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Versatile roles for histones in early development

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Abstract

The nuclear environment changes dramatically over the course of early development. Histones are core chromatin components that play critical roles in regulating gene expression and nuclear architecture. Additionally, the embryos of many species, including *Drosophila*, Zebrafish, and *Xenopus* use the availability of maternally deposited histones to time critical early embryonic events including cell cycle slowing and zygotic genome activation. Here, we review recent insights into how histones control early development. We first discuss the regulation of chromatin functions through interaction of histones and transcription factors, incorporation of variant histones, and histone post-translational modifications. We also highlight emerging roles for histones as developmental regulators independent of chromatin association.

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Introduction

Histones are the core components of eukaryotic chromatin. Two pairs of each of the four nucleosomal histones (H2A, H2B, H3, and H4) form an octamer that wraps ~146 base pairs of DNA to form a nucleosome while the linker histone H1 controls nucleosome spacing and larger chromatin conformation [1–3]. Histone occupancy, positioning, exchange, and post-translational modifications (PTMs) regulate transcription initiation and elongation, and incorporation of specific histone variants or PTMs can mark genomic features such as sites of DNA damage or heterochromatin [4–6].

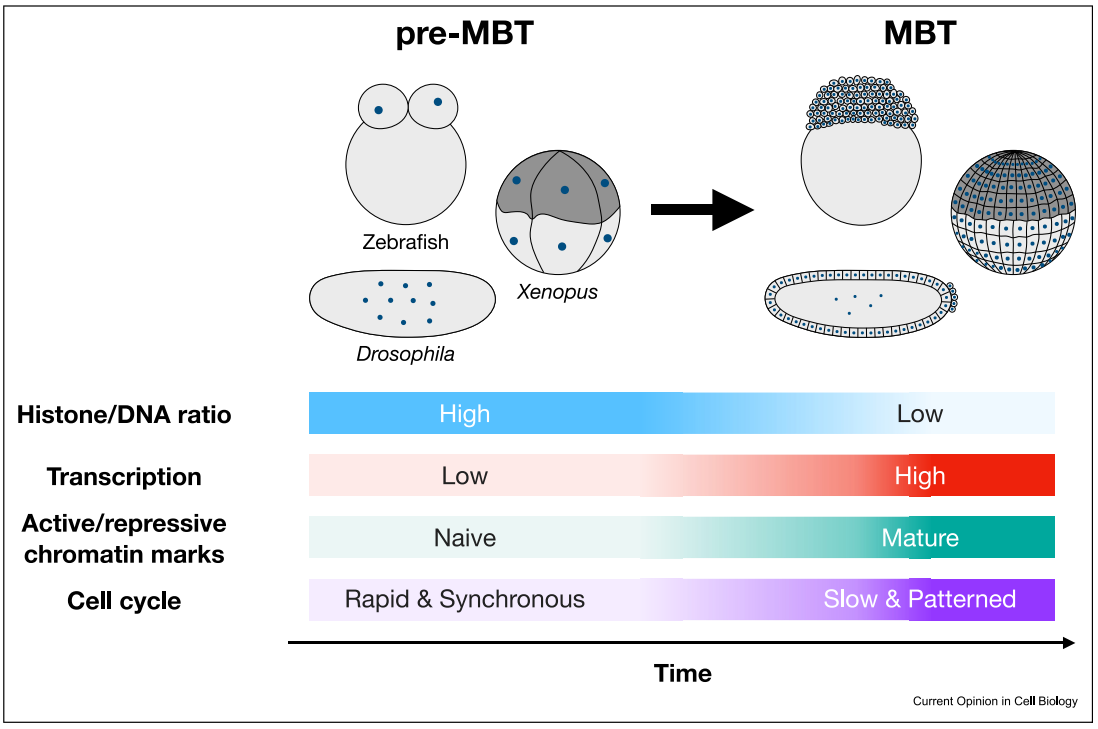
The chromatin landscape changes dramatically during development. In sexually reproducing species, the sperm pronucleus must first be decondensed and then fused with the maternal pronucleus during the process of fertilization. In most species, the initial chromatin state is "naive"; transcription is largely inactive, there is a relative absence of histone PTMs, and the three-dimensional organization of the genome is not well established. As development progresses, cells within the embryo acquire cell type-specific transcriptional profiles, PTMs, and chromatin architecture [7,8]. Prior to zygotic genome activation (ZGA), development is driven by maternally deposited components. ZGA coincides with cell cycle remodeling in many species and this coordinated transition is referred to as the mid-blastula transition (MBT) [9–11] (Figure 1). How the MBT is temporally regulated has been a fascinating and important question in developmental biology.

