Anim. Reprod., v.12, n.1, p.52-61, Jan./Mar. 2015

Molecular mechanisms of transcriptional and chromatin remodeling around embryonic genome activation

Y.S. Bogliotti, P.J. Ross[1]

Department of Animal Science, University of California-Davis, Davis, CA, USA.

Abstract

The early stages of embryonic development in all metazoans are characterized by profound remodeling of chromatin organization and transcriptomics. This remodeling involves modifying two differentiated cells (oocyte and sperm) into a totipotent embryonic configuration capable of forming all the specialized cells that make up the adult animal. The early cleavage stages of preimplantation animal embryos occur in the absence of active transcription and relies on maternal factors stored in the cytoplasm of the oocyte during oogenesis. Chromatin and transcriptomic remodeling during preimplantation development are key for the initiation of embryonic transcriptional activity at species-specific stage, yet the exact mechanisms that trigger embryonic genome activation (EGA) are still unknown. Evidence of key transcriptional regulators and permissive chromatin configuration accompanied by specific epigenetic marks has been revealed as some of the mechanisms needed for EGA in the past few years. In this review, we will revisit the latest advances in the understanding of the mechanisms involved in the activation of the embryonic genome across several species, focusing on data generated by next generation sequencing technologies.

Keywords: DNA methylation, EGA, epigenetic remodeling, histone modifications, preimplantation embryos, RNA-seq.

Introduction

Embryonic development begins when the sperm and the oocyte fuse during fertilization to form a 1-cell embryo (a.k.a. zygote). Interestingly, in all animal embryos, the early stages of preimplantation development occur in the absence of transcription. During this period of transcriptional quiescence, development relies on maternal proteins and mRNAs that are stored in the cytoplasm of the oocyte during oogenesis (Tadros and Lipshitz, 2009). These maternal products drive basic biosynthetic processes, direct the first mitotic divisions, and prepare the embryo for activation of its genome and becoming totipotent.

The transition from maternal to embryonic control of development does not happen suddenly, but in successive waves of increasing intensity until the major activation of the embryonic genome occurs. This transition has been designated with different names across the literature: maternal to embryonic control of development (MET), zygotic genome activation (ZGA), embryonic genome activation (EGA), and midblastula transition (MBT) in drosophila embryos. In this review, we will use EGA to refer to the point in which massive transcription from the embryonic genome occurs as well as the point in which activation is strictly necessary to proceed with the developmental program as demonstrated by experiments in which embryos grown in the presence of the transcriptional inhibitor α-amanitin stop developing.

EGA occurs in mice at the 2-cell stage (Schultz, 1993), in pigs at the 4-cell stage (Jarrell *et al*., 1991), in humans by the 4- to 8-cell stages (Braude *et al*., 1988), and in bovine by the 8- to 16- cell stages (Memili and First, 2000). In mammals, EGA occurs relatively early in development (in terms of the number of cell divisions) compared to lower species such as zebrafish, xenopus, or drosophila, where EGA occurs after 10, 13, or 14 cycles of cell division respectively (Newport and Kirschner, 1982a). Nonetheless, in terms of time after fertilization, EGA occurs before in lower species (2 to 5 h post fertilization) than in mammals (one to three days, depending on the species). Despite the differences in timing of EGA across species, its major features, such as the degradation of maternal mRNA and proteins and the massive transcriptional activation of the embryonic genome are conserved in all metazoans (Schultz, 2002; Schier, 2007; Walser and Lipshitz, 2011).

EGA is a key event during early (preimplantation) development, as demonstrated by the developmental block when transcription is inhibited from the embryonic genome; however, the specific mechanisms involved in this process are still poorly understood. The general hypothesis is that in order for EGA to take place, the early embryo must remodel its transcriptionally oppressive chromatin configuration into a permissive totipotent configuration and that many factors (maternal and embryonic) are involved in this process. Gene transcription can be regulated by the availability of molecular machinery (e.g. RNA polymerase, general transcription factors, activators, repressors) as well as by accessibility to the genome modulated by chromatin configuration (Maston *et al*., 2006; Schulze and Wallrath, 2007). Chromatin configuration can be modified in order to change the

[1]Corresponding author: pross@ucdavis.edu
Received: July 10, 2014
Accepted: August 13, 2014

transcriptional status of a given loci in multiple ways including, ATP-dependent chromatin remodeling enzymes, epigenetic modifications (e.g. DNA methylation and histone post-translational modifications), and the inclusion of histone variants, among others.

A better understanding of EGA mechanisms will lead to a clearer conception of the achievement of totipotency and later differentiation. In this review, we will discuss the latest information on the molecular mechanisms involved in EGA in different species, focusing on data generated by next generation sequencing technologies and large-scale analysis.

Transcriptional remodeling

Preimplantation development is a complex process that includes dramatic changes on transcriptomic profiles. Part of this complexity is given by the establishment of the embryonic genome, which has to progress from a differentiated and transcriptionally silent state to a totipotent and transcriptionally active state in a precise and timely manner. The mechanisms and regulators of this transition are still largely unknown.

As the newly formed embryo is loaded with maternally-derived mRNAs, the blastomeric cytoplasm may contain three different types of transcripts: purely maternal, purely embryonic, and a combination of both, making the analysis of transcript abundance hard to interpret. Transcriptional changes during EGA have been studied by several means such as analysis of tritiated uridine incorporation to assay newly synthesized RNAs, and embryo culture in the presence of a transcriptional inhibitor (e.g. α-amanitin) to evaluate the time at which development stops in the absence of EGA. Also, transcriptional changes have been studied using relative abundance of specific genes by PCR through preimplantation development and lately using microarray technologies. Microarray technologies offered the most comprehensive study of the transcriptome of early embryos until the RNA sequencing (RNA-seq) technique came about. RNA-seq has numerous advantages over microarrays, including a greater dynamic range, less background, and higher sensitivity. Also, RNA-seq allows the discrimination of parent-specific transcriptomic changes using single-nucleotide polymorphisms (SNP). Using intron reads it also allows for the uncovering of earliest embryonic transcribed genes. In this section, we will discuss the latest findings on transcriptome profile around EGA (section "Transcriptomic dynamics: lessons from RNA-seq analysis**"**) as well as the role of key master regulators on this process (section "Master Regulators of EGA**"**).

