

The maternal-to-zygotic transition: a play in two acts

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All animal embryos pass through a stage during which developmental control is handed from maternally provided gene products to those synthesized from the zygotic genome. This maternal-to-zygotic transition (MZT) has been extensively studied in model organisms, including echinoderms, nematodes, insects, fish, amphibians and mammals. In all cases, the MZT can be subdivided into two interrelated processes: first, a subset of maternal mRNAs and proteins is eliminated; second, zygotic transcription is initiated. The timing and scale of these two events differ across species, as do the cellular and morphogenetic processes that sculpt their embryos. In this article, we discuss conserved and distinct features within the two component processes of the MZT.

Introduction

“*What’s past is prologue*”

Shakespeare, *The Tempest* (II.i)

The maternal genome controls virtually all aspects of early animal development. Maternal mRNAs and proteins, which are loaded into the egg during oogenesis, implement basic biosynthetic processes in the early embryo, direct the first mitotic divisions, and specify initial cell fate and patterning. As development proceeds, two processes are triggered that together form the maternal-to-zygotic transition (MZT): first, a subset of the maternal mRNAs is eliminated; second, the transcription of the zygotic genome begins. Initially, the destruction of maternal mRNAs is accomplished by maternally encoded products. However, zygotic transcription leads to the production of proteins and microRNAs (miRNAs) that provide feedback to enhance the efficiency of maternal mRNA degradation. In addition, among the earliest mRNAs synthesized de novo in the embryo are transcriptional activators that enhance the efficiency of zygotic transcription. The net result is that the control of development is transferred from the maternal to the zygotic genome.

The purpose of this primer is to review the events that constitute the MZT in representatives of different branches of the metazoan (see Glossary, Box 1) evolutionary tree in which the MZT has been most extensively studied (Fig. 1): echinoderms (*Strongylocentrotus purpuratus*), nematodes (*Caenorhabditis elegans*), insects (*Drosophila melanogaster*), fish (*Danio rerio*), amphibians (*Xenopus laevis*) and mammals (*Mus musculus*). The MZT also occurs in plants, but this is not considered here (reviewed by Baroux et al., 2008). We begin with an overview of the major developmental events that occur during the MZT. We then examine what is known about maternal transcript destabilization and the

Box 1. Glossary

Cis-element

A defined DNA or RNA region that mediates transcription in the case of DNA, or processes such as translation, stability, export and localization in the case of RNA. Cis-elements are bound by trans-factors, which can be proteins or RNAs

Compound chromosomes

Chromosomes with their arms reassorted relative to their centromeres. For example, for a metacentric chromosome that normally has a left arm (L) and a right arm (R), giving the composition L:R, compound chromosomes might have two right arms attached to one centromere (R:R) and two left arms attached to another centromere (L:L). A diploid cell would still have two left arms and two right arms. However, meiosis can result in gametes (and thus, after fertilization, embryos) that lack both left arms or both right arms. More complex compound chromosomes also exist.

Gap phase

A pause in the cell cycle between DNA synthesis (S phase) and mitosis (M phase). The gap between M and S phase is termed G1, and that between S and M phase, G2.

Gene ontology (GO) analysis

Classification system whereby genes that encode products contributing to a common cellular component, or implementing a particular molecular or biological function, share a corresponding ‘GO term’ designating them as such. This facilitates the bioinformatic identification of trends, in which genes of a particular class are similarly regulated.

Male pronuclear remodeling

The morphological and biochemical modifications, such as the replacement of protamines with histones, that occur after fertilization and that transform a dormant sperm nucleus into a functional male pronucleus.

Metazoan

A eukaryotic multicellular animal (excluding single-celled protozoa and multicellular sponges). Metazoa are subdivided into Bilateria (animals with bilateral symmetry) and Radiata (animals with radial symmetry). Bilateria can be further subdivided, based on particular developmental distinctions, into deuterostomes (e.g. echinoderms, such as sea urchins, and chordates, such as tunicates and vertebrates) and protostomes (e.g. Ecdysozoa, such as arthropods and nematodes).

Parthenogenesis

The development of an egg without fertilization, common in some insects and arthropods.

Protamines

Small, basic, arginine-rich proteins that are often found associated with DNA in sperm nuclei.

Syncytial divisions

Nuclear divisions without cytokinesis. Syncytial divisions can occur after fertilization in the common cytoplasm of some early embryos, notably in many insects.

Trans-factor

A protein or small RNA that binds a cis-element and mediates some aspect of nucleic acid regulation.

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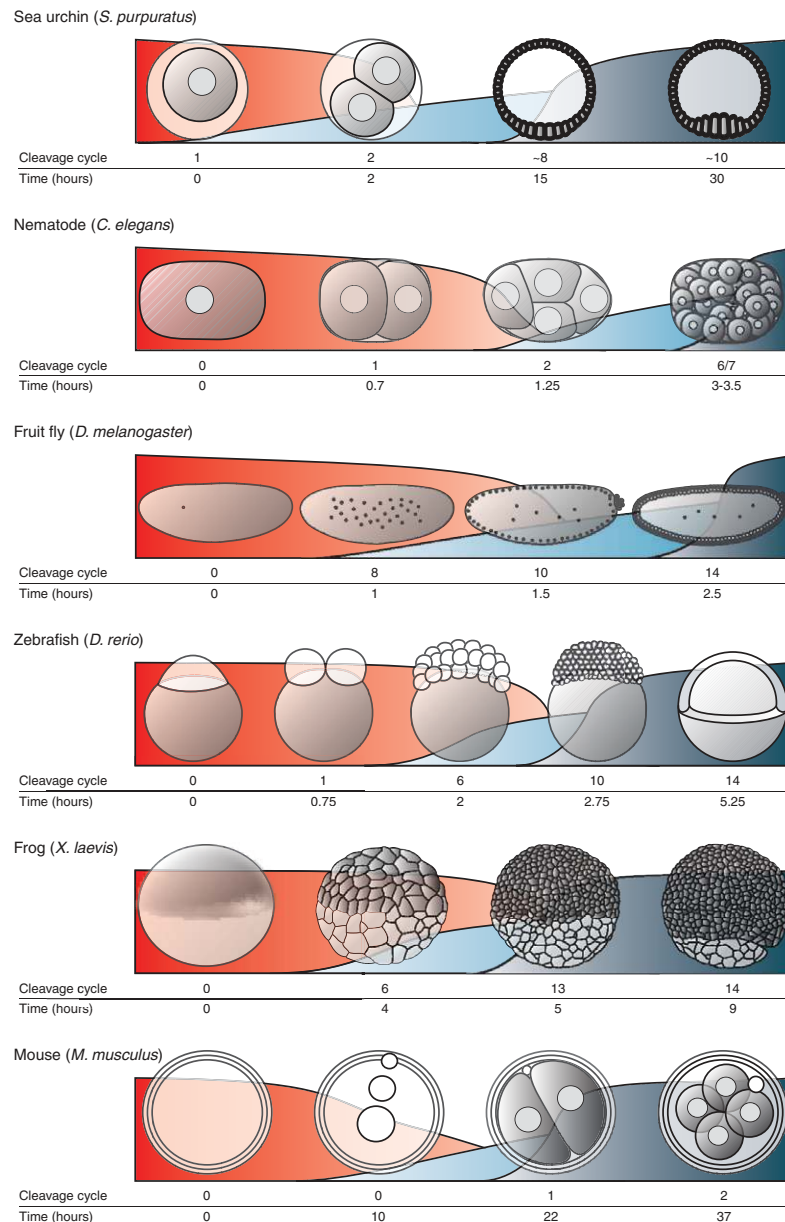


