Cell. Mol. Life Sci. 65 (2008) 212 – 227
1420-682X/08/020212-16
DOI 10.1007/s00018-007-7295-4

Cellular and Molecular Life Sciences

Review

From the beginning: the basal transcription machinery and onset of transcription in the early animal embryo

M. Zurita*, E. Reynaud and J. Aguilar-Fuentes

Department of Developmental Genetics and Molecular Physiology. Institute of Biotechnology, National Autonomous University of México, Av. Universidad 2001, Cuernavaca Morelos 62250 (México), Fax: 52 777 3172388, e-mail: marioz@ibt.unam.mx

Received 28 June 2007; received after revision 7 August 2007; accepted 11 September 2007
Online First 15 October 2007

Abstract. Transcription onset in the early animal embryo is a fundamental process required for proper embryonic development. Depending on the species, transcription onset occurs at what specifically appears to be different developmental stages. However, studies in early embryos from different animal models have shown that components of the basal transcription machinery play fundamental and highly regulated roles at the onset of transcription. The state of the basal transcription machinery in the embryo seems to be equivalent in different organisms at transcription onset. The dynamic balance between putative activators and repressors as well as the chromatin/cytoplasmic ratio seem to be coordinated with basal transcription factors in order to activate zygotic transcription. Here we discuss and compare the regulation of the basal transcription machineries and their activation in early embryos of different model organisms.

Keywords. Transcription, animal embryo, development, zygotic gene activation.

Introduction

The transition dynamics of a transcriptionally active oocyte to a silenced one and transcription activation in the early animal embryo after fecundation are fascinating phenomena. They require that the basal transcription machinery and the chromatin structure be modulated at different levels so that transcription can be tuned on in the embryo, a mechanism coordinated with the specific developmental characteristics of each animal and generally established by maternal factors [1]. Although many clues have been uncovered as to how this process might be regulated, definitive answers remain elusive. Over the past 20 years, researchers have learned a great deal about the function of the transcription machinery involved in eukaryotic cells. However, signals and pathways responsible for activating these mechanisms for the first time at the beginning of animal development are still not completely understood. As we have mentioned, the earliest events in animal embryo development are controlled by maternal factors produced during oogenesis. However at specific developmental times that are characteristic for each species, *z*ygotic *g*ene *a*ctivation (ZGA) becomes fundamental for proper embryo development. Here we review and compare what is known regarding the role of the basal transcription machinery at transcription onset in embryos of four classical animal models in which it has been best studied: *Drosophila, Caenorhabditis elegans, Xenopus laevis* and the mouse.

* Corresponding author.

A quick overview of the basal transcription machinery for mRNA synthesis in eukaryotic cells

Research for the identification and functional characterization of the components required for RNA polymerase II (RNPII) transcription has generated a detailed view of how this process occurs, although it is far from finalized. Since in this review we discuss what is known of the role of some RNPII transcription factors in ZGA, we will start by describing in general fashion, the function of some of the more relevant factors during transcription activation. However, for a more detailed discussion, we recommend two excellent recent reviews [2, 3].

The pre-initiation-complex

Many factors are involved in RNA synthesis in eukaryotic cells. Transcription of mRNA involves the assembly of multiple protein factors responsible for the formation of the pre-initiation-complex (PIC) [2–4] (Fig. 1). The principal general factors involved in assembly of the PIC are TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH, RNPII and Mediator. These factors, together with RNPII, are required to initiate transcription [3]. A summary of the PIC components and its protein composition are presented in Table 1. The function of each individual factor has been experimentally determined, mostly in reconstituted *in vitro* assays [3]. TFIIA functions to stabilize binding of TFIID to the target DNA. It can also act as an anti-repressor and coactivator [5]. TFIIB helps determine the position of transcription initiation, stabilizes the TBP-TATA complex, is required for the recruitment of TFIIF and selects the transcription initiation site [6, 7]. TFIID contains the TATA-binding protein (TBP) and at least 14 TBP-associated factors (TAFs), although in human cells about 20 different TAFs have been found. These factors collectively bind DNA proximal to the transcription start site (at the TATA box motif in some promoters) and act as a platform for the incorporation of TFIIA and TFIIB. Among the components of TFIID, some TAFs show tissue-specific variants, and some animals have TBP-related factors (TLFs) that have specific and in some cases equivalent functions to TBP [2, 4, 8]. Some TAFs can also be found in complexes other than TFIID, and TBP can also be found as a component of complexes involved in transcription activation by RNPI and RNPIII such as SL1 and TFIIIB, respectively [2, 9]. In addition, TFIID may work as a coactivator and several enzymatic activities have been found, such as protein kinase, ubiquitin-activating and -conjugating activities as well as histone acetyl transferase activity. All these activities are necessary at some point in different promoters for their transcriptional activation [2–4]. TFIIE stabilizes the open complex, recruits TFIIH and facilitates the formation of an initiation complex allowing promoter clearance [10]. TFIIF acts in concert with other basal factors to recruit RNPII and is involved in the formation of the open complex [11]. TFIIF is also involved in the recruitment of TFIIE and TFIIH. TFIIF together with TFIIB participates in the selection of the transcription initiation site and facilitates RNPII elongation [12]. TFIIH is a multifunctional complex that also participates in DNA repair and cell cycle control [13]. During transcription initiation, TFIIH facilitates the formation of the open complex by the action of two ATP-dependent helicases XPB and XPD and the Cdk7 kinase subunit that phosphorylates the RNPII large-subunit carboxy-terminal domain (CTD) allowing the polymerase to escape from the promoter. Recently the p44 subunit of TFIIH has also been reported to have a ubiquitin ligase activity [14].

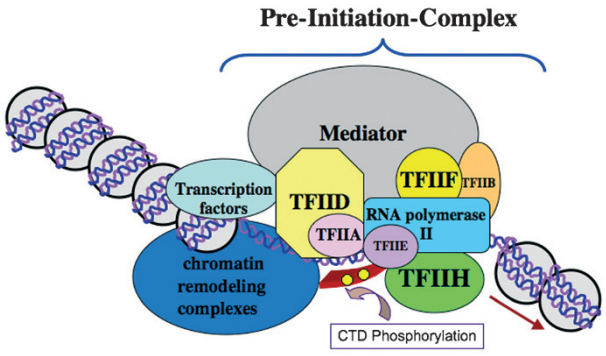

Figure 1. Schematic representation of the RNA polymerase II pre-initiation complex (PIC). Based on different experimental conditions, two models have been proposed for assembly of the PIC on class II gene promoters (RNPII promoters). In the first model, the different basal factors are sequentially assembled on the promoter. The second model postulates the existence of an RNPII holoenzyme containing most of the basal factors except TFIID, with promoter recognition by TFIID triggering recruitment of the holoenzyme to the promoter. The figure represents the PIC at the moment of the open complex, when a bubble is formed in the DNA and the RNPII large subunit CTD is phosphorylated. In brief, the components and functions of PIC are as follows: TFIIA blocks TAF1 transcription inhibition and may also participate as positive and negative regulator; TFIIB is important to define the transcription initiation site; TFIID helps the binding of TFIIB and RNPII and is the target of repressors and activators; TFIIF stabilizes the binding of RNPII with the promoter and is important for the open complex formation; TFIIE stabilizes the open complex and interacts with TFIIH; TFIIH is fundamental for the melting of the DNA around the transcription initiation site and phosphorylates the RNPII large-subunit CTD domain; Mediator binds the RNPII and acts as transducer of activators and repressors to the PIC. For more details consult the text and Table 1. Tissue-specific transcription factors and chromatin remodeling complexes are not considered to be part of the PIC.

The RNPII large-subunit CTD domain

The function of all the above-mentioned factors allows RNPII to initiate and elongate transcription.

Table 1. Components of the RNPII Pre-initiation-complex.

Factor	Number of subunits	Functions
TFIIA	3	stabilizes TBP-TATA complex; co-activator and anti-repressor
TFIIB	1	binds TBP; selection of the transcription Initiation site; binds RNPII
TFIID	TBP TAFs (around 14)	promoter binding factor. Recognition of promoter elements; histone-acetyl-transferase activity; ubiquitin activating and conjugating activities
TFIIE	2	formation of the initiation complex; interacts with TFIIH; facilitates RNPII promoter escape
TFIIF	2	binds RNPII; recruits TFIIE and TFIIH; participates in RNPII promoter escape and elongation.
TFIIH	10	allows RNPII promoter escape and elongation; helicase, ATPase, kinase and ubiquitin ligase activities. Also involved in DNA repair and cell cycle control
RNPII	12	transcription initiation, elongation and termination; CTD can be phosphorylated, ubiquinated and glycosylated.
Mediator	12–24	positive and negative modulator; transduces signals from different transcription factors to the pre-initiation complex.

RNPII is composed of 12 subunits that are highly conserved in all eukaryotic cells. RNPII was first identified by Roeder and Rutter in 1969 [15] together with RNPI and RNPIII. Five of the RNPII subunits are also present in RNPI and RNPIII. The CTD of the largest RNPII subunit contains tandem repeats of the heptapeptide, YSPTSPS. The number of repeats of this amino acid sequence differs among species, with the *Drosophila* and human CTDs containing 42 and 52 repeats, respectively. The CTD may be phosphorylated at two serine residues (S2 and S5) of each repeat. It is generally accepted that a non-phosphorylated CTD is involved in PIC assembly and transcription initiation, while the hyper-phosphorylated form is required for transcription elongation. Phosphorylation and dephosphorylation of the CTD occur during the assembly and disassembly of the PIC, and different phosphorylation patterns are required to recruit the capping, splicing, transcription termination and 3'-end processing factors during transcription. Thus, the stage of RNPII-CTD phosphorylation in a given promoter may define the transcriptional status of the associated gene [16, 17].

