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Report

Zygotic Resetting of the HISTONE 3 Variant Repertoire Participates in Epigenetic Reprogramming in *Arabidopsis*

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Summary

In most eukaryotes, the HISTONE 3 family comprises several variants distinguished by their amino acid sequence, localization, and correlation with transcriptional activity [1–3]. Transgenerational inheritance of epigenetic information carried by histones is still unclear [4, 5]. In addition to covalent histone modifications, the mosaic distribution of H3 variants onto chromatin has been proposed to provide a new level of epigenetic information [6]. To study the transmission of patterns of H3 variants through generations, we combined transcriptional profiling and live imaging of the 13 H3 variants encoded by the *Arabidopsis* plant genome [7]. In comparison with somatic cells, only a restricted number of H3 variants are present in male and female gametes. Upon fertilization, H3 variants contributed by both gametes are actively removed from the zygote chromatin. The somatic H3 composition is restored in the embryo by de novo synthesis of H3 variants. A survey of *Arabidopsis* homologs of animal H3 chaperones suggests that removal of parental H3 from the zygote nucleus relies on a new mechanism. Our results suggest that reprogramming of parental genomes in the zygote limits the inheritance of epigenetic information carried by H3 variants across generations.

Results and Discussion

Expression of the HISTONE THREE RELATED Gene Family in Somatic Cells

The *Arabidopsis* genome comprises 15 *HISTONE THREE RELATED* (*HTR*) genes (http://www.chromdb.org/) that fall into four classes: a canonical H3.1 encoded by five genes (*HTR1*, *HTR2*, *HTR3*, *HTR9*, and *HTR13*), hereafter referred to as H3.1; a canonical H3.3 encoded by three genes (*HTR4*, *HTR5*, and *HTR8*), hereafter referred to as H3.3; the unusual H3 variants encoded by four genes (*HTR6*, *HTR10*, *HTR14*, and *HTR15*) [7, 8]; and a divergent centromeric H3 variant (CENH3/HTR12) [9]. Transcripts encoded by the remaining genes, *HTR7* and *HTR11*, have not been detected, and these are considered as pseudogenes [8]. We analyzed the expression of the entire HTR gene family by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) in seedlings (see Figure S1A available online). We could not detect any of the noncanonical H3 variants (encoded by *HTR6*, *HTR10*, *HTR14*, and *HTR15*) in seedlings (Figure S1A); transcripts of the three other classes of *HTR* genes (H3.1, H3.3, and CENH3) were detected in somatic cells of seedlings (Figure S1A).

To further detail H3 variant expression pattern, we generated transgenic plant lines expressing H3 variants tagged with fluorescent proteins (FP) expressed under the control of their endogenous promoter. We first analyzed the dynamics of tagged H3.1 and H3.3 in the root dividing cells (Figures S1B–S1I). In contrast to the ubiquitous expression of H3.3-FP variants (Figures S1F and S1G), H3.1-FP variants were expressed in a salt-and-pepper distribution (Figures S1B–S1E and S1J) reminiscent of cell-cycle-regulated H3.1 in animals [2, 10]. Subnuclear localization of tagged H3.1 and H3.3 variants is markedly distinct. H3.3 variants are uniformly dispersed in the nucleoplasm, in agreement with an euchromatic pattern (Figure S1I), and H3.1 variants accumulate at foci corresponding to chromocenters, which represent the constitutive heterochromatin [11] (Figures S1H and S1J show distribution of HTR2-CFP and HTR13-CFP, respectively, which are similar to what we observed for all other H3.1-FP fusion proteins). At mitosis, tagged H3.1 and H3.3 segregated with the chromosomes (arrowheads in Figures S1C and S1F). Hence, the distinct distribution of tagged H3 variants might reflect the expected association of H3.3 with transcriptionally active genes in euchromatin, whereas H3.1 is expected to associate with silent genes in heterochromatin [2, 10]. Because tagged H3 variants are incorporated in chromatin, their distributions likely reflect the distribution of corresponding endogenous H3 variants. The limited impact of fluorescent tags on H3 variant incorporation has also been documented in animal cells [1]. In conclusion, these results suggest that most genes encoding H3 variants are expressed in somatic cells and that the cytological patterns of H3.1 and H3.3 variants in chromatin reflect their conserved association with heterochromatin and euchromatin, respectively.