Fig. 1. A comparative overview of the maternal-to-zygotic transition (MZT) in several model organisms. Key embryonic stages for each model organism are depicted schematically above the corresponding cleavage cycle and time after fertilization. The red curves represent the degradation profiles of destabilized maternal transcripts in each species. The light and dark blue curves illustrate the minor and major waves, respectively, of zygotic genome activation. The last embryonic stage presented for each organism is the developmental point at which there is a major requirement for zygotic transcripts.

subsequent activation of the zygotic genome. Our focus is on the dynamics and the scale of these events, their underlying molecular mechanisms and their functions.

Stage directions: an overview of early embryogenesis

“We will draw the curtain and show you the picture”
Shakespeare, *Twelfth Night* (I.v)

To place the events of the MZT in context, we describe the setting in which it occurs – activated eggs and early embryos – with a focus on major developmental and cell cycle hallmarks.

Egg activation

Egg cells are suspended both in their metabolic activity and in their cell cycle, the latter at a particular stage of meiosis that varies from species to species. Egg activation comprises a multitude of events, triggered in response to external stimuli, which bring the mature egg cell out of its suspended state. Egg activation is necessary – and, in some cases, also sufficient – for the initiation of embryogenesis (reviewed by Horner and Wolfner, 2008). In echinoderms, nematode worms and many vertebrates, fertilization is the trigger for egg activation. In other species, such as insects that undergo parthenogenesis (see Glossary, Box 1), fertilization is not required; instead, changes in the ionic environment, pH, or mechanical stimulation trigger activation. In

non-parthenogenetic insects, such as *D. melanogaster*, egg activation also occurs independently of, and prior to, fertilization, and involves a combination of osmotic and mechanical stimulation. Egg activation always results in a rise in intracellular calcium, waves of which initiate a signal-transduction cascade that brings about the resumption of meiosis, male pronuclear remodeling (see Glossary, Box 1) and cytoskeletal (and, for externally developing embryos, eggshell) rearrangements, as well as alterations in gene regulation at both the post-transcriptional and post-translational level.

Early cleavage divisions and the mid-blastula transition

In most metazoans, following the completion of meiosis and the fusion of the male and female pronuclei, synchronous and rapid cell cycles ensue (Fig. 1), often without gap phases (see Glossary, Box 1). In mammalian and nematode embryos, however, the early mitotic divisions are asynchronous. In most species, complete or nearly complete cytokinesis follows every cleavage. In *D. melanogaster* and many other insects, however, the synchronous early cleavage cycles occur without cytokinesis, producing nuclei that migrate through the yolk to the periphery of the embryo, where they undergo additional synchronous syncytial divisions (see Glossary, Box 1). The early mitotic cycles range in length from 8 minutes in flies, to 15 minutes in zebrafish, to 12 hours in mice.

The early mitoses eventually produce a blastula or ‘ball of cells’ (in most amphibians and echinoderms) or a peripheral layer of blastoderm nuclei (in many insects) that occupies the same volume of cytoplasm as the original unfertilized egg (Fig. 1). In echinoderms, *X. laevis* and zebrafish, the synchronous divisions are followed by asynchronous cleavages, with the introduction of gap phases during a developmental event termed the ‘mid-blastula transition’ (MBT; see Box 2). In *D. melanogaster*, the MBT also involves the introduction of a gap phase, which permits the invagination of plasma membrane between the syncytial blastoderm nuclei to form the cellular blastoderm. The MBT is followed by the onset of gastrulation, during which the germ layers of the embryo – endoderm, mesoderm and ectoderm – form through a combination of cell migration, ingression and invagination.

Act 1: maternal transcript destabilization

“If it were done, when ’tis done, then ’twere well
It were done quickly.”

Shakespeare, *Macbeth* (I.vii)

The first event of the MZT is the elimination of maternal transcripts. Here, we discuss the scale, mechanisms and functions of this process.

Dynamics and scale of maternal transcript destabilization

Estimates of the fraction of the protein-coding genome represented as maternal mRNAs range from 40% in the mouse (Wang et al., 2004), to 65% in *D. melanogaster* (Lecuyer et al., 2007; Tadros et al., 2007), to 75% in *S. purpuratus* (Wei et al., 2006). The elimination of a subset of these transcripts is the first event of the MZT (Figs 1 and 2). Destabilized mRNAs range from 30% of maternal mRNAs in *C. elegans* (Baugh et al., 2003), to 33% in mice (Hamatani et al., 2004), to 35% in *D. melanogaster* (De Renzis et al., 2007); corresponding microarray-based data are unavailable for echinoderms and amphibians. Notably, these transcripts remain in oocytes for days, weeks or even months prior to their elimination, which occurs in a matter of hours; hence, this represents a cataclysmic change in the stability of a significant fraction of the maternal transcript pool.

