Restarting life: fertilization and the transition from meiosis to mitosis

Dean Clift and Melina Schuh

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, is reintroduced; cell division shifts from being extremely asymmetric to symmetric; genomic imprinting is selectively erased and re-established; and protein expression shifts from translational control 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 before birth (FIG. 1a). It is generally thought that oocytes are not replenished after birth, but this dogma of developmental biology has recently been challenged1,2. The stored oocytes have already undergone meiotic DNA replication and recombination that ensures the genetic diversity of potential offspring (FIG. 1b). These 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 provide the oocyte with precursors of macromolecules through gap junctions3. 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 the oocyte (FIG. 1c). 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.

In the oviduct, the sperm binds to the zona pellucida, which is 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 assemblies around the now diploid zygotic genome. A series of mitotic cell divisions then produces 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 (FIG. 1a). The two subsequent cell divisions generate 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 extra-embryonic tissue that is required to support embryonic 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 first review 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 processes in humans.

**Fertilization**

Fertilization involves the fusion of sperm and egg cells, and this event was first observed by Oscar Hertwig in the nineteenth century (BOX 1).

**Sperm–egg binding.** Sperm cells initially bind to the zona pellucida of the egg (FIG. 2a), which is made up of just a few glycoproteins (that is, zona pellucida sperm-binding protein 1 (ZP1) to ZP3 in mice and ZP1 to ZP4 in humans), yet the precise molecules that mediate mammalian sperm–egg binding have remained elusive. Early studies identified ZP3 as a potential primary sperm...
Polyspermy

An egg is fertilized by more than one sperm.

Tobacco etch virus protease technology (TEV protease technology).

Using this technique, the recognition sequence for the TEV protease is introduced into proteins so that they may be artificially cleaved in vivo by ectopic expression of TEV protease.

How is an entirely new human being generated following sexual intercourse? This question has evoked human thinking since ancient times. Hippocrates (460–370 years BCE) argued that man and woman each contributed semen that mixed in the uterus to form the embryo, whereas Aristotle (384–322 years BCE) 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 seventeenth 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. Indeed, Harvey went so far as declaring ‘ex ovo omnia’, which means ‘everything from the egg’. Soon after, in 1677, Antonii van Leeuwenhoek built a microscope to study human semen and made the remarkable discovery of spermatozoa. Therefore, by the late seventeenth century, both key components of fertilization — the egg and sperm — had been realized, yet the relative contributions that each made to the embryo remained unclear for almost 200 years. In the early nineteenth century, Karl Ernst von Baer first confirmed the presence of the mammalian egg under the microscope, and Matthias Jakob Schleiden and Theodor Schwann postulated that egg and sperm are equivalent in that they are both cells. At around the same time, it became apparent that the widely observed phenomenon of heredity may involve factors that are contained within egg and sperm. Finally, it was in 1876 that Oscar Hertwig made the seminal discovery of fertilization in sea urchins. He observed that the nuclei of sperm and egg fused during fertilization, thereby providing a conceptual basis for genetic inheritance and settling the long-standing debate on the role of the egg and sperm in the generation of new life. Johannes Sobotta’s drawings of pronuclear fusion in the mouse from 1895 are remarkably accurate and could still be printed in any text book today (see the figure).

receptor, 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.

An alternative possibility is that the zona pellucida proteins together adopt a three-dimensional structure that presents a binding site for sperm. Such a binding site would be lost upon the cleavage of ZP2 that occurs after fertilization, providing a conceptual basis for genetic inheritance and settling the long-standing debate on the role of the egg and sperm in the generation of new life. These findings are consistent with a model in which sperm–egg binding depends on the cleavage status of ZP2.

In the future, the tobacco etch virus protease technology could be used to test whether ZP2 cleavage is sufficient to prevent sperm binding.

Zona pellucida glycoproteins are modified with oligosaccharides at Asp (N-linked) and/or Thr (O-linked) residues. A recent study of unfertilized human eggs found that most oligosaccharides terminate with the sialyl Lewis X tetrasaccharide motif. Consistent with a function of this motif in sperm–egg binding, sialyl Lewis X oligosaccharides or antibodies against it interfere with sperm–egg binding in vitro, and the removal of sialic acid from solubilized zona pellucida reduces its affinity for sperm. An important challenge for the future is to identify the sperm-bound receptor responsible for binding to the sialyl Lewis X motif.
Although a complete understanding of the 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. Further work will be necessary to test this model and how variations on a theme ensure species-specific sperm–egg interactions.