Mediator

Another complex that has recently been the subject of studies about transcription initiation by RNPII is Mediator. Mediator is a general cofactor that transduces signals from different transcription factors to the PIC. Mediator is composed of complexes comprising 11 to 14 different proteins in animals, or 24 in yeast. In human cells, two forms of Mediator have been identified, under different purification protocols [18]. Structural and biochemical studies have shown that Mediator has three structural modules, namely the head, middle and tail. The head module interacts with RNPII and is thought to modulate conformational changes in RNPII. The head also interacts with specific transcriptional activators. The middle contacts RNPII at the place where it intersects with the tail module. This interaction seems to allow conformational changes to take place in Mediator, facilitating recruitment of the RNPII. In addition, a subgroup of four proteins form a specific module that can sometimes be identified as component of Mediator. This module includes a cyclin-dependent kinase (CDK8) along with cyclin C, which modulates CDK8 kinase activity. CDK8 can phosphorylate serines 2 and 5 in the CTD as well as cyclin H (a component of TFIIH), inactivating the kinase activity present in TFIIH, suggesting that it negatively regulates the PIC. The Mediator complex that contains the CDK8 module can repress transcription in vitro and there is evidence that CDK8 may be involved in the negative regulation of specific genes. In addition, CDK8 also phosphorylates specific transcription factors having a locus-specific effect [19, 20]. Thus, Mediator may act as both a negative regulator and a stimulatory cofactor for transcription. In addition to its (CDK8) kinase activity, it has also been found to have histone acetyl transferase activity in yeast, but so far this has not been identified in other organisms [21–23]. Other multiprotein factors may also participate in general transcription. Examples of these are SAGA and SLIC complexes in yeast and the TBP-free TAFII-containing complex (TFTC) and STAGA in animals, which have multiple enzymatic activities and contain several TAFs that stimulate transcription by interacting with the PIC [for review see refs 2, 3].

From silencing to activation

During oogenesis, an important transition takes place at the level of gene expression, as transcriptionally active chromatin changes to silenced chromatin dur-

ing meosis. This implies that chromatin is modified and transcription factors, in general, are excluded from the compacted chromosomes. This is even more dramatic in spermatogenesis where the entire genome is shut down and hyper-compacted. Only after fertilization and at different developmental stages, specific for each species, is transcription activated again, but now in totally undifferentiated and totipotential cells, in some cases, as in mouse transcription, beginning at the two-pronuclei stage, while in the fly it starts after the 13th mitotic division.

The onset of transcription at ZGA

The analysis of different models has demonstrated that maternal factors are deposited in the oocyte cytoplasm with all the necessary components, including proteins and mRNAs, necessary for the transition from silent to transcriptionally active chromatin. For example, about 50% of the mRNAs encoded in the *Drosophila* genome are present in the early embryo by maternal contribution [24] and in mice, maternally deposited mRNA composes about 40% of the total genome encoded transcripts [25].

In the case of *Drosophila*, after pro-nuclei fusion, the embryo goes through 13 synchronic nuclear divisions without cell divisions, resulting in the formation of a syncytium. However, during the first 8 divisions, only the S and M phases of the cell cycle are completed and there is no evidence of transcription. During nuclear divisions 8–10, however, a weak transcriptional wave initiates and histone, GAP, pair rule and sex determination genes are expressed. After nuclear division 13, cellularization of the embryo takes place, the cellular blastoderm forms and widespread transcription is activated in somatic cells [26] (Fig. 2). The rate of mRNA transcription between the syncytial blastoderm and the cellular blastoderm are different as it has been clearly demonstrated that there is only low mRNA synthesis until the cellular blastoderm [27, 28], and that polyA$^+$ containing RNA transcription is dramatically increased in cellularized nuclei when compared to syncytial blastoderm nuclei [29, 28].

In the case of early embryo development of the nematode *C. elegans*, which has asymmetrical and asynchronous cell cleavages, maternal factors are segregated and then become involved in ZGA. The first signals of zygotic transcription are detectable at the four-cell stage (Fig. 2). Intriguing experiments showed that inactivation of RNPII using RNAi or α-amanitin only affected post-gastrulation transcription [30] (Fig. 2), thus indicating that maternally contributed factors mediate the development of *C. elegans* embryos until the 100-cell stage, without requiring any new transcripts.

An important point that has to be considered is that in *Drosophila* and *C. elegans*, the activation of transcription in germ cells occurs later in development than in the somatic cells. Transcriptional repression in the germ cells of both organisms is maintained at the level of RNPII elongation [31]. In the case of *Drosophila*, this repressed state in the germ cells requires the PCG peptide as well as Nanos and Pumillo [32]. In *C. elegans*, transcriptional repression requires the product of the gene PIE-1 and the Nanos homologs NOS-1 and NOS-2 [33]. The repressed state in the germ cells correlates with the absence of trimethylated lysine 4 in the histone H3 (H3K43me) in the germ cell chromatin [31], which is a marker for active chromatin, linked to RNPII elongation and suppression of cryptic transcription [34].

Xenopus has been a classical vertebrate model for the study of transcription onset in the early embryo. In 1935, Lanclos and Hamilton [35] showed that high transcriptional activity occurred during *Xenopus* oogenesis, but transcription was inactivated at oocyte maturation. Similar to post-fertilization *Drosophila* embryos, the *Xenopus* embryo undergoes 11 rapid mitotic cycles that are essentially transcription free [36], with maternally contributed factors mediating embryonic development. Although a small amount of transcriptional activity can be detected after the sixth mitotic division in *Xenopus* embryos [36], global ZGA begins at the mid-blastula transition (MBT), when the embryo is at the 4000-cell stage (Fig. 2). At this point, a shift from synchronous to asynchronous divisions takes place. Therefore, for the African frog also, two transcriptional waves can be postulated.

In mammals, ZGA occurs at different developmental stages depending on the species. In mouse, high transcriptional activity occurs during early oogenesis, then after germinal vesicle breakdown, the oocytes arrest at metaphase II and no transcription is detected [37]. This process takes about 14 h; transcription can then be detected only about 10 h later, after fertilization and only when the pro-nuclei are already formed. During this time, several cytoplasmic processes take place, including the translation and systematic degradation of maternally deposited mRNAs [38]. From the one-cell stage to the two-cell stage, a degradation of about 90% of the stored RNA in the oocyte takes place [39]. Thus, some of the basal transcription factors or their corresponding mRNAs are maternally contributed and used to activate embryo transcription. For still unknown reasons, most of the transcription that occurs at the one-cell stage happens in the male pro-nuclei [39] (Fig. 2). A recent review indicates that the mouse genome is activated in the embryo at the two-cell stage [40]; however, there is evidence that the first signals of transcription, al-

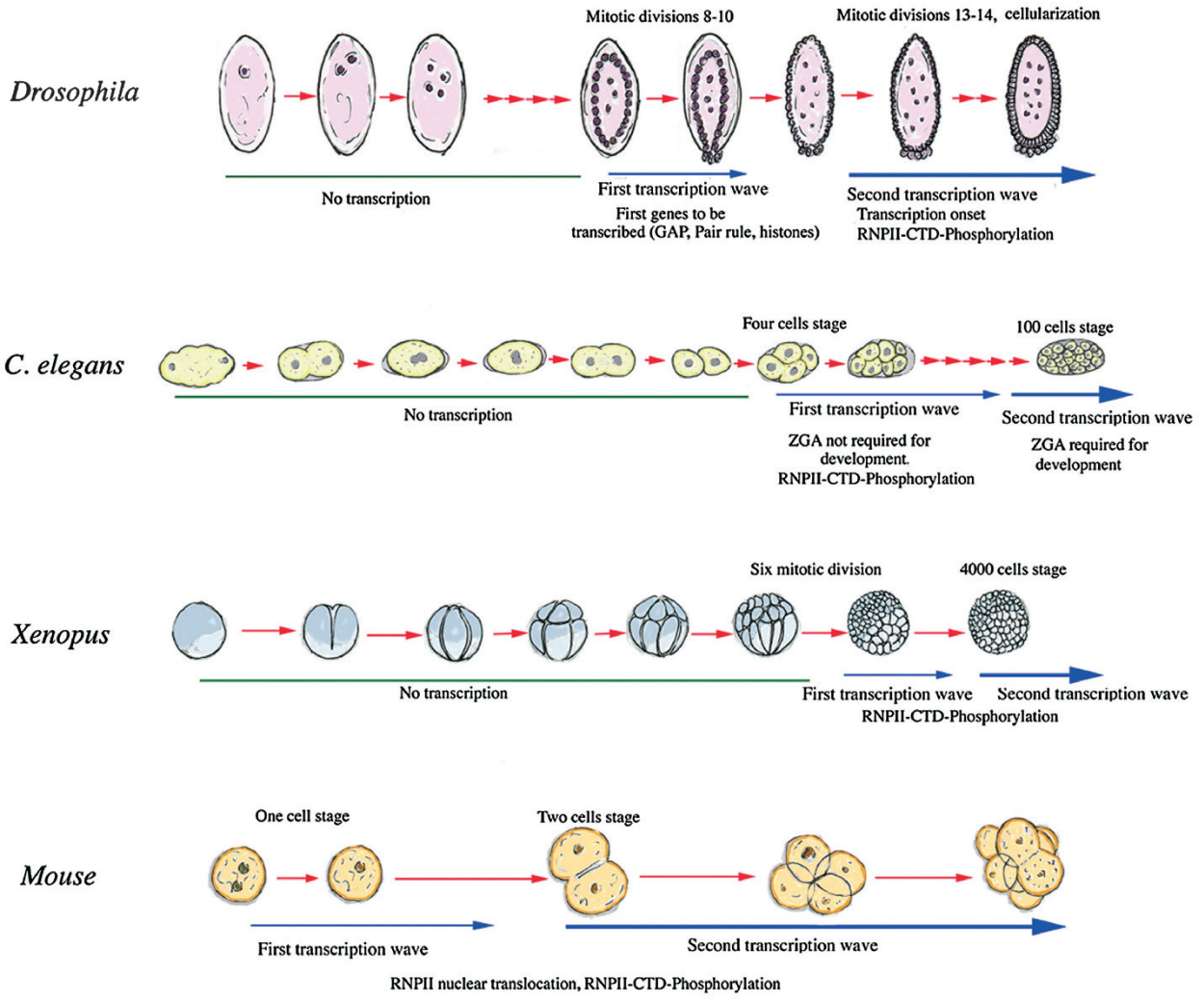

Figure 2. Comparative diagram of transcription onset in *Drosophila*, *C. elegans*, *Xenopus* and mouse during the early developmental stages. In the case of the fly, *C. elegans* and *Xenopus*, the initial mitotic divisions occur without transcription. In the mouse embryo, transcription initiates at the one-cell stage, at the male pro-nuclei, in a process that requires several hours. All organisms seem to have two transcriptional waves. The first one only involves a few selected genes, and the second establishes somatic gene expression.

though at low levels, occur even before the fusion of the two pro-nuclei [38]. Therefore, also in the mouse embryo, two transcriptional stages have been identified, a minor transcriptional wave at the one-cell stage and a second major transcriptional wave at the two-cell stage [41]. This conclusion is supported by experiments that have shown that at the one-cell stage, there is an important RNPII-dependent incorporation of bromouridine triphosphate (BrUTP), and at two-cell stage, the RNA synthesis has a clear increase in BrUTP incorporation. The BrUTP incorporation during the one-cell stage is only 40% of the total BrUTP incorporation at the two-cell stage, again indicating two different stages of transcriptional activation. The higher levels of BrUTP incorporation acquired in the two-cell stage are maintained in subsequent developmental stages [41, 42]. Therefore in the mouse, the basal transcription machinery is ready to activate, after fertilization, the transcription of characteristic genes such as those encoding heat shock proteins, transcription factors, components of the translational machinery and factors involved in splicing [38, 41, 42].

