each other in the 8 cell stage and undergo compaction to form a solid ball of cells known as morula. The two subsequent cell divisions generate two different populations of cells; those that occupy the inside of the embryo, which contribute to the embryo proper, and those that occupy the outside, which will give rise to the extraembryonic tissue that is required to support embryo development in the uterus. At the 32 cell stage, a fluid-filled cavity begins to form inside the embryo. This cavity continues to grow as the embryo matures into a blastocyst. By this time, the embryo has migrated into the uterus. The blastocyst hatches from the zona pellucida and implants into the uterine wall, where the embryo continues to develop (FIG. 1a).

The transition from egg to embryo is perhaps one of the most dramatic and complex cell transformations in human biology: two highly differentiated gametes fuse, and the resulting cell, the zygote, is capable of dividing to generate all the cells of the human body. In this review we discuss the remarkable changes to the cellular machinery that govern this transition. We begin by reviewing our current knowledge of the fertilization process, with emphasis on sperm-egg binding and exit from meiotic arrest. We then discuss the recent advances in our understanding of the egg to embryo transition, with particular focus on the shift from meiosis to mitosis. Fertilization-triggered changes to chromosomes, the microtubule spindle, cell division symmetry and gene expression regulation will all be discussed. We mainly focus on mammals, but refer to findings from non-mammalian organisms when relevant for understanding the human condition.

Fertilization

Sperm-egg binding

Oscar Hertwig first recognised that fertilization involves the fusion of sperm and egg cells (BOX 1). Sperm initially bind to the egg's zona pellucida[4] (FIG. 2a), which is made up of just a few glycoproteins, ZP1 to ZP3 in mice and ZP1 to ZP4 in humans, yet the precise molecules that mediate mammalian sperm-egg binding have remained elusive to researchers. Early studies identified ZP3 as a potential primary sperm receptor[5,6], however, it seems unlikely that ZP3 alone is sufficient for sperm binding, because mouse eggs in which mouse ZP3 is replaced with the human ZP3 are unable to bind human sperm[7].

An alternative possibility is that the zona pellucida proteins together adopt a three-dimensional structure that presents a binding site for sperm[8]. Such a binding site would be predicted to be lost upon the cleavage of ZP2 that occurs after fertilization[9], providing a possible mechanism for preventing polyspermy. Indeed, the protease responsible for ZP2 cleavage, Ovastacin, was recently identified. Ovastacin is a component of cortical granules, which fuse with the egg plasma membrane and release their contents after fertilization[10]. Crucially, sperm can bind to 2-cell embryos isolated from mice expressing non-cleavable

ZP2, but not to wild type embryos whose ZP2 is presumably cleaved. These findings are consistent with a model in which sperm-egg binding depends on the cleavage status of ZP2[11, 12] (FIG. 2b). In the future it would be interesting to utilize the tobacco etch virus (TEV) protease technology[13] to test if ZP2 cleavage is sufficient to prevent sperm binding.

The zona pellucida proteins are glycoproteins modified with oligosaccharide sequences at asparagine (N-linked) and/or threonine (O-linked) residues[4]. A recent study of unfertilized human eggs found that the majority of oligosaccharides terminated with the Sialyl-Lewis^X tetrasaccharide motif[14]. Consistent with a function of the Sialyl-Lewis^X motif in sperm-egg binding, Sialyl-Lewis^X oligosaccharides or anti Sialyl-Lewis^X antibodies interfere with sperm-egg binding *in vitro*, and the removal of sialic acid from solubilized zona pellucida reduces its affinity for sperm[14]. An important challenge for the future will be to identify the sperm-bound receptor responsible for binding to the Sialyl-Lewis^X motif.

Although a complete understanding of mammalian sperm-egg interaction is still lacking, one model that encompasses much of the available data is that the three-dimensional architecture of the zona pellucida, which depends on the cleavage state of ZP2, serves to present oligosaccharide chains in a way that permits sperm binding[15] (FIG. 2b.). Further work will be necessary to test this model, and how variations on a theme ensure species-specific sperm-egg-binding. Once bound, the sperm releases a specialised secretory vesicle, the acrosome, which contains a mix of hydrolytic and proteolytic enzymes that once released pave a way through the zona pellucida promoting plasma membrane fusion[16].

Ca^{2+} triggers exit from meiosis

The fusion of the sperm with the egg's plasma membrane triggers the completion of the second meiotic division and the transition to mitosis. How does the sperm trigger this transition? The sperm induces a rise in free Ca^{2+} in the egg, which was first observed more than 30 years ago in medaka and sea urchin eggs and subsequently confirmed in all species studied to date[17, 18]. In mammals, a series of Ca^{2+} oscillations triggers a temporally ordered sequence of events, including the release of cortical granules, the completion of the second meiotic division, translation of maternal mRNAs and ultimately the transition from meiosis to mitosis[19, 20]. The increase in Ca^{2+} in mammalian eggs is likely to be initiated by the phospholipase C-zeta (PLCζ)[21], which is introduced into the egg by the sperm. PLCζ promotes the production of inositol 1,4,5-trisphosphate (IP$_3$), which then binds to receptors on the endoplasmic reticulum, causing the release of Ca^{2+}[22, 23] (FIG. 2c). Recent studies have shed light on how the increase in Ca^{2+} triggers the exit from meiosis. All vertebrate eggs, including human eggs, arrest in metaphase of the second meiotic division while they await fertilization. This arrest is mediated by an activity termed cytostatic factor (CSF) that was first found by Masui and Markert in 1971[24]. An essential mediator of CSF activity is Emi2. Emi2 maintains metaphase II arrest by inhibiting the anaphase-promoting complex / cyclosome (APC/C), which targets cyclin B and the separase inhibitor securin for degradation[25-29]. A recent study revealed that Emi2 also regulates APC/C activity during the early mitotic divisions of Xenopus embryos[30], and it will be interesting to investigate why the function of Emi2 as an APC/C regulator continues beyond meiosis into the mitotic divisions. The increase in intracellular Ca^{2+} upon fertilization activates Ca^{2+}/calmodulin-

dependent protein kinase II (CaMKII), which phosphorylates Emi2[26]. Emi2 is further phosphorylated by Plk1[26, 27, 31], and subsequently targeted for degradation by the SCF ubiquitin ligase[31] (FIG. 2c). This leads to the activation of the APC/C, destruction of cyclin B and securin, elimination of half of the sister chromatids into the second polar body and the formation of the female pronucleus.