Transcriptomic dynamics: lessons from RNA-seq analysis

Using an RNA-seq approach, Park *et al*. analyzed thetranscriptional profile of preimplantation in-vitro fertilized and parthenogenetic mice embryos, including α-amanitin treated embryos on the experimental design. They found that the transcriptional program after fertilization includes mRNAs from the embryonic genome as well as maternal and sperm-derived factors, and identified some potential key transcription factors involved in the transcriptional initiation of the embryonic genome: *Foxd1*, *Nkx2-5*, *Sox18*, *Myod1*, and *Runx1* (Park *et al*., 2013).

In spite of the limitation on material from human samples, a very interesting paper was published last year that showcased the results from single cell RNA-seq of embryos at different stages of development. A great advantage of single-cell RNA-seq is that it can expose the variability and potential from specific functions of individual cells within an embryo. The authors of this paper observed a significant up-regulation of 2495 genes at the time of EGA that were enriched in RNA metabolism and translation, ribonucleoprotein complex biogenesis, and ribosome biogenesis, as well as in chromosome organization, cell division, and DNA packaging, indicating that the epigenetics and cell-cycle are regulated by the embryonic genome as soon as it becomes activated (Yan *et al*., 2013). Another comprehensive single-cell RNA-seq study compared mouse and human preimplantation embryos, showing that the transcriptomes of these two species have comparable functionality at equivalent developmental stages, and that their transcriptomes can be described with a succinct number of functional modules (i.e. clusters of genes that are co-expressed in a specific stage; Xue *et al*., 2013). Moreover, using weighted correlation network analysis, the authors found several hub genes (key genes within each network) with likely crucial roles during preimplantation development. Among them, KPNA7 was identified in human and mouse pre-major EGA networks, agreeing with previous reports that showed developmental abnormalities in *Kpna7*-deficient mice (Rother *et al*., 2011; Xue *et al*., 2013).

A comprehensive transcriptome analysis around EGA in cattle was recently reported (Graf *et al*., 2014). The authors used hybrid *in-vitro* fertilized embryos (*Bos taurus* oocytes x *Bos indicus* sperm) to differentiate paternal and maternal transcripts and implemented three different strategies to identify transcripts of embryonic origin. These included genes absent in oocytes that became expressed in embryos, transcripts from the paternal allele, and incompletely spliced transcripts (denoting active transcription). The largest proportion of up-regulated genes was found at the 8-cell stage, coinciding with EGA. Among the first embryonic genes to be expressed was *HNRNPA2B1*, which is known to interact with SOX2, a key transcription factor for embryonic stem cell pluripotency (Masui *et al*., 2007), as well as KLF17, which can activate/suppress transcription (van Vliet *et al*., 2006), and the Nanoghomeobox (NANOG) coinciding with previous reports (Khan *et al*., 2012).

Gene ontology analysis of genes activated at EGA included RNA processing, translation, and transport enrichment (Graf *et al*., 2014).

The *in-vivo* and SCNT-derived pig embryo transcriptomes were analyzed and compared using SOLID mRNA sequencing. The authors found that EGA is 1 cycle delayed in SCNT embryos compared to fertilized embryos (it usually happens at the 4-cell stage). Moreover, hierarchical clustering of DNA binding protein transcripts, characteristic of EGA, appeared at the 4-cell stage for *in-vivo* embryos, and at the 8-cell stage for SCNT-derived embryos, corroborating a delayed EGA in SCNT pig embryos (Cao *et al*., 2014).

Transcriptome analyses of early embryos in non-mammalian species have also been reported (Vesterlund *et al*., 2011; Paranjpe *et al*., 2013; Tan *et al*., 2013). A comparative analysis of zebrafish, mouse, and drosophila early embryonic transcriptomes was recently published. The authors analyzed the structure of genes transcribed in early development and found that maternal genes are largely conserved among the divergent species, while the earliest embryonic genes are not, suggesting species-specific functions of embryonic genes during EGA and a central conserved maternal program across species (Heyn *et al*., 2014).

In brief, recent technological advances enabled several studies that managed to obtain the global transcriptome of blastomeres at different stages during preimplantation development. A common characteristic of all these studies is that the greatest change (up-regulation) in gene expression is seen at the time of the major genome activation. Also, the main categories of gene ontology analysis for embryonically-expressed genes coincide in RNA processing, translation, and chromatin remodeling with major categories. These studies are primarily oriented towards describing the general characteristics and functions of genes that undergo dynamic changes in expression during preimplantation development with the final goal of identifying key regulators that control the developmental program.

Master regulators of EGA

In the previous section, the global characteristics of gene expression in differently species during EGA were reviewed. However, the mechanisms governing the timed and coordinated activation of specific embryonic genes at EGA are largely unknown. In this section, recent advances in the discovery of key regulator genes that significantly affect the expression of embryonic genes at EGA in non-mammalian species will be discussed.