A relatively new and useful tool for the identification of the initial genes transcribed is the DNA microarray in *Drosophila.* These have been applied to mice and *C. elegans* but unfortunately in *Xenopus*, only very recently has robust genomic data become available. In the case of *C. elegans*, studies analyzing lineage-specific zygotic transcripts have shown that there are at least 275 genes that increase their transcription rate at the 12-cell stage, compared with the 4-cell stage [43]. This suggests that even if ZGA activation begins in *C. elegans* at the 4-cell stage, later in development,

higher transcriptional activity ensues. With this information it can be proposed that in *C. elegans* there are also two transcriptional waves as in the case of *Drosophila, Xenopus* and mice: the first wave at the 4-cell stage and a second wave of broad transcription at the 100 cell stage, required for the continuity of embryo development (Fig. 2).

Maternal mRNA degradation and ZGA

An important point that has only been considered recently is the switch between maternally encoded mRNAs and the activation of zygotic transcripts. In *Drosophila* it has been shown that maternal mRNA degradation takes place simultaneously with gene activation. In the fly, maternal mRNA degradation occurs by two mRNA degradation pathways. One occurs before the MBT and is maternally encoded and some maternal mRNA 3' untranslated regions (UTRs) contain cis-acting elements are required for this degradation pathway. The second mechanism is activated just before the MBT. Both mechanisms are essential for embryo development [44]. In the fly, also about 20 % of the degradation of these transcripts is dependent on the multitranslational regulator SMAUG, which is modulated by cell cycle regulators like PAN GU [24]. On the other hand, a recent elegant study in *Drosophila* using chromosome ablation and microarrays demonstrates that that expression of some of the first zygotic transcripts correlates with the concomitant maternal mRNA degradation. The zygotic transcripts are especially enriched in transcription factors that modulate the subsequent differentiation program. Interestingly, the earlier zygotic transcripts come from intronless genes that are regulated by a specific transcription factor that enhances its expression at ZGA [45]. Based on these results, De Renzis et al., [45] postulate that the absence of introns in the first genes that are transcribed has an evolutionary advantage that facilitates the fast mitotic divisions of the 10–13 stages. These results demonstrate a coordinated relationship between ZGA and maternal mRNA steady-state levels and suggest the role of a specific activator (we will discuss later the role of repressors and activators). The analysis of how this activator is regulated will be an interesting challenge for future experiments.

The mice early embryo also has been the subject of study to identify the first genes transcribed at ZGA. Two recent reports that use microarrays compare the transcript profile of one-cell to two-cell embryos treated with or without α-amanitin reported intriguing results. In one case, mRNA transcripts sensitive to α-amanitin in the one-cell stage were not found [46]. In the second, only one transcript sensitive to α-amanitin was detected [47]. A possible explanation is that in the α-amanitin-treated embryos, BrUTP incorporation at the one-cell stage is due to the transcription of polyA$^-$ RNPII-dependent RNAs like small nuclear RNA, small nucleolar RNA and histone transcripts. It is also possible that in the methods used, mRNAs with very short polyA$^+$ tails could not be detected. Based on these results, it can be speculated that *de novo* transcription (RNPII-dependent BrUTP-RNA incorporation) in the one-cell-stage embryo only includes a small fraction of the total mature mRNAs detected in the microarray experiments. It is likely that most of them are maternal transcripts that encode components used in mRNA metabolism and protein synthesis that are required for the embryo to continue and maintain development from the one cell to the two cell stage, but not further, since about 90 % of the oocyte-stored RNA is degraded by the end of the two-cell stage [42, 48] and later developmental stages [47].

Recently the role of microRNA (miRNA) in the transition from maternal to zygotic mRNA has been the focus of attention. For example, in early zebrafish embryos, the degradation of some maternal mRNAs is controlled by a miRNA that is expressed at ZGA. This miRNA also promotes deadenylation of target mRNAs, suggesting that this pathway may have some control over the translation from maternal to zygotic transcripts in the early embryo [49]. During mouse oogenesis, miRNAs are expressed and stored in the mature oocyte. Indeed Dicer is required for oocyte maturation [50]. Intriguingly, about the 60 % of the maternal miRNAs are degraded between the one-cell to two-cell embryo [51], a stage in which most of the maternal mRNAs are also degraded. Later, at the four-cell stage, miRNA levels rise again. These interesting results suggest a dynamic role for the miRNA machinery at the transition from maternal to zygotic transcripts and thus is supported by the finding that Dicer-deficient embryos were not able to develop from the one-cell to the two cell stage [51]. Therefore, the miRNA and RNAi machineries may have a role in the transition from maternal to zygotic mRNA in the early animal embryo.

Although these studies identify the transition from maternal to zygotic RNA, it is important to point out that it is not only maternal RNA that is stored in the early embryo, but maternal proteins as well, that in many cases function in early and late developmental stages. This is the case of homozygous lethal mutants in several basal transcription factors in *Drosophila,* in which the protein stored in the oocyte allows embryo development until larval stages [52, 53]. Related to this point, some maternal transcripts and their translated protein products are present and act to ensure transcription during the first cell division in mice. One of these, cyclin A2, modulates the kinase activity of

CDK2 (CycA2-CDK2), as shown by studies revealing that inactivation of cyclin A2 inhibited transcriptional activation at the one-cell stage [54]. Although the CycA2-CDK2-specific transcription-activating targets are not yet known, it seems possible that this kinase activity is required to activate transcription factors that may include members of the basal transcription machinery.

PIC players and CTD phosphorylation at ZGA

A number of researchers have sought to analyze ZGA in different models by identifying the components and dynamics of the basal transcription machinery and its influence in CTD phosphoryaltion. Immunostaining, RNAi and protein ablation have been used in early embryos to study how PIC formation is regulated at transcription onset.

Nuclear translocation of PIC components

Different studies have collectively shown that the subunits of RNPII, as well as other components of the basal transcription machinery, are maternally contributed to the cytoplasm of the early embryo and are translocated from the cytoplasm to the nuclei just before ZGA. For example, in *Drosophila*, the RNAPII large subunit can be detected in the nuclei of the syncytial blastoderm at mitotic cycle 7, but its phosphorylated active form is detected only at the cellular blastoderm stage, which correlates with the high transcriptional activity seen at this stage [55]. In the fly also, TBP may be initially detected in the nuclei at mitotic cycle 8, just before expression of the first zygotic genes [56]. On the other hand, TFIIH, which is also deposited in the early embryo cytoplasm by maternal contribution, can be detected in the cytoplasm of the syncytial blastoderm before the onset of transcription [57]. However, not until after nuclear division 9 are the core and CDK-activating kinase of TFIIH translocated into the nuclei, where they are positioned at active gene promoters for ZGA [57].

As in other animals, the mouse oocyte contains large amounts of RNPII; the levels of phosphorylated CTD decrease during oocyte maturation and increase after fertilization at the end of the one-cell stage, showing good correlation with ZGA [58]. Interestingly, a transitory phosphorylated form of the large RNPII subunit may be detected prior to zygotic transcription [58]. Most of the transitory phosphorylated form is cytoplasmic, while the hypo-phosphorylated form is present both in the cytoplasm and the nucleus and the persistent form of hyper-phosphorylated RNPII is mostly nuclear. Translocation of RNPII from the cytoplasm to the nuclei takes place at the late one-cell stage, about 9 h after fertilization and preferentially in the male pro-nucleus. This nuclear translocation does not require the *de novo* synthesis of RNA or proteins, and therefore follows a program established during oocyte development. Based on these results, it seems logical to believe that the nuclear translocation of RNPII and its CTD phosphorylation are a major level of developmental regulation for ZGA in the mouse embryo [58]. In addition, components of the TFIID factor like TBP and TAF1 are practically undetectable in the pro-nuclei after fertilization. TBP can be identified inside the male mouse pro-nuclei after 4 h post-fertilization and TAF1 after 6 h. Therefore, the nuclear localization of TBP and TAF1 together with RNPII translocation from the cytoplasm to the nuclei correlates with ZGA. The evidence observed in *Drosophila* and mice suggests that activation of the basal transcription machinery is a limiting step for ZGA [59, 60]. However, these experiments cannot distinguish if the regulated translocation of the basal transcription machinery causes transcription onset or, due to transcriptional activation, the basal transcription components accumulate in the early embryo nuclei.

TAF's and Mediator in CTD phosphoryaltion

C. elegans is probably the organism in which the most studies on the role and dynamics of the basal transcription machinery at ZGA have been done. The CTD-phosphorylated RNPII form can be detected in the somatic nuclei at the four-cell stage. The relationship between CTD phosphorylation and some TAFs has been documented. For example, TAF5 can be identified in the nuclei at the two-cell stage, whereas TAF10 and TAF11 are present in four-cell-stage nuclei [61]. By the time gastrulation occurs, all three of these maternally derived TAFs are located inside the nuclei. RNAi inactivation of these three TAFs and TFIIB showed that Ser5 CTD phosphorylation was reduced by TFIIB RNAi, only slightly affected by TAF5 RNAi, and not affected by TAF10 and TAF11 RNAi, while Ser2 phosphorylation was largely reduced by inactivation of TFIIB or TAF5 and partially reduced by inactivation of TAF10 and TAF11 [61]. These results indicate that Ser5 phosphorylation, which is critical to open-complex formation, requires TFIIB and has some need for TAF5, but not TAF11 and TAF10, whereas Ser2 phosphorylation, which is required for RNPII-based elongation, requires TAF5 and TFIIB and has some need for TAF10 and TAF11. On the other hand, TAF5 is required for general gene expression, while TAF10 and TAF11 are only involved in the expression of a fraction of genes at these stages. This may correlate with the observation that TAF5 can be found inside nuclei at the two-cell stage,

while TAF10 and TAF11 do not show nuclear localization until the four-cell stage. In future experiments, it could be interesting to determine if the early nuclear localization of TAF5 is related to its broad role in transcription. Intriguingly, the TAF10 and TAF11 transcriptionally associated genes were found to be metazoan-specific genes [50], suggesting that loci-specific functions may be conferred to different TFIID-related complexes during ZGA.

