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REVIEW

New insights into the maternal to zygotic transition

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ABSTRACT

The initial phases of embryonic development occur in the absence of *de novo* transcription and are instead controlled by maternally inherited mRNAs and proteins. During this initial period, cell cycles are synchronous and lack gap phases. Following this period of transcriptional silence, zygotic transcription begins, the maternal influence on development starts to decrease, and dramatic changes to the cell cycle take place. Here, we discuss recent work that is shedding light on the maternal to zygotic transition and the interrelated but distinct mechanisms regulating the onset of zygotic transcription and changes to the cell cycle during early embryonic development.

KEY WORDS: Cell cycle, Embryonic development, Gap phases

Introduction

The first steps of embryonic life are a dramatic period during animal development, involving complex processes such as fertilisation, the completion of meiosis and the very first cell divisions in the embryo. This early stage of embryonic development occurs in the absence of *de novo* transcription and is initially controlled by maternal mRNAs and proteins that are deposited in the egg during oogenesis (Tadros and Lipshitz, 2009; Marlow, 2010). Following this period of transcriptional quiescence, zygotic transcription commences and the maternal control of development begins to decline (Giraldez et al., 2006). This period of embryonic development is also marked by dramatic changes to the cell cycle. For example, in developing *Xenopus laevis* embryos there are 12 synchronous cell divisions occurring at 30-min intervals, with alternating S and M phases and no G1 or G2 phases. After these 12 rapid divisions, the length of S phase increases, G1 and G2 phases intervene between M and S, and there is a concomitant slowing of the cell cycle and a loss of synchrony (Newport and Kirschner, 1982a). DNA replication also changes during this period, with it initiating at regular intervals in the genome initially and then, when S phase length increases, replication is directed to specific sites within the genome (Hyrien et al., 1995; Lemaitre et al., 1998). Large-scale zygotic transcription begins around this time, and cells simultaneously begin to move and then become susceptible to apoptosis, for example as a result of DNA damage incurred during cleavage replications (Newport and Kirschner, 1982a; Stack and Newport, 1997; Ikegami et al., 1999). Similar events also occur in the zebrafish embryo, which initially performs ten rapid and synchronous cell cycles before undergoing cell cycle remodelling, commencing bulk zygotic transcription and initiating cell movements (Kane and Kimmel, 1993). Zebrafish embryos also show a similar response to cleavage stage DNA damage (Ikegami et al., 1999). During *Drosophila* development, the embryo undergoes 13 nuclear divisions, without cell division, forming a syncytium. The cell cycle lengthens at cycle 14, cellularisation occurs, and bulk zygotic transcription begins (Edgar and Schubiger, 1986). The time of zygotic transcription initiation varies in other species that have been studied. For example, during mouse embryonic development zygotic transcription commences at the two-cell stage (Hamatani et al., 2006). Despite these differences this is an evolutionarily conserved event, as all species initially start embryonic development with a period of transcriptional quiescence followed by the activation of zygotic transcription.

This period of development has, confusingly, been referred to both as the maternal to zygotic transition (MZT) and as the midblastula transition (MBT). The distinction between these terms is that the MZT is a period of development starting just after fertilisation, when maternal transcripts first begin to be eliminated, spanning the initiation of transcription and cell cycle changes, and ending in the point at which cells become susceptible to apoptosis (Fig. 1) (Stack and Newport, 1997; Tadros and Lipshitz, 2009). By contrast, the MBT is a precise developmental point, which occurs during the MZT (Fig. 1), at which there are dramatic changes to the cell cycle and, coincidently, bulk zygotic transcription is observed. The MBT also marks the point after which cells begin to move and become susceptible to apoptosis.

The features of the MZT and its evolutionary conservation have been well reviewed by Tadros and Lipshitz (2009). Here, we focus on work carried out since that review, in zebrafish, *Xenopus* and *Drosophila*, which is beginning to uncover the distinct but interrelated mechanisms that control the initiation of zygotic transcription and the remodelling of the cell cycle.

The onset of transcription during the MZT

One of the defining characteristics of the MZT is the initiation of zygotic transcription. Over the last few decades several models have been proposed to explain the mechanisms controlling this event. However, more recently, advances in sequencing technologies have provided insights into the timing and mechanism underlying zygotic transcription initiation, which we will discuss below.

Mechanisms and models of transcription initiation

Several models have been proposed to explain the onset of zygotic transcription in the early embryo. Previous work demonstrated that when plasmid DNA was injected into early *Xenopus* embryos it was transiently transcribed at stages when there is no zygotic transcription. After transcription of the plasmid, it was silenced, only for transcription to reinitiate at the normal point of zygotic transcription initiation. This key experiment demonstrated that the early embryo is competent to perform transcription – it does not lack core components of the transcription apparatus – but that DNA is normally held in a state in which transcription cannot occur. This suggests that there are transcriptional repressors within the early embryo that maintain genomic DNA in a state that is