Since the 1990s, it has been recognized that histones repress early zygotic transcription [12–14], but the mechanistic basis remains elusive. In this review, we discuss recent findings on histone control of early development. We first review recent progress that has provided new insights into the classical role of histones as chromatin components in regulating ZGA and cell cycle remodeling. We then also discuss an unusual, chromatin-independent signaling mechanism that is critical for timing the MBT.

Histone supply and ZGA regulation

In several species with large externally fertilized eggs such as *Drosophila*, Zebrafish, and *Xenopus*, large pools of histones are maternally loaded into the egg prior to fertilization to support rapid pre-MBT development. This results in unusually high histone-to-DNA ratios and excess non-DNA-bound histones. For example, in *Xenopus*, a pool of histones sufficient to chromatinize 20,000 genomes is synthesized during oogenesis [15]. In Zebrafish, the embryo accumulates more than 3000 genomes worth histones by the 1-cell stage [16]. In *Drosophila*, zygotic histone null mutant embryos can

Figure 1



Mid-blastula transition (MBT) and histone dynamics. Upon fertilization, the early embryos of many species, including *Drosophila*, Zebrafish, and *Xenopus*, undergo a developmental transition after a species-specific stereotypical number of cleavage divisions. This transition involves remodeling and lengthening of the cell cycle and zygotic genome activation (ZGA) characterized by the onset of activation of zygotic genes and establishment of the mature chromatin state. Histones are maternally deposited into the embryos during oogenesis and are progressively incorporated into DNA, resulting in a decreasing Histone/DNA ratio. Histones have been implicated in regulating the timing of several critical events of the MBT, including transcription activation, chromatin maturation, and cell cycle slowing.

produce ~7000 diploid nuclei using maternally provided histone stores [17]. These excess early histones are imported into the nucleus, resulting in unusually high nuclear histone concentrations [16,18]. Nuclear histone concentrations decrease as the increasing number of nuclei dilute the maternal pool during the repeated cleavage divisions [16,18,19].

