Awakening of the zygotic genome by pioneer transcription factors

Wataru Kobayashi[1,2] and Kikuë Tachibana[1,2*]

[1] Department of Totipotency, Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152, Martinsried, Germany

[2] Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Vienna Biocenter, Dr. Bohr Gasse 3, 1030, Vienna, Austria

*Correspondence should be addressed to K.T. (email: tachibana@biochem.mpg.de)

Abstract

After fertilization, the genome of the totipotent embryo is transcriptionally inactive and then initiates bursts of transcription termed zygotic genome activation (ZGA). Despite the fundamental importance of initiating an embryonic transcription program for the start of life, the essential regulators and molecular mechanisms triggering ZGA in most organisms are poorly understood. One mechanism centers on pioneer factors that function in cellular reprogramming and differentiation. Recent studies revealed that not only a single but multiple pioneer factors bind cooperatively to the genome to open chromatin, resulting in changes of epigenetic modifications and triggering ZGA. Here, we review recent insights into the functions of pioneer factors during ZGA and discuss the potential relevance to 3D chromatin organization during embryonic development.

Short title

Zygotic genome activation triggered by pioneer transcription factors

Keywords

Zygotic genome activation, Pioneer transcription factor, Totipotency, Chromatin structure, Topologically associating domain

Introduction

Embryonic development begins with the fertilized egg that is generated by the fusion of two terminally differentiated germ cells, sperm and egg (oocyte). A newly formed one-cell embryo (zygote) is totipotent, which is the developmental potential to form all cell types including extra-embryonic tissues and a complete organism [1,2]. It is thought that the reprogramming capacity is provided by maternal products (RNA and/or protein) derived from the oocyte, since oocyte cytoplasm is sufficient to reprogram somatic nuclei as demonstrated by John Gurdon's somatic cell nuclear transfer experiment [3]. In mammals, the embryo undergoes a series of cleavage divisions, progressing to 2-cell, 4-cell, 8-cell, 16-cell, morula and blastocyst stages. The totipotent potential gradually decreases during cleavage divisions until reaching a pluripotent or differentiated state (Figure 1a) [1,2].

How reprogramming to a totipotent state is achieved is a long-standing question. The transition from an egg to an embryo is accompanied by degradation of maternal RNA and protein, post-translational regulation and epigenetic reprogramming [4,5]. In this review, we focus on reprogramming that is mediated by a specialized class of transcription factors called pioneer factors (Figure 1b). We use the term pioneer as referring to transcription factors that fulfill these criteria: 1) binding to their (partial) motif in closed chromatin, 2) required for local chromatin opening *in vivo*, and 3) binding to nucleosomes *in vitro* [6-10]. Some pioneer factors also bind to condensed chromosomes and are retained during mitosis as "book markers" but it is not clear if this extends to all pioneer factors [11]. How precisely pioneers open chromatin remains poorly understood but it is thought that chromatin remodelers and epigenetic regulators are recruited to facilitate the process.

Pioneer factors are essential for early embryonic development and zygotic genome activation (ZGA) in several species. Here, we review recent studies that have provided further insights into ZGA regulation by pioneer factors during early embryonic development.

Multiple pioneer factors are required to trigger ZGA

The natural reprogramming that occurs after fertilization is thought to be driven by maternal products that act upon the transcriptionally inactive zygotic maternal and paternal genomes. Subsequently, transcription is activated, and control of embryonic development is handed over to the zygotic genome. The transition of developmental control from the oocyte to the embryo is known as the maternal-to-zygotic transition (MZT), which encompasses both degradation of maternal products and ZGA [1,2,4,5]. The latter can occur in two or more waves, starting with

low levels of transcription during minor ZGA and later bursts of transcription called major ZGA (Figure 1a) [12,13].