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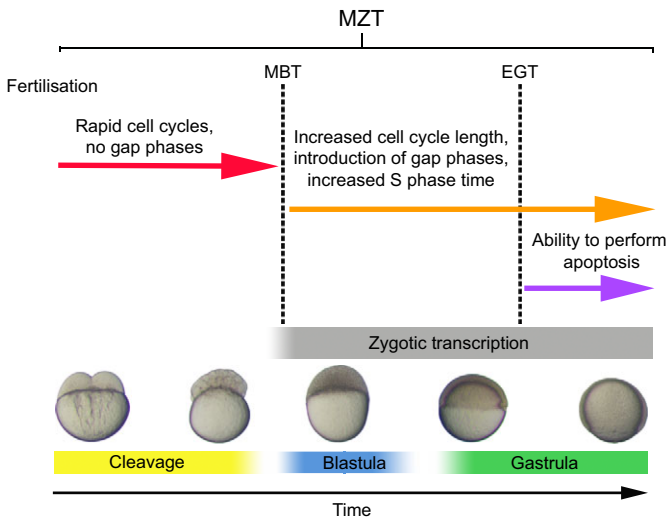

Fig. 1. The maternal to zygotic transition. The maternal to zygotic transition (MZT) spans a period of early embryonic development starting just after fertilisation. During the cleavage stages of early development there is no transcription and the cell cycles, which lack gap phases, are rapid. At a specific point during the MZT, known as the midblastula transition (MBT), transcription commences and cell cycles begin to lengthen due to the introduction of gap phases and an increase in the length of S phase. The early gastrula transition (EGT) then marks a point at which embryos acquire the ability to perform apoptosis. Images of zebrafish embryos are shown to highlight the different developmental stages.

incompatible with transcription. The nucleocytoplasmic model takes these observations into account. It proposes that there is a transcription repressor in the early embryo, which becomes titrated out by subsequent cell divisions. In *Xenopus* and zebrafish embryos the early cell divisions are reductive, whereby the volume of the embryo remains constant such that each cell division doubles the ratio of nucleus to cytoplasm (Newport and Kirschner, 1982a,b; Kane and Kimmel, 1993). The increasing ratio of nucleus (or DNA) to cytoplasm would titrate out the repressors such that, at a critical point, genomic DNA would become relieved of its repressed state and zygotic transcription could begin. In support of this model, increasing the DNA content of an embryo by injecting large amounts of plasmid DNA, or inducing polyspermy, can lead to earlier transcription (Newport and Kirschner, 1982a).

A second model proposes that fertilisation sets in motion a molecular clock that regulates the events surrounding the MZT. Support for this model comes from the observation that the degradation of Cyclin A and E1 proteins depends on time after fertilisation and not on the ratio of nucleus to cytoplasm (Howe et al., 1995; Howe and Newport, 1996; Stack and Newport, 1997). Similarly, work in *Drosophila* suggests that the majority of zygotic transcription is not dependent on the nucleus to cytoplasm ratio, but rather on time after fertilisation (Lu et al., 2009).

Another model proposes that although the embryo attempts to perform transcription, the machinery controlling DNA replication during the early rapid cell cycles leads to transcription being aborted. Support for this model comes from the observation that, in *Xenopus* and *Drosophila*, blocking embryos at cell cycles before normal zygotic transcription leads to premature zygotic transcription (Edgar and Schubiger, 1986; Kimelman et al., 1987). However, additional regulation must exist as it was shown that blocking *Drosophila* embryos at early time points did not lead to premature transcription (Edgar and Schubiger, 1986).

Individually, these different models can describe specific observations surrounding the events of the MZT. However, we will discuss how recent work is beginning to bring these different observations into a more complete model of the MZT.

Maternal control of the MZT

Recently, sequencing technologies have been used to address the mechanism controlling zygotic transcription initiation. Harvey and colleagues used RNA sequencing and single nucleotide polymorphisms to discriminate between transcription from different alleles in the developing zebrafish embryo and thus distinguish between maternal and paternal mRNAs (Harvey et al., 2013). Using the appearance of paternal mRNAs as a marker of zygotic transcription, this approach revealed that there is widespread post-transcriptional regulation of maternal mRNAs before zygotic transcription begins. Thus, maternal mRNAs, which are deposited in the egg during oogenesis, are held in an inactive state by proteins bound to cytoplasmic polyadenylation elements in their 3′ UTRs (Mendez and Richter, 2001; Groisman et al., 2002; Harvey et al., 2013). When required, such maternal mRNAs, including those encoding regulators of the cell cycle such as Cyclin B1 (Groisman et al., 2000, 2002), are released from their inactive state (Mendez and Richter, 2001). Fertilisation also sets in train a different type of post-transcriptional regulation in which maternal mRNAs gradually become polyadenylated and then translated prior to zygotic transcription initiation (Lee et al., 2013).

Among the class of maternal mRNAs that gradually become polyadenylated and translated after fertilisation in the zebrafish embryo are those encoding transcription factors such as Nanog, Pou5f1 (Pou5f3 – ZFIN) and Sox19b (Harvey et al., 2013; Lee et al., 2013). Pou5f1 binds to specific genomic loci before zygotic transcription begins (Leichsenring et al., 2013), suggesting that it primes certain genes to be zygotically expressed. Morpholino knockdown of *nanog* in maternal and zygotic *pou5f1* mutants, or with quadruple knockdown of SoxB1 family members (*sox2*, *sox3*, *sox19a* and *sox19b*), causes a developmental arrest of embryos that resembles the arrest caused by treatment with the RNA polymerase II inhibitor α-amanitin (Kane et al., 1996; Lee et al., 2013), and indeed zygotic transcription initiation is significantly disrupted in such embryos, suggesting that the accumulation of these maternal factors is essential for zygotic transcription initiation (Lee et al., 2013; Leichsenring et al., 2013). These findings might represent an overlap with the induction of pluripotent stem cells and of pluripotency in embryonic stem cells, where orthologues of *nanog*, *pou5f1* and the Sox genes play essential roles (Niwa et al., 2000; Takahashi and Yamanaka, 2006; Masui et al., 2007).

