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海外特別研究員としての派遣期間を終了しましたので、下記のとおり報告いたします。
なお、下記及び別紙記載の内容については相違ありません。

記

1. 用務地（派遣先国名）用務地：プリンストン大学（国名：米国）

2. 研究課題名（和文）※研究課題名は申請時のものと違わないように記載すること。
初期胚におけるタイマーとしてのヒストンの機能とその制御機構

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Lewis-Sigler Institute for Integrative Genomics, Princeton University

5. 所期の目的の遂行状況及び成果…書式任意 **書式任意（A4 判相当 3 ページ以上、英語で記入も可）**
（研究・調査実施状況及びその成果の発表・関係学会への参加状況等）
（注）「6. 研究発表」以降については様式 10－別紙 1～4 に記入の上、併せて提出すること。

Abstract

This study uncovers how the supply of chromatin components, namely histone H3, affects the nuclear and chromatin environment during early embryonic development through quantitative live imaging and mathematical modeling. First, I construct an endogenously-regulated, photo-switchable histone H3-Dendra2 reporter and use this tool to characterize changes in the percent of H3 in the nucleus and on chromatin during the syncytial blastoderm stages of *Drosophila* embryogenesis. I find that in the first observable cycle, nearly 60% of the total nuclear H3 reporter is not bound to chromatin and that this percentage falls dramatically over the course of the cleavage stage. I construct a mathematical model that demonstrates that the observed accumulation and loss of free nuclear H3 is consistent with titration of a finite pool of maternally provided H3 by an exponentially increasing number of nuclei. Moreover, I show that these changes in H3 availability correspond to a large (40%) reduction in the total amount of H3 on chromatin, which is compensated by the increased incorporation of variant histone H3.3. These data indicate a massive change in the chromatin composition exactly as the zygotic genome is becoming active.

Introduction

In many species with large, externally developing eggs, the embryo goes from a single fertilized cell to thousands of cells in just a few hours. These rapid divisions are highly dependent on maternally provided components and accomplished by alternating directly between S-phase and M-phase without intervening gap phases or large-scale transcription. During the final part of the rapid cleavage phase, the chromatin environment changes dramatically. The zygotic genome becomes gradually activated, nucleosomes become more precisely positioned on promoters, and topologically associating domains become detectable for the first time. In *Drosophila*, late replicating regions emerge as the cell cycles gradually lengthen and shortly afterward heterochromatin marks begin to appear. The supply of chromatin components relative to the exponentially increasing amount of DNA may play a role in these changes. Altering the ratio of histones to DNA affects the timing of zygotic genome activation in both *Xenopus* and zebrafish embryos at the equivalent developmental stage. It is therefore imperative that we understand how histones are supplied to nuclei in the cleavage embryo and if the method of their supply results in changes to nuclear and chromatin composition during early embryogenesis.

With the exception of the early embryo, bulk production of histones is tightly coupled to S-phase so that the histone supply matches the demands of the newly synthesized DNA. These histones are termed “replication-coupled” histones while variant histones that mark specific chromatin features such as sites of active transcription or DNA damage are typically replication independent. In these more typical cell cycles, nearly all histones are incorporated into chromatin and excess pools are rapidly degraded .The histone supply is likely highly regulated because accumulation of excess histones is cytotoxic and insufficient histone supply during S-phase results in replication fork stalling, DNA damage, and improper chromatin condensation during mitosis. However the unusually short S-phase duration and lack of gap phases in the early embryo necessitate a different strategy for histone protein production. In this case, the mother provides large stores of both histone RNA and protein in the cytoplasm during oogenesis to facilitate the rapid cleavage cycles. For the replication-coupled histones, these maternally provided RNAs and proteins are sufficient to last the embryo through the early cleavage cycles and zygotic histone mutants arrest in S-phase of cycle 15. Here, I investigated how cytoplasmic histone stores are partitioned into nuclei to allow for the rapid genome replication and remodeling of the chromatin environment in the early *Drosophila* embryo.

Results

Construction of an endogenously-regulated H3-Dendra2 reporter

In order to study histone dynamics in the early embryo, I constructed a histone reporter designed to reproduce the behavior of the replication-coupled histone pool. In *Drosophila melanogaster*, replication-coupled histones are encoded by a cluster of ~100 tandem repeats located near the centromere of chromosome 2L. Due to the repetitive nature of the histone locus, endogenous tagging of histone genes is not feasible. Therefore, I decided to introduce a “101st” repeat, where H3 is tagged with a green-to-red photo-switchable fluorescent protein, Dendra2 (Figure 1A). This 5-Kb repeat region is required to recruit many of the endogenous histone regulators. Importantly, the H3-Dendra2 reporter was constructed to retain the endogenous H3 3’ UTR including the regulatory stem loop. With this new tool, I examined the import, export, accumulation, and chromatin association of H3.