ZELDA and STAT

A very important discovery about the mechanisms of early embryonic development and embryonic genome activation was made with the discovery of a key activator of the embryonic genome in drosophila: Zelda (Liang *et al*., 2008). Zelda (zld) is a zinc-finger maternally-derived protein that binds to CAGGTAG and related sequences (termed TAG-team sequences) present in many regulatory regions of early-activated genes in drosophila (ten Bosch *et al*., 2006). Embryos lacking zld (zld-/-) are phenotypically similar to wild type embryos before cycle 14 (major EGA), but show severe abnormal morphology after that. About 70% of the activated genes at 1-2 h in-wild type embryos are down-regulated in zld-/-, including genes involved in cellular blastoderm formation, sex determination, dorso-ventral development, and pattern formation. Another cohort of genes (mainly maternally expressed) becomes up-regulated in zld-/- embryos. These results demonstrate that zld is a key regulator of EGA in drosophila (Liang *et al*., 2008). Two papers were published at the same time studying the possible mechanism by which zld may act during EGA to coordinate the expression of many genes (Harrison *et al*., 2011; Nien *et al*., 2011). Using a chromatin immune precipitation-microarray (ChIP-chip) approach combined with expression analysis, the authors found that zld binds to more than 2000 TSS in 1-2 h embryos and also binds to many hotspot genomic regions (i.e. regions where multiple transcription factors bind; modENCODE Consortium *et al*., 2010). Moreover, although many genes fail to be activated in zld-/- embryos, others (principally those associated with pattern formation) are just delayed, indicating that zld regulates the timing within the segmentation gene network (Nien *et al*., 2011). Using a ChIP-seq approach at different developmental stages (before, cycle 8, during, cycle 13, and after, cycle 14, major EGA) and expression analysis data, Harrison *et al*. showed that zld binds to thousands of target sites prior to major EGA (Harrison *et al*., 2011). When they analyzed genomic regions usually bound by 21 known TFs involved in patterning, they found that a large fraction of them were also bound by zld at cycle 8. Correlation analysis between zld binding sites and chromatin accessibility data (DNAseI hypersensitivity assay in embryos at cycle 14; Thomas *et al*., 2011) showed that the regions where zld binds at cycle 8 strongly correlate with accessible chromatin at cycle 14. The authors proposed a model for the zld mechanism for activating the embryonic genome in which zld binds to most of CAGGTAG sites (64% of these sites are bound by zld at cycle 8) while chromatin is in a fairly accessible state (before EGA); as development proceeds, and chromatin accessibility diminishes (acquiring patterns of accessible/inaccessible regions) zld-marked loci remain open, facilitating the binding of embryonically-expressed specific TFs that zld binding sites are enriched in hotspots. Although great advances have been made in our understanding of the mechanism of action of Zelda during drosophila development, the

exact mechanism of action still remains elusive.

In 2011, Tsurumi *et al*. described an additional key regulator of EGA in drosophila: STAT92E (Tsurumi *et al*., 2011). STAT92E is required for the expression of embryonic genes, affecting a 78.2% of expressed genes at major EGA (down-regulated genes in embryos lacking STAT92E,STAT92E-/-). Fifty percent of the embryonic genes affected in $zld^{-/-}$ embryos were also down-regulated in STAT92E-/- embryos, suggesting that these genes might be co-regulated by STAT and Zelda. A co-transfection experiment in which an embryonically-expressed gene (dpp) affected by Zelda and STAT was tested by luciferase activity, showed that dpp activation was: 22 fold when exposed to STAT, 48 fold when exposed to Zelda, whereas the combination of Zelda and STAT up-regulated dpp expression by 230 fold. This indicates that these two transcription factors could act synergistically *in-vivo* to up-regulate the transcription of many early embryonically-expressed genes (Tsurumi *et al*., 2011). These results evidence the complexity and importance of the transcriptional networks at the onset of embryonic transcription.

Pou5f1, Nanog, and SoxB1

In vertebrates, more specifically in zebrafish embryos *Pou5f1, Nanog, and Soxb1* are key regulators of EGA (Lee *et al*., 2013; Leichsenring *et al*., 2013). RNA-seq analysis of zebrafish embryos during EGA showed that these 3 transcription factors are the most expressed ones before the major activation of the embryonic genome. Loss of *Nanog, Pou5f1, and SoxB1* during early development resulted in 77% reduction of transcription of strictly embryonic genes 4 h post fertilization (hpf). ChIP-seq analysis of *Nanog* showed that 74% of the earliest transcribed genes are bound to this transcription factor (including the micro RNA gene miR-430; Lee *et al*., 2013). This result is interesting since the embryonic expression of *miR-430* in zebrafish embryos is responsible for the clearance of several hundred maternal messages (Giraldez *et al*., 2006). Lee *et al*. found that miR-430 is activated by *Nanog* together with *Pou5f and SoxB1*, linking maternal regulation of embryonic gene expression with clearance of maternal mRNAs (Lee *et al*., 2013). Therefore, the degradation of maternal mRNA is one more time shown to be key for EGA. Although it is likely that many factors (most of them still unknown) act in coordination to activate a specific network of genes at EGA, some of them have been already discovered as well as some of their targets and mechanisms of action.

DCP1A/DCP2

An equivalent key transcription factor for EGA has not been found in mammals to date. However, there are some maternally-derived genes with key roles in the correct activation of the embryonic genome in mammals. *DCP1A* and *DCP2* are two maternally-derived mRNAs that are recruited during oocyte maturation via cytoplasmic polyadenylation elements in mice and are involved in the degradation (by de-capping) of maternally-derived mRNAs (Mendez and Richter, 2001). As mentioned before, one of the highlights of EGA for all animal species is the degradation of maternal messengers. Inhibition of *DCP1A/DCP2* translation during oocyte maturation by siRNA or morpholinos showed a decrease of 50% in embryonically-expressed genes at the 2-cell stage in mice (EGA). This result shows that the degradation of maternally-derived transcripts is necessary for the activation of the embryonic genome, while DCP1A and DCP2 proteins play key roles in the correct activation of zygotic genes during EGA in a mammalian species (Ma *et al*., 2013).

Chromatin remodeling

The profound remodeling in the embryonic transcriptome described above is accompanied by a critical chromatin remodeling during the early stages of embryonic development, which, in part, is believed to be responsible for the observed transcriptomic dynamics. The role of chromatin in the activation of the embryonic genome was exposed in the early 80 s with the introduction of a plasmid containing a coding gene in xenopus early embryos. The experiments showed that the plasmid became transcribed soon after it was introduced in the cleavage embryo, but then it inactivates and remains silenced until EGA, demonstrating that the embryo does not lack an active transcriptional machinery, but that changes in the chromatin are regulating transcription from the early embryo genome (Newport and Kirschner, 1982b). Since that moment, many papers were published on the role of chromatin remodeling during early development. General chromatin configuration during early development and its implications in the activation of the embryonic genome will be reviewed in section “Chromatin structure”, while evidence of epigenetic mark modifications associated with EGA will be discussed in section “Epigenetic modifications”.