Box 2. MZT versus MBT

The literature on early metazoan embryogenesis is rife with inconsistencies and confusion regarding the terms ‘maternal-to-zygotic transition’ (MZT) and ‘mid-blastula transition’ (MBT). Here, we use the following definition of the MZT: the period that begins with the elimination of maternal transcripts, continues through the production of zygotic transcripts and ends with the first major morphological requirement for zygotic transcripts in embryonic development. One key difference between this definition and those used previously [for example, that adhered to by Baroux et al. (Baroux et al., 2008)] is the idea that the MZT spans a period rather than being a point in time. Another important distinction relates to the starting point of the MZT, which we propose is egg activation rather than fertilization. In *D. melanogaster*, the elimination of maternal transcripts begins immediately upon egg activation, prior to and independently of any zygotic input (Bashirullah et al., 1999; Tadros et al., 2003). This also appears to be true in the majority of model organisms in which transcript elimination has been studied (see text). Conversely, the term ‘MBT’ was first used to describe a developmental event in amphibian embryogenesis in which, after the first 11 or 12 synchronous cleavage divisions, the cell cycle lengthens, gap phases appear and mitoses become desynchronized (Gerhart, 1980). Since then, researchers have applied the term to analogous stages in other animals, such as fish (Kane and Kimmel, 1993) and flies (Blankenship and Wieschaus, 2001). It is important to note that, although the MBT roughly coincides with the major activation of the zygotic genome in these organisms, the same does not appear to hold true in mammals.

Transcript destabilization is achieved through the combined action of at least two types of degradation activity. The first, ‘maternal’, activity is exclusively maternally encoded and functions upon egg activation in the absence of zygotic products. The second, ‘zygotic’, activity requires zygotic transcription. In *D. melanogaster* (Fig. 2A), owing to the uncoupling of egg activation and fertilization, the maternal activity can be observed separately from the zygotic one (Bashirullah et al., 1999; Tadros et al., 2003). Genome-scale analyses have shown that over 20% of maternal transcripts are destabilized by this maternal activity (Tadros et al., 2007). The zygotic activity further destabilizes these transcripts and eliminates an additional 15% of maternal mRNAs, which results in the overall elimination of 35% of maternal transcripts by the end of the MZT (De Renzis et al., 2007).

In the mouse, a large fraction of the maternally supplied mRNAs is degraded by the two-cell stage (Piko and Clegg, 1982), and fertilization triggers at least some of this destabilization (Alizadeh et al., 2005). Gene expression profiling experiments have provided evidence for what are probably the maternal and zygotic degradation activities (Hamatani et al., 2004) (Fig. 2B): some maternal transcripts are degraded soon after fertilization (e.g. cluster 9); others show a later and very rapid decrease that coincides with the major onset of transcription at the two-cell stage (e.g. cluster 7). Genome-scale transcript analyses in zebrafish (Fig. 2C) (Ferg et al., 2007; Mathavan et al., 2005) and *C. elegans* (Baugh et al., 2003) also show destabilization profiles that are consistent with maternal and zygotic degradation activities.

Distinct groups of mRNAs are enriched in the stable versus unstable maternal subsets. For example, in *D. melanogaster*, Gene Ontology (GO) term analysis (see Glossary, Box 1) has shown that the stable subset is highly enriched for transcripts related to RNA transactions (binding, metabolism, translation), consistent with a requirement for these processes both during the MZT and beyond.

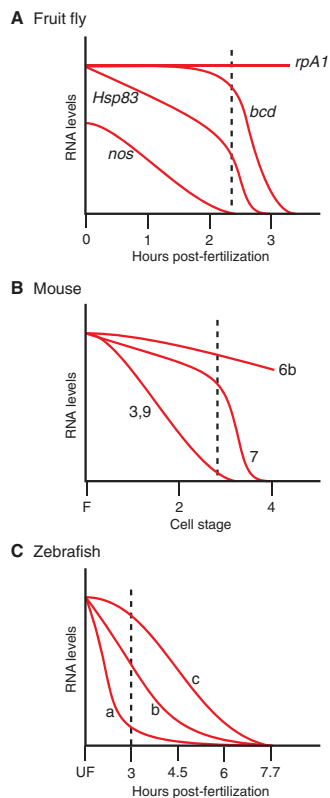


Fig. 2. Degradation profiles of maternal transcripts during the MZT. Presented are profiles of degrading maternal transcripts (red) based on published data from three different model systems. **(A)** Four subsets of maternal transcripts have been characterized in *D. melanogaster*: stable mRNAs (e.g. *rpA1*; *RpLP2* – FlyBase); mRNAs targeted solely by the maternal (e.g. *nos*) or the zygotic (e.g. *bcd*) degradation pathways; and those targeted by both (e.g. *Hsp83*) (Bashirullah et al., 1999; Surdej and Jacobs-Lorena, 1998). **(B)** In the mouse, genomic profiling (Hamatani et al., 2004) has revealed clusters of maternal transcripts that appear to degrade with kinetics that correspond to the subsets identified in *D. melanogaster*: clusters 3 and 9 degrade in the absence of significant zygotic transcription; degradation of cluster 7 transcripts appears to accelerate coincident with the onset of the major zygotic genome activation (ZGA) wave, whereas cluster 6b transcripts most resemble the stable subset. **(C)** In zebrafish embryos, transcripts that degrade have been assigned to three different groups according to whether the majority of their degradation occurs (a) before, (b) during or (c) following ZGA (Mathavan et al., 2005). In each case, the dotted black line denotes the timing of the first major wave of ZGA.

By contrast, unstable transcripts are enriched for GO terms related to the cell cycle (Tadros et al., 2007), the possible functional significance of which is discussed in the section on the function of maternal transcript destabilization. In the mouse, too, unstable maternal transcripts are enriched for GO terms related to the cell cycle; however, GO terms related to RNA metabolism, RNA binding and protein synthesis are enriched in zygotically synthesized transcripts rather than in maternal transcripts (Hamatani et al., 2004). This difference might reflect the very rapid early development of fly embryos (requiring pre-loaded RNA and protein synthetic machinery); in the more slowly developing mammalian embryo, there is plenty of time for the zygotic production of these components.