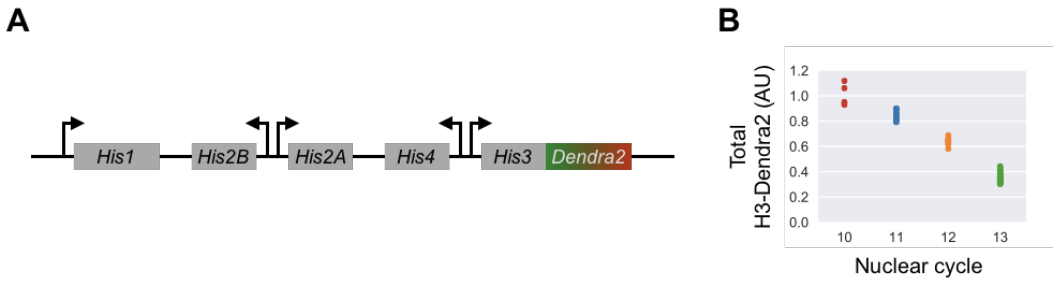

Figure 1. (A) Schematic of the H3-Dendra2 reporter in the context of a single HisC cluster. A green-to-red photo-switchable protein, Dendra2, was inserted into C-terminal of *His3* before the endogenous UTR. (B) Total H3-Dendra2 intensities (AU) at the end of each cell cycle. The total volume and nuclear H3 decrease with developmental progression. (Figures adapted from Shindo & Amodeo, *Current Biology* (2019).)

Total H3 in the nucleus decreases with each cell cycle

First, I sought to characterize how the nuclear composition may change over the course of early embryogenesis. In *Drosophila*, the rapid cleavages take place without cytokinesis (and are therefore known as nuclear cycles) resulting in a syncytium. After the 8th nuclear cycle (NC8), the nuclei migrate to the surface where they divide as a syncytial blastoderm for four more cycles until the pausing and cellularization in NC14. In NC10–13, I measured the total volume and brightness of the nuclear H3-Dendra2 at the end of each cycle by z-stack imaging with a laser scanning confocal microscope. The final nuclear volume decreased with each division. However, the total quantity of H3-Dendra2 in the nucleus fell even faster resulting in ~65% reduction in total nuclear H3-Dendra2 between NC10 and NC13 (Figure 1B). There are two possible mechanisms that can account for this change in total nuclear H3. There must either be a pool of non-DNA bound H3 in the nucleus of the earlier cycles, or the amount of H3 on chromatin must be falling. However, these mechanisms are not mutually exclusive.

Free nuclear H3 is reduced with each cycle

To test if there exists free histone H3 in the nucleus, I photo-activated nuclear H3-Dendra2 in late S phase of NC11 and observed the behavior of the red protein by z-stack time-lapse imaging. After nuclear envelope breakdown, I observed a drop of the total H3-Dendra2 signal within ~1 min, indicating the presence of non-DNA bound H3. This pool quickly rejoined cytoplasmic pools during mitosis by diffusion and therefore must not retain a significant interaction with the chromatin during mitosis. These observations indicate the accumulation of large pools of free histone H3 in the nuclei of the early embryo. I also found that the free pool size decreased with each cycle. Given that nuclear export of H3 was negligible, changes in nuclear import must explain the changes in free nuclear H3 observed between the different cell cycles.

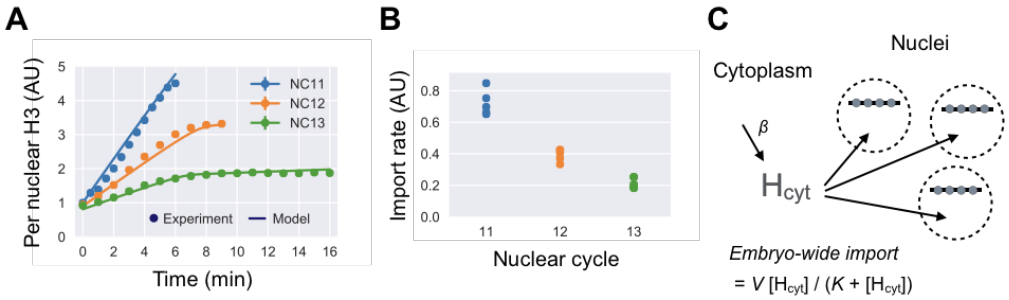

Figure 2. (A) Total nuclear H3-Dendra2 intensities over time for average individual nuclei. Lines and points represent simulated time course and experimental data, respectively. (B) Rates of H3-Dendra2 nuclear import as measured by initial slopes of the increase in nuclear intensities in (A). (C) Schematic of a model for histone nuclear import. I assumed that the rate of nuclear import is approximated by the Michaelis-Menten equation. (Figures adapted from Shindo & Amodeo, Current Biology (2019).)