Interestingly, the maternal transcripts identified as being polyadenylated were strongly conserved between *Xenopus tropicalis* and zebrafish. Thus, of the 286 *X. tropicalis* genes thought to be polyadenylated within three hours of fertilisation, 254 (89%) were also classified by Aanes et al. (2011) as being maternal transcripts subject to polyadenylation in zebrafish (Aanes et al., 2011; Collart et al., 2014). As in the zebrafish, the products of polyadenylated maternal mRNAs in *X. tropicalis* are necessary for the proper activation of zygotic transcription: treatment of embryos with cordycepin, which blocks polyadenylation, prevented the normal activation of many, but not all, zygotically activated genes. One maternal factor that is crucial for the correct initiation of transcription during the *Xenopus* MZT is the T-box transcription factor VegT (Skirkanich et al., 2011). Interestingly, however, the orthologues of *nanog*, *pou5f1* and *sox19b* are unlikely to be key players in *Xenopus*, as no *Xenopus* orthologue of *nanog* has yet

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been identified and the *Xenopus* orthologue of *pou5f1* (*Oct91*) cannot be detected maternally (Collart et al., 2014). Of the Sox genes, *Sox1* and *Sox2* were both detected at low levels maternally before being strongly activated at around the time of the MBT, and only *Sox3* has significant levels of maternal transcripts in *X. tropicalis*. More work is required to determine whether different genes take on the roles of *nanog*, *pou5f1* and the Sox genes in *Xenopus* or whether there are more fundamental differences between the species. The Ventx genes have been proposed to take on some *nanog*-like activities in *Xenopus*, but these are not expressed maternally, and instead are activated strongly at around the time of the MBT (Scerbo et al., 2012).

These recent findings present similarities with zygotic transcription initiation in the *Drosophila* embryo, where the maternal factor Zelda (Vielfaltig – FlyBase) prepares specific genes to be the first to be expressed during the MZT (Liang et al., 2008; Harrison et al., 2011; Nien et al., 2011). In embryos lacking *Zelda* function, zygotic transcription initiation and cellularisation are disrupted.

Collectively, these results demonstrate that in the early embryo certain transcription factors are in limited supply and the post-transcriptional regulation of maternal mRNAs is required to accumulate these factors, which are required for correct zygotic transcription initiation.

The timing of transcription initiation

Recent studies have also provided insight into the timing of zygotic transcription initiation. By synchronising embryonic development by means of *in vitro* fertilisation (IVF), and then performing RNA sequencing on embryos collected every 15 min, it was demonstrated that zygotic transcription begins after ten cell cycles in zebrafish (Harvey et al., 2013). This is consistent with radioactive UTP incorporation experiments (Kane and Kimmel, 1993), but the conclusion differs from recent work, which used 4-thio-UTP incorporation as a marker of zygotic transcription, that suggests that the transcription of protein-coding genes begins three cell cycles earlier, at the seventh cell cycle (Heyn et al., 2014). We do not yet understand this apparent discrepancy; however, analysing 4-thio-UTP incorporation in IVF synchronised embryos would address this problem. Zebrafish embryos comprise one cell for the first 45 min after fertilisation and subsequent cell cycles are only 15 min long. Therefore, one-cell embryos collected after natural matings could have a maximum 45 min difference between the time that they were fertilised. Subsequently, the differences observed in when zygotic transcription commences could be due to the use of embryos derived from natural matings or IVF.

In the absence of transcription, cell cycle progression depends on the translation of maternal mRNAs such as *cyclin b1* (Groisman et al., 2002) and, in line with this, treatment of zebrafish embryos with the translation inhibitor cycloheximide blocks cell cycle progression. During normal zebrafish MZT, transcription initiates after ten cell cycles, but blocking embryos after seven cell cycles (at the 128-cell stage) nevertheless permits some zygotic genes (e.g. *vox*) to be expressed normally while others (e.g. *claudin e*) fail to initiate transcription (Fig. 2) (Harvey et al., 2013). Thus, for some genes, the ability to be activated during the MZT is established before the 128-cell stage, whereas for others it is established later than this. As discussed above, the post-transcriptional regulation of specific maternal mRNAs could explain the differences in those genes that are or are not expressed in embryos blocked at the 128-cell stage. Those zygotic genes that are expressed in the presence of cycloheximide are activated around the time that transcription begins in control embryos. This indicates that after the 128-cell stage cell cycles and, subsequently, the changes in the nuclear to cytoplasmic ratio are not necessary for the initiation of zygotic transcription. These observations suggest that zygotic transcription initiation is dependent on a set time post fertilisation.