From meiotic to mitotic chromosomes

Upon fertilization, the parental genomes must be reprogrammed for totipotency, and the cell cycle machinery must switch from meiotic (reductional) to mitotic (equational) chromosome segregation. In this section we will discuss recent work that has begun to provide a molecular understanding of fertilization-triggered changes to chromosomes.

Chromatin remodelling

At fertilization, the maternal genome is contained within individualised chromosomes arrested in metaphase II. The paternal genome is tightly compacted for packaging into the sperm head. This is achieved by replacing most nucleosomes with protamines that are rich in positively charged amino acids which form a nucleoprotamine complex with the negatively charged DNA[32]. Therefore in the zygote, there initially exists a striking difference in the chromatin state of the parental genomes that must be resolved to ensure accurate segregation of chromosomes during the first mitotic division. Almost immediately after fertilization this disparity begins to be resolved: the increase in intracellular Ca^{2+} triggers exit from meiosis and the formation of a female pronucleus, whilst the sperm genome undergoes decompaction. Protamines are rapidly removed from the sperm pronucleus and the DNA is re-wrapped around nucleosomes that contain the histone H3 variant H3.3, which is replaced with canonical histone H3 during DNA replication[33, 34] (FIG. 3a). Although now equivalently structured in nucleosomes, the two pronuclei retain some parent-specific histone methylation patterns, particularly at pericentromeric heterochromatin regions, which are equilibrated gradually during the first embryonic divisions[35].

For the zygote to acquire totipotency, the parental genomes must also undergo extensive epigenetic reprogramming, which involves global DNA demethylation. The sperm genome is highly methylated compared to that of the egg[36], perhaps reflecting terminal differentiation and dense chromatin packaging within the sperm. Within hours of fertilization, however, the sperm pronucleus undergoes rapid active demethylation prior to DNA replication[37, 38]. Recently, this was found to be mediated by the oxidation of 5-methyl cytosine by the DNA dioxygenase Tet3[39-41], yet the precise mechanisms involved remain poorly understood. Significantly, Tet3-null mice show developmental failure, suggesting that active paternal demethylation is crucial for proper embryonic development[41]. Maternal chromatin is largely protected from this initial active demethylation by the action of the DNA binding protein PGC7[42]. PGC7 binds to H3K9 methylation that is present mainly on maternal chromatin[43, 44]. During the cleavage stages of the early embryo, both parental genomes undergo passive, replication-dependent demethylation[45]. Methylation finally reaches a minimum at the blastocyst stage, prior to cell lineage specification[46] (FIG. 3a).

DNA demethylation in the embryo, however, must not go to completion. A small proportion of mammalian genes are imprinted, meaning that they are expressed from only one of the two parental chromosomes. Imprinted genes are established by differential methylation marks laid down in the sperm and egg during gametogenesis[47], which must be maintained during epigenetic reprogramming in the embryo. Imprinted loci marked by H3K9 methylation recruit PGC7, which protects against active demethylation immediately after fertilization[44]. A chromatin-modifier complex that assembles via the interaction of ZPF57 and TRIM28 also ensures that imprinted loci are spared from passive demethylation during blastomere cleavage stages[48-50].

Chromosome Segregation

Not only the chromatin itself is remodelled upon fertilization, but also the physical linkages between chromosomes have to be reorganized to support the reductional segregation of homologous chromosomes during the first meiotic division, the segregation of sister chromatids during the second meiotic division and finally the equational segregation of replicated sister chromatids during the first mitotic division of the embryo (FIG. 1b). An elegant set of experiments has found that the transition from meiosis to mitosis involves a dramatic change in the composition of the chromosomal cohesin complex[13]. The multiprotein cohesin complex forms a ring that entraps sister chromatids following DNA replication. Chromosome segregation in anaphase is triggered by cleavage of cohesin's kleisin subunit by the protease separase. This destroys the cohesin ring and therefore sister chromatid cohesion[51]. Mitotic cohesin complexes contain the Scc1 kleisin subunit. In meiotic cohesin complexes, Scc1 is largely replaced by the meiosis-specific Rec8 protein, which is essential for reductional chromosome segregation[52]. By engineering mice to express versions of Scc1 and Rec8 that can be artificially cleaved by the TEV protease, it was possible to test the relative contributions of Scc1- and Rec8-containing cohesin complexes in holding sister chromatids together before and after fertilization. Artificial Rec8 cleavage could trigger meiosis II chromosome segregation in the egg but not mitotic chromosome segregation in the zygote, whereas Scc1 cleavage had the opposite effect[13]. Therefore, the switch from meiotic to mitotic cohesin complex occurs immediately after fertilization in the zygote (FIG. 3b).

How might such a rapid switch be facilitated? The transition from egg to embryo occurs in the absence of transcription (see below). Thus, it seems likely that some proteins required for the mitotic divisions are already present before fertilization. In fact, Scc1 protein is abundant in oocytes[53, 54] and so may be readily available for loading onto chromosomes during DNA replication in the zygote. A recent genome-wide polysome profiling study revealed a 10-fold increase in translation of the mitotic cohesin proteins STAG1 and STAG2 in eggs compared to prophase oocytes[55]. This suggests that translational upregulation of the mitotic machinery just before fertilization may also contribute to the transition from meiosis to mitosis. Because all Rec8 protein is likely cleaved by separase in meiosis II, and subsequently degraded by the N-end rule pathway[56], meiotic cohesin complexes may be rapidly inactivated after fertilization, further facilitating a rapid switch to mitosis in the zygote.

Intriguingly, however, it seems that not all aspects of mitotic chromosome segregation are adopted immediately in the zygote. Mitotic cells lose chromosome cohesion in a step-wise manner: cohesion between chromosome arms is eliminated during prophase, whereas centromere cohesion is protected, giving rise to the characteristic X-shape (or V-shape in telocentric species) of metaphase chromosomes[57, 58]. Metaphase chromosomes of zygotes, however, retain chromosome arm cohesion[13]. Furthermore, mitotic chromosome spreads during early embryonic divisions suggest that arm cohesion may not be lost until the 8-cell stage of the embryo[59]. This raises the interesting question as to how cohesin regulatory mechanisms are re-tuned during the egg to embryo transition.