H3.3 Variants Represent the Main Class of HTR Proteins Present in Male and Female Gametes

Quantitative RT-PCR analyses showed that all classes of H3 variants were expressed in reproductive tissues (anthers, pistils, and siliques) (Figure S1A). We documented the expression of H3 variants in male and female gametes using the collection of FP-tagged H3 variants (Figure 1). The male germline differentiates during pollen development through an asymmetric division producing the large vegetative cell and a smaller generative cell. The generative cell undergoes another mitosis, producing two sperm cells. We found that only a limited subset of H3 variants were expressed during male gametogenesis (Figures 1A–1D) [8, 12], in comparison to the nine H3 variants detected in somatic cells. The vegetative cell only expressed the tagged H3.3 variants HTR5-GFP (Figure 1A) and HTR8-CFP (Figure 1C) and the unusual variant

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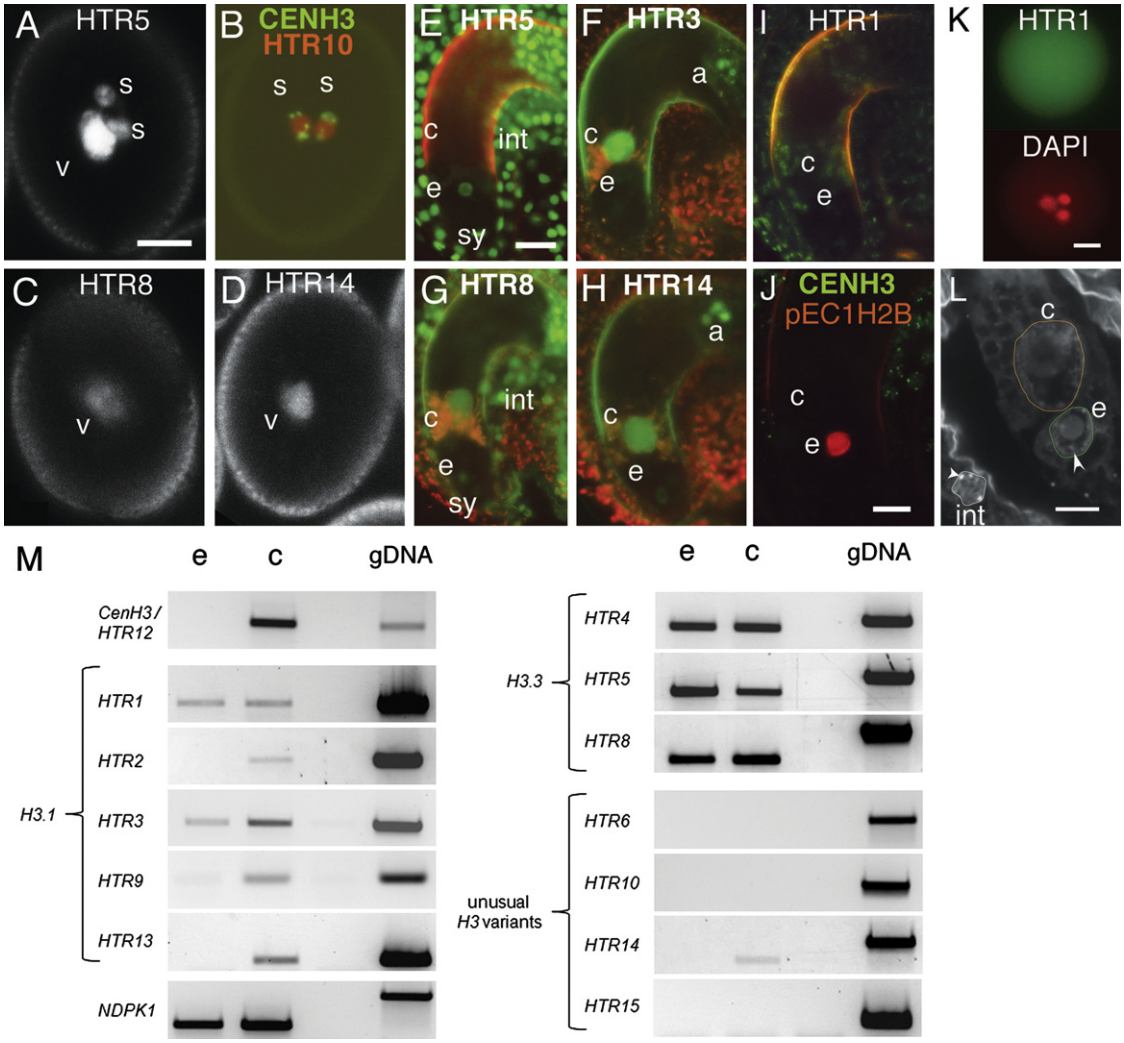

Figure 1. Distinct Repertoires of H3 Variants Are Expressed in Male and Female Gametes in *Arabidopsis*

(A–D and K) Male gametes in pollen.

(E–J and L) Female gametes in embryo sacs.

(A) HTR5-GFP in sperm cells (s) and vegetative cell (v) nucleus.

(B) HTR10-RFP (red) and CENH3-GFP (green) in sperm cell nuclei (s).

(C) HTR8-CFP in vegetative cell nucleus (v).

(D) HTR14-CFP in vegetative cell nucleus (v).

(E) HTR5-GFP in the nucleus of the egg cell (e), the synergids (sy), the central cell (c, weak signal), and the ovule integument (int) cell nuclei.

(F) HTR3-CFP in central cell (c) and antipodal (a) nuclei.