Mechanisms of maternal transcript destabilization

The exact link between calcium signaling during egg activation and maternal transcript destabilization remains unclear, in part because mutations that affect calcium signaling are pleiotropic, which makes their recovery in genetic screens based on maternal transcript stability rare (Tadros et al., 2003). Nonetheless, work in *D. melanogaster* has identified a post-translational and post-transcriptional cascade triggered upon egg activation that functions in transcript destabilization. Following egg activation, the Pan gu (PNG) Ser/Thr kinase complex promotes the translation of an RNA-binding protein, Smaug (SMG) (Tadros et al., 2007). SMG acts as a specificity factor, binding maternal transcripts that contain cis-elements (see Glossary, Box 1), known as SMG recognition elements (Smibert et al., 1996). SMG recruits the CCR4/POP2/NOT-deadenylase complex to these target transcripts, thus prompting the removal of their poly(A) tail, the first and rate-limiting step in mRNA degradation (Semotok et al., 2005; Semotok et al., 2008). SMG is essential for eliminating the majority of unstable maternal transcripts (Tadros et al., 2007), although it is not yet clear what fraction of these is directly bound by SMG.

In *D. melanogaster*, computational analyses have identified two additional cis-elements that are enriched in destabilized maternal transcripts (De Renzis et al., 2007): one resembles PUF-family binding sites and could, in principle, be bound by Pumilio (PUM), a post-transcriptional regulator implicated in both translational repression and destabilization of mRNAs; the other resembles AU-rich cis-elements (AREs), which mediate either transcript stabilization (through binding of HuR proteins) or destabilization (through binding of AUF1). To date, the potential roles of PUM and ARE-binding proteins in maternal transcript destabilization in *D. melanogaster* have not been effectively tested. In *X. laevis*, however, the ARE-mediated pathway (Voeltz and Steitz, 1998) works with the Embryonic Deadenylation Element Binding Protein (EDEN-BP) (Paillard et al., 1998) to trigger the deadenylation of maternal transcripts upon fertilization via the recognition of two distinct types of AU-rich cis-elements. EDEN-BP is in a complex with 158 mRNAs that are enriched for the EDEN cis-element (Graindorge et al., 2008). Intriguingly, the addition of calcium to cell-free extracts induces an unidentified kinase-phosphatase cascade that results in both EDEN-BP dephosphorylation and a concomitant increase in its deadenylation activity (Detivaud et al., 2003). This might explain how EDEN-BP, which is present at equivalent levels in oocytes and early embryos (Paillard et al., 1998), is activated in response to fertilization.

Notably distinct from the maternal degradation pathways in other systems, fertilization-induced deadenylation in *X. laevis* does not trigger decay until after the onset of zygotic transcription (Audic et al., 1997; Duval et al., 1990; Voeltz and Steitz, 1998), which indicates a requirement for a zygotically produced factor that acts after deadenylation has taken place. However, in the absence of genome-wide profiling data on transcript stability, it remains unclear whether this is the general pattern for destabilized maternal mRNAs in *X. laevis*.

Temporal coupling of deadenylation and destabilization does occur in the zygotic degradation pathway in *X. laevis*. Here, *Cyclin A1* and *Cyclin B2* transcripts are targeted for elimination through discrete regions in their 3'UTRs (Audic et al., 2001; Audic et al., 2002). Although the trans-factors that mediate this process are unknown, preliminary evidence indicates that it might be accomplished through the binding of a miRNA (Guo et al., 2008). Indeed, mounting evidence suggests that miRNAs are mediators of

the zygotic degradation pathway. For example, the zygotic expression of *miR-430* in zebrafish embryos is responsible for the clearance of several hundred maternal messages (Giraldez et al., 2006). *miR-430* requires additional, zygotically produced, non-miRNA factors to degrade a subset of its targets (Ferg et al., 2007). The *D. melanogaster miR-309* family of miRNAs, which are synthesized zygotically in a SMG-dependent manner, participate in the destabilization of several hundred maternal mRNAs in a manner analogous to that of zebrafish *miR-430* (Benoit et al., 2009; Bushati et al., 2008).

Beyond these studies, it has been shown in *D. melanogaster* that genomic loci that map to different chromosome arms are required for the destabilization of distinct subsets of maternal mRNAs (De Renzis et al., 2007). Thus, it is likely that there are several distinct zygotic degradation activities.

Functions of maternal transcript destabilization

In the absence of mutations that specifically abrogate maternal transcript destabilization, it has been difficult to demonstrate functions for this process in development – assuming there are any. One possibility is that the elimination of a significant fraction of maternal mRNAs might be necessary to prevent abnormal mRNA dosage in the embryo, representing the maternal counterpart to the zygotic lethality caused by chromosomal segmental aneuploidy (Lindsley et al., 1972). If so, then the elimination of a significant fraction of all maternal mRNAs – rather than the elimination of a specific maternal mRNA (or set of mRNAs) – might be required.

Alternatively, the elimination of specific maternal mRNAs could be essential for early development. For example, the elimination of ubiquitously distributed maternal mRNAs might permit the patterned transcription of their zygotic counterparts to direct spatially and temporally localized control. Consistent with this hypothesis, genome-scale analyses have determined that the zygotic transcripts that replace their uniformly distributed maternal counterparts tend to be expressed in restricted patterns much more frequently than does the average gene (De Renzis et al., 2007). This particular role would be permissive rather than instructive, as maternal transcript elimination would have no function other than to permit patterned zygotic transcripts to exert their influence. For example, maternal *string* mRNA, which encodes a *D. melanogaster* homolog of the cell cycle regulator Cdc25, is eliminated throughout the embryo by the end of the MZT, and is then replaced by patterned zygotic expression that correlates with mitotic domains in the gastrulating embryo (Edgar and O'Farrell, 1990; Foe, 1989). The degradation of ubiquitous maternal cell cycle mRNAs might be required to permit these patterned mitoses.

A third possibility is that the elimination of maternal mRNAs is instructive rather than permissive. For example, a gradual decrease in maternal cell cycle mRNA levels in early *D. melanogaster* embryos might direct the gradual increase in mitotic cycle length and the pause at interphase 14 that allows cellularization. Consistent with this hypothesis, increasing or decreasing the maternal dosage of the *string* and *twine* genes – and thus of maternal *string* and *twine* mRNA levels – results in an increase or decrease, respectively, in the number of nuclear cycles that occur prior to blastoderm cellularization (Edgar and Datar, 1996). Also consistent with this hypothesis, embryos produced by *smaug* mutant females, in which cell cycle mRNAs such as *Cyclin A* and *B* fail to be eliminated, continue to undergo very rapid nuclear cycles without slowing and pausing as in wild-type embryos (Benoit et al., 2009).