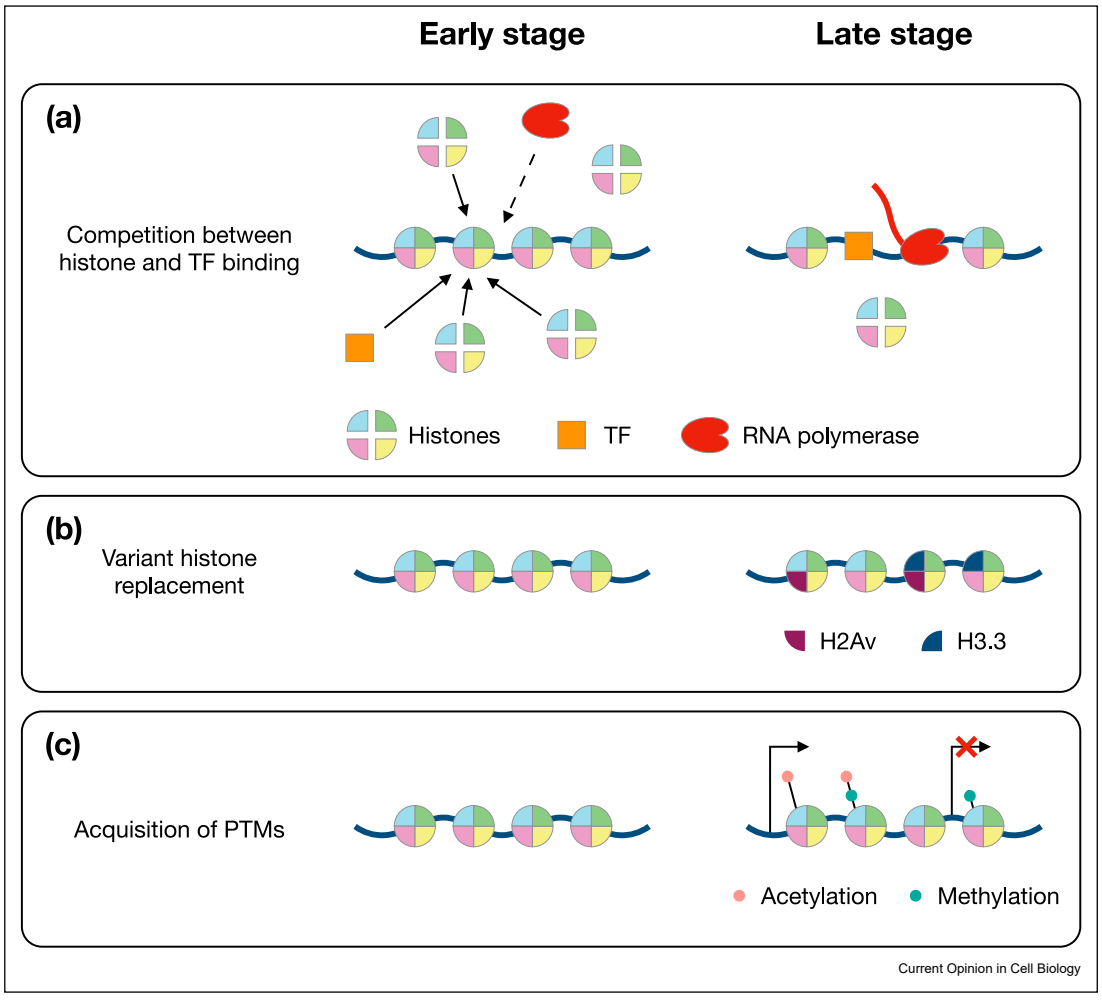
Histone concentrations regulate key cellular events at the MBT. In *Xenopus* embryos, changing the pool size of histones results in corresponding changes in the timing of ZGA and cell cycle remodeling [20]. In Zebrafish, histones compete with key early transcription factors (TFs) (Figure 2a), such as Pou5f3 and Sox19b for chromatin binding [16]. These TFs are pioneer factors that can evict nucleosomes [21,22], suggesting that the pioneering activity may be important to compete with the hyper-abundant early histones. In *Drosophila*, both over- and under-expression of maternally provided histones result in corresponding delay and advancement in ZGA timing and cell cycle remodeling [23]. Together, these results demonstrate a common theme of histone regulation of ZGA.

Dynamics of variant histone composition during the MBT

The so-called canonical core histones, namely H2A, H2B, H3, H4, and H1, are inserted throughout the genome during DNA replication and are referred to as replication-dependent histones. Histone variants have different amino acid sequences than replication-dependent histones and are incorporated in specialized chromatin contexts, including sites of active transcription, DNA damage, and heterochromatin [6,24,25]. In contrast to the replication-dependent histones, variant histones are incorporated at specific sites during other phases of the cell cycle. The histone variants have distinct transcriptional regulations, PTMs, and chaperones [26].

Histone variants show dramatic turnover during ZGA (Figure 2b). For example, the amount of H2Av on chromatin increases more than two-fold during ZGA in *Drosophila* [27]. Similarly, replication-dependent H3 that predominates in early cell cycles is replaced by variant histone H3.3 in the final cycles leading up to ZGA [18]. In contrast, species- and early embryo-specific H1

Figure 2



Histone regulation of ZGA. (a) Excess histones compete with TFs for DNA binding and suppress transcription in early stages. TFs eventually outcompete histones as histone concentrations decrease in later cycles. **(b)** The histone composition of chromatin dynamically changes during early embryogenesis. For example, in *Drosophila*, incorporation of variant histones H2Av and H3.3 into chromatin increases in the final cleavage divisions leading up to the MBT. **(c)** Early chromatin is relatively depleted for histone PTMs. Many histone PTM marks associated with active transcription and gene silencing increase over the MBT.

variants predominate before the MBT and are eventually replaced by replication-dependent H1 in somatic cells in *Drosophila* [28], Zebrafish [29,30], and *Xenopus* [31–33]. In Zebrafish, an oocyte-specific H2Af1o variant supplied in the early embryo becomes depleted before the ZGA [34]. Understanding whether the replacement of these variant histones occurs at specific genomic regions, such as sites of active transcription and heterochromatin requires further investigation. It may also be interesting to determine if distinct variant species are incorporated into the same nucleosome.