From meiotic to mitotic spindle

Chromosome segregation is mediated by microtubules, which assemble a bipolar spindle structure that captures and aligns chromosomes before distributing them equally between daughter cells during cell division. In somatic cells, the major microtubule organising centre (MTOC) is the centrosome, an organelle consisting of a pair of centrioles surrounded by a cloud of pericentriolar material[60]. Centrosomes are instrumental in spindle assembly during mitosis, with each centrosome forming one of the poles of the bipolar spindle (FIG. 4c). Female meiotic cells, however, are unique in that they do not contain canonical centriole-containing centrosomes[61]. In this section we discuss the mechanism of acentrosomal spindle assembly in mammalian oocytes, and how the transition from acentrosomal to centrosomal spindle assembly is achieved after fertilization.

Acentrosomal spindle assembly in oocytes

In almost all species studied to date, including humans, centrosomes are absent from oocyte meiotic spindles[61]. Electron microscopy of mouse oocytes showed that centrioles are present in fetal oocytes at pachytene stage, but not thereafter, indicative of active centriole elimination during oogenesis[62]. However, the underlying mechanisms of centriole elimination still remain poorly understood. A recent study in *C. elegans* identified a role for the mRNA stabilizing factor CGH-1 in this process[63], although factors directly affecting centriole elimination have yet to be identified.

So how do mammalian oocytes assemble a meiotic spindle without centrosomes? At the onset of meiotic maturation, mouse oocytes contain numerous acentriolar MTOCs (aMTOCs) that exhibit similar microtubule nucleation properties as centrosomes[64]. These aMTOCs appear to consist of an electron dense material[62], and contain gamma-tubulin and pericentrin, suggesting that they may be similar to the pericentriolar material of centrosomes, although their exact composition and structure is unclear. Upon nuclear envelope breakdown, GTP-bound Ran promotes a massive increase in microtubule nucleation from aMTOCs[64, 65]. The microtubules then self-assemble into a bipolar spindle through the action of the plus-end directed motor protein Kinesin-5, with aMTOCs distributed to spindle poles[64, 66] (FIG 4a). An important challenge for the future will be to address whether human oocytes employ a similar mechanism of acentrosomal spindle assembly.

Transition to centrosomal spindle assembly

When and how does the transition from acentrosomal to centrosomal spindle assembly occur following fertilization? During mouse development, this transition is a gradual process. In rodents, sperm centrioles degenerate during spermatogenesis, and the sperm brings little or no centrosome material into the egg at fertilization[67, 68]. The first three embryonic divisions in the mouse are meiosis-like, with the spindle assembling from multiple aMTOCs similar to the oocyte[69-73]. During the subsequent three divisions (morula-blastocyst), the number of aMTOCs from which the spindles are assembled gradually decreases, and some cells begin to show centrin-positive staining[73], consistent with the first appearance of centrioles[72, 74]. Also the spindle morphology gradually adopts the typical mitotic appearance: the length of the spindle decreases and the spindle poles become more focussed as the embryo develops (FIG. 4b,d). Finally, in the blastocyst, all cells assemble a spindle from two centrin-positive MTOCs, indicative of a typical centrosome-driven mitosis[73]. At about the same time, maintenance of spindle bipolarity shifts from kinesin-5-dependent to – independent[75], consistent with the observation that centrosomal spindles do not rely on kinesin-5 to maintain bipolarity[76]. Therefore, the transition from the meiotic to mitotic spindle machinery occurs gradually in mice, and involves the *de novo* production of centrioles in the blastocyst (FIG. 4d).

Whether a similar gradual transition occurs during human development, however, is unclear. In humans, sperm centrioles are not completely lost, and so the human sperm delivers one intact and one partially degenerate centriole to the egg at fertilization[77]. Indeed, centrioles can be detected at spindle poles in the human zygote[78], suggestive of a centrosomal mechanism of spindle assembly and therefore a switch from meiotic to mitotic spindle function immediately after fertilization. However, whether the human zygotic spindle is assembled exclusively by a centrosomal mechanism, or if residual aMTOCs from the egg continue to contribute to spindle assembly in the embryo has yet to be tested. In this regard, it is intriguing that sperm centrioles are not required for the initial embryonic divisions, because human parthenotes develop to the blastocyst stage[79, 80].

From asymmetric to symmetric divisions

One of the most dramatic transitions that is triggered by fertilization is the switch from the extremely asymmetric meiotic divisions of the oocyte to the symmetric division of the one cell embryo. In this section, we will review our current understanding of the molecular and cellular machineries that support these two contrasting types of divisions at the transition from meiosis to mitosis.

Asymmetric spindle positioning in oocytes

The meiotic divisions of the oocyte are the most asymmetric divisions that are known in mammals. They are essential to form a large egg, which contains sufficient storage material for embryo development. To divide asymmetrically, mammalian oocytes have to move the spindle from their centre to the cortex using an actin-dependent mechanism[81] (reviewed in[82]). Several recent studies in mouse oocytes showed that asymmetric spindle positioning requires a dynamic actin network that fills the cytoplasm and is nucleated by cooperation

between the actin nucleators Formin-2, Spire1 and Spire2[83-87] (FIG. 5a,c). The actin network includes Rab11a-positive vesicles as building blocks which recruit the network's nucleators and assemble actin filaments from the vesicles' surface[88, 89]. The vesicles also recruit the motor protein myosin-Vb, which continuously transports the vesicles along the network's actin filaments towards the plasma membrane[88] and drives the dynamics of the actin network[89] (FIG. 5d).

Recent work has shed light on how the vesicle-actin network might mediate asymmetric spindle positioning. The spindle poles locally contract the network's actin filaments in a myosin light chain kinase-dependent manner and thereby pull on the network[85]. This pulling could couple the spindle to the outward-directed movement of the Rab11a-positive vesicles and their associated actin filaments (FIG. 5d). Consistent with this model, Rab11a-positive vesicles[89], myosin Vb[89], Myosin light chain kinase activity[85] and the dynamics of the vesicle-actin network[89] are required for asymmetric spindle positioning. In addition, Mos[90, 91], Cdc42[92] and the Arp2/3 complex[93-95] have been implicated in asymmetric spindle positioning, but their precise function remains to be investigated.