In addition, studies using RNAi against TAF1 and TAF2 in early embryos have shown that both proteins are required for mRNA transcription of several genes in early *C. elegans* embryos and, furthermore, the total RNPII-CTD phosphorylation levels were dramatically reduced as a consequence of the RNAi knock-down [62]. Altogether these studies indicate that an important component for transcription activation in the *C. elegans* embryo is TFIID, although it seems that there are TAFs that are dispensable for the expression of some genes at ZGA while others are not. On the other hand, the demonstration that TFIID -pecific TAFs like TAF1 and TAF2 are necessary for gene expression at ZGA in *C. elegans,* at least for most of the genes that have been analyzed, is intriguing, since it has been demonstrated that these proteins are not required for the expression of about 86 % and 97 % of yeast genes, respectively [63]. This implies that the complexity of ZGA in metazoans requires the ordered action of TFIID including the sequential use of most of its TAFs.

A recent study in *Xenopus* on a new TBP-related factor named TBP2, which is only present in vertebrates, shows that in some genes it has redundant roles with TBP during ZGA in regulating their expression, but not in others [64]. Knock-down and ChIP experiments demonstrated that TBP2 is positioned in promoters of genes expressed in early embryos, in particular at the elongation factor 1a promoter. TBP2 is required for the transcription of a subset of genes in the early embryo and can substitute the role of TBP in some promoters, but it is restricted to early development. Therefore, different TBPs may have various functions in controlling different genes at the point of ZGA.

On the other hand, both in the mouse embryo as in *Xenopus*, it seems that some TBP-related factors can substitute or have a redundant TBP function for the expression of some genes in early development. Homozygous early embryos that have been knocked out for the TBP gene have active transcription by RNPII [65]. In these embryos, the TBP-related factor 3 (TRF3) appears to be one of the redundant factors involved in RNPII transcription at ZGA. TRF3 is expressed in oocytes and early embryos, and ChIP experiments demonstrated that although there is selectivity for some promoters between TBP and TRF3, depending on their relative amount, these factors can be redundant for the activation of some promoters at ZGA [65]. However, in these TBP knock-out experiments, the presence of enough TBP deposited in the egg by maternal contribution may mask the TBP requirements in the early embryo.

In *C. elegans*, CTD phosphorylation at Ser2 and Ser5 is affected when the highly conserved RGR-1 subunit of the Mediator complex is knocked down [66]. Therefore, the connection that has been reported between Mediator activities and CTD phosphorylation in yeast is also a key element in ZGA in *C. elegans* [66]. In addition, a TBP-like factor (CeTLF) was found to be located in nuclei at the two-cell stage and its inactivation by RNAi prevented CTD phosphorylation and the expression of specific early patterning genes. The fact that it is present in similar amounts with TBP suggests that it has a specific function in the activation of specific genes at ZGA [67, 68].

CTD kinases

Studies of the kinases responsible for phosphorylating the CTD have identified the involvement of CDK9, which together with cyclin T forms the p-TEFb complex and phosphorylates Ser2 of the CTD [69]. It has been proposed that this modification coordinates the capping and elongation of RNPII transcripts. Notably, p-TEFb is essential for the expression of early embryonic genes and the phosphorylation of Ser 2 in the CTD and the elongation factor SPT-5. Experiments in *C. elegans* have shown that p-TEFb and SPT-4/SPT-5 have opposing functions during RNPII elongation, and p-TEFb is thought to mediate several different post-initiation pathways [69]. However, it is not yet known how p-TEFb functions in these differential pathways. In addition, the role of p-TEFb in early gene expression has not yet been analyzed in the four-cell *C. elegans* embryo, when ZGA occurs.

Another kinase identified as being involved in CTD phosphorylation is CDK7, which phosphorylates the CTD Ser5 and is a component of TFIIH [13, 70, 71]. Since zygotic transcripts are not required in the early *C. elegans* embryo until the 100-cell stage, a conditional mutant and RNAi were used to determine the roles of CDK7 in embryonic transcription and cell cycle control. Interestingly, CDK7 inactivation at the 50-cell stage led to developmental arrest, demonstrating that CDK7 plays a role in cell cycle control. Similar results have been found in *Drosophila* either using Cdk7 mutants or by the microinjection of early embryos with anti-CDK7 antibodies [57, 72–74]. In addition, a conditional CDK7 mutant showed changes

in the early expression patterns of the zygotic genes, reduced CTD phosphorylation and a longer cell cycle [73, 74]. Thus, in the early *C. elegans* and in *Drosophila* embryos, CDK7 appears to participate in both cell cycle control and transcription onset [72–76]. Future studies will be required to determine how CDK7 simultaneously and differentially participates in both functions at the same time.

Based on the analysis of the dynamics of CTD phosphorylation during *C. elegans* oocyte silencing and its subsequent transcription activation in the embryo, Walker and colleagues [77] proposed a very attractive model that suggests that transcription activation is prepared during oocyte maturation. This model is based on experiments that show that RNAi inhibition of the CTD phosphatase Fcp-1 in oocytes that have entered diakinesis, and which are inactive in transcription, accumulate large amounts of Ser 5-phosphorylated CTD in the nuclei. Intriguingly, RNAi inhibition of components in the ubiquitination pathways produces the same effect. In addition, RNAi inhibition of Cdk7 and other components of the PIC, together with the inhibition of Fcp-1, does not show CTD Ser5 phosphorylation, suggesting that the CTD phosphorylation occurs in the context of the PIC. Thus this model proposes that at the beginning of diakinesis, gene expression is silenced due to aborture transcription processes. The basal transcription machinery, however, is already positioned at gene promoters that require rapid activation at ZGA. Supporting this hypothesis is the fact that when oocyte maturation is stimulated, there is also an increase in the Ser5-phosphorylated CTD. Another important point contributed by this work is that the balance and regulation between CTD phosphatases and CTD kinases plays a major role in ZGA. However, other components of the PIC are required for CTD phosphorylation and it has not been demonstrated that phosphorylated RNPII and the rest of the basal transcription machinery are positioned in chromatin that is going to be transcribed at ZGA. Demonstrating this will be a major task, since the system is highly dynamic.

In addition, substantial evidence indicates that CTD phosphorylation is a key aspect in the initiation of mRNA synthesis in *Xenopus* [78]. After fertilization, the CTD is rapidly dephosphorylated in the *Xenopus* embryo. This unphosphorylated state is maintained throughout several divisions, and then the CTD is rapidly phosphorylated at the MPT [79]. Although the dynamics and roles of the TFIIH and p-TEFb complexes have not yet been analyzed in *Xenopus*, experiments in other models have shown that these factors play fundamental roles in CTD phosphorylation. Additional work will be required to examine the mechanisms governing post-fertilization CTD dephosphorylation in *Xenopus* and other organisms.

Taking together the accumulated knowledge about the dynamics and functions of PIC components at ZGA, it is clear that PIC formation is a key element for the transition of a silent to an active genome. On the other hand, the CTD phosphorylation is an important marker that determines the transcription activation state in the animal embryo. However, techniques with more resolution, such as chromatin immunoprecipitation, will be required to identify the PIC status in specific genes at ZGA.

Repressors, activators and the chromatin/cytoplasm ratio

The regulation of PIC components to activate transcription has to be coordinated with the modulators that selectively activate gene expression. One of the more interesting hypotheses about transcription activation in early embryo development has been the influence of repressors and activators. In general, these ideas come from studies in which the introduction of exogenous DNA accelerates ZGA, probably by the titration of a global repressor as well as the fact that some activators are necessary for the transcriptional activation of specialized genes. In initial studies, specific exogenous DNA templates capable of being assembled as nucleosomes were injected into *Xenopus* embryos, in an effort to determine at what point in development they were transcribed [80]. These experiments revealed that templates carrying RNPIII promoters were transcribed prior to the MBT and further suggested that their transcription was activated by a shift in the ratio between chromatin and transcription factors [80]. An interpretation of this model suggests that as cell division progresses, increased DNA assembly into chromatin titrates a repressor (i.e. a global repressor, histones or some other structural chromatin factor), allowing transcription of the newly relaxed chromatin.

This hypothesis was challenged by Almouzni and Wolfe [81], who demonstrated that the introduction of an exogenous DNA template containing a RNPII promoter (CMV), was only transcribed after the MBT. Interestingly, when they simultaneously injected their promoter construct along with a non-specific DNA (e.g. λ DNA), they observed histone titration but no transcription from the RNPII promoter. However, they observed transcription following co-injection of template DNA along with non-specific DNA and TBP. They also corroborated that the activation of RNPIII promoters can occur before the MBT in the absence of TBP, but showed that the

RNPII promoter required TBP [81]. To complement these experiments, the authors used the minimal adenovirus E4 promoter under the control of the yeast GAL4 transcriptional activator and found that introduction of exogenous GAL4 activated transcription of the exogenous DNA template prior to the MBT. Based on these observations, it was proposed that activation of transcription in the early *Xenopus* embryo is not only due to the DNA-based titration of repressors, but is also mediated by the limiting activity of transcription factors, such as TBP [81]. This interesting hypothesis seems to explain the observed results and may indicate that TBP acts as a limiting factor for activating transcription at the MBT in the *Xenopus* system. However, activation of the minimal adenovirus E4 promoter by GAL4 did not require extra TBP under their experimental conditions.

Supporting the hypothesis that TBP may be a limiting factor, another study showed that TBP protein levels are minimal in the early embryo and increase prior to the MBT, when ZGA begins [82]. Therefore, beside the DNA/chromatin ratio, the levels of active components of the basal transcription machinery (e.g. TBP) are also important for mediating transcription activation at the MBT. Interestingly, TBP is essential for continued development after gastrulation, and a TBP-like factor (TLF/TRF2) is essential for continued development after the MBT [83]. However, it was also shown that transcription is repressed before the MBT, even if a transcriptional activator is positioned at its DNA-binding element; this seems to indicate that the activator is unable to recruit the basal transcription machinery at this developmental stage. After MBT, the histone/DNA ratio approaches its normal somatic value and the PIC can be assembled [84]. Therefore, it can be concluded that the ratio of transcriptional activators and repressed chromatin is a major determinant for ZGA in *Xenopus*.