H3 nuclear import halves with each cell cycle and is biphasic in NC13, consistent with a titration model

To measure histone nuclear import, I imaged non-photoswitched, green, H3-Dendra2 accumulation in the nucleus and calculated the total nuclear intensities for the duration of NC11–13 (Figure 2A). At NC11, nuclear levels of H3-Dendra2 increased almost linearly over time, indicating a constant import rate. In contrast, I found biphasic dynamics of H3 nuclear import at NC13, where levels of nuclear H3-Dendra2 increased at an almost constant rate for the first 6–7 min followed by a plateau at approximately twice the initial H3-Dendra2 level for the remaining 9–10 min until the end of the nuclear cycle. To better characterize the dynamics of H3

nuclear accumulation, I calculated import rates by measuring initial slopes of nuclear H3-Dendra2 intensities. I found that the initial import rates decreased by a factor of two from NC11 to NC12, and again from NC12 to NC13 (Figure 2B). Therefore, given the fact that the number of blastoderm nuclei doubles with each nuclear cycle, the total, embryo-wide H3 initial import rate is almost constant from NC11 through NC13. These observations suggest that the histone import capacity of the total embryo may be fairly static and that H3 concentrations may become limiting over the blastoderm cycles as the demand for chromatin components exponentially increases. To quantitatively test this idea, I constructed a simple kinetic model for histone nuclear import (Figure 2C), recapitulating the dynamics of H3 nuclear accumulation in all cell cycles, including the decrease in excess nuclear H3 from NC11 through NC13 and the biphasic dynamics at NC13 (Figure 2A).

H3.3 replaces H3 on chromatin during the early cell cycles

Given the dynamic changes in nuclear H3 availability, I wondered if the chromatin composition might also change during the early cell cycles. To test this, I compared the total H3-Dendra2 intensities during mitosis 10, 11, 12, and 13. I found that in addition to losing free nuclear H3, the amount of H3 incorporated into chromatin decreased by ~10% in each cycle between NC10 and NC12 with an even larger loss, resulting in a final H3-Dendra2 intensity that was 60% of initial NC10 levels, at the end on NC13 (Figure 3A). One interpretation of the marked loss of H3-Dendra2 from chromatin is that there is a global loss of nucleosomes during this period. However, previous ChIP-seq analysis using a pan-H3 antibody that recognizes both H3 and H3.3 found that the amount of total H3 isoforms on chromatin remains relatively constant between NC8 and NC14. Therefore, I reasoned that the incorporation of H3.3 variant may compensate for the loss of H3. To directly test this, I created an endogenously-tagged H3.3-Dendra2 using CRISPR-Cas9 genome editing. I found that H3.3 on chromatin increased with each cell cycle (Figure 3B) in contrast to H3 that decreased during the early cell cycles. The complementary behavior of H3 and H3.3 strongly suggests a model in which H3.3 is replacing H3 on chromatin during the early embryogenesis.

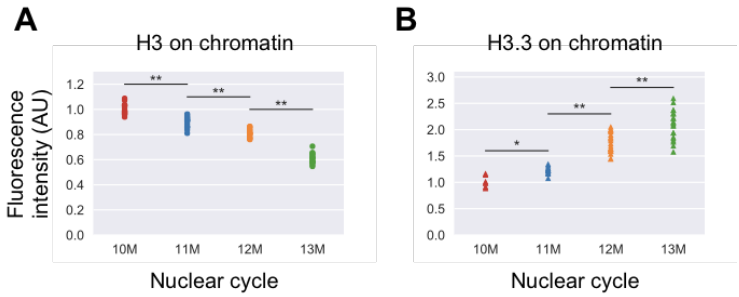

Figure 3. (A) Total fluorescent intensities of H3-Dendra2 on mitotic chromosome were measured in mitosis 10, 11, 12, and 13. Total H3-Dendra2 intensities on chromatin decrease with each cell cycle. (B) Total fluorescence intensities of mitotic H3.3-Dendra2 as in (A). Data are normalized to the average intensity of mitosis 10. Asterisks denote *P* values from two-tailed t-tests where *P < 1e-2 and **P < 1e-4. (Figures adapted from Shindo & Amodeo, Current Biology (2019).)

Conclusions

In the early cell cycles, large pools of free H3 accumulates in the nucleus. These pools become depleted in later cycles with a corresponding reduction of H3 on chromatin. The H3.3 variant ultimately replaces H3 on chromatin. These findings have profound implications for the nuclear and chromatin environment during the early embryogenesis, where large-scale chromatin remodeling including the onset of the late replicating regions, the activation of major zygotic transcription, and the formation of topologically associating domains all occur.

Publications and conference presentations

Yuki Shindo & Amanda A. Amodeo, Dynamics of Free and Chromatin-Bound Histone H3 during Early Embryogenesis. *Current Biology* **29**, 359–366 (2019).

Yuki Shindo & Amanda A. Amodeo. Dynamics of free and chromatin-bound histone H3.2 and H3.3 yield insights into chromatin changes during early embryogenesis. *2018 ASCB/EMBO Annual Meeting*, San Diego, USA (December 8–12, 2018).

Yuki Shindo & Amanda A. Amodeo. Dynamics of histone nuclear import in the early *Drosophila* embryo. *59th Annual Drosophila Research Conference*, Philadelphia, USA (April 11–15, 2018).