A previous study suggested that the spindle might be pushed to the cortex by an actin cloud at the back of the spindle[96]. However, the actin reporter that was used in this study was later found to be unsuitable for labeling intracellular actin structures in oocytes[97]. The actin cloud signal might have represented autofluoresence from FAD^{2+} in the oocyte's mitochondria instead[98], which are clustered around the spindle[99] (FIG. 5b). Consistent with autofluorescence, the oocyte's prominent actin cortex was not labeled in the figure and movie that were shown as evidence for the actin cloud (Figure 3c-g and Movie 5[96]). Other reporters for actin in live oocytes as well as staining of fixed oocytes with fluorescent phalloidin do not detect an actin cloud pushing the spindle, but reveal the extensive actin network that fills the oocyte (FIG. 5a,c)[83, 85-87].

Also the metaphase II spindle needs to be positioned asymmetrically before polar body extrusion. The metaphase II spindle forms close to the cortex because it assembles from the spindle half that remains in the egg upon polar body extrusion. The maintenance of the metaphase II spindle at the cortex is actin-dependent and requires Rac1[100] and the Arp2/3 complex[97] (FIG. 5e). The Arp2/3 complex becomes locally activated in the cortical region overlying the spindle where it nucleates a thick cortical actin layer termed 'actin cap'[101, 102]. The Arp2/3-dependent actin cap is essential for cytoplasmic flows that have been suggested to push the metaphase II spindle against the cortex[97]. Analogously, Arp2/3-dependent cytoplasmic flows have also been suggested to promote the late stages of asymmetric spindle positioning during meiosis I[103].

Spindle centration in the embryo

In stark contrast to the asymmetric meiotic divisions, the first mitotic division after fertilization needs to be symmetrical to equally distribute storage material between the two blastomeres of the 2-cell embryo. How is this rapid switch to spindle centration achieved? A key step towards centering the first mitotic spindle is the migration of the male and female pronuclei towards the zygote's centre (FIG. 5f,g). The movement of both the male and the female pronucleus is microtubule-dependent. In most species, including humans, the male

pronucleus is associated with the centrosome that is contributed by the sperm during fertilization. The centrosome nucleates a large microtubule aster, termed sperm aster[104]. The female pronucleus is likely to associate with the sperm aster and to move towards the male pronucleus in a dynein-dependent manner[101,106,107]. But how do both pronuclei move towards the zygote's centre? Early studies suggested that pushing of sperm aster microtubules against the zygote's cortex could move the male pronucleus towards the centre (reviewed in[104]). However, cortical interactions are dispensable for pronuclear movement in sanddollar eggs[105], and microtubules emanating from the sperm aster are too short to interact with the cortex in large zygotes such as those of *Xenopus* and zebrafish[106, 107]. Several recent studies support a model in which pulling by dynein that is anchored throughout the zygote mediates the movement of the male pronucleus towards the zygote's centre[106-109]. In *C. elegans* embryos, dynein has been suggested to be anchored on intracellular organelles[109], but also the endoplasmic reticulum or other cytoskeletal structures could serves as sites for dynein anchoring[107]. How is the pronucleus moving inwards if dynein is homogenously distributed throughout the cytoplasm? A length-dependent microtubule pulling mechanism[105] could explain this point: the sperm aster's microtubules are longer towards the centre of zygote so that a higher number of dynein motors should pull the aster inward rather than outward[106, 110] (FIG. 5g).

Upon nuclear envelope breakdown, the first mitotic spindle assembles. In *C. elegans* embryos, astral microtubules interact with the polarized cortex to position the mitotic spindle. In *Xenopus* and zebrafish zygotes, the spindle's astral microtubules are too short to reach the cortex, but cytoplasmic dynein has been suggested to help position the spindle[106]. Whether dynein centers the spindle in mammalian one cell embryos, remains to be investigated. It is also unclear if the actin-dependent mechanisms that drive asymmetric spindle positioning in mammalian oocytes are completely switched off in the one cell embryo. Interestingly, data from mouse[111] and pig[112] zygotes suggest that actin is also required for pronuclear movement and spindle centration[113], but the mechanism by which actin promotes spindle centration in mammalian embryos is still unclear.

As the distinct cell lineages of the early embryo develop, asymmetry needs to re-established (reviewed in[114]). It will be interesting to analyze whether the spindle positioning mechanisms that act in the oocyte and one cell embryo are adapted to promote symmetric and asymmetric spindle positioning during later stages of early embryo development as well.

Control of gene expression

Transcription comes to a halt towards the end of the growth phase of the oocyte prior to maturation, when the chromatin is highly condensed and surrounding the nucleolus[115]. It is not until after fertilization, at the 2-cell stage in mice[116] or 4-cell stage in humans[117], that transcription fully resumes. Therefore, oocyte maturation, fertilization and the multitude of cellular modifications that drive the transition from egg to embryo all occur in the absence of transcriptional regulation. In this section, we discuss how posttranscriptional control of gene expression in mammalian oocytes ensures the storage and timely activation of maternal factors necessary for the egg to embryo transition. We further discuss the mechanisms by

which the regulation of gene expression is transferred from maternal to embryonic control after fertilization.

Translational control

During oocyte growth, the genome is actively transcribed. Some transcripts are readily translated into proteins[118]. However, many transcripts must be stockpiled and their translation prevented until oocyte maturation or until after fertilization. Indeed, there is a dramatic difference in polyribosome-associated mRNAs between prophase and MII oocytes[55], and between MII oocytes and zygotes[119], suggestive of stage-specific translation initiation of a multitude of transcripts.

Many oocyte mRNAs contain cytoplasmic polyadenylation elements (CPEs) in their 3′ untranslated region[120]. These CPEs recruit a translation-repressing complex of CPE-binding protein (CPEB) and its binding partner Maskin[121]. Such transcripts appear to be stored in oocyte-specific subcortical aggregates that may be functionally related to P bodies[122]. These dormant mRNAs can then be activated for translation by phosphorylation of CPEB, which occurs at the onset of oocyte maturation. This displaces Maskin and promotes the assembly of the translation initiation complex[121]. The importance of CPEB-mediated translation regulation is underscored by CPEB1-depleted mouse oocytes, which exhibit severe defects in oocyte development[123].