Interestingly, early studies showed that ZGA is not initiated simultaneously in all nuclei of the *Drosophila* embryo [85]. For example, the *fushi taratzu* gene (*ftz*) is initially transcribed in some nuclei at mitotic cycles 8–9, with *ftz* transcription seen in an increasing number of nuclei during later rounds of division. It has been shown that the ratio between the number of nuclei and the concentration of a repressing transcription factor is critical for triggering zygotic transcription in *Drosophila*. This is the case for *ftz*, which is repressed by *tramtrack*. It has been proposed that as the nuclei/cytoplasm ratio increases across sequential nuclear divisions, a titration effect stochastically decreases the effective concentration of the transcriptional repressor in each nucleus, allowing gradual transcription activation [85]. This model is highly attractive if we assume that all the genes activated at the point of ZGA have a titratable repressor which may be specific or not. Since it is obvious that many genes do not have a known specific repressor, and this model implies that the repressor binds to the control region of genes, it seems alternatively possible that a global repressing chromatin structure exists in the earliest embryo and eventually becomes titrated through successive nuclear divisions, thus obviating the need for specific repressor factors

In *Drosophila*, some of the genes encoding the earliest zygotic transcripts (e.g. those involved in sex determination and patterning) share a DNA motif called the TAGteam. This motif is required for their expression at mitotic cycles 8–10, suggesting the action of an activator [86]. Interestingly, in the previously mentioned work in *Drosophila* by De Renzis et al. [45], a similar element was identified upstream of the gene promoter encoding the early transcribed genes. This *cis* motif is about seven base pairs long and is recognized by the bicoid stability factor (BSF), which was previously identified as a factor that binds the *bicoid* mRNA. BSF can activate transcription in early embryos in constructs containing this 7-bp motif upstream of a reporter gene, demonstrating that it operates at ZGA. This discovery is highly relevant, since for the first time the existence of a general activator for ZGA has been demonstrated. Since putative BSF homologs are present in other organisms, it will be imperative to know if BSF has an equivalent function in early vertebrate embryos.

Taking into account the above-mentioned repressor and activators activity, it has been proposed that the balance between repressors and activators may determine the early expression of some genes [86]. However, while these are important arguments for the regulation of genes expressed in specific cells of the early embryo, additional work will be required to determine how these processes are coordinated with the basal transcription machinery. In addition, it is important to note that in the fly, most of the genes activated in the syncytial nuclei at mitotic cycle 10 continue to be expressed during and after cellularization, suggesting that establishment of molecular memory around some promoters allows continued transcription. Interestingly, TFIID is maintained in active promoters during mitosis, and the presence of maternal BSF [45] in the different early fly embryo stages suggests that a memory mark may be established [87].

To make the process a little more complex in the fly, the overexpression in *Drosophila* embryos of the Nanos (Nos) protein, which is a translational repressor of *Hunchback* mRNA, required for the establishment of posterior embryo development, appears to have a global repressing role in transcription by

reducing the CTD phosphorylation levels in early somatic cells [88]. These data suggest that Nos may inhibit the translation of factors required for CTD phosphorylation in the posterior embryo, thus maintaining reduced levels of global transcription that may be required for posterior development. However, which factors involved in CTD phosphorylation are regulated by Nos are unknown. Some of the possible Nos targets could be mRNAs encoding different TFIIH subunits. However, it has been demonstrated that different TFIIH protein subunits are already present in the early embryo by maternal contribution [57]. Therefore, it is also possible that the Nos targets may be mRNAs encoding other components of the basal transcription machinery required for RNPII activation and, as a consequence, a reduction in phosphorylated CTD levels. Thus in *Drosophila*, Nos not only participates in the regulation of ZGA in the germ cells, but also in somatic cells.

An intriguing hypothesis making a link between maternal mRNA degradation and activators like BSF that bind both RNA and DNA, could be that the activator is titrated by maternal RNA in the early embryo and that when it becomes degraded, the activator is released and thus allowed to activate transcription. A simple experiment to test this hypothesis could be to enhance maternal mRNA degradation and thus analyze whether ZGA becomes accelerated under these conditions.

Chromatin and epigenetic markers at transcription onset

Transcriptional activation in the embryo requires additional processes beyond the coordinated activation of the basal transcription machinery. For example, epigenetic changes occur in chromatin during the transition from a transcriptionally silent to a transcriptionally active genome. During early *Xenopus* development, DNA methylation is required for transcriptional silencing before the MBT, and inactivation of maternal DNA methyltransferases was shown to cause transcriptional activation two cell divisions earlier than normal [89]. In addition, the promoters of genes that are activated at the MBT, such as TFIIA and c-Myc, show reduced methylation at this stage, whereas repetitive DNA elements remain methylated [90]. These data collectively indicate that DNA methylation plays an important role in the onset of transcription in the early embryo.

Global chromatin changes at the onset of transcription have been observed in early *Drosophila.* During the first nuclear divisions, the condensed chromatin preferentially contains the high-mobility-group protein, HMG-D, but not the H1 histone. In contrast, H1 accumulates after nuclear division 7, when the nuclei become more compact and transcription is activated [91]. Therefore, a correlation exists between the HMG-D/H1 and nuclei/cytoplasm ratios at the point of ZGA. It has been speculated that this change in chromatin composition is needed because more condensed chromatin is required to permit the next nuclear divisions. However, during these stages, the chromatin structure around the activated genes must be relaxed, or at least in a configuration that allows activators and the basal transcription machinery to access their promoters.

The heterochromatin domains in the chromosomes of the *Drosophila* embryo seem to be established early in development. It has recently been discovered that a complex that contains the proteins Su(var)3-3 and Su(var) 3-9, which are a histone H3-lysine 4 demethylase and a histone H3-lysine 9 methylase respectively, coordinate the demethylation of lysine 4 and the methylation of lysine 9 of histone 3. These H3 modifications are part of the signals that determine the limits between heterochromatin and euchromatin during cellularization [92]. Therefore, at the same time as transcription activation, the epigenetic boundaries between transcribed and silenced chromatin have to be established (Fig. 3).

Chromatin rearrangements, nucleosome modifications and changes in protein composition have also been shown to occur before zygotic transcription in mammals [93]. It is intriguing that the male pronucleus is transcriptionally active before the female pro-nuclei. To achieve this, male pro-nuclei suffer a drastic chromatin remodeling process including the change from protamines for histones that are maternally supplied. During this process, histone modifications seem to be required to activate transcription [92]. For example, in the one-cell mice embryo, there are differences in the amount of histone H3-lysine 9 methylation between male and female pro-nuclei. In contrast to the female pro-nuclei, the male pronucleus has practically no H3-Lys9 methylation and its H3-Lys4 methylation is increased, correlating with its higher transcriptional activity [94, 95]. Later, at the four-cell stage, the H3-Lys9 methylation is similar between the two parental genomes. In addition, histone acetylation is increased in the male pronuclei and at the same time reduced in the female one. This process occurs by the action of histone acetyl transferases and deacetylases supplied by the oocyte [94, 95].

A recent study using a combination of techniques including Cre-loxP gene targeting and RNAi demonstrated that the SWI/SNF2 remodeling complex subunit, BRG1 (a homologue of the Brahma protein