Current studies clarified that multiple pioneer factors are required to drive ZGA. Several scenarios can be considered for how pioneer factors trigger ZGA (Figure 2): 1) One master pioneer factor binds to its motif, 2) multiple pioneer factors, each with their own motif, target different loci in the genome, 3) multiple pioneer factors bind to the same motif and function redundantly, 4) multiple pioneer factors independently bind to their own motif on the same locus, and 5) multiple pioneer factors interdependently bind to their respective motifs. Multiple pioneer factors may function to make ZGA more robust, synchronous and protect against lethality from a single gene mutation. More than one transcription factor may be required to bind nucleosomes to promote eviction and chromatin opening, as proposed by Mirny [14]. Consistent with this model, recent studies from single-molecule footprinting suggested that transcription factor co-occupancy frequently occurs at *cis*-regulatory elements with high levels of nucleosome binding [15••,16••]. How transcription factor co-occupancy mechanistically leads to histone eviction from nucleosomes remains an important question.

Zelda and additional pioneer factors drive ZGA in flies

Zelda is the first identified essential activator of the zygotic genome in any organism [17]. Interestingly, the conservation of Zelda orthologs is limited to the Pancrustacea lineage including insect clade [18]. Zelda is required for transcription of hundreds genes during ZGA, and its absence leads to embryonic lethality [17]. It has been reported that enhancers generally have a strong intrinsic nucleosome barrier [15,16,19,20]. Therefore, transcription factors are required to overcome this nucleosome barrier to trigger ZGA. Zelda binds to *cis*-regulatory elements and establishes accessible chromatin for other factors to bind the DNA, suggesting that it functions as a pioneer factor *in vivo* [19,21,22]. Consistent with this, purified recombinant Zelda binds nucleosomal DNA with sequence specificity *in vitro* [23,24]. Furthermore, the establishment of some active histone modifications depends on Zelda-binding [25]. Thus, Zelda fulfills the defining criteria of pioneer factors. Unlike other pioneer factors, it does not appear to “bookmark” chromatin in mitosis [26], albeit the lack of detection on mitotic chromatin does not provide unequivocable evidence for its absence. The continued pioneer activity of Zelda is required for maintaining accessible chromatin regions [24], which is consistent with recent findings that continuous SWI/SNF activity regulates chromatin

openness [27,28]. Thus, continuous pioneer and chromatin remodeling activities may be necessary to maintain chromatin accessibility in interphase.

Although Zelda is considered to be a master regulator of ZGA, it is dispensable for opening chromatin regions enriched for GA di-nucleotides repeats [19,22]. This finding indicated that other transcription factors regulate chromatin accessibility on GA di-nucleotides repeats. These repeats are bound by GAGA-associated factor (GAF) and Chromatin-linked adaptor for male-specific lethal protein (CLAMP), and both function as activators of ZGA [29••,30••]. GAF maintains chromatin accessibility by recruiting chromatin remodelers independently of Zelda [29••]. In contrast, CLAMP and Zelda interdependently regulate each other's chromatin binding and function redundantly to mediate chromatin accessibility and ZGA [30••]. Further studies will be needed to reveal how Zelda and other transcription factors cooperatively trigger ZGA.

Pluripotency factors control ZGA in zebrafish

The regulators of ZGA in zebrafish are closely linked to the pluripotency gene regulatory network. Nanog, Pou5f3 (OCT4 homolog) and Sox19b (SoxB1 family) are required for triggering ZGA [31,32]. Combined loss of these factors results in a developmental arrest before gastrulation and a failure to activate zygotic genes [32,33••]. A recent pre-print demonstrated the importance for all three factors (NPS) by abrogating ZGA in a triple maternal-zygotic (MZ) *nanog*$^{-/-}$*;pou5f3*$^{-/-}$*;sox19b*$^{-/-}$ mutant [33••]. Triple mutant analysis revealed that NPS synergistically establish chromatin accessibility at more than half of active enhancers, and loss of enhancer activity is correlated with a reduction of transcription [33••]. OCT4 and SoxB1 family members from other organisms have been shown to bind nucleosomes *in vitro* [34], demonstrating that they function as pioneer factors. Cryo-EM studies revealed that OCT4-SOX2, SOX2 or SOX11 binding to nucleosomes locally distorts DNA [35•,36•]. The nucleosome-bound Sox11 further showed the rotation of the N-terminal H4 tail, which functions to stabilize higher-ordered chromatin structure [35•], thereby providing detailed insights into how pioneer factors might initiate local chromatin opening. Therefore, at least two of the three transcription factors function as pioneer factors for zebrafish ZGA. Remarkably, the restoration of Nanog in NPS mutants is sufficient to rescue chromatin opening [33••], indicating that Nanog may also function as a pioneer factor. Biochemical and structural analysis of Nanog may provide insights into cooperative chromatin opening by NPS.