A key question is how are different mRNAs activated for translation at different times during oocyte maturation? Recently it was shown that CPEB1 activates the translation of the RNA-binding protein Dazl[55]. Dazl in turn directs its own translation as well as that of an additional subset of mRNAs that are important for meiotic spindle assembly and early embryonic development[55]. The sequential activation of two RNA binding proteins, CPEB1 and Dazl, may therefore provide one mechanism by which mammalian oocytes temporally regulate the translation of different subsets of transcripts[55]. An important challenge for the future will be to determine how fertilization triggers additional changes to the translational landscape in the zygote[119].

Eliminating the maternal stockpile

The handover from maternal to embryonic control of gene expression requires that the stockpile of maternal transcripts is significantly reduced. However, oocyte mRNAs are inherently stable, remaining in the oocyte for up to a few weeks prior to ovulation[124]. There must therefore be a dramatic change in the stability of the maternal transcript pool at some point during the fertilization process. It turns out that the degradation of excess maternal mRNA begins at the onset of oocyte maturation[125], and continues beyond fertilization, with mRNA levels reaching a minimum at the 2-cell stage in mice[126] (FIG. 6). In fact, maternal transcripts are degraded in a timely and selective manner. During oocyte maturation, transcripts involved in processes related to earlier events such as oocyte growth and prophase arrest are rapidly degraded, whereas those transcripts important for maintaining the metaphase II arrest remain intact[127]. Similarly, transcripts involved in meiotic processes, but not embryonic development, are rapidly degraded upon fertilization[128].

How are maternal transcripts degraded in mammals? Work from Schultz and colleagues have identified a critical function for the RNA-binding protein Mys2 in this process. Mouse oocytes lacking Mys2 contain less total mRNA and the stability of reporter mRNAs injected into these oocytes is substantially reduced, suggesting that Mys2 promotes global mRNA stability in oocytes[129]. A clue that regulation of Mys2 may play a role in mRNA degradation came from the finding that Mys2 is phosphorylated in a Cdk1-dependent manner during meiotic maturation[130].

Importantly, expression of a non-phosphorylatable Mys2 prevents maturation-induced mRNA degradation, whereas expression of a phosphomimetic mutant can induce mRNA degradation even in prophase-arrested oocytes[130]. Therefore, phosphorylation of Mys2 during meiotic maturation may inactivate Mys2 and trigger a transition from mRNA stability to instability[129]. Further work is required to elucidate the mechanism by which Mys2 binding confers mRNA stability and how phosphorylation inactivates Mys2 during oocyte maturation. What regulates the selectivity of transcript degradation during the oocyte to embryo transition also remains completely unknown[127, 128].

Reactivating the genome

The final step in the transition from maternal to embryonic control of gene expression involves the activation of transcription from the newly formed embryonic genome, a process termed zygotic genome activation. The vital importance of zygotic genome activation for embryo development was first realised in the 1970s, when it was found that inhibition of transcription using the RNA polymerase inhibitor α-amantin caused mouse embryos to arrest at the 2-cell stage[131, 132]. Subsequent studies have shown that zygotic genome activation begins in the zygote, where there is a low level of transcription primarily from the paternal pronucleus[133], followed by a major wave of transcription in the 2-cell embryo[116] (FIG. 6).

In general, transcription is associated with an 'open' chromatin state, which allows transcription factors to easily access the DNA[134]. Therefore, it has long been proposed that chromatin remodelling plays a key role in the initiation of zygotic genome activation[135]. Consistently, mouse embryos lacking the maternal copy of Brg1, the catalytic subunit of the SWI/SNF chromatin remodelling complex, arrest at the 2-cell stage with reduced transcription of 30% of expressed genes[136]. Brg1-depleted embryos also show decreased H3K4 dimethylation, which is a marker for transcriptionally active chromatin[136]. The localization of both Brg1 and the catalytic subunit of the ISWI chromatin remodelling complex, SNF2H, is perturbed in embryos where TIF1α (transcription intermediary factor 1α) is inhibited, and such embryos exhibit misregulated transcription of a subset of genes in the zygote[137]. Therefore, chromatin remodelling by maternal complexes appears to be an important step for zygotic genome activation in mice. The identification of additional maternal factors essential for zygotic genome activation will be necessary to further our understanding of how the genome is reactivated after fertilization.

Conclusions and perspectives

Recent work has begun to shed light on the complexity of the cellular programmes that transform two highly specialized cells, the sperm and the egg, into a totipotent mitotic embryo. Reprogramming takes place on all levels: the highly condensed chromatin of the sperm head is re-wrapped around nucleosomes; genomic imprinting is selectively erased and re-established; protein expression shifts from translational control back to transcriptional control; linkages between sister chromatids are remodelled to support the switch from reductional meiotic to equational mitotic divisions; the centrosome, which is absent from the egg, needs to be reintroduced; and the axis of cell division is shifted from extremely asymmetric to symmetric. And all this is triggered by the fusion of the sperm with egg, causing an increase in intracellular Ca^{2+} levels.

How abrupt or gradual these transitions are achieved, varies for each aspect. Several features of mitosis are adopted immediately in the zygote, for example mitotic cohesin complexes immediately replace their meiotic counterparts and cell division rapidly switches from asymmetric to symmetric. Other mitotic features develop more gradually. For example, the switch from translational to transcriptional control of protein expression is gradually achieved over the first mitotic divisions; and the switch to canonical mitotic centrosomal spindle assembly is not observed until the blastocyst stage during mouse development.

Although parts of the complex transition puzzle are beginning to emerge, we are still far from having a clear picture. What are the molecules and mechanisms involved in re-packaging the paternal genome? How is methylation at imprinted regions preserved during global reprogramming? What are the mechanisms responsible for degradation of maternal transcripts? How is transcription re-initiated in the embryo? These are just a few of the many unanswered questions that will need to be addressed in the future.

Exploring the mechanisms that drive the reprogramming of the highly specialized meiotic germ cells towards a totipotent embryo is not only of great interest from a fundamental science point of view, but also directly relevant to understanding the development of embryonic stem cells and their potential for therapeutic use in the future[138]. Independently of where you come from, the transition from meiosis to mitosis certainly represents an exciting and underexplored field for future research.

Glossary

Zona pellucida   a specialized extracellular matrix that forms a thick coat surrounding the egg.

Polyspermy   an egg is fertilized by more than one sperm.