Chromatin structure

ATP-dependent chromatin remodeling

Human brahma-related gene 1 (*BRG1*) is a maternal-effect gene capable of changing chromatin structure that regulates genome activation in mice (Bultman *et al*., 2006). BRG1 protein is the catalytic subunit of the chromatin remodeling complex SWI/SNF in mammals. BRG1 has DNA-dependent ATPase activity and can alter the conformation and position of nucleosomes, thus altering gene expression patterns.

BRG1-depleted oocytes can mature and be fertilized, but mice embryos lacking this protein (BRG1-/-) arrest at the 2-cell stage (EGA) indicating that BRG1 plays a key role in the activation of the embryonic genome in that species. Moreover, transcriptional activity of embryos lacking BRG1 is reduced by 30%, and the down-regulated gene categories are enriched in transcription, RNA processing, and cell cycle regulation (previously shown to be transcribed from the embryonic genome at EGA). The effect of this maternal gene on EGA is very interesting and evidences that chromatin configuration is key for the correct activation of the embryonic genome. The exact mechanisms of action of BRG1 during early development are still unknown. However, the possibility that a lack of BRG1 can modify epigenetic marks at a global level was tested. Interestingly, histone acetylation levels in BRG1 -/- embryos were not significantly different than wild type embryos at the 2-cell stage. However, an active methylation mark (dimethyl H3K4) was also tested, and the global levels of this mark were significantly reduced (~61%) in BRG1-/- embryos versus wild type embryos. BRG1 binds to acetylated histone tails. Since the authors found that the global levels of histone acetylation are not changed in BRG1-/- embryos, they proposed a model in which BRG1 acts downstream or in parallel to histone acetyl transferases (HATs) and maternally SWI/SNF and histone acetylation converge to stimulate the specific methylase of H3K4me2 (MLL) to mark loci for their posterior transcription (Bultman *et al*., 2006).

Enhancers and looping

Similar to promoter regions, enhancers play key roles in gene regulation. Most animal transcription factors (TFs) recognize short degenerate DNA sequences near most genes (Wunderlich and Mirny, 2009). Therefore, the complex gene expression patterns during development and differentiation could be hard to imagine if only promoter sequences and TFs were involved in regulating the whole transcriptome. Enhancers are the most common regulatory regions in higher eukaryotes (Heintzman *et al*., 2009). Interestingly, enhancers can control promoters that are located quite distant from themselves, and be positioned in virtually any location respect to its target promoter: upstream/downstream and in intronic regions of target/neighboring genes. Chromosome conformation studies have provided evidence of looping (Kadauke and Blobel, 2009). Chromatin loops are physical interaction between distal loci, and enhancer/promoter interactions have been shown to form loops (Kadauke and Blobel, 2009). In drosophila, higher order interactions have been shown to occur for the developmentally regulated Hox genes (Ronshaugen and Levine, 2004). The complexity of gene patterns during early development opens the possibility that loops can be involved in the activation of a coordinated network of embryonic genes at EGA. The formation of loops has not been analyzed in preimplantation embryos to date. Studies of chromatin conformation structure during EGA in different species are required to contribute to the understanding of the transcriptional networks activated at this critical developmental time and their mechanism. Remarkably, BRG1 is implicated in loop formation at the beta-globin locus (Kim *et al*., 2009). Since Brg1-/- mice embryos fail to correctly activate the embryonic genome (Bultman *et al*., 2006) it is tempting to consider that possibly looping formation is at least in part involved in the correct and coordinated activation of the embryonic genome in mice.

Chromatin accessibility

Using a DNAase I hypersensitivity assay approach, it was shown in early drosophila embryos that global chromatin accessibility changes during early development (Thomas *et al*., 2011) and that developmental regulators are quantitatively correlated with DNA accessibility sites in the genome in the early embryo (Li *et al*., 2011). Combining information from DNase Ihypersensitivity assayand ChIP-chip for 21 developmental regulators, it was shown that 61% of DHSs contained at least one of the analyzed developmental factors bound and that only 7% of non-DHSs sites were associated with a ChIP-chip region (Li *et al*., 2011). These results suggest that developmental regulators are expressed in sufficient amounts in the early embryo and that it is the chromatin configuration what would determine the binding and expression at a specific developmental stage. Analysis of open chromatin by DNase Ihypersensitivity assays have not been done in mammalian early embryos to date, likely due to the limited material availability.

Epigenetic modifications

In the previous section, the global chromatin structure and some of the potential modifiers of this structure during early development were discussed. Here, we will focus on chemical modifications on the chromatin that can change gene expression without changing the DNA sequence and that once established can be maintained after cell division (epigenetic marks). We will focus on two main types of epigenetic marks: DNA methylation and histone post-translational modifications, and review what is known about their remodeling during early development and implications in the activation of the embryonic genome.

DNA methylation

DNA methylation (DNAme) is a repressive epigenetic mark that consists of the addition of a methyl group to the fifth carbon position of cytosine residues in