### Ca²⁺ triggers exit from meiosis

When binding to the egg is established, the sperm releases a specialized secretory vesicle, the acrosome, which contains a mix of hydrolytic and proteolytic enzymes that pave the way through the zona pellucida and promote plasma membrane fusion. The fusion of the sperm with the plasma membrane of the egg 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 Ca²⁺ in the egg, which was first observed more than 30 years ago in medaka (Oryzias latipes) and sea urchins and subsequently confirmed in all species studied to date. In mammals, a series of Ca²⁺ 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. The increase in Ca²⁺ levels in mammalian eggs is likely to be initiated by phospholipase Cζ (PLCζ), which is introduced into the egg by the sperm. PLCζ promotes the production of inositol-1,4,5-trisphosphate (Ins(1,4,5)P₃), which then binds to receptors on the endoplasmic reticulum (ER), causing the release of Ca²⁺ (REFS 22, 25) (FIG. 2c). Recent studies have shed light on how the increase in Ca²⁺ 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 the activity of cytostatic factor (CSF) that was first found by Masui and Markert in 1971 (REF. 24). An essential mediator of CSF activity is EMIL2 (early mitotic inhibitor 2). EMIL2 maintains metaphase II arrest by inhibiting the APC/C (anaphase-promoting complex; also known as the cyclosome), which targets cyclin B and the separase inhibitor securin for degradation. A recent study revealed that emil2 also regulates APC/C activity during the early mitotic divisions of Xenopus laevis embryos. It will be interesting to investigate why the function of EMIL2 as an APC/C regulator continues beyond meiosis into the mitotic divisions. The increase in intracellular Ca²⁺ levels upon fertilization activates Ca²⁺/calmodulin-dependent protein kinase II (CaMKII), which, together with Polo-like kinase 1 (PLK1), phosphorylates early mitotic inhibitor 2 (EMIL2). The SCF (SKP2–cullin 1–F-box protein) ubiquitin ligase complex then targets EMIL2 for destruction, which leads to the release of EMIL2-mediated inhibition of the APC/C (anaphase-promoting complex; also known as the cyclosome). This allows the APC/C to promote exit from meiosis. The rise in intracellular Ca²⁺ levels 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. 14. © (1998) Oxford Journals.
Chromatin remodelling. At fertilization, the maternal genome is contained within individualized 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 which are rich in positively charged amino acids and form a nucleoprotamine complex with the negatively charged DNA. 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 Ca²⁺ levels triggers exit from meiosis and the formation of a female pronucleus, while 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 [13, 14] (Fig. 5a). Although now equivalently structured in nucleosomes, the two pronuclei retain some parent-specific histone methylation patterns, particularly at pericentromeric heterochromatin regions that are equilibrated gradually during the first embryonic divisions [15].

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 with that of the egg [16], perhaps reflecting terminal differentiation and dense chromatin packaging within the sperm. Within hours of fertilization, however, the sperm pronucleus undergoes rapid active demethylation before DNA replication [17, 18]. Recently, this was found to be mediated by the oxidation of 5-methylcytosine by the DNA dioxygenase ten–eleven translocation 3 (TET3) [19–21], yet the precise mechanisms involved remain poorly understood. Importantly, Tet3-null–mice show developmental failure, suggesting that active paternal demethylation is crucial for proper embryonic development [22]. Maternal chromatin is largely protected from this initial active demethylation by the action of the DNA-binding protein PGC7 (primordial germ cell protein 7; also known as DPPA3 or Stella) [23]. PGC7 binds to histone H3 methylated at Lys9 (H3K9me2), a methylation mark that is present mainly on maternal chromatin [24, 25]. During the cleavage stages of the early embryo, both parental genomes undergo passive, replication-dependent demethylation [26]. Methylation finally reaches a minimum at the blastocyst stage, before cell lineage specification [27] (Fig. 5a).