TEV protease technology   technique of introducing the consensus sequence for the tobacco etch virus (TEV) protease into proteins so that they may be artificially cleaved *in vivo* by ectopic expression of TEV protease.

Anaphase Promoting Complex/ Cyclosome	an E3 ubiquitin ligase that triggers anaphase by ubiquitinating cyclin B and securin, targeting them for proteolysis by the proteasome.
Separase	a cysteine protease that triggers chromosome segregation by cleaving chromosomal cohesin complexes
Pronucleus	the haploid nucleus of either sperm or egg within the zygote
Reductional chromosome segregation	During meiosis I, pairs of homologous chromosomes are segregated so that each daughter cell contains half the number of chromosomes as the parent.
Equational chromosome segregation	During meiosis II and mitosis, sister chromatids are segregated so that each daughter cell contains the same number of chromosomes as the parent.
Totipotency	the ability of a cell to divide and produce all the differentiated cells of a complete organism.
Polysome	a cluster of ribosomes bound to an mRNA molecule, indicative of active translation.
N-end rule pathway	a ubiquitin-proteosome proteolysis pathway that regulates the half-life of cellular proteins based on their N-terminal amino acid residue.
Telocentric	centromeres located at the ends of chromosomes close to telomeres

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Box 1

History of the discovery of fertilization

How is an entirely new human being generated following sexual intercourse? This question has evoked human thinking since ancient times. Hippocrates (460-370 BC) argued that man and woman each contributed semen that mixed in the uterus to form the embryo, whereas Aristotle (384-322 BC) favoured a more male-centred view that the woman merely provided fertile ground for the male seed to grow. These ideas dominated thinking until the 17th century, when the combined work of William Harvey, Jan van Horne, Jan Swammerdam, Neils Stensen, Regner de Graaf and Francesco Redi led to the theory that all female organisms, including humans, produced eggs[139]. Indeed, Harvey went so far as declaring "Ex Ova Omnia", or everything from the egg[140]. Soon after, in 1677, Antonii van Leeuwenhoek built a microscope to study human semen and made the remarkable discovery of spermatozoa[141]. Therefore by the late 17th century, both key components of fertilization – the egg and sperm – had been realised, yet the relative contributions that each made to the embryo remained unclear for almost 200 years. In the early 19th century, Karl Ernst von Baer first confirmed the presence of the mammalian egg under the microscope[142], and Schleiden and Schwann postulated that egg and sperm are somewhat equivalent in that they are both cells. At around the same time it was becoming apparent that the widely observed phenomenon of heredity must somehow involve factors that are contained within egg and sperm respectively[143]. Finally, it was in 1876 that Oscar Hertwig made the seminal discovery of fertilization in sea urchins[144]. Hertwig observed that the nuclei of sperm and egg fused during fertilization[144], thereby providing a conceptual basis for genetic inheritance and settling the long standing debate over the role of the egg and sperm in the generation of new life.
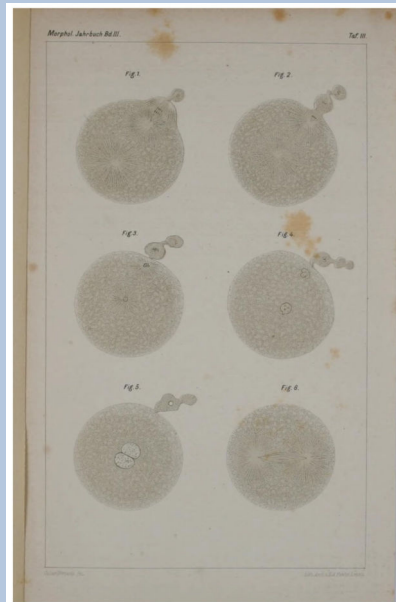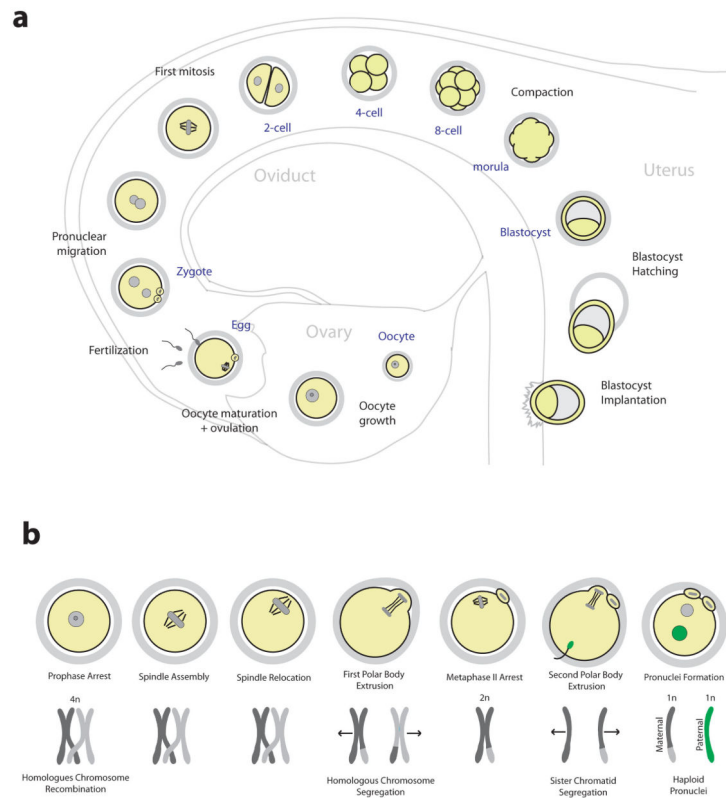
Figure 1. From oocyte to embryo