ZGA is accompanied by several epigenetic chromatin changes including the deposition of histone acetylation H3K27ac at active enhancers. In zebrafish, H3K27ac precedes active transcription during ZGA [37-39]. Both H3K27ac and H3K18ac are reduced in embryos lacking NPS, suggesting that these factors are required for recruiting histone acetyltransferase to activate transcription [33••]. Whether they do so directly or indirectly remains to be determined.

Blackbox of mammalian ZGA regulators

ZGA in mammals is less well understood compared to flies or zebrafish. This is possibly due to both the lack of evolutionary conservation of embryonic pioneer factors and the challenges of *de novo* identification using scarce material. Several transcription factors involved in ZGA have been reported. The Nfya subunit of the Nfy complex promotes transcription of a subset of ZGA genes and is required for development beyond the morula stage [40]. Nfya contributes to maintain accessible promoters, suggesting that it functions as a pioneer factor [41,42]. *In vitro* assays demonstrated that the Nfy complex binds and distorts nucleosomes via the DNA-binding domain, which is structurally similar to the histone-fold domains of H2B and H2A [43,44]. In addition, the double homeobox proteins Dux (mice) and DUX4 (human) are expressed before major ZGA and activate a 2-cell-embryo-like transcription program in embryonic stem cells (2C-like cells) [45,46]. Dux knockout embryos display minimal effects on ZGA and the knockout mice are viable [47,48], which indicates that Dux is not essential for embryogenesis. Human DUX4 recruits histone acetyltransferase P300 through its C-terminus and induces local chromatin opening in human myoblast cells [49], suggesting that DUX4 may function as a pioneer factor. Whether it directly binds nucleosomes *in vitro* remains to be shown. How the majority of the mammalian embryonic genome is transcriptionally activated remains unknown.

Chromatin organization during early embryonic development

An intriguing aspect of ZGA is that it is accompanied by changes in 3D chromatin organization [2,50-54]. Interphase chromosomes are folded into loops and topologically associating domains (TADs) by a cohesin-dependent mechanism. Loop extrusion is hypothesized to proceed until encountering a boundary, which is generated by binding of the zinc finger transcription factor CTCF to its cognate motif [55-57]. The strength of a boundary is measured

as TAD insulation. Genomes also segregate into A/B compartments, which correlate with active and repressive histone modifications.

Several Hi-C analyses of embryos revealed that chromatin organization changes during early embryonic development [50-54,58]. Whilst loops and TADs are generated in the mouse zygote within hours after fertilization, TAD insulation is initially weak and becomes stronger at ZGA [50-52]. A similar phenomenon has been observed in most species, with the exception of zebrafish [59]. Interestingly, transcription is not required for chromatin reorganization at ZGA in flies and mice [50,51,53,54]. A notable exception are human embryos, where TAD establishment coincides with CTCF expression during ZGA and requires transcription [53]. CTCF is required but appears not to be sufficient for TAD establishment in the absence of transcription [53], implying that another aspect of ZGA priming might be important for chromatin reorganization.