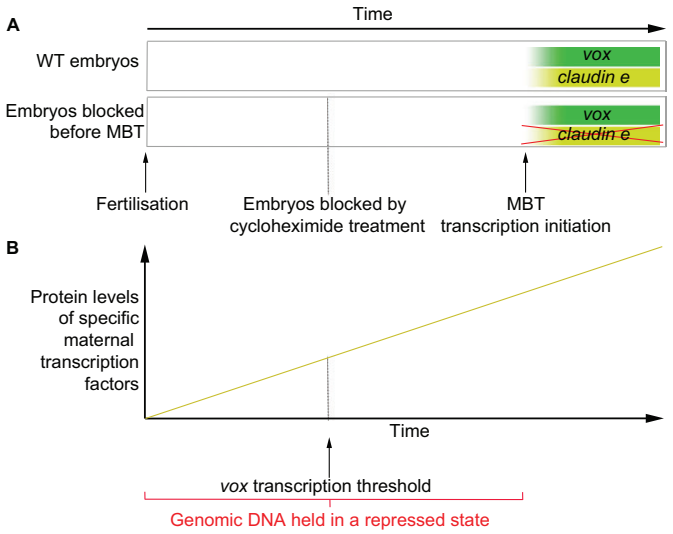

Fig. 2. Zygotic transcription initiation. (A) In wild-type (WT) zebrafish embryos, transcription of the genes *vox* and *claudin e* initiates after ten cell cycles, at the MBT. When cell cycle progression is blocked at the seventh cell cycle (using the translation inhibitor cycloheximide) *vox* transcription initiates at the MBT whereas *claudin e* transcription fails to initiate. This indicates that the proteins required for transcription are established as early as the seventh cell cycle for *vox*, but after this time for *claudin e*. Also, even though the correct transcription factors have been established by the seventh cell cycle, transcription of *vox* does not commence until the normal time of zygotic transcription initiation (MBT). As the cells are blocked at the seventh cell cycle, after this point the nuclear to cytoplasmic ratio is not critical for the timing of transcription initiation. (B) Following fertilisation, maternal mRNAs become polyadenylated and translated, leading to the gradual accumulation of specific maternal transcription factors. As *vox* continues to initiate transcription at the MBT in embryos exposed to cycloheximide, this suggests that, prior to the MBT, specific maternal transcription factors have achieved a threshold required to initiate transcription. However, even though this threshold has been achieved, transcription does not initiate until the MBT because genomic DNA is held in a state that is incompatible with transcription.

An integrated model for transcription initiation

Collectively, these results provide a detailed model for how zygotic transcription begins during the MZT (Fig. 3). Genomic DNA must first be released from a repressed state (in accordance with the nucleocytoplasmic model). Correct transcription initiation then depends on two events. Recent work demonstrates that specific transcription factors are in limited supply in the early embryo. After fertilisation, maternal mRNAs must be polyadenylated and translated to accumulate the correct repertoire of transcription factors needed for zygotic transcription. In addition, even though the embryo accumulates these factors as early as the 128-cell stage in zebrafish, transcription does not begin until a set time post fertilisation, when genomic DNA becomes compatible with transcription (i.e. the clock model). This clock is not dependent on the nucleocytoplasmic ratio and, similarly, is not dependent on the translation of maternal mRNAs, as zygotic transcription can still commence in embryos treated with the translation inhibitor cycloheximide.

What underlies the repressed state of genomic DNA prior to the MBT and the control of this maternal clock? Chromatin regulation might be the key to this. The chromatin modifications histone H3 lysine 4 methylation (H3K4me3) and histone H3 lysine 27

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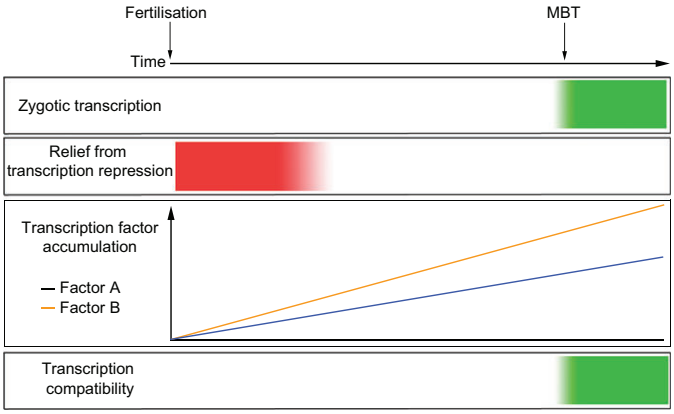

Fig. 3. An integrated model to explain the onset of zygotic transcription. The initiation of bulk zygotic transcription commences at the MBT. Initiation of transcription is dependent first on genomic DNA being relieved from a repressed state. Initially, specific transcription factors are in limited supply and maternal mRNAs must be polyadenylated and translated to accumulate these factors above a specific threshold. Different transcription factors, which are required for the transcription of specific genes, accumulate during this period. Even though the embryo may have acquired the correct concentration of specific transcription factors, transcription still does not commence until a set time after fertilisation when genomic DNA becomes transcriptionally compatible.

methylation (H3K27me3), which are normally associated with transcriptionally active and repressed genes, respectively, are unlikely to play a role: these marks do not appear in the zebrafish or *Drosophila* embryo until the time of zygotic transcription initiation (Barski et al., 2007; Vastenhouw et al., 2010; Chen et al., 2013). Although these specific marks might therefore regulate the zygotic expression of particular genes, they are unlikely to act as global regulators of transcriptional quiescence prior to zygotic transcription initiation or to global activation of transcription thereafter. By contrast, whole-genome bisulphite sequencing has recently shown that DNA methylation is tightly regulated in the early zebrafish embryo (Jiang et al., 2013; Potok et al., 2013). Before the onset of zygotic transcription, the methylation state of specific paternal alleles is inherited from one cell division to the next. However, although the methylation state of maternal DNA is inherited until the 16-cell stage, it is then discarded and resolves to resemble the paternal methylation state by the time that zygotic transcription begins. This raises the possibility that a mechanism exists in the early embryo to distinguish between maternal and paternal alleles, but also that alleles must reach equal methylation levels for transcription to commence.