a | An overview of preimplantation development. Following ovulation, eggs are fertilized in the oviduct to form the zygote. After several mitotic divisions, the embryo undergoes compaction to form the morula. A fluid-filled cavity develops inside the embryo forming the blastocyst, which hatches from the zona pellucida to implant into the uterine wall. **b** | Stages of oocyte maturation and corresponding chromosome configuration. Following nuclear envelope breakdown the spindle assembles, relocates to the oocyte surface and segregates half the homologous chromosomes into a polar body. A spindle assembles around the remaining chromosomes and the egg arrests in metaphase II awaiting fertilization. Upon sperm binding, the egg segregates half of the sister chromatids into a second polar body. The resulting zygote contains haploid pronuclei from the mother and the father.
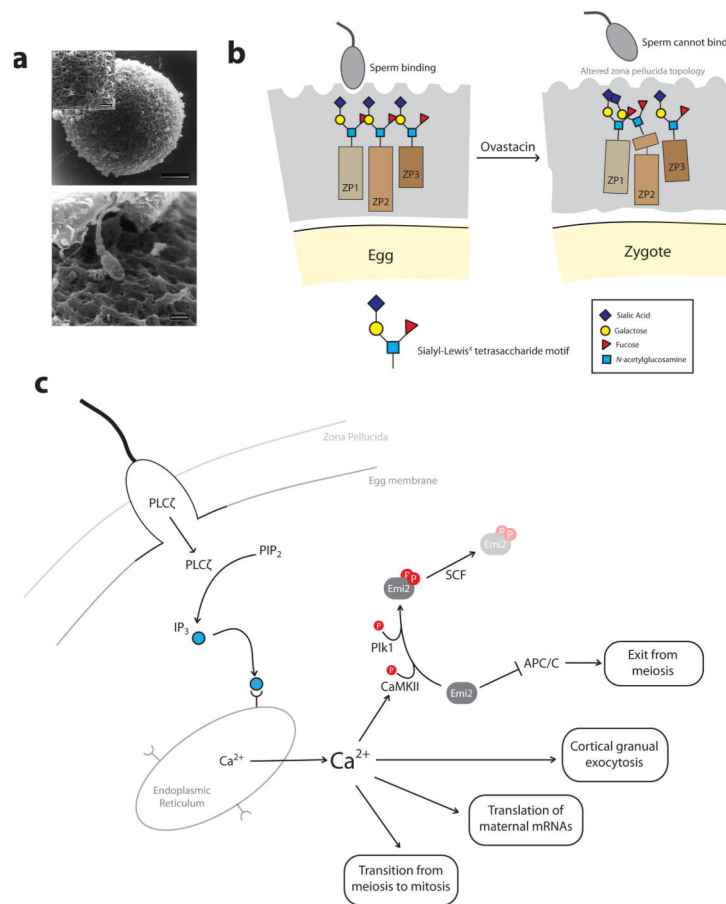
Figure 2. Fertilization: Sperm-egg binding and exit from meiosis

a | Scanning electron microscopy of a human egg surrounded by the zona pellucida (top). A human sperm can be seen approaching the surface of the zona pellucida (bottom). **b** | Schematic of sperm-egg binding. The zona pellucida (grey) consists of glycoproteins that are modified with oligosaccharide chains. Sperm can bind to the zona pellucida of unfertilized eggs. Following fertilization, cleavage of ZP2 by ovastacin renders the zona pellucida non-permissive for sperm binding. **c** | Following sperm-egg membrane fusion, sperm-derived PLCζ promotes the production of IP_3, which binds to receptors on the endoplasmic reticulum causing release of Ca^{2+}. Ca^{2+} activates CaMKII which, together with Plk1, phosphorylates Emi2 promoting its targeting for destruction by the SCF ubiquitin ligase. This releases Emi2-mediated inhibition of the APC/C, allowing the APC/C to promote exit from meiosis. The rise in intracellular Ca^{2+} also triggers cortical granule exocytosis, translation of maternal mRNAs and ultimately the transition from meiosis to mitosis. Images in part **a** are reproduced, with permission, from REF. 145 © (1998) Oxford Journals.
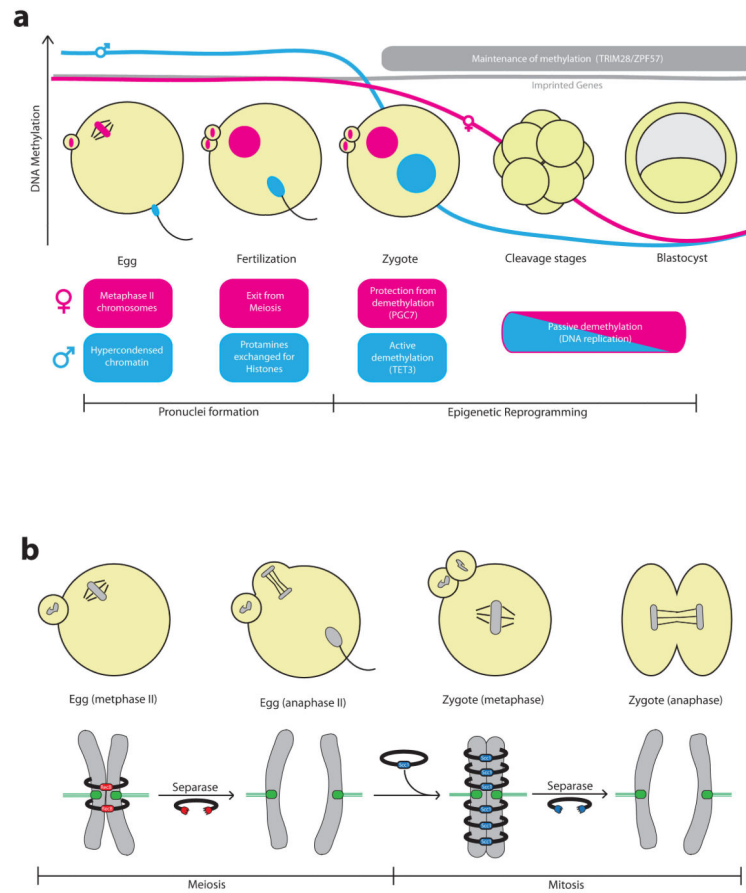
Figure 3. Transition from meiotic to mitotic chromosomes