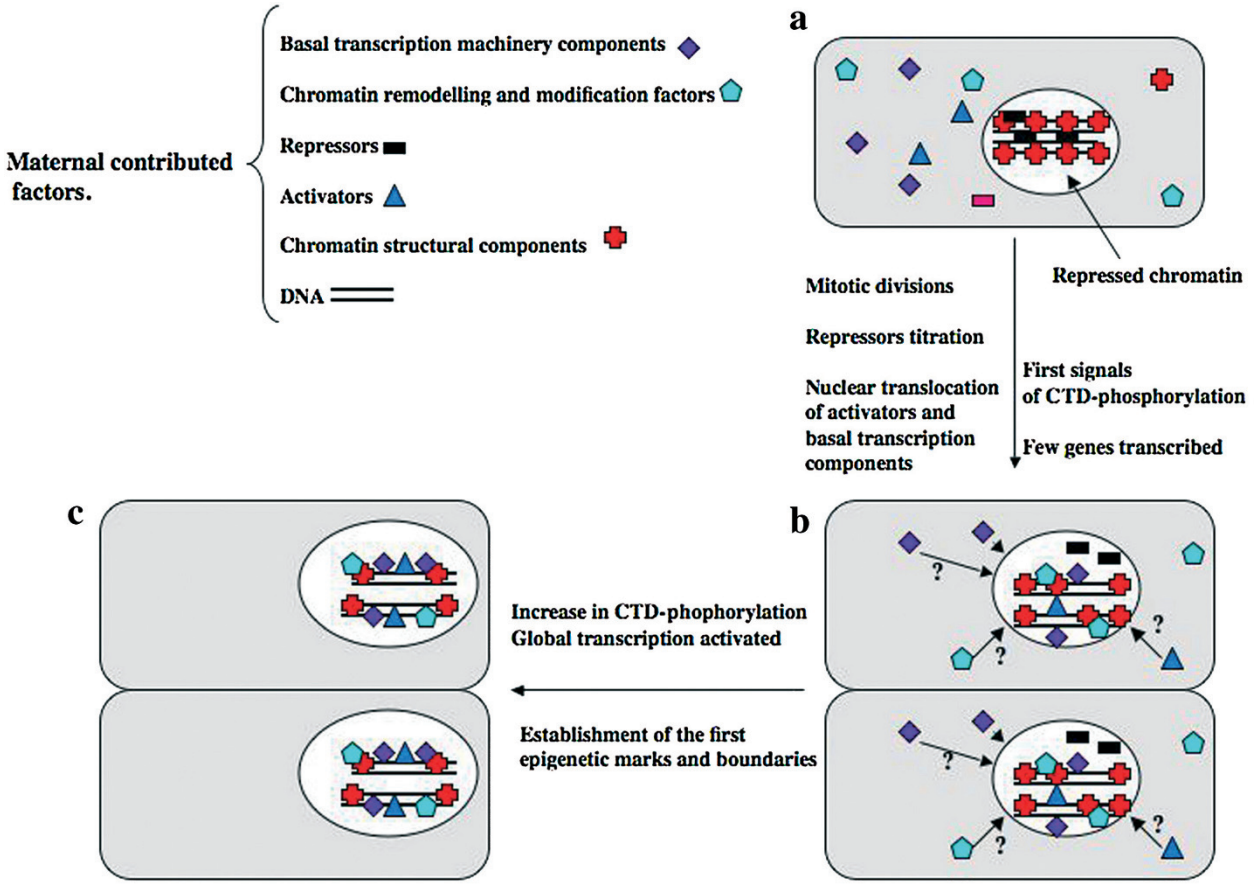

Figure 3. General mechanisms for transcription onset in animal embryos. (*a*) Maternal factors required for the activation of transcription are deposited in the egg cytoplasm, both in the form of mRNA and protein. These factors include components of the basal transcription machinery, chromatin remodeling and modification factors, transcriptional repressors, transcription activators and chromatin structural components (histones and other chromatin proteins). At this stage, no transcriptional activity is detected and the chromatin is in a repressed state. (*b*) During the first stage of transcription activation, specific repressors and structural chromatin components are titrated by nuclear division, while transcription activators and components of the basal transcription machinery (e.g. RNPII) are translocated into the nuclei. In some cases, such as *Drosophila*, the basal factors translocate at various nuclear division stages, but are all present inside the nuclei at the time of transcription onset, when the CTD is phosphorylated and transcription elongation takes place at a few specific zygotic genes. (*c*) Later in development and in some organisms at the MBT, global transcription is activated, correlating with increased CTD phosphorylation levels. At this stage, the first epigenetic marks are established.

in *Drosophila*), was required for activation of zygotic transcription in mice [96]. The transcribed genes comprised cell cycle regulators, transcription factors and components of the RNA-processing machineries, and inactivation of BRG1 led to embryonic arrest at the two-cell stage. Reduced BRG1 was concomitant with reduced methylation of Lys4 in histone H3, which marks transcriptionally active chromatin. No global reduction in histone acetylation was detected, but only a few acetylation markers were tested. A more detailed analysis will be required to determine if reduced BRG1 activity affects other histone modifications. Based on these results, a model has been proposed in which the BRG-containing SWI/SNF chromatin-remodeling complex acts together with histone acetyl transferases to methylate K4 in histone H3 and activate transcription via the PIC [96]. This model of ZGA is attractive, but does not yet account for coordination with other chromatin-remodeling factors and the basal transcription machinery. Indeed, there is evidence that other factors participate with the chromatin-remodeling machinery at ZGA in mice. One of these factor is the transcription intermediary factor 1α (TIF-1α) that is required for the proper chromatin localization of BRG1, SNF2H and the RNPII [97]. TIF-1α has been identified as a transcription regulator and it can be associated with chromatin-remodeling factors [98]. TIF-1α ablation using RNAi affects the expression of genes that are activated just after ZGA in the mouse one-cell embryo. These genes include proteins that may be involved in RNA processing [97]. It is intriguing that in the microarray assay studies for the identification of α-amanitin-sensitive mRNAs at

the one-cell stage, none of these transcripts were identified [47, 48].

Conclusions and perspectives

Even though ZGA occurs at different developmental stages in various species, it seems that the factors required for transcription activation, including components of the basal transcription machinery (e.g. RNPII), chromatin-remodeling factors and histone modification complexes, are in general deposited during oogenesis. In *Drosophila* and *Xenopus* and perhaps other organisms, unknown factors may govern ZGA by regulating translocation of maternally derived factors from the cytoplasm to the nuclei. Another commonality is that CTD phosphorylation and its subsequent effects on transcription are central cues that determine the basal transcription factor status in ZGA in all animals. A paradox is that in *Drosophila*, CTD phosphorylation is not detected at the mitotic cycles 8–10 in the early embryo, when the first zygotic genes are transcribed. However, since only few genes are transcribed at this point, it is possible that the immunostaining experiments have not been sensitive enough to detect the few RNPII large-subunit molecules that are actually phosphorylated at the CTD. We think that this is probably the case, since together with the RNPII, CDK7 is positioned at the promoters of the genes to be transcribed at mitotic cycles 8–10 [53].

The four organisms discussed here seem to have two transcriptional waves during ZGA. In the first wave, transcription of a selected few genes occurs early in development (at the one-cell stage in mice, mitotic division rounds 8–10 in the fly, the four-cell stage in *C. elegans* and the sixth mitotic division in the frog); these genes are likely required for the continuance of cell division and/or for the activation of the second transcription wave, which involves the expression of a larger number of genes (Figs 2, 3). Although the possibility that the higher levels of transcription in the second wave are a consequence of a general and continuous increase of transcription during development cannot be discarded, the finding in *Drosophila* of a gene activator specific for the early stages supports the two-wave hypothesis.

Although various studies suggest that DNA/chromatin and/or DNA/repressor ratios control transcription activation during early embryonic development, the generality of this model is not yet fully accepted in all animal models. Future work will be required to assess the participation of activators for transcription of large gene sets, as well as the coordination of repressors and activators with the basal transcription machinery and various chromatin remodeling factors (Fig. 3).

The more we learn about the onset of transcription in different animal models, the more similar the pathways appear. The observed differences like the different transcriptional activation timings, seem largely superficial, and probably reflect an escalation effect caused by differences in cytoplasm volume and the chromatin/cytoplasm ratio. For example, we have to take into account that in the fly, the activator BSF is produced during oogenesis, and is only active until mitotic division 10. When this ratio is low at the time of fertilization, more synchronous replication cycles will be required to trigger induction of global and space/time-regulated somatic transcription. To address this possibility, it would be interesting to study ZGA carefully in organisms that have very small chromatin/cytoplasm ratios, such as chicken and zebrafish. Another possible basis for the observed differences in transcription onset among species could be evolutionary adaptation of the basal transcription machinery to various developmental conditions and/or the development of species-specific transcription factors (activators and repressors) responsible for coordinating the balance between transcriptionally permissive or repressive chromatin.

It will also be valuable to study how factors that activate ZGA at the correct time are coordinated with the basal transcription machinery, and how chromatin structure and its regulation determines the activation of a somatic nucleus introduced into an early embryo [99]. The latter experiments may help us understand why so few cloned organisms develop to maturity, and may be of primary importance for future progress in somatic cell cloning, dedifferentiation and tissue engineering. Therefore, new studies using updated genomic approaches (e.g. ChIP on chip and new imaging techniques) will be useful for investigating early transcription requirements at the genome-wide level, as well as for identifying and characterizing the roles of global repressors, activators and components of the basal transcription machinery during ZGA.

Before ZGA some maternal mRNAs are degraded while others can remain until late developmental stages, particularly in *Drosophila.* Therefore, the mechanisms involved in the selectivity of mRNA degradation and the possible role of miRNA and RNAi machineries are just beginning to be explored. On the other hand, we know very little about the genes that are differentially transcribed in the two transcriptional waves at ZGA. Therefore similar microarray experiments to those performed in one cell and two cell mice embryos, in the presence and absence of α-amanitin, will be highly relevant if they are integrated with ChIP on chip experiments.

In summary, from the information discussed here, three models compatible with the available data can be proposed. (1) There are general transcription repressors and activators that are deposited during ovogenesis either as mRNA or as proteins and they are translated or postranslationally modified in an orderly way during early development to activate gene transcription. (2) There are general transcription repressor or repressors that are simply titrated as the chromatin/cytoplasmic ratio diminishes. (3) Components of the basal transcription machinery are deposited in the egg cytoplasm, their translocation into the nuclei is then tightly regulated in order to start zygotic transcription. It is important to stress that these possible mechanisms are not mutually exclusive and all of them may be involved in some form in ZGA. These models open several exciting new questions. For example: How is the nuclear translocation of the PIC components regulated before ZGA? How are the cell cycle and ZGA coordinated? What is the role of chromosome condensation in gene expression during early nuclear divisions? How does the activator BSF initiate ZGA? Is there a mechanism that links the RNAi machinery with maternal mRNA degradation and P-body formation? Thus the problem of how ZGA is achieved in the early animal embryo still requires many answers and is far from resolved. However with all the accumulated knowledge in these four model organisms and the viability of new techniques, the time is ripe to look forward to the solution of this problem.

Acknowledgements. We apologize to authors whose work could not be cited because of space limitations. M. Z. gratefully acknowledges financial support from the Howard Hughes Medical Institute, CONACyT and PAPIIT/UNAM.