In line with a crucial role for chromatin regulation in controlling the MZT, the DNA methyltransferase Dnmt1 has been suggested to control transcriptional repression in the early embryo, and morpholino knockdown of *Xenopus Dnmt1* resulted in the premature expression of some genes (Stancheva and Meehan, 2000; Dunican et al., 2008). However, loss of zygotic *dnmt1* function during zebrafish development had no apparent effect on the MZT as embryos develop normally until 84 hpf (Anderson et al., 2009; Goll et al., 2009).

Genes activated during the MZT and their participation in a gene regulatory network

Similar to the studies discussed above, sequencing-based methods have been used to study the onset of transcription in *X. tropicalis*, with samples taken for RNA sequencing at intervals of 30 min from fertilisation, through the MBT and up to the early gastrula stage (Collart et al., 2014). This high-resolution study allows one to infer with more confidence the structure of gene regulatory networks in the embryo as well as to make comparisons with other species. This work revealed, as in the zebrafish, a post-fertilisation wave of polyadenylation of maternal transcripts (as well as some genes that were deadenylated), and this was followed by a broad wave of zygotic transcription that began at the seventh cleavage and extended beyond the MBT at the twelfth cleavage. Importantly, the high temporal resolution of this study indicates that there is no sudden onset of gene expression during the MBT in *Xenopus*. Rather, there is a broad wave of zygotic transcription that begins at the seventh cleavage and extends beyond the MBT at the twelfth cleavage. In addition, there is no evidence within this broad wave of transcription for distinct ‘early’ and ‘late’ components as proposed by Tadros and Lipshitz (2009).

This broad wave of transcription is enriched, perhaps not surprisingly, for genes involved in the regulation of transcription, stem cell maintenance, and axis patterning and development, and it is probable that the genes expressed in this wave are early components of the genetic regulatory networks that underlie the development of different regions and tissues of the embryo. Furthermore, in *Xenopus* additional genes involved in these genetic regulatory networks are likely to be expressed in a second wave of zygotic transcription: this wave occurs a significant time after the first wave, and experiments show that it includes some genes that are regulated by transcription factors activated in the preceding wave. Thus, Brachyury and Mixer regulate genes such as *Gdf3*, *Plod2* and *Msgn1*, and *Cer1* and *Gata5*, respectively. Gene ontology analysis of genes expressed in this second wave shows an enrichment of genes involved in the control of translation and in the early steps of organogenesis, including those involved in the development of the heart and the kidney, consistent with the idea that the embryo is now establishing the genetic regulatory networks that lead to the formation of specific cell types in the embryo.

This scenario is analogous to findings from recent *Drosophila* studies. Although the bulk of zygotic transcription commences around the time of cellularisation, a small number of genes are expressed prior to this (Edgar and Schubiger, 1986; De Renzis et al., 2007; Ali-Murthy et al., 2013). Genetic analysis demonstrates that these earliest expressed genes are establishing a gene regulatory network that controls subsequent events during the *Drosophila* MZT. The nuclei in *engrailed* mutant embryos divide asynchronously prior to cellularisation and, similarly, loss of the linker histone variant BigH1 leads to mitotic defects and early embryonic lethality (Perez-Montero et al., 2013).

Changes in the cell cycle at the MBT

Another dramatic event to occur during the MZT is the change in cell cycle behaviour (Fig. 1). If, at least in *Xenopus*, there is no sharp onset of transcription the same cannot be said of the change in the cell cycle at the MBT, when the switch from rapid synchronous cell divisions to slower metachronous cell divisions is sudden (Fig. 4). The mechanisms regulating the cell cycle changes at the MBT are distinct from those regulating the onset of transcription, and recent studies have demonstrated that the cell cycle changes are regulated differently in different organisms. The nuclear to cytoplasmic ratio plays a pivotal role in controlling this event, but recently the molecules controlling this mechanism are coming into focus.

Mechanisms regulating changes to the cell cycle

In *Xenopus* and zebrafish, it is generally believed that the cue for cell cycle remodelling is provided by the ratio of nucleus to cytoplasm.

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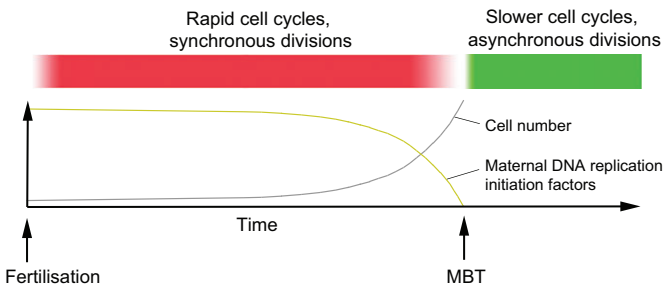

Fig. 4. Cell cycle changes during the MZT. After fertilisation and the completion of meiosis, the embryo enters a state in which the cell cycles are rapid and lack gap phases. These early cell divisions are synchronous and, therefore, cell number increases exponentially. Simultaneously, maternal stores of the DNA replication initiation factors Cut5, Recq4, Treslin and Drf1 decrease, leading to a slowing of S phase at the MBT and, subsequently, an increase in cell cycle length via the introduction of gap phases.