We hypothesize that pioneer factors contribute to 3D chromatin reorganization as loop extrusion barriers during embryonic development (Figure 3). Transcriptional reprogramming is linked to the dynamics of TAD boundary and A/B compartment switching [60]. Indeed, pioneer factors such as OCT4 and C/EBPα correlate with accelerated topological remodeling of compartmentalization and TAD insulation during somatic cell reprogramming [61]. In plants, where CTCF is absent, transcription factor motifs are enriched at the boundaries of TADs [62]. More directly relevant to embryonic development, Zelda is required for establishing a subset of TADs [54]. This suggests that either opening of chromatin by Zelda provides access for other transcription factors or insulators (other than CTCF) to bind to chromatin or Zelda itself is a barrier to loop extrusion (Figure 3). In taking this concept further, we consider the possibility that CTCF functions as a pioneer factor in establishing boundaries and that this might be a general feature of *bona fide* pioneer factors. Further studies will be needed to test whether pioneer activity is important for 3D genome reorganization during ZGA.

Concluding remarks

Fertilized eggs rapidly reprogram the epigenome and 3D chromatin organization to acquire a totipotent state. In this review, we summarized how pioneer factors function as activators of zygotic genomes in different species. The key pioneer factor(s) for triggering ZGA in mammals remains unknown. Multiple pioneer factors function redundantly in other species, raising the possibility that a similar network might function in mouse and human. Extensive mapping of transcription factor binding sites during embryonic development will be needed to inform

functional experiments for elucidating the molecular mechanisms underlying ZGA. A combination of embryology, genetics, genomics, and biochemistry will shed light on how pioneer factors trigger ZGA and generate a totipotent embryo.

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Declarations of interest

The authors declare no conflict of interest.

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Figure legends

Figure 1 Overview of embryonic development and zygotic genome activation. (**a**) Embryonic development in mammals. In mice, zygote and two-cell embryos are considered to be totipotent, and this potential gradually decreases during the cleavage divisions. After fertilization, the clearance of maternal products is coordinated with the activation of zygotic transcription. The low level of transcription called minor ZGA occurs, followed by the burst of transcription called major ZGA. (**b**) Activation of transcription triggered by a pioneer factor. Transcription factors bind regulatory elements including promoters and enhancers, and drive gene expression. However, most transcription factor cannot access their motifs in closed chromatin. Pioneer factors scan and bind to their target sequences on nucleosomal DNA, establishing an accessible chromatin domain that facilitates the recruitment of additional factors, such as other transcription factors, histone modifiers and chromatin remodelers.

Figure 2 Models for how one or more pioneer factors establish accessible chromatin domain to trigger ZGA. To simplify a model, we show one target nucleosome on the regulatory element. (**a**) One master pioneer factor binds to regulatory element and trigger ZGA. Master pioneer factor (magenta) binds to its motif (magenta region on nucleosome) and establish chromatin accessibility. (**b**) Multiple pioneer factors (pTFs) bind at different genomic loci. Both pTF1 (magenta) and pTF2 (green) bind to their respective motif (magenta or green on nucleosome) to activate transcription. (**c**) Multiple pTFs which targets the same motif, bind the same locus and function redundantly. Either pTF1(magenta) or pTF2 (orange) binds to the same motif (magenta on nucleosome) to establish chromatin accessibility. (**d**) Multiple pTFs bind independently on regulatory element to establish chromatin accessibility. Both pTF1 (magenta) and pTF2 (green) bind to their respective motif (magenta or green on nucleosome) on the same locus and promote chromatin accessibility. (**d**) Multiple pTFs bind sequentially on regulatory element and regulate interdependently each other's binding. In this model, either pTF1 (magenta) or pTF2 (green) binds initially to their respective motif (magenta or green on nucleosome) and alter chromatin structure to allow secondary pTF binding.

Figure 3 Model of 3D chromatin reorganization triggered by pioneer factor. (1) Cohesin-mediated loop extrusion generates loops and TADs. Pioneer factors bind their target motif in chromatin. (2) Pioneer factors lead to nucleosome depletion to establish accessible chromatin. (3) Model 1: Pioneer factors generate accessible chromatin that allows insulators (e.g. CTCF) to bind and establish domain boundaries. Model 2: Pioneer factors are barriers to loop extrusion and directly establish domain boundaries.