1 Davidson, E. H. (1986) Gene Activity in the early development. Academic Press Orlando.
2 Thomas, M. C. and Chiang C-M. (2006) The general transcription machinery and general cofactors. Crit. Rev. Biochem. Mol. Biol. 41, 105–178.
3 Hahn, S. (2004) Structure and mechanism of the RNA polymerase II transcription machinery. Nat Struct. Mol. Biol. 11, 394–403.
4 Buratowski, S., Hahn, S., Guarente, L., and Sharp, P. A. (1989) Five intermediate complexes in transcription initiation by RNA polymerase II. Cell 56, 549–561.
5 Solow, S., Salunek, M., Ryan, R. and Liberman, P. M. (2001) TAF250 phosphorylates human transcription factor IIA on serine residues important for TBP binding and transcription activity. J. Biol. Chem. 276, 15886–15892.
6 Orphanides, G., Lagrange, T., and Reinberg, D. (1996) The general transcription factors of RNA polymerase II. Genes Dev. 10, 2657–2683.
7 Ranish, J. A., Yudkosvsky, N., and Hahn, S., (1999) Intermediates in formation and activity of the RNA polymerase II preinitiation complex holoenzyme recruitment and a postrecruitment role for the TATA box and TFIIB. Genes Dev. 13, 49–63.
8 Hiller, M. A., Lin, T. Y., Wood. C. and Fuller, M. T. (2001) Developmental regulation of transcription by tissue-specific TAF homolog. Genes Dev. 15, 1021–1030.
9 McMahom, S. B., Van Buskirk, H. A., Dugan, K. A., Copeland, T. D., and Cole, M. D. (1998) The novel ATM-related protein TRAP is an essential cofactor for the c-Myc and E2F oncoprotiens. Cell 94, 363–374.
10 Goodrich, J. A. and Tijian, R. (1994) Transcription factors IIE and IIH and ATP hydrolysis direct promoter clearance by RNA polymerase II. Cell 77, 145–156.
11 Ghazy, M. A., Brodie, S. A., Ammerman, M. L., Ziegler, L. M. and Ponticelli, A. S. (2004) Aminoacid substitutions in yeast TFIIF confer upstream shifts in transcription initiation and altered interactions with RNA polymerase II. Mol. Cell. Biol. 24, 10975–10985.
12 Shilatifard, A., Conoway, R .C., and Conoway, J. W. (2003) The RNA polymerase II elongation complex. Annu. Rev. Biochem. 72, 693–715.
13 Zurita, M. and Merino, C. (2003) The transcriptional complexity of the TFIIH complex. Trends Genet. 19, 578–584.
14 Takagi, Y., Masuda, C. A., Chang, W. H., Komori, H., Wang, D., Hunter, T., Joazeiro, C. A. and Kornberg, R. D. (2005) Ubiquitin ligase activity of TFIIH and the transcriptional response to DNA damage. Mol. Cell 18, 237–243.
15 Roeder, R. G. and Rutter, W. J. (1969) Multiple forms of DNA-dependent RNA polymerase in eukaryotic organisms. Nature 224, 234–237.
16 Meinhart,A., Kamenski, T., Hoeppner, S., Baumli, S. and Cramer P. (2005) A structural perspective of CTD function. Genes Dev. 19, 1401–1415.
17 Lee, T. I. and Young, R. A. (2000) Transcription of eukaryotic protein coding genes. Annu. Rev. Genet 34, 77–137.
18 Konberg, R. D. (2005) Mediator and the mechanism of transcriptional activation. Trends Biochem. Sci. 30, 235–239.
19 Loncle, N., Boube, M., Joulia, L., Boschiero, C., Werner, M., Cribbs, D. l. and Bourbon, H.-M. (2007) Distinct roles for mediator Cdk8 module subunits in Drosophila development. EMBO J. 26, 1045–1054.
20 Elmlund, H. Barazanenok, V. Lindahl, M., Samuelsen, C. O., Koeck, P. J., Holmerg, S., Hebert, H. and Gustafsson, C. M. (2006) Cyclin dependent kinase 8 modulate sterically blocks Mediator interactions with RNA polymerase II. Proc. Natl. Acad. Sci. USA 103, 15788–15793.
21 Conoway, R. C., Sato, S., Tomomori-Sato, C., Yao, T., and Conoway, J.W (2005) The mammalian Mediator complex and its role in transcriptional regulation. Trends Biochem. Sci. 30, 250–255.
22 Malik, S. and Roeder, R. G. (2005) Dynamic regulation of pol II transcription by the mammalian Mediator complex. Trends Biochem. Sci. 30, 256–263.
23 Kim, Y. J. and Lis, J. T. (2005) Interactions between subunits of *Drosophila* Mediator and activator proteins. Trends Biochem. Sci. 30, 245–249.
24 Tadros, W., Goldman, A. L., Babak, T., Menzies, F., Vardy, L., Orr-Weaver, T., Hughes, T. R., Westwood, J. T., Smibert, C. A. and Lipshitz, H. (2007) SMAUG is a major regulator of maternal mRNA destabilization in *Drosophila* and its translation is activated by the PAN GU kinase. Dev. Cell 12, 143–154.
25 Wang, Q. T., Piotrowska, K., Ciemerych, M. A., Milenkovic, L., Scott, M., Davis, R. W. and Zernicka-Goetz, M. (2004) A genome-wide study of gene activity reveals developmental signaling pathways in the preimplantation mouse embryo. Dev Cell 6, 133–144.
26 Yasuda, G. K. and Shubiger, G. (1992) Temporal regulation in the early embryo: is MBT too good to be true. Trends Genet. 8, 124–127.
27 McKnight, S. L. and Miller, O. L. (1976) Ultrastructural patterns of RNA synthesis during early embryogenesis of *Drosophila melanogaster.* Cell 8, 305–319.

28 Edgar, B. A. and Schubiger, G. (1986) Parameters controlling activation during early *Drosophila* development. Cell 44, 871–877.
29 Lamb, M. M. and Larid, C. D. (1976) Increase in nuclear poly(A)-containing RNA at syncytial blastoderm in *Drosophila melanogaster* embryos. Dev. Biol. 52, 31–42.
30 Powell-Coffman, J. A., Knight, J. and Wood, W. B. (1996) Onset of *C. elegans* gastrulation is blocked by inhibition of embryonic transcription with RNA polymerase antisense RNA. Dev. Biol. 178, 472–483.
31 Strome, S. and Lehmann, R. (2007) Germ versus soma decisions: lessons from flies and worms. Science 316, 392–393.
32 Santos, A. C. and Lehmann, R. (2004) Germ cell specification and migration in Drosophila and beyond. Curr. Biol. 14, 578–589.
33 Seydoux, G. and Braun, E. (2006) Pathway to totipotency: lessons from germ cells. Cell 127, 891–904
34 Huarte, M., Lan, F., Kim, T., Vaughn, M. W., Zaratiegui, M., Martienssen, R. A., Buratowski, S. and Shi, Y. (2007) The fission yeast jmj2 reverses histone H3 lysine 4 trimethylation. J. Biol. Chem. 282, 21662–21670.
35 Lanclos, K. D. and Hamilton, T. H. (1975) Translation of hormone-induced messenger RNA in amphibian oocytes. I. Induction by estrogen of messenger RNA encoded for vitellogenic protein in the liver of the male African clawed toad (Xenopus laevis). Proc. Natl. Acad. Sci. USA 72, 3934–3938.
36 Maller, J. L., Gross, S. D., Schwab, M. S., Finkielstein C. V., Taieb, F. E. and Oian Y. W. (2001) Cell cycle transitions in early *Xenopus* development. Novartis Found. Symp. 237, 58–73.
37 Bacharova, R (1985) Gene expression during oogenesis and oocyte development in mammals. Dev. Biol. 1, 453–524.
38 Aoki, F., Worrad, D. M. and Schultz, R. M. (1997) Regulation of transcriptional activity during the first and second cell cycles in the pre implantation mouse embryo. Dev. Biol. 181, 296–307.
39 Schultz, R. M. (2002) The molecular foundations of the maternal to zygotic transition in the preimplantation embryo. Hum. Reprod. Update 8, 323–331.
40 Schier, A. F. (2007) The maternal-zygotic transition: death and birth of RNAs. Science 316, 406–407.
41 Forlani, S., Bonnerot, C., Capgra, S. and Nicolas, J. F. (1998) Relief of a repressed gene expression state in the mouse 1-cell embryo requires DNA replication. Development 125, 3153–3166.
42 Aoki, E. and Schultz, R. M. (1999) DNA replication in the 1 cell mouse embryo: stimulatory effect of hystone acetylation. Zygote 7, 165–172.
43 Robertson, S. M. , Shetty, P. and Lin, R. (2004) Identification of lineage-specific zygotic transcripts in early *Caenorhabditis elegans* embryos. Dev. Biol. 276, 493–507.
44 Bashirullah, A., Halsell, S. R. Cooperstock, R., Kloc, M., Karaiskakis, A., Fisher, W. W., Fu, W. Hamilton, J. K., Etkin, L. D. and Lipshitz, H. D. (1999) Joint action of two RNA degradation pathways controls the timing of maternal transcript elimination at the midblastula transition in Drosophila melanogaster. EMBO J. 18, 2610–2660.
45 De Renzis, S., Elemento, O., Tavazoie, S. and Wieschaus, E. F. (2007) Unmasking activation of the zygotic genome using chromosomaldeletions in the *Drosophila* embryo. PLOS Biol. 5, 1036–1052.
46 Zeng, F. and Schultz, R. M. (2005) RNA transcript profiling during zygotic gene activation in the preimplantation mouse embryo. Dev. Biol. 283, 40–57.
47 Hamatani, T., Carter, M. G. Sharov, A. A. and Ko, M. S.H. (2004) Dynamics of global gene expression changes during mouse preimplantation development. Dev. Cell 6, 117–131.
48 Evsikiv, A. V. Graber, J. H., Brockman, J. M., Hempl, A., Holbrook, A. E., Singh, P., Eppig, J. J., Solterm D. and Knowles, B. (2006) Cracking the egg: molecular dynamics and evolutionary aspects of the transition from the fully grown oocyte to embryo. Genes Dev. 20, 2713–2722.
49 Giraldez, A. J., Mishima, Y., Rihel, J., Grocock ,R. J., Van Dongen, S., Inoue, K., Enright, A. J. and Schier, A. F. (2006) Zebrafish MIR-430 promotes deadenylation and clearance of maternal mRNAs. Science 312, 75–79.
50 Murchison, E. P., Stein, P., Xuan, Z., Pan, H., Scultz, R. M. and Hannon, G. J. 2007. Critical roles for Dicer in the female germline. Genes Dev. 21, 682–693.
51 Tang, F., Kaneda, M., O'carroll, D., Hajkova, P., Barton, S. C., Sun, A., Lee, C., Tarakhovsky, A., Lao, K. and Surani, M. A. (2007) Maternal microRNAs are essential for mouse zygotic development. Genes Dev. 21, 644–648.
52 Merino, C., Reynaud, E., Vazquez, M. and Zurita, M. (2002) DNA repair and transcriptional effects of mutations in TFIIH in *Drosophila* development. Mol. Biol. Cell 13, 3246–3256.
53 Gutierrez, L., Zurita, M., Kennison, J. A. and Vazquez, M. (2003) The *Drosophila* trithorax group gene tonally (tna) interacts genetically with the Brahma remodeling complex and encodes an SP_RING finger protein. Development 130, 343–345.
54 Hara, K. T., Oda, S., Naito, K., Nagata, M., Schultz , R. M. and Aoki, F. (2005) Cyclin A2-CDK2 regulates embryonic gene activation in 1-cell mouse embryos. Dev. Biol. 286, 102–113.
55 Seydoux, G. and Dunn, M. A. (1997) Transcriptionally repressed germ cells lack a subpopulation of phosphorylated RNA polymerase II in early embryos of *Caenorhabditis elegans* and *Drosophila melanogaster.* Development 124, 2191–2201.
56 Wang. Z. and Lindquist, S. (1998) Developmentally regulated nuclear transport of transcription factors in *Drosophila* embryos enable the heat shock response. Development 125, 4841–4850.
57 Aguilar-Fuentes, J., Valadez-Graham,V. Reynaud E, and Zurita M. (2006) TFIIH trafficking and its nuclear assembly during early *Drosophila* embryo development. J. Cell Sci. 119, 3866–3875.
58 Bellier, S., Chastant, S., Adenot, P., Vincent, M., Renard, J. P. and Bensaude O. (1997) Nuclear translocation and carboxi-terminal domain phosphorylation of RNA pol II delineate the two phases of zygotic gene activation in mammalian embryos EMBO J. 16, 6250–6262.
59 Wang, K., Sun, F. and Sheng, H. Z. (2006) Regulated expression of TAF-1 in cell mouse embryos. Zygote 14, 209–215
60 Worrad, D. M., Ram, P. T. and Schultz, R. M. (1994) Regulation of gene expressionin the mouse oocyte and early preimplantation embryo: developmental changes in Sp1 and TATA box-binding protein, TBP. Development 120, 2347–2357.
61 Walker, A. K., Rothman, J. H., Shi, Y. and Blackwell, T. K. (2001) Distinct requirements for C. elegans TAF(II)s in early embryonic transcription. EMBO J. 20, 5269–5279.
62 Walker, A. K., Shi, Y. and Blackwell, T. K. (2004) An extensive requirement for transcription factor IID-specifi TAF-1 in *Caenorhabditis elegans* embryonic transcription. J. Biol. Chem. 279, 15339–15347.
63 Shen, W. C., Bhaumik, S. R., Causton, H. C., Simon, I., Zhu, X., Jennings, E. G., Wang, T. H., Young, R. A. and Green, M. R. (2003) Systematic analysis of essential yeast TAFs in genome-wide transcription and preinitiation complex assembly. EMBO J. 22, 395–402.
64 Jallow, Z., Jacobi, U. G., Weeks, D. L., Dawid, I. B. and Veenstra, G. J. C. (2004) Specialized and redundant roles of TBP and a vertebrate-specific TBP paralog in embryonic gene regulation in *Xenopus.* Proc. Natl. Acad. Sci. USA 101, 15325–15330.
65 Yang Y., Cao J., Huang L., Fang, H. Y. and Sheng, H. Z. (2006) Regulated expression of TATA-binding-related factor 3 (TRF3) during early embryogenesis. Cell Res. 16, 610–621.
66 Shim, E.Y., Walker, A.K. and Blackwell, T. K. (2002) Broad requirement for the mediator subunit RGR-1 for transcription in the *Caenorhabditis elegans* embryo. J. Biol. Chem. 277, 30413–304136.
67 Dantonel, J-C., Quintin, S., Lakatos, L., Labouesse, M., and Tora, L. (2000) TBP-like factor is required for embryonic polymerase II transcription in *C. elegans.* Mol. Cell 6, 715–722.