Xenopus eggs can be constricted soon after fertilisation, such that the embryo develops with effectively half the normal volume of cytoplasm, and this results in a reduction in the number of synchronous and rapid cell cycles. Furthermore, in zebrafish, cell cycle lengthening occurs one cleavage later in haploid embryos compared with normal diploid embryos, and one cleavage early in tetraploid embryos (Kane and Kimmel, 1993). In addition, partial enucleation of a zebrafish embryo can lead to a single nucleus re-establishing itself in a larger than normal cytoplasmic volume, and this, as expected, causes an extension of the synchronous and rapid cell cycles (Newport and Kirschner, 1982a; Kane and Kimmel, 1993). Finally, and consistent with the idea that the depletion of some maternal factor(s) triggers cell cycle changes, the introduction of G2 gap phases in both the *Xenopus* and zebrafish embryo is a transcription-independent event (Newport and Kirschner, 1982a; Dalle Nogare et al., 2009).

However, what are the factors that are limiting in the cytoplasm of the embryo and that, when depleted, result in an increase in the length of S phase? Two groups have recently used the *Xenopus* embryo to address this question. Zegerman and colleagues noted that the elongation of the cell cycle at the MBT in *Xenopus* coincides with a reduction in the density and synchrony of DNA replication initiation events (Collart et al., 2013). This suggested that replication factors might themselves be limiting at the MBT. Following on from this, these authors measured the abundance of DNA replication initiation factors in the *Xenopus* embryo, and found four factors – Cut5, RecQ4, Treslin and Drf1 – the mRNA and protein levels of which decreased during early development, while others remained constant or increased (Fig. 4). Although the titration model poses that levels of the limiting factor(s) might remain constant, any factor that decreases in concentration as development proceeds would make it a particularly strong candidate because the ratio of nucleus to cytoplasm would increase more significantly if the denominator decreased while the numerator increased. With this in mind, Zegerman and colleagues carried out experiments in *Xenopus* egg extracts and showed that the addition of Cut5, RecQ4, Treslin and Drf1 to such extracts caused an increase in DNA synthesis. They also found that increasing the number of sperm nuclei in the extract, which effectively mimics the effects of cell division in the early embryo, caused a reduction in DNA synthesis in each nucleus, and that this reduction was prevented by the addition of the four replication factors. Meanwhile, experiments *in vivo* demonstrated that overexpression of the four factors caused an almost twofold increase in replication origin firing, and rapid synchronous cell divisions were shown to persist beyond the twelfth cell cycle. By contrast, depletion of the four factors by antisense morpholino oligonucleotides caused premature lengthening of the cell cycle. Taken together, these results indicate that titration of at least these four replication factors regulates the initiation of DNA replication and thus in turn cell cycle length at the MBT in *Xenopus*.

The authors also explored two additional questions. First, to what extent is lengthening of the cell cycle associated with the onset of transcription? RNA sequencing-based experiments using *Xenopus* embryos overexpressing Cut5, RecQ4, Treslin and Drf1 showed that, as well as extending the period of rapid synchronous cell divisions, the four factors caused the expression of some zygotic genes to be delayed. This suggests a link between the cell cycle and the onset of transcription and is consistent with the conclusion from work in zebrafish that more than one mechanism regulates the onset of transcription during the MZT (Fig. 3). The second question was more fundamental: it is known that inhibition of cell division at early gastrula stages has little effect on morphogenesis or development to the tailbud stages (Cooke, 1973), but what of embryos in which the rapid and synchronous cell divisions continue beyond the twelfth cell cycle? *Xenopus* embryos overexpressing Cut5, RecQ4, Treslin and Drf1 proved to be inviable, failing to complete gastrulation and dying before neurula stages. Therefore, the authors proposed that this developmental defect might be due to an increase in rates of origin activation caused by overexpression of the four factors. To test this hypothesis, they partially depleted the pre-replication complex protein Cdc6, which is required for replication licensing, in embryos that were also overexpressing the four factors. Survival of these embryos was indeed improved compared with embryos overexpressing the four factors alone, demonstrating that correct regulation of the rate of initiation of DNA replication is crucial for normal development.

A second group also investigated the effect of the nucleus to cytoplasm ratio on the rate of DNA replication in *Xenopus* egg extracts (Murphy and Michael, 2013). By titrating sperm chromatin, they found that there is an increase in the time required to replicate sperm nuclei at high nucleus to cytoplasm ratios and, consistent with the work of Collart et al. (2013), that this is caused by a decrease in replication origin activation. Murphy and Michael further demonstrated that the protein phosphatase PP2A, along with its regulatory subunit B55α, is a limiting factor in egg extracts for the initiation of DNA replication at high nucleus to cytoplasm ratios. The authors therefore proposed that during early development the increasing nucleus to cytoplasm ratio effectively titrates out PP2A-B55α, resulting in an increase in the length of S phase that may result in an increase in the length of the cell cycle and thus the cell cycle changes at the MBT. However, more work will be necessary to determine the relative contributions of PP2A-B55α and the four replication factors to the regulation of initiation of DNA replication as well as to the cell cycle changes at the MBT. In particular, it will be important to understand how a decrease in the rate of initiation of DNA replication, with an associated increase in the length of S phase, ultimately leads to a complete remodelling of the cell cycle with the appearance of gap phases and with slower asynchronous cell divisions.