a | Schematic of chromatin remodelling during fertilization and early embryo development. Following fertilization, the egg exits from meiosis and assembles a haploid pronucleus. At the same time the sperm genome, which enters the egg in a highly compact state, undergoes decompaction. Sperm protamines are replaced by histones and the male haploid pronucleus is formed. Maternal and paternal genomes are methylated during gametogenesis, including parental-specific methylation at imprinted genes. The sperm pronucleus undergoes rapid active demethylation in the zygote mediated by Tet3. The maternal pronucleus is largely protected from this active demethylation by the action of PGC7. Following pronuclear fusion, the zygotic genome undergoes passive demethylation during the blastomeres cleavage stages, reaching a minimum in the blastocyst. The maintenance of methylation at imprinted regions depends on the ZPF57/TRIM28 DNA-binding complex. **b** | Sister chromatids in the egg are held together by Rec8-containing cohesin complex. Rec8 cleavage by separase triggers second polar body extrusion following fertilization. Scc1-containing cohesin complexes are loaded onto chromosomes immediately in the zygote, and Scc1 cleavage by separase triggers sister chromatid segregation during the first mitotic division.
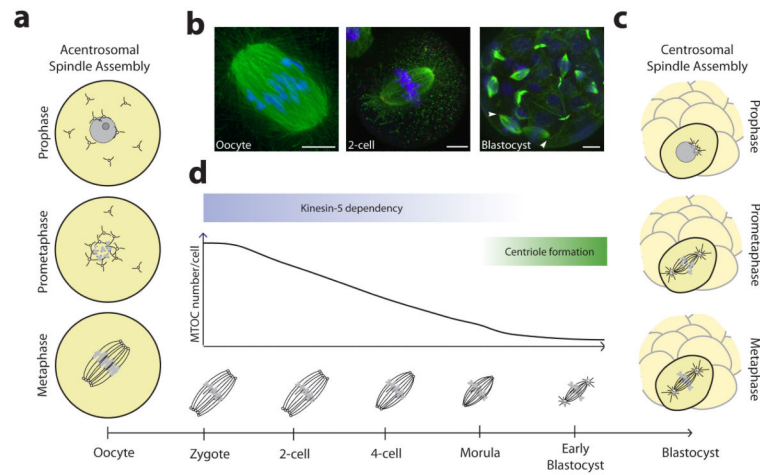
Figure 4. Transition from acentrosomal to centrosomal spindle assembly
a | Acentrosomal spindle assembly in mouse oocytes is driven by multiple acentriolar microtubules organising centres (aMTOCs) that self-assemble a bipolar spindle. **b** | An acentrosomal spindle with unfocused poles in the mouse oocyte (left) and 2-cell embryo (middle). Cells in the blastocyst assemble from centrosomes and have focused spindle poles as indicated by arrow heads (right). Scale bars: 10 μm. **c** | Centrosomal spindle assembly involves the separation of two centrosomes that act as MTOCs and form the spindle poles. **d** | The transition from acentrosomal to centrosomal spindle assembly during mouse development is a gradual process that is completed by the blastocyst stage. Image of oocyte in part **b** is courtesy of the author D.Clift. Images of 2-cell embryo and blastocyst in part **b** are reproduced, with permission, from REF. 73 © (2012) Rockefeller University Press.
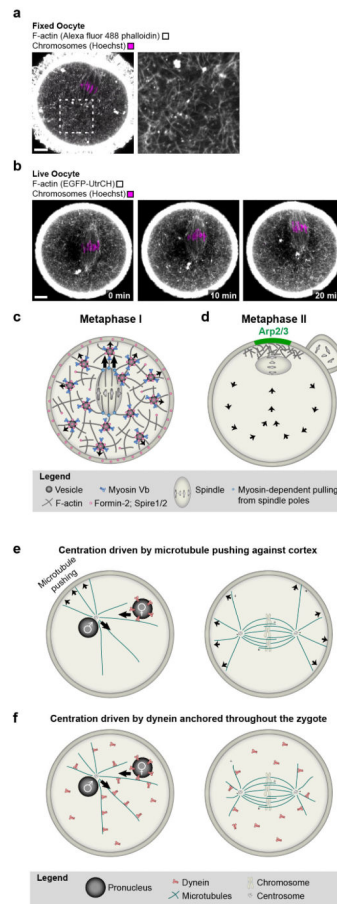
Figure 5. Transition from asymmetric to symmetric spindle positioning

a | A cytoplasmic F-actin network mediates asymmetric spindle positioning during meiosis I in mouse oocytes. F-actin (grey; Alexa fluor 488 phalloidin) and chromosomes (magenta; Hoechst) in fixed mouse oocyte. Scale bar: 10 μm. **b** | Autofluorescence (green) around spindle region and chromosomes (magenta; Hoechst) in fixed mouse oocyte. Scale bar: 10 μm. **c** | F-actin (grey; EGFP-UtrCH) and chromosomes (magenta; Hoechst) in live oocyte during asymmetric spindle positioning. Scale bar: 10 μm. **d** | Model for vesicle-actin network mediated asymmetric spindle positioning during the first meiotic division **e** | Model for how the Arp2/3-complex helps to maintain the metaphase II spindle in cortical proximity while the egg awaits fertilization. **f** | Model for centration of pronuclei and first mitotic spindle by pushing of astral microtubules against the cortex. **g** | Model for centration of pronuclei and first mitotic spindle driven by dynein that is anchored throughout the cytoplasm.
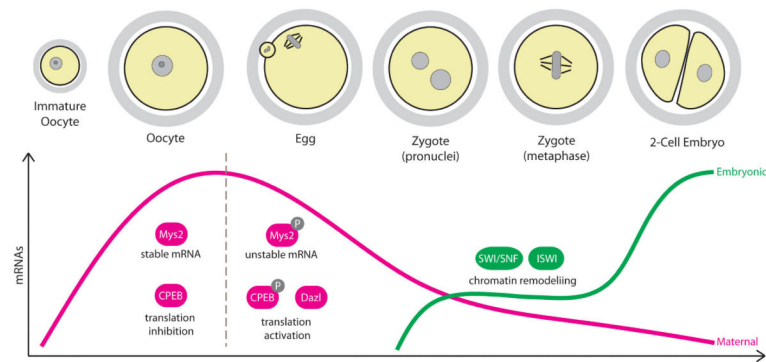
Figure 6. Regulation of gene expression

During the growing phase, oocytes accumulate large amounts of mRNA necessary to drive the transcriptionally silent phase of oocyte maturation and fertilization. Global mRNA stability in the oocyte is ensured by the RNA-binding protein Mys2 and many mRNAs are held in a translationally-repressed state by binding of CPEB to their 3′UTRs. Maternal mRNAs begin to be translated and/or degraded during oocyte maturation, which involves the phosphorylation of Mys2 and CPEB, and mRNA levels reach a minimum at the 2-cell stage. Transcription from the embryonic genome is initiated firstly in the zygote followed by a robust activation of transcription in the 2-cell embryo, and involves extensive chromatin remodelling.