Although histone variants play important roles in marking specific sites within the chromatin, the functional relevance of the change of histone variants at the MBT remains elusive. In *Drosophila*, H3.3 mutants can develop normally if provided with replication-dependent H3 under the control of H3.3's *cis*-regulatory elements [35,36]. Likewise, current research suggests that the loss of *Drosophila* H1 variant dBigH1 can be compensated by replication-dependent H1 [37]. It may be that in *Drosophila* the amino acid difference between replication-dependent and variant histones is less important than simply having enough of each histone at the correct stage. However, histone variants have demonstrable roles in Zebrafish and *Xenopus* MBT. In Zebrafish, perturbations to installation of the H2A variant H2A.Z lead to inappropriate activation or repression of genes that are marked by H2A.Z containing placeholder nucleosomes [38]. In *Xenopus*, H3.3 is essential for proper gastrulation immediately after the MBT and cannot be rescued by replication-dependent

H3 [39,40]. Rescue of the H3.3 knock-down by H3/H3.3 chimeric proteins revealed that the H3.3-specific S31 residue is critical for proper development, indicating that the amino acid differences between replication-dependent histones and variants do matter in some circumstances. What determines a specific requirement of variant histones during early development requires further study.

Bulk changes in the state of histone PTMs during the MBT

Given the well-established roles for PTMs in transcription regulation, it is unsurprising that the genome acquires many novel PTMs at the onset of ZGA (Figure 2c). ChIP-chip/seq and more recently CUT&RUN/CUT&TAG techniques have allowed for extensive mapping of sites for PTMs over the course of early development [41–47]. The contributions of the various histone PTMs are too numerous to discuss fully here, we encourage the reader to refer to a recent review [7] for further details. The genome-wide atlas of histone PTMs has dramatically increased our understanding of developmental dynamics of histone PTM landscapes as well as their associations with *cis*-regulatory elements. However, the temporal resolution of these analyses is relatively low compared to the timescale of gene expression in rapidly developing embryos, and our understanding about temporal regulation of PTMs and transcription control remains limited.

Live-imaging probes for chromatin modifications allow for more precise temporal analysis of the changing PTM landscape [48]. For example, live-imaging of both H3K27ac and the elongation form of RNAPol2 using Fab-based fluorescent probes revealed that acquisition of H3K27ac precedes the onset of the earliest zygotic gene transcription in Zebrafish [49]. The effect of H3K27ac on transcription in the early embryo is global since injection of the writer (P300) and reader (Brd4) of H3K27ac results in premature expression of thousands of genes [50]. Chemical inhibition of P300 and Brd4 abolishes transcription and in turn blocks gastrulation, suggesting that a gain of H3K27ac is an essential step to initiate the zygotic developmental program in Zebrafish [50]. However, mouse embryonic stem cells (mESCs) do not appear to share this requirement for H3K27ac for proper transcription. It may be that in mice, the different developmental program or genome architecture allows for the functional compensation for loss of H3K27ac by other features, such as H3K4me1, and acetylation at other residues [51]. Similarly, catalytically-dead mutants for H3K4me1 methyltransferase can result in normal development and transcription in *Drosophila* and mESCs, although the mutants manifest developmental abnormalities under stress conditions [52,53]. An important open question is to disentangle the contributions of each PTM and crosstalk between PTMs to ZGA and development in different species.