Recent studies by the Wieschaus, O'Farrell and Großhans groups have begun to shed light on the mechanism that regulates cell cycle changes in *Drosophila* (Di Talia et al., 2013; Farrell and O'Farrell, 2013; Sung et al., 2013). Briefly, it was shown that the early zygotic transcription of a subset of genes can target the Cdc25 phosphatase Twine for destruction, and that loss of Twine allows the

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accumulation of inhibitory phosphorylation on Cdk1, the activity of which is required for mitosis in *Drosophila*. Therefore, in contrast to *Xenopus*, zygotic transcription is required to regulate cell cycles changes in *Drosophila*. However, at present it is not clear what role the nucleus to cytoplasm ratio plays in regulating the cell cycle changes in *Drosophila*. The studies by the Wieschaus and O'Farrell groups indicate that the nucleus to cytoplasm ratio triggers the onset of transcription of the genes required for Twine destruction (Di Talia et al., 2013; Farrell and O'Farrell, 2013). The study by the Großhans group, however, suggests that the destruction of Twine occurs independently of changes to the nucleus to cytoplasm ratio (Sung et al., 2013). In addition, as mentioned above, a previous study in *Drosophila* has demonstrated that the majority of zygotic transcription is not dependent on the nucleus to cytoplasm ratio, but rather on time post fertilisation (Lu et al., 2009).

Differences in cell cycle regulation before and after the MBT

As mentioned above, the MBT is marked by dramatic remodelling of the cell cycle, including the introduction of gap phases, and a fundamental change in the manner in which DNA replication is regulated. Prior to the MBT, DNA replication initiates at regular intervals within karyomeres (individual membrane-bound chromosomes), whereas initiation occurs in a site-specific manner within somatic nuclei post MBT (Hyrien et al., 1995; Lemaitre et al., 1998). Are there qualitative alterations to the regulation of the cell cycle that might accompany these changes? Krude and colleagues have recently demonstrated that the cell cycle differs fundamentally before and after the MBT, in that small non-coding Y RNAs, which are essential for the initiation of DNA replication in vertebrate somatic cells (Christov et al., 2006; Krude et al., 2009), are not required for DNA replication before the MBT (Collart et al., 2011). Thus, *Xenopus* and zebrafish embryos depleted of Y RNAs develop normally until the MBT, at which point they fail to replicate their DNA and die before gastrulation. The MBT thus marks a switch between Y RNA-independent and Y RNA-dependent regulation of DNA replication, and understanding this change offers an opportunity both to explore Y RNA function and to understand more about the remodelling of the cell cycle at the MBT. The best clue at the moment comes from the observation that a factor present in *Xenopus* egg extract is sufficient to overcome the requirement for Y RNAs for DNA replication. The dominant activity of this factor is presumably lost at the MBT, perhaps owing to an increase in the nucleus to cytoplasm ratio, suggesting that it might be one of the limiting DNA replication factors identified in the two reports described above.

Clearly, in light of the studies by the Zegerman, Krude and Michael groups, a thorough understanding of the regulation of the initiation of DNA replication in the early embryo (Nordman and Orr-Weaver, 2012), and how this changes, will be fundamental to further our understanding of the mechanisms that regulate cell cycle changes at the MBT.

Conclusions

While several models have existed to explain distinct features of the transitions between maternal and zygotic function, recent publications are now beginning to build a collective story of the mechanisms that control this process. The nucleocytoplasmic model demonstrates that, although the embryo is capable of performing transcription, genomic DNA is held in a state that is incompatible with transcription (Newport and Kirschner, 1982a,b). However, relief from this repression is still not sufficient for the correct initiation of zygotic transcription. Instead, through the post-transcriptional regulation of maternal mRNAs, maternal transcription factors must accumulate. Also, fertilisation sets in motion a clock that defines when transcription commences (Fig. 3). The post-transcriptional regulation of maternal factors is also a crucial determinant of changes to cell cycle behaviour. As maternal stores of key DNA replication factors become depleted, the cell cycles change to become asynchronous with longer S phases and G1 and G2 phases.

We now have a clearer understanding of the molecular mechanisms controlling these early transitions, although several questions remain to be answered. Some of the transcription factors required for the activation of zygotic transcription have been identified, but the proteins that establish transcriptional quiescence and then hold genomic DNA in a repressed state remain largely elusive (Fig. 2). To systematically identify these factors will require a detailed study of not just the MZT but also of when transcription is silenced during oogenesis. What properties might these factors have? Changing the absolute concentration of these factors during the MZT could result in premature or delayed zygotic transcription initiation. The establishment of transcriptional quiescence and, subsequently, the maturation of oocytes is likely to be disrupted in the absence of these factors. It is possible that the establishment of transcriptional quiescence occurs at a point in oogenesis when the factors controlling nuclear reprogramming are present (Gurdon, 1962). Therefore, it is interesting to speculate that, similar to the discovery of the factors controlling induced pluripotency (Takahashi and Yamanaka, 2006; Takahashi et al., 2007), the repressive factors that establish transcriptional quiescence during oogenesis could induce transcriptional quiescence in normally transcriptionally active cells. Recent work has suggested a molecular overlap between the factors that can establish pluripotency and transcriptional regulation during the MZT (Lee et al., 2013; Leichsenring et al., 2013). In mouse and human cells the addition of four factors – Oct3/4 (Pou5f1), Sox2, c-Myc and Klf4 – induces differentiated cells to become pluripotent. Recent work suggests that a proposed zebrafish orthologue of *Oct4*, *pou5f1*, is in limited supply in the early embryo. Maternal *pou5f1* must be polyadenylated and translated to then bind to specific regions of the genome for the correct initiation of zygotic transcription (Lee et al., 2013; Leichsenring et al., 2013).