However, the genome of the embryo is not entirely demethylated. 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 [28], and they 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 [29]. A chromatin-modifying complex that assembles via the interaction of the zinc-finger protein ZPF57 and TRIM28 (tripartite interaction motif 28; also known as KAP1 or TIF1β) also ensures that imprinted loci are spared from passive demethylation during blastomere cleavage stages [30–32].

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 revealed that the transition from meiosis to mitosis involves a dramatic change in the composition of the chromosomal cohesin complex [33]. This multiprotein complex forms a ring that entraps sister chromatids following DNA replication. Chromosome segregation in anaphase is triggered by cleavage of the cohesin subunit kleisin by the protease separase. This destroys the cohesin ring and therefore sister chromatid cohesion [34]. Mitotic cohesin complexes contain the sister chromatid cohesion 1 (SCC1) kleisin subunit. In meiotic cohesin complexes, SCC1 is largely replaced by meiosis-specific REC8, which is essential for reductional chromosome segregation [35]. 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 artificial SCC1 cleavage had the opposite effect [13]. Therefore, the switch from the 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 is abundant in oocytes [36–38] 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 (stromal antigen 1) and STAG2 in eggs compared with prophase oocytes [39]. 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 probably cleaved by separase in meiosis II, and subsequently degraded by the N-end rule pathway [40], meiotic cohesin complexes may be rapidly inactivated after fertilization, further facilitating a rapid switch to mitosis in the zygote.
Intriguingly, it seems that not all aspects of mitotic chromosome segregation are adopted immediately in the zygote. For example, chromosome cohesion is lost in a step-wise manner. Whereas in mitotic cells cohesion is eliminated during prophase between chromosome arms but protected at the centromere giving rise to characteristic X-shaped (or V-shaped in telocentric species) metaphase chromosomes, zygotic metaphase chromosomes...
chromosomes retain chromosome arm cohesion. Furthermore, mitotic chromosome spreads during early embryonic divisions suggest that chromosome arm cohesion may not be lost until the 8-cell stage of the embryo. 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-organizing centre (MTOC) is the centrosome, an organelle consisting of a pair of centrioles surrounded by a cloud of pericentriolar material.

Centrosomes are instrumental in spindle assembly during mitosis, with each centrosome forming one of the poles of the bipolar spindle. Female meiotic cells, however, are unique in that they do not contain canonical centriole-containing centrosomes. The mechanisms of acentrosomal spindle assembly in mammalian oocytes and how the transition from acentrosomal to centrosomal spindle assembly is achieved after fertilization are being elucidated (FIG. 4).

**Acentrosomal spindle assembly in oocytes.** In almost all species studied to date, including humans, centrosomes are absent from oocyte meiotic spindles (FIG. 4a). Electron microscopy of mouse oocytes showed that centrioles are present in fetal oocytes at pachytene stage but not thereafter, indicative of active centriole formation. Acentrosomal spindle assembly is driven by multiple acentriolar microtubules-organizing centres (aMTOCs) that cluster together and self-assemble into a bipolar spindle (bottom left). Centrosomal spindle assembly involves the separation of two centrosomes that act as MTOCs and form the spindle poles (bottom right). The transition from acentrosomal to centrosomal spindle assembly during mouse development is a gradual process that is completed by the blastocyst stage. During this process, the number of MTOCs per cell gradually decreases, the spindle becomes shorter and the spindle poles more focused, the maintenance of spindle bipolarity becomes independent of kinesin 5 and centrioles assemble de novo in the early blastocyst. Image of the oocyte in part a courtesy of Dean Clift, Medical Research Council Laboratory of Molecular Biology (MRC LMB), Cambridge, UK. Images of the 2-cell embryo and the blastocyst in part a are reproduced, with permission, from REF. 73 © (2012) Rockefeller University Press.
elimination during oogenesis. However, the underlying mechanisms of centriole elimination still remain poorly understood. A recent study in Caenorhabditis elegans identified a role for the mRNA stabilizing factor CGH-1 (conserved germline helicase) in this process, 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. These aMTOCs consist of an electron dense material and contain γ-tubulin and pericentrin. This suggests 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. Microtubules then self-assemble into a bipolar spindle through the action of the plus end-directed motor protein kinesin 5, with aMTOCs distributed at spindle poles. An important challenge for the future will be to address whether human oocytes use 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. The first three embryonic divisions in the mouse are meiosis-like, with the spindle assembly from multiple aMTOCs similar to the oocyte. During the subsequent three divisions (morula to blastocyst), the number of aMTOCs from which the spindles are assembled gradually decreases, and some cells begin to show centrin-positive staining, which is consistent with the first appearance of centrosomes. Also, the spindle morphology gradually adopts the typical mitotic appearance: the length of the spindle decreases and the spindle poles become more focused as the embryo develops.