These hypotheses are not mutually exclusive. For example, the elimination of maternal cell cycle mRNAs could be both instructive (slowing the cell cycle) and permissive (allowing patterned mitoses after the MZT).

Finally, we note that, during the MZT, a subset of the destabilized maternal mRNAs is eliminated in a spatially regulated manner: they are protected from degradation in the germ plasm, where they function to specify certain aspects of germ cell fate and behavior. This degradation-protection mechanism for maternal transcript localization is present in *D. melanogaster* (Bashirullah et al., 1999; Bergsten and Gavis, 1999; Ding et al., 1993), *C. elegans* (Seydoux and Fire, 1994) and zebrafish (Kopranner et al., 2001). In *D. melanogaster*, several hundred mRNAs are localized to the germ plasm via this mechanism (Lecuyer et al., 2007).

Act 2: zygotic genome activation

“Our remedies oft in ourselves do lie”

Shakespeare, *All's Well That Ends Well* (I.i)

The second event of the MZT is the onset of zygotic transcription, commonly referred to as zygotic genome activation (ZGA). Here we discuss the scale, mechanism and function of ZGA.

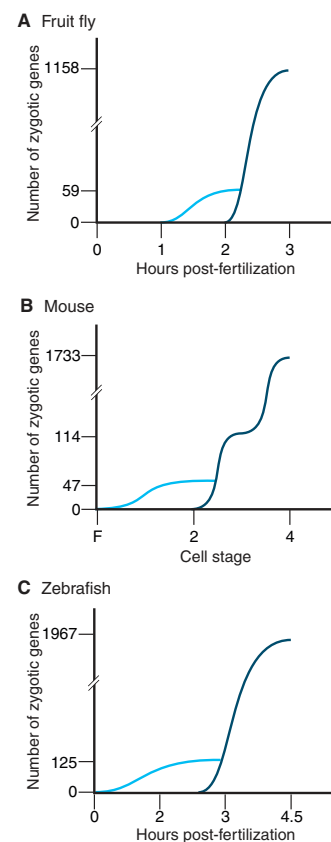


Fig. 3. Minor and major waves of zygotic genome activation during the MZT. The numbers of transcripts produced in the minor (light blue) and major (dark blue) waves of zygotic genome activation are presented. The schematics are based on data from (A) *D. melanogaster* (De Renzis et al., 2007), (B) mouse (Hamatani et al., 2004) and (C) zebrafish (Mathavan et al., 2005).

Dynamics and scale of zygotic genome activation

ZGA occurs in successive waves of increasing degree (see Figs 1, 3). In terms of the embryonic mitoses, mouse and sea urchin embryos begin ZGA the earliest, with the first wave of transcription commencing at the one-cell stage. Whereas BrUTP has been shown to be incorporated into the male pronucleus during the one-cell stage in the mouse (Aoki et al., 1997), genome-wide analyses have revealed only a single transcript, the production of which is sensitive to an RNA polymerase II inhibitor, α -amanitin, at this stage (Hamatani et al., 2004). *D. melanogaster* initiates its minor and major transcriptional waves during cleavage cycles 8 and 14, respectively. In terms of absolute time, however, the very rapid cleavage cycles of the early fly embryo mean that ZGA begins several hours earlier than in the mouse. In *D. melanogaster*, transcription has been detected prior to the first wave of ZGA: genome wide, 30 genes are predicted to be transcribed during the first few nuclear cleavages, within the first 30 minutes after fertilization (Lecuyer et al., 2007).

Microarray-based expression profiling studies – supplemented in *D. melanogaster* by genome-wide fluorescence in situ hybridization analyses (Lecuyer et al., 2007) – have begun to uncover the scope of ZGA: up to 15% of the genome in *D. melanogaster* (Benoit et al., 2009; De Renzis et al., 2007; Lecuyer et al., 2007), over 12% in zebrafish (Mathavan et al., 2005) and over 15% in mice (Hamatani et al., 2004). These values are likely to be underestimates (see Box 3). These genes can be subdivided into two categories: some are strictly zygotic, whereas others are loaded maternally and then re-expressed in the embryo. In sea urchins, 24% of the mRNAs in the early embryo are strictly zygotic (Wei et al., 2006), in *D. melanogaster*, a third fall into this category (De Renzis et al., 2007), whereas in *C. elegans*, only 11% are strictly zygotic (Baugh et al., 2003).

In *D. melanogaster*, the strictly zygotic mRNA set is highly enriched for transcription factors (De Renzis et al., 2007). Some of these are responsible for the rapid establishment of the body plan during the syncytial and cellular blastoderm stages, whereas some others might be responsible for maintaining or inducing subsequent waves of ZGA. Transcription factor-encoding transcripts are also enriched in the pool of mRNAs present in early sea urchin embryos (Samanta et al., 2006); these are expressed in distinct waves at the two-cell stage, the early blastula stage, the early gastrula stage and beyond (Wei et al., 2006). Interestingly, in the sea urchin, although transcripts that encode signaling receptors are present in the maternal pool, those encoding their ligands are strictly zygotic (Wei et al., 2006), probably because the latter are expressed in a spatially regulated manner to specify cell fate and position in the embryo. As mentioned above, in the mouse, ZGA transcripts are enriched for genes involved in RNA transactions (Hamatani et al., 2004; Zeng et al., 2004).

Mechanism and timing of ZGA onset

Four mechanisms that are not mutually exclusive could account for the timing of ZGA onset.