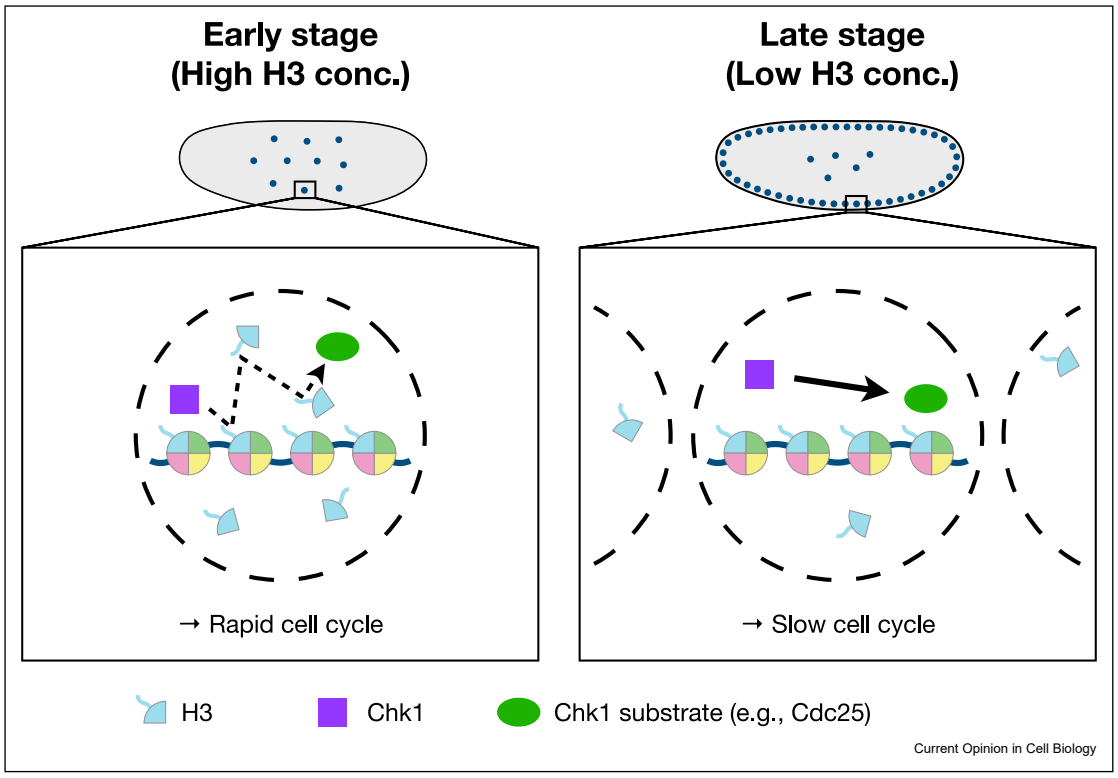
In parallel with the onset of zygotic transcription, heterochromatin is established during the MBT. Live-imaging of a fluorescently-labeled H3K9me2 Fab, a PTM characteristic of constitutive heterochromatin, in *Drosophila* showed that bulk levels of H3K9me2 increase during repeated cleavage division cycles leading up to the MBT [54]. It also revealed that interphase durations, which lengthen leading up to the MBT, limit the extent of H3K9me2 deposition. This indicates that ZGA and cell cycle slowing are not independent processes at the MBT, but rather influence each other [54–57]. Together, these works provided quantitative insights into how bulk histone PTMs change during the MBT. Integrating the bulk PTM dynamics with chromatin states at individual loci will be one of the next challenges for researchers seeking to understand chromatin control of development.

Emerging roles of histones in cell cycle control, beyond chromatin regulation

In addition to their roles in transcription and chromatin regulation, histones have also been shown to control cell cycle remodeling at the MBT. Given the central role of histones as core chromatin components, it is tempting to think that histone control of cell cycle slowing would be mediated entirely through the action of downstream transcripts. Indeed, in *Drosophila*, transcription of the cell cycle regulator frs responds to changes in the size of the maternally loaded histone pool [23]. However, in many contexts histones play additional roles as more than chromatin components. The antibacterial properties of histones *in vitro* were reported, prior to their recognition as core chromatin components [58,59]. Surprisingly, a recent study showed that the histone H3-H4 tetramer has an enzymatic activity to catalyze cupric ion reduction [60]. New work on the early *Drosophila* embryo has found that histone H3 can regulate cell cycle progression independent of chromatin incorporation [61]. This study used truncated histones H3 and H4 that retain the N-terminal tail regions which are the sites of the majority of histone post-translational modifications but lack the histone fold domains required for chromatin incorporation. Expression of H3-tail significantly increased cell cycle progression and promoted extra divisions before MBT, indicating that H3-tail that is not incorporated into chromatin is sufficient to positively regulate the cell cycle progression. By contrast, H4-tail expression had no effect on the cell cycle.