the DNA (5-mC). This covalent modification occurs mostly within CpGdinucleotides by DNA methyltransferases (DNMTs). DNAme can exert its silencing effects by blocking the access to regulatory regions of a gene, but also by recruiting transcriptional repressors and/or chromatin modifiers to a specific locus. From immunostaining assays it was shown that, in mice, there is a global oxidation of the 5-mC to 5-hydroxymethylcytosine (5-hmC) in the paternal pronucleus (PN) soon after fertilization in the zygote, while the maternal PN remains at its initial levels of 5-mC (Inoue and Zhang, 2011; Iqbal *et al*., 2011; Wossidlo *et al*., 2011). The 5mC to 5-hmC conversion is catalyzed by the maternally-derived protein Ten-Eleven Translocation 3 enzyme (Tet3; Iqbal *et al*., 2011). Also, 5-hmC residues were detected until the 8-cell stage, suggesting that it remains on the paternally-derived genome without further metabolism and its loss is likely due to dilution by cell division (Inoue and Zhang, 2011). A passive mechanism (cell division in absence of maintenance methylase DNMT1) was believed to take place in the maternally-derived genome (Inoue and Zhang, 2011). However, a very striking paper was published recently in which sequencing evidence is given for DNA demethylation dynamics during early mice development. The authors used hybrid mice embryos to follow parent-of-origin DNAme changes at base-resolution during preimplantation development and found that oxidized forms of 5mC are found in both maternal and paternal methylomes in mice embryos. Also, they found that the paternal methylome and at least part of the maternal methylome undergo active demethylation during early embryonic development (Wang *et al*., 2014). Although this mark is remodeled during early development, and some of the mechanism and molecular players involved in its remodeling have been uncovered, the importance and role of DNAme remodeling for the achievement of EGA is still discussed. It is generally believed that repressive marks have to be removed to allow EGA to occur, but the specific loci affected by DNAme around EGA are still unknown. The importance of DNA methylation remodeling in early development is evidenced by knock out studies on modifiers of DNAme, in which embryonic lethality is observed (Li *et al*., 1992; Stancheva and Meehan, 2000; Stancheva *et al*., 2001, 2002; Payer *et al*., 2003). In non-mammalian species such as xenopus and zebrafish, the dynamics of DNAme in early embryos is not the same as in mice. Neither the maternal nor the paternal genomes of the Xenopus zygote undergo any dramatic remodeling of DNA methylation during the first few hours after fertilization (Stancheva *et al*., 2002). Xenopus embryos retain a high methylation level after fertilization (Veenstra and Wolffe, 2001; Stancheva *et al*., 2002; Bogdanovic *et al*., 2011) and show no correlation between promoter methylation and transcriptional repression (Bogdanovic *et al*., 2011). Zebrafish embryos undergo post-fertilization gametic demethylation and remethylation (Mhanni and McGowan, 2004). The role of DNAme remodeling was assessed in xenopus embryos in which the depletion of maternal xDnmt1 initiate zygotic transcription approximately two cell cycles before MBT, indicating a role of DNAme in the correct timing of EGA in this species (Stancheva and Meehan, 2000). In zebrafish, higher proportions of genes hypomethylated pre-EGA or at EGA are expressed after EGA compared to methylated genes. Thus, pre-EGA promoter hypomethylation correlates with enhanced expression potential after EGA (Andersen *et al*., 2012).

Histone post-translation modifications

In Eukaryotes, the DNA is packaged in chromatin inside the nucleus. The nucleosome constitutes the basic unit of chromatin and consists of a segment of DNA (~147 bp), wrapped twice around an octamer of histone core proteins (two copies of: H2A, H2B, H3, and H4; Kornberg, 1974). The amino terminal (N-terminal) portions of the proteins remain outside of the nucleosome core predisposing them to experience post-translational modifications (Luger and Richmond, 1998). The epigenetic modifications that occur in the histone proteins are post-translational modification (PTM). Histone PTMs occur primarily on the N-terminal tails and can include phosphorylations, ubiquitylations, sumoylations, acetylations and methylations, among others. Unlike DNA methylation, that is usually associated with silencing of gene expression, histone PTMs can cause either activation or repression of the associated genes depending on the specific modification and the specific amino acid residue modified.

Epigenetic modifications on histones have been shown to be remodeled during early development in several species (Pedersen and Helin, 2010). Methylation at lysine residues is one of the most studied marks and can signal either activation or repression, depending on the sites of methylation and the number of methyl groups added [mono- (me1), di- (me2) or tri- (me3)]. Importantly, histone methylation is a reversible modification regulated in a very specific way by histone lysine methyl-transferases (KMTs) and histone lysine demethylases (KDMs; Shi *et al*., 2004; Klose *et al*., 2006; Agger *et al*., 2007, 2008). It has been shown that KDMs play crucial roles at different stages of development: during gametogenesis, early embryonic development, cell differentiation, neural commitment and organogenesis (Pedersen and Helin, 2010). However, the specific mechanisms and moreover the specific targets of these enzymes during early development are still unknown.

In this section we will focus on two histone post-translational modifications at histone H3: the tri-methylation at lysine 27 and lysine 4 (H3K27me3 and H3K4me3). These epigenetic marks have been

associated with promoters of genes that are repressed and activated, respectively (Cheung and Lau, 2005). Moreover, both marks are associated with developmentally-regulated genes and are thought to bring about the establishment of lineage-specific gene expression programs during embryonic development (Surface *et al*., 2010). Immunofluorescence-based studies have shown that H3K4me4 and H3K27me3 have dynamic patterns during early mammalian embryonic development (Sarmento *et al*., 2004; van der Heijden *et al*., 2005; Ross *et al*., 2008; Gao *et al*., 2010; Black *et al*., 2012; Bogliotti and Ross, 2012; Canovas *et al*., 2012; Zhang *et al*., 2012). The remodeling of these marks around EGA indicates a possible role of them in EGA. Interestingly, these two marks were shown to co-exist at the same genomic loci in mammalian embryonic stem cells and were termed "bivalent domains". It is believed that these bivalent domains in a pluripotent cell generate a halted stage of transcription of the associated loci that is resolved upon differentiation becoming activated if it loses H3K4me3 and retains H3K27me3 and repressed if the opposite happens (Azuara *et al*., 2006; Bernstein *et al*., 2006; Mikkelsen *et al*., 2007).

The role of the bivalent domain in the activation of the embryonic genome has been analyzed in non-mammalian species only. A ChIP-chip and sequential ChIP-chip were done in zebrafish embryos to analyze the dynamics and presence of bivalent domains in early embryos across EGA (Vastenhouw *et al*., 2010; Lindeman *et al*., 2011). It was found that bivalent domains are present and change dynamically in early zebrafish embryos. Vastenhouw *et al*. found that bivalent domains after EGA were enriched in many non-transcribed (inactive) developmentally-regulated genes, as it happens in embryonic stem cells (Bernstein *et al*., 2006). Bivalent domains were also assessed in xenopus (Akkers *et al*., 2009; Schneider *et al*., 2011) and drosophila (Schuettengruber *et al*., 2009) early embryos. In these species, the presence of bivalent domains was not seen. This can be due to differences between species as well as in differences in experimental design. Genome-wide studies of H3K4me3 and H3K27me3 around EGA in mammals have not been done to date and are needed to understand if bivalent domains are a common feature of vertebrate early development and what are their implications for EGA.