Nucleocytoplasmic ratio

The effects of the nucleocytoplasmic ratio on the control of the embryonic cell cycle have been studied for more than a century (reviewed by Masui and Wang, 1998). The nucleocytoplasmic ratio model hypothesizes that a ZGA repressor is present in the cytoplasm of the early embryo, but is titrated by the increasing number of nuclei (or amount of chromatin) relative to the unchanging volume of cytoplasm. This model was proposed when

Box 3. Genome-wide expression profiling: some caveats

At any given point during the MZT, both maternal and zygotic versions of the same transcript can exist. In general, standard microarray-based analyses (or 'deep-sequencing' methods) cannot distinguish between these two types of transcript. This is particularly problematic when assessing both the extent and the mode of maternal transcript degradation, as well as the extent of zygotic transcription. For example, the observation that a transcript does not change in abundance from the start to the end of the MZT could suggest that it is a maternally contributed, stable mRNA. Another explanation, however, is that the maternally loaded transcript is destabilized but is replaced by zygotic transcription. In *D. melanogaster*, it has been possible to circumvent this difficulty by using large chromosomal deficiencies to remove simultaneously up to 40% of the genes from the embryo but not from the mother, thus eliminating the zygotic component of the mRNA signals read for these genes (De Renzis et al., 2007). This study convincingly showed that two thirds of zygotic transcripts also had a maternal contribution that would otherwise have masked their synthesis during ZGA. With respect to the degradation of maternally supplied transcripts, genome-wide expression profiling studies usually do not have enough temporal resolution to determine whether any specific maternal mRNA is eliminated by maternal and/or by zygotic degradation pathways (see text). This problem can be circumvented in part by removing the latter pathway either through the use of transcriptional inhibitors or, in *D. melanogaster*, by studying activated, unfertilized eggs in which the degradation of maternal mRNAs occurs in the absence of zygotic transcription (Tadros et al., 2007).

it was discovered that, in polyspermic *X. laevis* embryos, zygotic transcription is activated two cleavage divisions earlier than normal (Newport and Kirschner, 1982a; Newport and Kirschner, 1982b). These studies also demonstrated that the timing of *X. laevis* ZGA is not based on a specific number of cleavages, rounds of DNA replication or a 'clock' mechanism. Support for a nucleocytoplasmic ratio model also comes from zebrafish, where a mutation that blocks chromosome segregation but leaves cell division unaffected results in polyploid cells (with a very high local DNA concentration) in which transcription starts several cycles earlier than in wild-type cells (Dekens et al., 2003).

A candidate transcriptional repressor titrated by increases in chromatin levels is the *X. laevis* homolog of DNA methyltransferase (xDmmt1), the depletion of which results in premature zygotic transcription (Stancheva and Meehan, 2000). Surprisingly, the role of xDmmt1 as a transcriptional repressor appears to be independent of DNA methylation, as premature transcription can be rescued by a catalytically inactive version of the protein (Dunican et al., 2008). In flies, the maternally loaded transcription factor Tramtrack (TTK) is a titratable repressor. TTK represses the transcription of the segmentation gene *fushi tarazu* (*ftz*) during early cleavage cycles (Brown et al., 1991; Pritchard and Schubiger, 1996). Eliminating TTK-binding sites or reducing the amount of TTK results in premature *ftz* transcription, whereas TTK overexpression has the opposite effect. By contrast, the modulation of the nucleocytoplasmic ratio in haploid *D. melanogaster* embryos does not determine the timing of zygotic transcription (Edgar et al., 1986). This apparent contradiction has recently been resolved through gene expression profiling studies that show that distinct groups of transcripts are produced during ZGA: the timing of

activation of a minority (including *ftz*) depends on the nucleocytoplasmic ratio, whereas most transcripts are activated via a maternal-clock-type mechanism (Lu et al., 2009).

Maternal clock

A second model postulates that a cell cycle-independent ‘clock’ set in motion by the events of egg activation or fertilization triggers ZGA by activating or producing the transcriptional machinery, or by derepressing zygotic gene transcription. In *X. laevis*, evidence for a maternal clock that regulates events during the MZT came from observations that the timing of Cyclin A and Cyclin E1 protein destruction is independent of the nucleocytoplasmic ratio (Howe et al., 1995; Howe and Newport, 1996). The clock requires the translation of maternal mRNAs, as the administration of a translational inhibitor, cycloheximide, blocks the vast majority (83%) of ZGA in mice (Hamatani et al., 2004). One such mRNA that must be translated during the one-cell stage to permit ZGA is cyclin A2 (*Ccna2*) (Hara et al., 2005). The role of the Cdk2-Ccna2 cell cycle complex in ZGA, however, remains unclear.

In *D. melanogaster*, gene expression profiling has shown that the majority of ZGA depends on absolute time or developmental stage rather than on the nucleocytoplasmic ratio (Lu et al., 2009). SMG, which is translated upon egg activation and is required for the destabilization of the majority of maternal transcripts (Tadros et al., 2007), is essential for the vast majority of high-level transcription during ZGA (Benoit et al., 2009). The SMG-dependent destruction of maternal mRNAs could time ZGA onset (Benoit et al., 2009) (Fig. 4). For example, SMG is required for the destruction of maternal *ttk* mRNA. Thus, during early embryogenesis, two processes might influence the transcriptional repression by TTK: first, as the nucleocytoplasmic ratio increases, TTK protein would be titrated; second, the SMG-dependent destruction of *ttk* mRNA, along with TTK protein turnover would lead to a progressive decrease in TTK protein levels. Additional support for a clock mechanism comes from the live imaging of *D. melanogaster* embryos in which cyclins had been depleted by RNA interference (RNAi), thereby blocking nuclei from entering mitosis (McClelland and O’Farrell, 2008): centrosome division continued with a period that lengthened over successive cycles despite the constant nucleocytoplasmic ratio.

Post-translational processes might also form part of the ZGA ‘timer’. For example, phosphorylation, nuclear shuttling and protein destabilization regulate ZGA onset in *C. elegans* (Güven-Ozkan et al., 2008). TAF-4, a crucial component in the assembly of the transcription factor-II D (TFIID) and the RNA polymerase II pre-initiation complex, is sequestered into the cytoplasm by the zinc finger proteins OMA-1 and OMA-2 during the one- and two-cell stages (Fig. 4). This inhibitory activity of the OMA complex is turned on by its fertilization-dependent phosphorylation. In a uniquely clear-cut example of the link between maternal product clearance and the onset of ZGA, the phosphorylation of OMA-1 and OMA-2 is carried out by MBK-2 (Güven-Ozkan et al., 2008), the same kinase that is responsible for the degradation of many maternal proteins (Stitzel et al., 2006). In fact, this phosphorylation event ultimately marks OMA-1 and OMA-2 for destruction, thereby allowing ZGA to commence in the somatic nuclei of four-cell embryos (Güven-Ozkan et al., 2008).