In the early embryos, cell cycle remodeling requires the activation of the checkpoint kinase, Chk1, which phosphorylates downstream targets to slow and eventually stop the cell cycle. Canonical substrates for Chk1 include cell cycle regulators, such as Wee1 and Cdc25,

Figure 3



Role of histone H3 beyond chromatin in the MBT. Cell cycle slowing at the MBT requires the activation of the Chk1 kinase that pauses cell cycle progression. Given its hyper-abundance, histone H3 acts as a competitive Chk1 inhibitor independent of chromatin association. The reduction in nuclear H3 concentrations during the cleavage division cycles results in an increase in Chk1 activity, thereby coupling the timing of cell cycle slowing and developmental progression.

which directly control the activity of the Cdk1-cyclin B complex that drives cell cycle progression. Interestingly, it was reported that Chk1 also phosphorylates the H3-tail [62]. This led to a hypothesis that histone H3 may outcompete Chk1's other substrates for Chk1 binding in the early embryo because of the unusually large amounts of maternally provided histones. *In vitro* kinase assays revealed that phosphorylation of relevant Chk1 substrates, Cdc25, was reduced by the presence of H3-tail at physiological concentrations. Measurements of a Chk1 sensor in the living embryos also showed that excess H3-tail decreases Chk1 activity *in vivo*. Mutation of the Chk1 phosphosite in the endogenous H3 protein advanced cell cycle slowing. Together, these data indicate that histone H3 acts as a competitive Chk1 inhibitor independent of chromatin incorporation. Since Chk1 has not been observed to interact with other histones than H3, this competitive inhibition mechanism is H3-specific, consistent with the observation that H4-tail had no effect on the cell cycle. Because excess histones become depleted as the embryo undergoes the repeated cleavage divisions, this mechanism allows coupling of developmental progression to the timing of Chk1 activation and cell cycle remodeling (Figure 3) [61].

In principle, similar competitive inhibition mechanisms could affect any histone interaction partners, not just Chk1. Interestingly, it has been shown that histone H3-tail is cleaved in some species and cell types and this histone clipping is involved in gene expression and cellular differentiation [63–66]. This could allow H3-tail signaling to be modulated independent of the amount of histone on chromatin, possibly providing a broader dynamic range for signaling by free histones. How and whether extra-chromosomal histones play a role in other species and cellular contexts is an interesting area for future study. Together, we propose that histones should be seen as a multi-faceted molecule that acts not only as chromatin components but also as regulatory molecules critical for early development.

Concluding remarks and outlook

In this review, we have discussed the versatile roles for histones in early development. Some of these include well-established pathways for chromatin control of genome accessibility and transcription. However, we also highlight emerging functions of histones independent of chromatin incorporation. We have focused on the

early embryonic stages of the *Drosophila*, Zebrafish, and *Xenopus* model systems, which undergo coordinated cell cycle slowing and ZGA at the MBT. It remains to be seen if a similar framework plays a role in other organisms including mice and humans, in which ZGA is not coupled to cell cycle slowing. Another key question is if and how histones contribute to the spatial patterning of ZGA to regulate differentiation. Finally, given the numerous cellular processes that are regulated by histones, a major open question will be to address how the different histone signals are coordinated in normal development and if such coordination goes awry in disease. For example, changes in a histone signal that affects the cell cycle may indirectly alter transcription through changing interphase durations that limit the transcriptional time window; and vice versa, where changes in transcription may remodel the cell cycle. Histone biosynthesis and nuclear import may have a more global impact on cellular physiology than we had previously recognized because they are common to the multiple histone pathways. Thus, a major challenge in the field will be to understand at the systems-level the mechanisms and principles for histone signal integration and its physiological consequences.

Author contributions

Conceptualization, Y.S. and A.A.A.; Writing – Original Draft, Y.S., M.G.B., and A.A.A. Writing – Review & Editing, Y.S., M.G.B. and A.A.A.

Conflict of interest statement

Nothing declared.

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