Conclusion

Early development is a very complex period in the development of an organism. Many events take place (e.g. EGA) and, to date, many of the mechanisms involved are still unknown. Based on current evidence, it seems that the coordinated action of many factors would act together to activate the embryonic genome. The presence of maternal factors loaded in the cytoplasm of the egg during oogenesis, early embryonically-transcribed genes, chromatin organization, and epigenetic modifications are needed to create a totipotent embryo capable of activating the genome and proceeding with its developmental program.

References

Agger K, Cloos PA, Christensen J, Pasini D, Rose S, Rappsilber J, Issaeva I, Canaani E, Salcini AE, Helin K. 2007. UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development. *Nature*, 449:731-734.

Agger K, Christensen J, Cloos PA, Helin K. 2008. The emerging functions of histone demethylases. *Curr Opin Genet Dev*, 18:159-168.

Akkers RC, van Heeringen SJ, Jacobi UG, Janssen-Megens EM, Françoijs KJ, Stunnenberg HG, Veenstra GJ. 2009. A hierarchy of H3K4me3 and H3K27me3 acquisition in spatial gene regulation in Xenopus embryos. *Dev Cell*, 17:425-434.

Andersen IS, Reiner AH, Aanes H, Alestrom P, Collas P. 2012. Developmental features of DNA methylation during activation of the embryonic zebrafish genome. *Genome Biol*, 13:R65.

Azuara V, Perry P, Sauer S, Spivakov M, JørgensenHF, John RM, Gouti M, Casanova M, Warnes G, Merkenschlager M, Fisher AG. 2006. Chromatin signatures of pluripotent cell lines. *Nat Cell Biol*, 8:532-538.

Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, Jaenisch R, Wagschal A, Feil R, Schreiber SL, Lander ES. 2006. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell*, 125:315-326.

Black JC, Van Rechem C, Whetstine JR. 2012. Histone lysine methylation dynamics: establishment, regulation, and biological impact. *Mol Cell*, 48:491-507.

Bogdanovic O, Long SW, van Heeringen SJ, Brinkman AB, Gómez-Skarmeta JL, Stunnenberg HG, Jones PL, Veenstra GJ. 2011. Temporal uncoupling of the DNA methylome and transcriptional repression during embryogenesis. *Genome Res*, 21:1313-1327.

Bogliotti YS, Ross PJ. 2012. Mechanisms of histone H3 lysine 27 trimethylation remodeling during early mammalian development. *Epigenetics*, 7:976-981.

Braude P, Bolton V, Moore S. 1988. Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. *Nature*, 332:459-461.

Bultman SJ, Gebuhr TC, Pan H, Svoboda P, Schultz RM, Magnuson T. 2006. Maternal BRG1 regulates zygotic genome activation in the mouse. *Genes Dev*, 20:1744-1754.

Canovas S, Cibelli JB, Ross PJ. 2012. Jumonji domain-containing protein 3 regulates histone 3 lysine

27 methylation during bovine preimplantation development. *Proc Natl Acad Sci USA*, 109:2400-2405.

Cao S, Han J, Wu J, Li Q, Liu S, Zhang W, Pei Y, Ruan X, Liu Z, Wang X, Lim B, Li N. 2014. Specific gene-regulation networks during the pre-implantation development of the pig embryo as revealed by deep sequencing. *BMC Genomics*, 15:4.

Cheung P, Lau P. 2005. Epigenetic regulation by histone methylation and histone variants. *Mol Endocrinol*, 19:563-573.

Gao Y, Hyttel P, Hall VJ. 2010. Regulation of H3K27me3 and H3K4me3 during early porcine embryonic development. *Mol Reprod Dev*, 77:540-549.

Giraldez AJ, Mishima Y, Rihel J, Grocock RJ, Van Dongen S, Inoue K, Enright AJ, Schier AF. 2006. Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. *Science*, 312:75-79.

Graf A, Krebs S, Zakhartchenko V, Schwalb B, Blum H, Wolf E. 2014. Fine mapping of genome activation in bovine embryos by RNA sequencing. *Proc Natl Acad Sci USA*, 111:4139-4144.

Harrison MM, Li XY, Kaplan T, Botchan MR, Eisen MB. 2011. Zelda binding in the early *Drosophila melanogaster* embryo marks regions subsequently activated at the maternal-to-zygotic transition. *PLoS Genet*, 7:e1002266.

Heintzman ND, Hon GC, Hawkins RD, Kheradpour P, Stark A, Harp LF, Ye Z, Lee LK, Stuart RK, Ching CW, Ching KA, Antosiewicz-Bourget JE, Liu H, Zhang X, Green RD, Lobanenkov VV, Stewart R, Thomson JA, Crawford GE, Kellis M, Ren B. 2009. Histone modifications at human enhancers reflect global cell-type-specific gene expression. *Nature*, 459:108-112.

Heyn P, Kircher M, Dahl A, Kelso J, Tomancak P, Kalinka AT, Neugebauer KM. 2014. The earliest transcribed zygotic genes are short, newly evolved, and different across species. *Cell Rep*, 6:285-292.

Inoue A, Zhang Y. 2011. Replication-dependent loss of 5-hydroxymethylcytosine in mouse preimplantation embryos. *Science*, 334:194.

Iqbal K, Jin SG, Pfeifer GP, Szabo PE. 2011. Reprogramming of the paternal genome upon fertilization involves genome-wide oxidation of 5-methylcytosine. *Proc Natl Acad Sci USA*, 108:3642-3647.

Jarrell VL, Day BN, Prather RS. 1991. The transition from maternal to zygotic control of development occurs during the 4-cell stage in the domestic pig, *Sus scrofa*: quantitative and qualitative aspects of protein synthesis. *Biol Reprod*, 44:62-68.

Kadauke S, Blobel GA. 2009. Chromatin loops in gene regulation. *Biochim Biophys Acta*, 1789:17-25.

Khan DR, Dubé D, Gall L, Peynot N, Ruffini S, Laffont L, Le Bourhis D, Degrelle S, Jouneau A, Duranthon V. 2012. Expression of pluripotency master regulators during two key developmental transitions: EGA and early lineage specification in the bovine embryo. *PloS One*, 7:e34110.