Transcript abortion

A third model postulates that incomplete zygotic transcripts are aborted by the DNA replication machinery during the rapid early cleavage cycles. This is supported by experiments in *X. laevis*

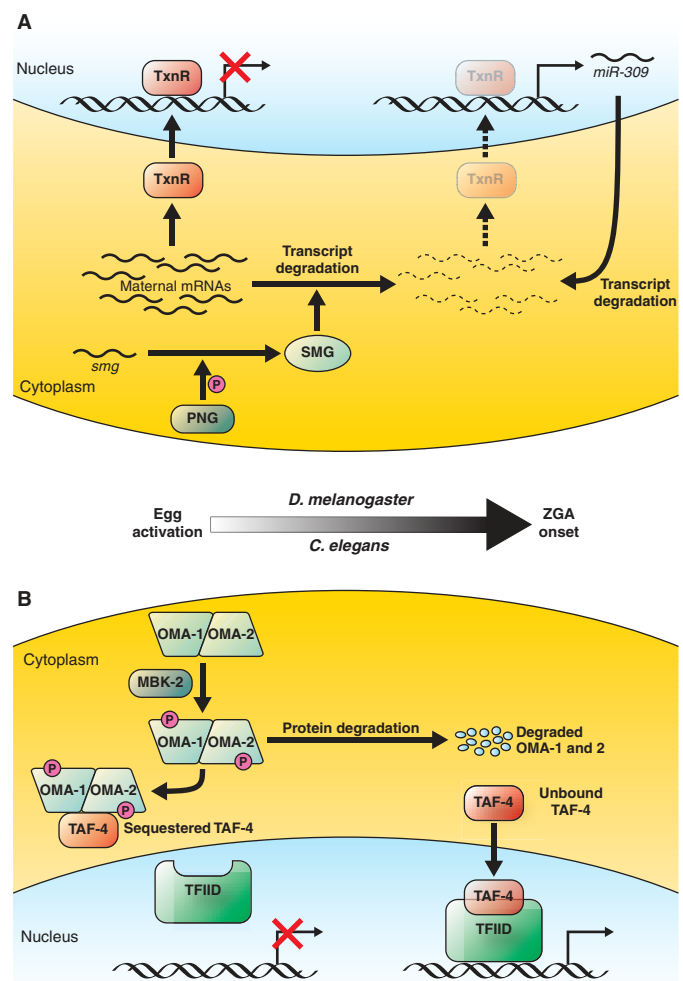


Fig. 4. Interplay between maternal transcript and protein destabilization and zygotic genome activation. (A) In *D. melanogaster*, maternally loaded transcriptional repressors (TxnR), such as TTK, are present in early embryos to maintain transcriptional quiescence.

Egg activation initiates the PNG kinase-mediated translation of Smaug (SMG), a sequence-specific RNA-binding protein that triggers the destabilization of a specific subset of maternal mRNAs. The accumulation of SMG protein is a component of the ‘maternal clock’, the activity of which in progressively eliminating maternal transcripts, including those that encode known transcriptional repressors such as TTK, ultimately reduces their levels below a threshold, thus permitting zygotic transcription to proceed. The ensuing transcription of components involved in the zygotic degradation activity, such as the *miR-309* family of microRNAs, accelerates destabilization and expands the repertoire of destabilized maternal transcripts. (B) In *C. elegans*, an analogous scenario plays out, this time at the post-translational rather than the post-transcriptional level, beginning with the egg activation-triggered phosphorylation of the zinc finger factors OMA-1 and OMA-2 by the kinase MBK-2. This induces OMA-1 and OMA-2 to sequester TAF-4, a component crucial for the assembly of the transcription factor-II D (TFIID) and the RNA polymerase II complex, in the cytoplasm. In the absence of TAF-4, TFIID cannot form the stable DNA-bound complex that is necessary for transcription, which therefore does not occur for the first two cleavage cycles. The phosphorylation of OMA-1 and OMA-2 causes their degradation by the four-cell stage, at which point TAF-4 is free to return to the nucleus and to mediate TFIID-mediated transcriptional activation of the zygotic genome. MBK, minibrain kinase; OMA, oocyte maturation defective; P, phosphorylation; PNG, Pan gu; TAF, TBP-associated transcription factor; ZGA, zygotic genome activation.

(Kimelman et al., 1987) and *D. melanogaster* (Edgar and Schubiger, 1986), in which the application of inhibitors that block the cell cycle (thus extending interphase) result in premature ZGA. Conversely, by studying a relatively large gene, it was demonstrated that progression through mitosis does, in fact, abort transcription (Shermoen and O'Farrell, 1991). This model predicts that the earliest wave of ZGA in flies, which begins when the mitotic cycles are only eight minutes long, would be enriched for relatively short genes. Indeed, the majority of early-expressing *D. melanogaster* genes lack introns and all encode small proteins (De Renzis et al., 2007). However, the fact that premature transcription is not elicited when the cell cycle is blocked prior to cycle 10 (Edgar and Schubiger, 1986) indicates that additional mechanisms function in very early embryos to prevent premature ZGA.

Chromatin regulation

A fourth model hypothesizes that zygotic chromatin is initially not competent for transcriptional activation (e.g. through histone modification). The effects of chromatin modifications on the onset of ZGA have been best studied in mice, where the first signs of transcription occur in the male pronucleus in the middle of the one-cell stage. Immediately after fertilization, the protamines (see Glossary, Box 1) that densely package the sperm DNA are replaced with maternally derived histones (Nonchev and Tsanev, 1990) that are more acetylated than those associated with the maternal pronucleus (Adenot et al., 1997; Santos et al., 2002). This increased acetylation is responsible for the differential transcriptional activity of the male and female pronuclei (Ura et al., 1997). Drugs that alter chromatin structure are able to induce premature gene expression (Aoki et al., 1997), which suggests that the timing of ZGA is accomplished in part by the temporal control of chromatin modifications. BRG1 (SMARCA4 – Mouse Genome Informatics), a component of SWI/SNF-related chromatin remodeling complexes, has been identified as a maternal factor that regulates ZGA (Bultman et al., 2006). SWI/SNF complexes are recruited by transcription factors to clear nucleosomal DNA. When BRG1 is depleted, the transcription of 30% of the genes activated during ZGA fails.