Finally, in the blastocyst, all cells assemble a spindle from two centrin-positive MTOCs, indicative of a typical centrosome-driven mitosis. At about the same time, maintenance of spindle bipolarity shifts from kinesin 5-dependent to -independent, which is consistent with the observation that centrosomal spindles do not rely on kinesin-5 to maintain bipolarity. 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.

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. Indeed, centrioles can be detected at spindle poles in the human zygote, 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.

**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. The molecular and cellular machineries that support these two contrasting types of divisions at the transition from meiosis to mitosis are being elucidated.

**Asymmetric spindle positioning in oocytes.** The meiotic divisions of the oocyte are the most asymmetric cell divisions that are known in mammals. They are essential to form a large egg, which contains sufficient storage material for embryonic development. To divide asymmetrically, mammalian oocytes have to move the spindle from their centre to the cortex using an actin-dependent mechanism (reviewed in Ref. 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 nucleator formin and the actin related protein 2/3 (ARP2/3) complex. The vesicles also recruit the motor protein myosin Vb, which continuously transports the vesicles along the actin filaments towards the plasma membrane and drives the dynamics of the actin network.

Recent work has shed light on how the vesicle–actin network might mediate asymmetric spindle positioning. The spindle poles locally contract the actin filaments in a myosin light chain kinase (MLCK)-dependent manner and thereby pull on the network. This pulling could couple the spindle to the outward-directed movement of the RAB11A-positive vesicles and their associated actin filaments. Consistent with this model, RAB11A-positive vesicles, myosin Vb, and MLCK activity and the dynamics of the vesicle–actin network are required for asymmetric spindle positioning. In addition, MOS, CDC42, and the actin-related protein 2/3 (ARP2/3) complex 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. However, the actin reporter that was used in this study was later found to be unsuitable for labelling intracellular actin structures in oocytes. Other reporters for actin in live oocytes as well as staining of fixed oocytes with phalloidin do not detect an actin cloud but reveal the extensive actin network that fills the oocyte.
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 part of the spindle 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 (REF. 98) and the ARP2/3 complex97 (FIG. 5c). 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’99,100. The ARP2/3-dependent actin cap is essential for cytoplasmic flows that have been suggested to push the metaphase II spindle against the cortex97. Analogously, ARP2/3-dependent cytoplasmic flows have also been suggested to promote the late stages of asymmetric spindle positioning during meiosis I101.

**Spindle positioning in the embryo.** In stark contrast to the asymmetric meiotic divisions, the first mitotic division after fertilization needs to be symmetric 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 positioning the first mitotic spindle is the migration of the male and female pronuclei towards the centre of the zygote (FIG. 5d,e). 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 which has been contributed by the sperm during fertilization. The centrosome nucleates a large microtubule aster, termed sperm aster\(^{102}\). The female pronucleus is likely to associate with the sperm aster and move towards the male pronucleus in a dynein-dependent manner\(^{99,103,104}\). But how do both pronuclei move towards the centre of the zygote? Early studies suggested that pushing of sperm aster microtubules against the cortex could move the male pronucleus towards the centre (reviewed in REF. 102) (FIG. 5d). 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 X. laevis and zebrafish\(^{103,104}\). Several recent studies support a model whereby pulling by dynein anchored throughout the zygote mediates the movement of the male pronucleus towards the centre\(^{103-107}\). In C. elegans embryos, dynein has been suggested to be anchored to intracellular organelles\(^{107}\), but also cytoskeletal structures could serve as sites for dynein anchoring\(^{104}\). How is the pronucleus moving inwards if dynein is homogenously distributed throughout the cytoplasm? A length-dependent microtubule pulling mechanism\(^{103}\) could explain this: the aster microtubules of the sperm are longer towards the centre of zygote so that a higher number of dynein motors should pull the aster inward rather than outward\(^{103,108}\) (FIG. 5e).