One factor that has been proposed to act as the transcriptional repressor during the *Xenopus* MZT is the DNA methyltransferase Dnmt1 (Stancheva and Meehan, 2000; Dunican et al., 2008). In *Xenopus* embryos injected with morpholinos targeting *Dnmt1*, the premature expression of some genes was detected. A more global analysis of zygotic transcription initiation in embryos in which maternal *Dnmt1* has been abolished would lend support to this factor being the elusive transcriptional repressor. Early embryonic development is normal in zebrafish *dnmt1* mutants, suggesting that zygotic *dnmt1* has little if any role in controlling the MZT (Anderson et al., 2009; Goll et al., 2009). However, the MZT has not been studied in zebrafish embryos in which both maternal and zygotic *dnmt1* function has been disrupted.

In addition, understanding the post-transcriptional regulation of maternal mRNAs will be crucial to uncover the mechanisms that control the coordinated changes in cell cycle behaviour and zygotic transcription initiation. Cytoplasmic polyadenylation elements (CPEs) in the 3′ UTRs of maternal mRNAs are essential for this post-transcriptional regulation, and the regulation of CPEs is required both to establish appropriate levels of the transcription factors that activate the first genes to be expressed during the MZT and for the regulation of cell division. How CPEs are regulated to produce these different patterns of polyadenylation remains to be uncovered; however, this offers a mechanism that coordinates cell cycle behaviour and zygotic transcription initiation. One possibility

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is that cell cycle-coupled polyadenylation patterns are controlled by both polyadenylation and deadenylation, but the factors controlling the deadenylation are unknown.

The post-transcriptional regulation of maternal mRNAs is essential for cell cycle progression and the accumulation of the transcription factors required for transcription initiation, but after genomic DNA has been relieved from its initially repressed state, the molecular clock that defines when zygotic transcription commences appears not to require the translation of maternal mRNAs or cell cycle progression. Based on recent findings, it is tempting to speculate that the regulation of DNA methylation is central to this control over the timing of zygotic transcription initiation (Jiang et al., 2013; Potok et al., 2013). For instance, whereas the methylation state of paternal alleles is maintained during early zebrafish development, the methylation state of maternal alleles is reprogrammed to match that of the paternal alleles, and the two alleles reach equal methylation levels coinciding with the normal period of zygotic transcription initiation. As the maternal and paternal alleles have different methylation levels, the absence of transcription cannot be due to DNA methylation levels per se. Rather, the ability to sense differences in the maternal and paternal alleles might control the timing of the MBT. In line with this, when zebrafish embryos were blocked at the 128-cell stage, some genes continue to be zygotically expressed, but only at the time that control embryos initiate zygotic transcription. Therefore, it would be of interest to determine whether the methylation levels continue to change in the absence of cell division.

The gradual depletion of maternal proteins controlling DNA replication also plays a key role during the MBT. This depletion in the early embryo triggers changes to DNA synthesis and ultimately the remodelling of the cell cycle at the MBT (Collart et al., 2013). This observation raises a new series of questions regarding the cell cycle changes during the MZT. For example, what controls the depletion of such maternal factors? Is it time post fertilisation or do these proteins become targeted for degradation once they are used during S phase?

The study of transcriptional regulation and cell cycle behaviour has produced many fascinating discoveries that have improved our understanding of development and disease. The MZT represents an extreme scenario of these processes and therefore, by studying this event, we will continue to improve our knowledge of transcription and cell cycle behaviour. For example, although the chromatin modification marks H3K4me3 and H3K27me3 associate with transcriptionally active and repressed zygotic genes, respectively, they play no role in transcriptional regulation prior to the MBT (Barski et al., 2007; Vastenhouw et al., 2010; Chen et al., 2013). How can we begin to uncover the mechanisms regulating the events of the MZT? With the availability of large numbers of loss-of-function mutations in zebrafish (Kettleborough et al., 2013) and amenable methods to disrupt gene function (Mali et al., 2013a,b), those conclusions that have been established using antisense morpholino oligonucleotides, which can have non-specific effects (Robu et al., 2007), would be strengthened by genetic analysis. As discussed, studying the establishment of transcriptional quiescence during oogenesis will improve our understanding of transcriptional regulation during the MZT. Although technological advances in sequencing have allowed us to identify the first genes to be expressed in the embryo, we know nothing of the heterogeneous state of cells within the embryo at those time points. In the future, single-cell RNA sequencing will allow us to determine if there is spatial restriction to zygotic transcription initiation, which might indicate additional mechanisms controlling this process.

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Competing interests

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