Kim SI, Bultman SJ, Kiefer CM, Dean A, Bresnick EH. 2009. BRG1 requirement for long-range interaction of a locus control region with a downstream promoter. *Proc Natl Acad Sci USA*, 106:2259-2264.

Klose RJ, Kallin EM, Zhang Y. 2006. JmjC-domain-containing proteins and histone demethylation. *Nat Rev Genet*, 7:715-727.

Kornberg RD. 1974. Chromatin structure: a repeating unit of histones and DNA. *Science*, 184:868-871.

Lee MT, Bonneau AR, Takacs CM, Bazzini AA, DiVito KR, Fleming ES, Giraldez AJ. 2013. Nanog, Pou5f1 and SoxB1 activate zygotic gene expression during the maternal-to-zygotic transition. *Nature*, 503:360-364.

Leichsenring M, Maes J, Mossner R, Driever W, Onichtchouk D. 2013. Pou5f1 transcription factor controls zygotic gene activation in vertebrates. *Science*, 341:1005-1009.

Li E, Bestor TH, Jaenisch R. 1992. Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell*, 69:915-926.

Li XY, Thomas S, Sabo PJ, Eisen MB, Stamatoyannopoulos JA, Biggin MD. 2011. The role of chromatin accessibility in directing the widespread, overlapping patterns of *Drosophila* transcription factor binding. *Genome Biol*, 12:R34.

Liang HL, Nien CY, Liu HY, Metzstein MM, Kirov N, Rushlow C. 2008. The zinc-finger protein Zelda is a key activator of the early zygotic genome in *Drosophila*. *Nature*, 456:400-403.

Lindeman LC, Andersen IS, Reiner AH, Li N, Aanes H, Østrup O, Winata C, Mathavan S, Müller F, Aleström P, Collas P. 2011. Prepatterning of developmental gene expression by modified histones before zygotic genome activation. *Dev Cell*, 21:993-1004.

Luger K, Richmond TJ. 1998. The histone tails of the nucleosome. *Curr Opin Genet Dev*, 8:140-146.

Ma J, Flemr M, Strnad H, Svoboda P, Schultz RM. 2013. Maternally recruited DCP1A and DCP2 contribute to messenger RNA degradation during oocyte maturation and genome activation in mouse. *Biol Reprod*, 88:11.

Maston GA, Evans SK, Green MR. 2006. Transcriptional regulatory elements in the human genome. *Annu Rev Genomics Hum Genet*, 7:29-59.

Masui S, Nakatake Y, Toyooka Y, Shimosato D, Yagi R, Takahashi K, Okochi H, Okuda A, Matoba R, Sharov AA, Ko MS, Niwa H. 2007. Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. *Nat Cell Biol*, 9:625-635.

Memili E, First NL. 2000. Zygotic and embryonic gene expression in cow: a review of timing and mechanisms of early gene expression as compared with other species. *Zygote*, 8:87-96.

Mendez R, Richter JD. 2001. Translational control by CPEB: a means to the end. *Nat Rev Mol Cell Biol*,

2:521-529.

Mhanni AA, McGowan RA. 2004. Global changes in genomic methylation levels during early development of the zebrafish embryo. *Dev Genes Evol*, 214:412-417.

MikkelsenTS, Ku M, Jaffe DB, Issac B, Lieberman E, Giannoukos G, Alvarez P, Brockman W, Kim TK, Koche RP, Lee W, Mendenhall E, O'Donovan A, Presser A, Russ C, Xie X, Meissner A, Wernig M, Jaenisch R, Nusbaum C, Lander ES, Bernstein BE. 2007. Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature*, 448: 553-560.

modENCODE Consortium *et al*. (173 collaborators). 2010. Identification of functional elements and regulatory circuits by *Drosophila* modENCODE. *Science*, 330:1787-1797.

Newport J, Kirschner M. 1982a. A major developmental transition in early *Xenopus* embryos: I. characterization and timing of cellular changes at the midblastula stage. *Cell*, 30:675-686.

Newport J, Kirschner M. 1982b. A major developmental transition in early *Xenopus* embryos: II. Control of the onset of transcription. *Cell*, 30:687-696.

Nien CY, Liang HL, Butcher S, Sun Y, Fu S, Gocha T, Kirov N, Manak JR, Rushlow C. 2011. Temporal coordination of gene networks by Zelda in the early *Drosophila* embryo. *PLoS Genet*, 7:e1002339.

Paranjpe SS, Jacobi UG, van Heeringen SJ, Veenstra GJ. 2013. A genome-wide survey of maternal and embryonic transcripts during *Xenopus tropicalis* development. *BMC Genomics*, 14:762.

Park SJ, Komata M, Inoue F, Yamada K, Nakai K, Ohsugi M, Shirahige K. 2013. Inferring the choreography of parental genomes during fertilization from ultralarge-scale whole-transcriptome analysis. *Genes Dev*, 27:2736-2748.

Payer B, Saitou M, Barton SC, Thresher R, Dixon JP, Zahn D, Colledge WH, Carlton MB, Nakano T, Surani MA. 2003. Stella is a maternal effect gene required for normal early development in mice. *Current Biol*, 13:2110-2117.

Pedersen MT, Helin K. 2010. Histone demethylases in development and disease. *Trends Cell Biol*, 20:662-671.

Ronshaugen M, Levine M. 2004. Visualization of trans-homolog enhancer-promoter interactions at the Abd-B Hox locus in the *Drosophila* embryo. *Dev Cell*, 7:925-932.

Ross PJ, Ragina NP, Rodriguez RM, Iager AE, Siripattarapravat K, Lopez-Corrales N, Cibelli JB. 2008. Polycomb gene expression and histone H3 lysine 27 trimethylation changes during bovine preimplantation development. *Reproduction*, 136:777-785.

Rother F, Shmidt T, Popova E, Krivokharchenko A, Hügel S, Vilianovich L, Ridders M, Tenner K, Alenina N, Köhler M, Hartmann E, Bader M. 2011. Importinalpha7 is essential for zygotic genome activation and early mouse development. *PloS One*, 6:e18310.