Apart from understanding what regulates the timing of ZGA onset, even less is known about the transcription factors that specifically mediate ZGA or the cis-acting elements through which these factors function. In *D. melanogaster*, variants of a 7-mer sequence called 'TAGteam' are overrepresented within 500 bp of the transcription start sites of the first wave of zygotic transcripts (ten Bosch et al., 2006). Two factors that bind TAGteam have been identified: Bicoid Stability Factor (BSF) and Zelda (ZLD; VFL – FlyBase) (De Renzis et al., 2007; Liang et al., 2008). Although the role of BSF is unclear, ZLD, a zinc-finger transcription factor, has been shown to be required for the first wave of ZGA in *D. melanogaster* embryos (Liang et al., 2008). Interestingly, both ZLD (Liang et al., 2008) and SMG (Benoit et al., 2009) are required for the transcription of the *miR-309* cluster, the products of which provide feedback to destabilize a subset of maternal mRNAs (Bushati et al., 2008). SMG and ZLD might function independently in ZGA: SMG to eliminate transcriptional repression, and ZLD to direct transcriptional activation.

Functions of ZGA

To assess the developmental role of ZGA, either transcription must be inhibited with drugs or genetic manipulations must be carried out that prevent some or all genes from being expressed after, but not before, fertilization. The results of experiments applying transcriptional inhibitors to early embryos have led to the conclusion that the first developmental requirements for

zygotic transcription occur significantly after the earliest detected traces of transcription, coinciding instead with the onset of the major wave(s) of ZGA. For example, after α -amanitin treatment, mouse embryos cleave to the two-cell stage, but the next cleavage is blocked (Braude et al., 1979). In *D. melanogaster*, similar treatment prevents cellularization, which normally coincides with the major ZGA wave (Edgar and Datar, 1996). Despite the fact that some transcription is first detected at the four-cell stage in *C. elegans* embryos (Seydoux and Fire, 1994; Seydoux et al., 1996), development can continue well into gastrulation – to about the sixth or seventh cell cycle – in the presence of α -amanitin (Edgar et al., 1994). In both frogs (Newport and Kirschner, 1982a) and zebrafish (Zamir et al., 1997), zygotic transcription is required for gastrulation immediately after the MBT.

A closer examination of the effects of transcriptional inhibitors has shown subtle earlier developmental defects. For instance, the first, minor ZGA wave is required in *D. melanogaster* embryos to establish subtle asymmetries in nuclear density along the anteroposterior axis as early as nuclear division cycle 11 (Blankenship and Wieschaus, 2001). Likewise, depleting the large subunit of RNA polymerase II in *C. elegans* embryos using RNAi results in defects in the division and migration of a pair of endodermal cells as early as the fifth cell cycle (Powell-Coffman et al., 1996).

Genetic strategies have led to the identification of specific zygotic genes that direct the developmental processes described above. The search for these genes has benefited from the existence of large chromosomal deficiencies and rearrangements in organisms such as flies and worms. In *D. melanogaster*, compound chromosomes (see Glossary, Box 1) have been used to remove the entire zygotic genome, one chromosome or chromosomal arm at a time (Merrill et al., 1988; Wieschaus and Sweeton, 1988). Following the use of smaller chromosomal deficiencies, these studies have shown that zygotic genes are not required during the early cleavage cycles and that a surprisingly small number of zygotic loci (eight, including *nullo*, which is one of the 30 earliest-expressed zygotic genes discussed above) are essential between cycle 14 and the onset of gastrulation. Similar experiments in worms, in which half of the zygotic genome was systematically removed, did not recover phenotypes as severe or as early as those observed after transcriptional inhibition (Storfer-Glazer and Wood, 1994). This might be attributable to genetic redundancy; alternatively, essential early-acting zygotic genes might reside in the untested half of the genome.

Conclusions

“what's done, is done.”

Shakespeare, *Macbeth* (III.ii)

To reach a full understanding of the mechanisms and functions of maternal transcript destabilization and zygotic genome activation during the MZT, it will be necessary to define the relationship of these components to each other. For example, even though we know that zygotic transcription is required for the degradation of a subset of maternal transcripts, it is not yet clear whether the converse is true. We now have genome-wide catalogs of maternal and zygotic transcripts for half-a-dozen animals, in some cases together with a certain knowledge of transcript dynamics during the MZT. Whereas analyses of these transcripts and their molecular and biological functions have been very informative, it would not be surprising, given the high degree of post-transcriptional regulation that occurs during early embryogenesis, if the transcriptome and the proteome did not correlate well during this stage. The regulation of maternal

products at the level of protein stability occurs in *X. laevis*, mice and *C. elegans*, where, for example, the successful exit from meiosis requires the ubiquitin-mediated degradation of several specific proteins upon egg activation (Lu and Mains, 2007; Rauh et al., 2005; Shoji et al., 2006). Catalogs of the proteome during the MZT are now beginning to appear (Gouw et al., 2009); initial results are consistent with the hypothesis that control of protein synthesis and stability are as important for early development as control of mRNA synthesis, stability and localization. Extensive post-translational modifications, such as changes in phosphorylation state, also occur during egg activation in frogs and sea urchins (Mochida and Hunt, 2007; Roux et al., 2006). Ultimately, therefore, to reach a full understanding of the players involved in the regulation of the MZT, we need to have accurate catalogs of the transcriptome and the proteome, as well as of post-translational modifications that are imposed on the proteome, before, during and after the MZT.

Genetic and RNAi-mediated analyses have led to significant advances in our understanding of the function of several RNAs and proteins that regulate and implement the MZT. However, many of these players have additional functions either during oogenesis or at other developmental stages; their phenotypes are thus pleiotropic and difficult to interpret. Reciprocally, genetic and functional redundancy could make it difficult to obtain any phenotype at all for some MZT components. RNAi-mediated knock down will be an important tool with which to assess the role of pleiotropic or redundant players, because this method can be used to exert temporal and spatial control on the removal of the RNAs encoding one, or even several, proteins. However, given that many of the maternal products that function during the MZT are loaded into the egg not just as RNA, but also as protein, knock-down approaches are of limited use in these cases as the elimination of the mRNA does not necessarily lead to reduced protein levels. The development and application of methods to remove proteins (Ditzel et al., 2003; Levy et al., 1999) and/or their post-translational modifications in a temporally and spatially regulated manner will thus be essential to future studies of the MZT, which are bound to uncover additional factors and novel mechanisms involved in this fundamental developmental event.

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