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 (FIG. 5d). In X. laevis and zebrafish zygotes, the astral microtubules of the spindle are too short to reach the cortex, but cytoplasmic dynein has been suggested to help position the spindle\(^{103}\). Whether dynein centres the spindle in mammalian one-cell embryos, remains to be investigated. It is also unclear whether 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\(^{109}\) and pig\(^{110}\) zygotes suggest that actin is also required for pronuclear movement and spindle positioning\(^{111}\), but the mechanism by which actin promotes the central position of the spindle in mammalian embryos is still unclear.

As the distinct cell lineages of the early embryo develop, asymmetry needs to be re-established (reviewed in REF. 112). It will be interesting to analyse 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 embryonic development as well.

**Control of gene expression**

Transcription comes to a halt towards the end of the growth phase of the oocyte before maturation, when chromatin is highly condensed and surrounds the nucleolus\(^{113}\). It is not until after fertilization, at the 2-cell stage in mice\(^{114}\) or 4-cell stage in humans\(^{115}\), that transcriptionally active oocytes ensure the storage and timely activation of maternal factors necessary for the egg to embryo transition. After fertilization, there is a switch from maternal to embryonic control of gene expression.

**Translational control.** During oocyte growth, the genome is actively transcribed. Some transcripts are readily translated into proteins\(^{116}\). However, many transcripts must accumulate and their translation prevented until oocyte maturation or until after fertilization. Indeed, there is a dramatic difference in polyribosome-associated mRNAs between prophase and meiosis II oocytes\(^{117}\), and between meiosis II oocytes and zygotes\(^{118}\), which suggests stage-specific translation initiation of a multitude of transcripts.

Many oocyte mRNAs contain cytoplasmic polyadenylation elements (CPEs) in their 3’ untranslated region (3’ UTR)\(^{118}\). These CPEs recruit a translation-repressing complex comprising CPE-binding protein (CPEB) and its binding partner maskin\(^{119}\). Such transcripts seem to be stored in oocyte-specific subcortical aggregates that may be functionally related to P bodies\(^{120}\). 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 translational initiation complex\(^{119}\). The importance of CPEB-mediated translation regulation is underscored by CPEB1-depleted mouse oocytes, which exhibit severe defects in oocyte development\(^{121}\).

A key question is how different mRNAs are activated for translation at different times during oocyte maturation. Recently, it was shown that CPEB1 activates the translation of the RNA-binding protein DAZL (deleted in azoospermia-like)\(^{35}\). DAZL in turn directs its own translation\(^{35}\) and that of an additional subset of mRNAs important for meiotic spindle assembly and early embryonic development\(^{35}\). The sequential activation of two RNA-binding proteins, CPEB1 and DSZL, may therefore provide one mechanism by which mammalian oocytes temporarily regulate the translation of different subsets of transcripts\(^{35}\). An important challenge for the future will be to determine how fertilization triggers additional changes to the translational landscape in the zygote\(^{127}\).

**Eliminating the stock of maternal transcripts.** The transition from maternal to embryonic control of gene expression requires that the amount of accumulated maternal transcripts is greatly reduced. However, oocyte mRNAs are inherently stable and remain in the oocyte for up to a few weeks before ovulation\(^{122}\). Thus, there must be a dramatic change in the stability of the maternal transcript...
The final step in the transition involves extensive chromatin remodelling (by SWI/SNF and imitator switch (ISWI). Firstly in the zygote followed by a robust activation of transcription in the 2-cell embryo. 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 (by SWI/SNF and imitation switch (ISWI)).