Sarmento OF, Digilio LC, Wang Y, Perlin J, Herr JC, Allis CD, Coonrod SA. 2004. Dynamic alterations of specific histone modifications during early murine development. *J Cell Sci*, 117:4449-4459.

Schier AF. 2007. The maternal-zygotic transition: death and birth of RNAs. *Science*, 316:406-407.

Schneider TD, Arteaga-Salas JM, Mentele E, David R, Nicetto D, Imhof A, Rupp RA. 2011. Stage-specific histone modification profiles reveal global transitions in the *Xenopus* embryonic epigenome. *PloS One*, 6:e22548.

Schuettengruber B, Ganapathi M, Leblanc B, Portoso M, Jaschek R, Tolhuis B, van Lohuizen M, Tanay A, Cavalli G. 2009. Functional anatomy of polycomb and trithorax chromatin landscapes in *Drosophila* embryos. *PLoS Biol*, 7:e13.

Schultz RM. 1993. Regulation of zygotic gene activation in the mouse. *Bio Essays*, 15:531-538.

Schultz RM. 2002. The molecular foundations of the maternal to zygotic transition in the preimplantation embryo. *Hum Reprod Update*, 8:323-331.

Schulze SR, Wallrath LL. 2007. Gene regulation by chromatin structure: paradigms established in *Drosophila melanogaster*. *Annu Rev Entomol*, 52:171-192.

Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, Casero RA, Shi Y. 2004. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell*, 119:941-953.

Stancheva I, Meehan RR. 2000. Transient depletion of xDnmt1 leads to premature gene activation in *Xenopus* embryos. *Genes Dev*, 14:313-327.

Stancheva I, Hensey C, Meehan RR. 2001. Loss of the maintenance methyltransferase, xDnmt1, induces apoptosis in *Xenopus* embryos. *EMBO J*, 20:1963-1973.

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

Surface LE, Thornton SR, Boyer LA. 2010. Polycomb group proteins set the stage for early lineage commitment. *Cell Stem Cell*, 7:288-298.

Tadros W, Lipshitz HD. 2009. The maternal-to-zygotic transition: a play in two acts. *Development*, 136:3033-3042.

Tan MH, Au KF, Yablonovitch AL, Wills AE, Chuang J, Baker JC, Wong WH, Li JB. 2013. RNA sequencing reveals a diverse and dynamic repertoire of the *Xenopus tropicalis* transcriptome over development. *Genome Res*, 23:201-216.

ten Bosch JR, Benavides JA, Cline TW. 2006. The TAGteam DNA motif controls the timing of *Drosophila* pre-blastoderm transcription. *Development*, 133:1967-1977.

Thomas S, Li XY, Sabo PJ, Sandstrom R, Thurman RE, Canfield TK, Giste E, Fisher W, Hammonds A, Celniker SE, Biggin MD, Stamatoyannopoulos JA. 2011. Dynamic reprogramming of chromatin

accessibility during *Drosophila* embryo development. *Genome Biol*, 12:R43.

Tsurumi A, Xia F, Li J, Larson K, LaFrance R, Li WX. 2011. STAT is an essential activator of the zygotic genome in the early *Drosophila* embryo. *PLoS Genet*, 7:e1002086.

van der Heijden GW, Dieker JW, Derijck AA, Muller S, Berden JH, Braat DD, van der Vlag J, de Boer P. 2005. Asymmetry in histone H3 variants and lysine methylation between paternal and maternal chromatin of the early mouse zygote. *Mech Dev*, 122:1008-1022.

vanVliet J, Crofts LA, Quinlan KG, Czolij R, Perkins AC, Crossley M. 2006. Human KLF17 is a new member of the Sp/KLF family of transcription factors. *Genomics*, 87:474-482.

Vastenhouw NL, Zhang Y, Woods IG, Imam F, Regev A, Liu XS, Rinn J, Schier AF. 2010. Chromatin signature of embryonic pluripotency is established during genome activation. *Nature*, 464:922-926.

Veenstra GJ, Wolffe AP. 2001. Constitutive genomic methylation during embryonic development of *Xenopus*. *Biochim Biophys Acta*, 1521:39-44.

Vesterlund L, Jiao H, Unneberg P, Hovatta O, Kere J. 2011. The zebrafish transcriptome during early development. *BMC Dev Biol*, 11:30.

Walser CB, Lipshitz HD. 2011. Transcript clearance during the maternal-to-zygotic transition. *Curr Opin Genet Dev*, 21:431-443.

Wang L, Zhang J, Duan J, Gao X, Zhu W, Lu X, Yang L, Zhang J, Li G, Ci W, Li W, Zhou Q, Aluru N, Tang F, He C, Huang X, Liu J. 2014. Programming and inheritance of parental DNA methylomes in mammals. *Cell*, 157:979-991.

Wossidlo M, Nakamura T, Lepikhov K, Marques CJ, Zakhartchenko V, Boiani M, Arand J, Nakano T, Reik W, Walter J. 2011. 5-Hydroxymethylcytosine in the mammalian zygote is linked with epigenetic reprogramming. *Nat Commun*, 2:241.

Wunderlich Z, Mirny LA. 2009. Different gene regulation strategies revealed by analysis of binding motifs. *Trends Genet*, (10):434-440.

Xue Z, Huang K, Cai C, Cai L, Jiang CY, Feng Y, Liu Z, Zeng Q, Cheng L, Sun YE, Liu JY, Horvath S, Fan G. 2013. Genetic programs in human and mouse early embryos revealed by single-cell RNA sequencing. *Nature*, 500:593-597.

Yan L, Yang M, Guo H, Yang L, Wu J, Li R, Liu P, Lian Y, Zheng X, Yan J, Huang J, Li M, Wu X, Wen L, Lao K, Li R, Qiao J, Tang F. 2013. Single-cell RNA-Seq profiling of human preimplantation embryos and embryonic stem cells. *Nat Struct Mol Biol*, 20:1131-1139.

Zhang A, Xu B, Sun Y, Lu X, Gu R, Wu L, Feng Y, Xu C. 2012. Dynamic changes of histone H3trimethylated at positions K4 and K27 in human oocytes and preimplantation embryos. *Fertil Steril*, 98:1009-1016.