68 Kaltenback, L., Horner, M- A., Rothman, J. H. and Mango, S. E. (2000) The TBP-like factor CeTLFG is required to activate RNA polymerase II transcription during *C. elegans* embryogenesis. Mol Cell 6, 705–713.

69 Shim, E. Y., Walker, A. K., Shi, Y. and Blackwell T K. (2002) CDK-9/cyclin T (P-TEFb) is required in two postinitiation pathways for transcription in *C. elegans* embryo. Genes Dev. 16, 2135–2146.

70 Guo, Z. and Stiller, J. W. (2004) Comparative genomics of cyclin-dependent kinases suggest co-evolution of the RNAP II C-terminal domain and CTD-directed CDKs. BMC Genom. 20, 69–76.

71 Kanin, E. I., Kipp, R. T., Kung, C., Slattery, M., Viale, A., Hahn, S., Shokat, K. M. and Ansari A. Z. (2007) Chemical inhibition of the TFIIH-associated kinase Cdk7/Kin28 does not impair global mRNA synthesis. Proc. Natl. Acad. Sci. USA 104, 5812–5817.

72 Chen J., Larochelle, S., Li, X. and Suter, B. (2003) Xpd/Ercc2 regulates CAK activity and mitotic progression. Nature 424, 228–232.

73 Larochelle, S., Chen, J. Knights, R. Pandur, J., Morcillo, P., Erdiument-Bromage, H., Tempst, P., Suter, B, and Fisher, R. P. (2001) T-loop phosphorylation stabilizes the CDK7-cyclin H-MAT1 complex in vivo and regulates its CTD kinase activity. EMBO J. 20, 3749–3759.

74 Leclerc, V. Raisin, S. and Leopold, P. (2000) Dominant-negative mutants reveal a role for the Cdk7 kinase at the mid-blastula transition in *Drosophila* embryos. EMBO J. 19, 1567–1575.

75 Fisher, R. P. (2005) Secrets of a double agent: CDK7 in cell cycle control and transcription. J. Cell Sci. 118, 5171–5180.

76 Wallenfang, M. R. and Seydoux, G. (2002) cdk-7 is required for mRNA transcription and cell cycle progression in *Caenorhabditis elegans* embryos. Proc. Natl. Acad. Sci. USA 99, 5527–5532.

77 Walker, A. K., Boag, P. R. and Blackwell, T. K. (2007) Transcription reactivation steps stimulated by oocyte maturation in *C. elegans*. Dev. Biol. 304, 382–393.

78 Palancade, B., Dubois, M. F., Dahmus,d Bensaude, O. (2001) Transcription-independent RNA polymerase II dephosphorylation by the FCP1 carboxy-terminal domain phosphatase in Xenopus laevis early embryos. Mol. Cell Biol. 21, 6359–6368.

79 Palancade, B., Bellier, S., Almounzi, G. and Bensaude, O. (2001) Incomplete RNA polymerase II phosphorylation in Xenopus laevis early embryos J. Cell Sci. 114, 2483–2489.

80 Newport, J. and Kirschner, M. (1982) A major developmental transition in early Xenopus embryos. II. Control of the onset of transcription. Cell 30, 687–696.

81 Almounzi, G. and Wolfe, A. P. (1995) Constraints on transcriptional activator function contribute to transcriptional quiescence during early Xenopus embryogenesis. EMBO J. 1752–1665.

82 Veenstra, G. J., Destree, O. H. and Wolffe, A. P. (1999) Translation of maternal TATA-binding protein mRNA potentiates basal but not activated transcription in Xenopus embryos at the midblastula transition. Mol. Cell. Biol. 19, 7972–7982.

83 Veenstra, G. J., Weeks, D. L. and Wolffe, A. P. (2000) Distinct roles for TBP and TBP-like factor in early embryonic gene transcription in Xenopus. Science 290, 2312–2315.

84 Prioleau, M. N., Buckle, R. S. and Mechali, M. (1995) Programming of a repressed but committed chromatin structure during early development. EMBO J. 14, 5073–5084.

85 Pritchard, D. K. and Schubiger, G. (1996) Activation of transcription in *Drosophila* embryos is a gradual process mediated by the nuclei cytoplasmic ratio. Genes Dev. 10, 1131–1142.

86 Ten Bosch, J. R., Benavides, J. A. and Cline, T. W. (2006) The TAGteam DNA motif controls the timing of *Drososphila* preblastoderm transcription. Development 133, 1967–1977.

87 Christova, R. and Oelgeschläger, R. (2002) Association of human TFIID-promoter complexes with silenced mitotic chromatin in vivo. Nat Cell Biol 4, 79–82.

88 Deshpande, G., Calhoum, G. and Jinks, T. M., Polydorides, A. D. and Scheld P. (2005) Nanos downregulates transcription and modulates CTD phosphorylation in the soma of the early *Drosophila* embryos. Mech. Dev. 122, 645–657.

89 Stancheva, I., El-Maarri, O., Walter, J., Niveleau, A. and Meehan, R,R. (2002) DNA methylation at promoter regions regulates the timing of gene activation in *Xenopus laevis* embryos. Dev. Biol. 243, 155–165.

90 Stancheva, I. and Meehan, R. R. (2000) Transient depletion of xDnmt1 leads to premature gene activation in *Xenopus* embryos. Genes Dev. 14, 131–324.

91 Ner, S. and Travers, A. (1994) HMG-D, the *Drosophila melanogaster* homologue of HMG 1 protein, is associated with early embryonic chromatin in the absence of histone H1. EMBO J. 13, 1817–1822.

92 Rudolph, T., Yonezawa, M., Lein, S., Heidrich, K., Kubicek, S., Schafer, C., Phalke, S., Walther, M., Schmidt, A., Jenuwein, T. and Reuter, G. (2007) Heterochromatin formation in *Drosophila* is initiated through active removal of H3K4 methylation by the LSD1 homolog SU(VAR)3-3. Mol. Cell 26, 103–115.

93 Schultz, R. M. and Worrad, (1995) Role of chromatin structure in zygotic gene activation in the mammalian embryo. Semin. Cell Biol. 6, 201–208

94 Yoshida, N., Brahmajosyula, M., Shoji, S., Amanai, M. and Perry, A. C. (2007) Epigenetic discrimination by mouse metaphase II oocytes mediates asymmetric chromatin remodeling independently of meiotic exit. Dev. Biol. 301, 464–477.

95 Liu, H., Kim, J.M and Aoki, F. (2004) Regulation of histone H3 lysine 9 methylation in oocytes and erly pre-implantation embryos. Development 131, 2269–2280.

96 Bultman, S. J., Gebuhr, H. P., Svoboda, P., Shultz, R. M. and Magnuson, T. (2006) Maternal BRG1 regulates zygotic genome activation in the mouse. Genes Dev. 20, 1744–1754.

97 Torres-Padilla, M. E. and Zernicka-Goetz, M. (2006) Role of TIFa as modulator of embryonic transcription in the mouse zygote. J. Cell Biol. 174, 329–338.

98 Germain-Desprez, D., Bazinet, M., Bouvier, M. and Aubry, M. (2003) Oligomerization of transcriptional intermediate factor 1 regulators and interaction with ZNF74 nuclear matrix protein reveled by bioluminescence resonance energy transfer in living cells. J. Biol. Chem. 278, 2367–22373.

99 Inoue, K., Ogonuki, N., Miki, M., Noda, S., Kim, J. M., Aoki, F., Miyoshi, H. and Ogura, A. (2006) Inefficient reprogramming of the hematopoietic stem cell genome following nuclear transfer. J. Cell Sci. 119, 1985–1991.