Reactivating the genome. The final step in the transition from maternal to embryonic control of gene expression involves transcriptional activation of the newly formed embryonic genome, a process termed zygotic genome activation. The vital importance of zygotic genome activation for embryonic development was first realized in the 1970s, when it was found that inhibition of transcription using the RNA polymerase inhibitor α-amanitin caused mouse embryos to arrest at the 2-cell stage. Subsequent studies have shown that zygotic genome activation begins in the zygote, which exhibits low transcriptional activity primarily from the paternal pronucleus, and that this is followed by a major wave of transcription in the 2-cell embryo (FIG. 6).

In general, transcription is associated with an open chromatin state that allows transcription factors to easily access the DNA. Therefore, it has long been proposed that chromatin remodelling has a key role in the initiation of zygotic genome activation. Consistently, mouse embryos lacking the maternal copy of BRG1 (also known as SMARCA4; the catalytic subunit of the SWI/SNF chromatin remodelling complex) arrest at the 2-cell stage with reduced transcription of 30% of expressed genes. BRG1-depleted embryos also show decreased H3K4 dimethylation, which is a marker for transcriptionally active chromatin.

The localization of both BRG1 and the catalytic subunit of the ISWI (imitation switch) chromatin remodelling complex, SNF2H (also known as SMARCA5), is perturbed in embryos in which TIF1a (transcription intermediary factor 1a) is inhibited, and such embryos exhibit misregulated transcription of a subset of genes in the zygote. Therefore, chromatin remodelling by maternal complexes seems 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 at 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. All these events are triggered by the fusion of the sperm with egg, which causes an increase in intracellular Ca²⁺ levels.

How abrupt or gradual these transitions are achieved varies for each of these processes. Several features of mitosis are adopted immediately in the zygote, for example mitotic cohesin complexes replace their pool at some point during the fertilization process. The degradation of excess maternal mRNAs begins at the onset of oocyte maturation and continues beyond fertilization, with mRNA levels reaching a minimum at the 2-cell stage in mice (FIG. 6). In fact, maternal transcripts are degraded in a timely and selective manner. During oocyte maturation, transcripts involved in earlier processes such as oocyte growth and prophase arrest are rapidly degraded, whereas transcripts important for maintaining the metaphase II arrest remain intact. Similarly, transcripts involved in meiotic processes, but not embryonic development, are rapidly degraded upon fertilization.

How are maternal transcripts degraded in mammals? A crucial function for the RNA-binding protein MYS2 in this process was identified. Mouse oocytes lacking MYS2 contain less total mRNA, and the stability of reporter mRNAs injected into MYS2-depleted oocytes is substantially reduced, which suggests that MYS2 promotes global mRNA stability in oocytes. A clue that regulation of MYS2 may have a role in mRNA degradation came from the finding that MYS2 is phosphorylated in a cyclin-dependent kinase 1 (CDK1)-dependent manner during meiotic maturation. Importantly, expression of a non-phosphorylatable MYS2 mutant prevents maturation-induced mRNA degradation, whereas expression of a phosphomimetic mutant can induce mRNA degradation even in prophase-arrested oocytes. Therefore, phosphorylation of MYS2 during meiotic maturation may inactivate MYS2 and trigger the transition from mRNA stability to instability. 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.
meiotic counterparts, and cell division rapidly switches from being asymmetric to being 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 few 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 procedure are beginning to emerge, we are still far from having a clear picture. What are the molecules and mechanisms involved in repacking the paternal genome? How is methylation at imprinted regions preserved during global reprogramming? What are the mechanisms responsible for the degradation of maternal transcripts? How is transcription reinitiated 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 for basic research, but it is also relevant to understanding the potential of developing embryonic stem cells and their application in regenerative medicine.

Suggests a microtubule-length-dependent pulling mechanism to position the pronucleus in the center of the zygote.


A comprehensive analysis of gene expression during mouse development showing that maternal transcript degradation and zygotic gene activation occur in sequential stages.


Harvey, W. & Whitteridge, G. Disputations touching the generation of animals (Blackwell Scientific, 1981).


Acknowledgements
Dean Clift and Melina Schuh have received financial support from the European Community’s Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 241548.

Competing interests statement
The authors declare no competing financial interests.
Copyright of Nature Reviews Molecular Cell Biology is the property of Nature Publishing Group and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.