(G) HTR8-CFP in the nucleus of the central cell (c), the egg cell (e, weak signal), and the synergids (sy).

(H) HTR14-CFP in the central cell (c) and antipodal nuclei (a), but not in the egg cell nucleus.

(I) HTR1-CFP is not expressed in the female gametes.

(J) CENH3-GFP is not detected in the central cell nucleus (c) or the egg cell nucleus (e), which is marked by H2B-RFP driven by the egg cell-specific promoter *pEC1*.

(K) HTR1-CFP is not expressed in pollen. Although DAPI clearly stains nuclei (red) of the sperm cells and the vegetative cell, only strong nonspecific fluorescence (green) is visible despite of a marked increase in the detector gain.

(L) Feulgen staining of the ovule. Chromocenters in the nucleus of ovule integument cells (int) are easily recognizable (arrowhead). In the embryo sac, the chromocenters are also distinguishable, although less marked in the egg cell nucleus (arrowhead). In contrast, the central cell nucleus does not show chromocenters.

Autofluorescence was used to visualize the contours of the embryo sac in (E)–(J). Scale bars represent 10 μm in (A)–(D), (J), and (K) and 15 μm in (E)–(I) and (L). The following abbreviations are used: v, vegetative cell; s, sperm cell; c, central cell; e, egg cell; int, ovule integuments; sy, synergids; a, antipodals.

(M) Expression analyses of the *HTR* gene family by RT-PCR in isolated egg cells (e) and central cells (c). *HTR* genes are classified into four classes [7]: a centromeric variant (CENH3/HTR12), typical H3.1, H3.3 variants, and unusual H3 variants. A control experiment with genomic DNA (gDNA) is shown. The standard used *NUCLEOSIDE DIPHOSPHATE KINASE TYPE 1* (*NDPK1*; At4g09320), which shows about 7.5% more transcripts in central cells than in the egg cell.

Further details on expression of H3 variants in somatic tissues are provided in Figure S1. Further details on the dynamics of HTR5 and HTR8 expression during ovule maturation are provided in Table S1.

HTR14-CFP (Figure 1D), whereas the two sperm cells expressed HTR5-GFP (Figure 1A), the unusual variant HTR10-RFP, and CENH3-GFP, which labeled the five centromeres (Figure 1B). Transcripts of *H3.1* genes and corresponding tagged H3.1-FP were not detected in anthers containing mature pollen (Figure 1K; Figure S1A). Our data thus strengthen previous observations that the sperm cell chromatin is enriched in three classes of H3 variants: the male germline-specific HTR10 (also known as AtGMH3) [8, 12], CENH3 [9], and H3.3 (Figure 1). The male gamete chromatin thus becomes distinct from the chromatin of the nongametic lineage represented by the vegetative cell during pollen development.

Using RT-PCR analyses from isolated female gametes [13] and transgenic lines expressing the tagged H3 variants, we completed the H3 variant expression analyses in the embryo sac where the female gametes, the egg cell, and the central cell differentiate. The fertilized egg cell generates the zygote, and the fertilized central cell generates the endosperm, which nurtures the embryo development [14]. RT-PCR expression studies showed that the maturing central cell expressed a broader repertoire of H3 variants than the maturing egg cell (Figure 1M). The central cell expressed H3.1 and H3.3 from several *HTR* genes and the unusual variant *HTR14* (Figure 1M). Upon maturation of the central cell, the fusion protein HTR5-GFP showed a gradual decline in signal intensity, whereas HTR8-CFP signal intensity increased (Figures 1E and 1G; Table S1). In contrast, the egg cell showed no detectable fluorescence for any of the tagged H3.1 variants (HTR1, HTR2, HTR3, and HTR13) (Figures 1F and 1I; HTR1 and HTR3 were representative of the entire class), although low levels of transcripts of *HTR1* and *HTR3* were detected in isolated maturing egg cells (Figure 1M). Maturing egg cells expressed high levels of H3.3 (Figure 1M). During egg cell maturation, HTR5-GFP fluorescence increased while HTR8-CFP signal decreased to become undetectable in a large fraction of mature egg cells (Figures 1E and 1G; Table S1). The unusual H3 variant HTR14 was detected in the central cell only, but not in the mature egg cell (Figures 1H and 1M). Surprisingly, although egg cell chromatin featured chromocenters (Figure 1L), neither *CENH3* transcripts nor CENH3-GFP was detected in egg cells (Figures 1J and 1M). The biological meaning of this observation is unclear. In contrast, the central cell chromatin did not show discernable chromocenters (Figure 1L), which could explain why CENH3-GFP was not detected although CENH3 transcripts were abundant (Figures 1J and 1M). Altogether, these data indicate that the egg cell chromatin contains predominantly, if not exclusively, H3.3 at maturity.

In conclusion, the chromatin of sperm cells and egg cells appears to be devoid of H3.1 at maturity. Because H3.1 synthesis is tightly linked with DNA synthesis during the S phase of the cell cycle [15], the absence of H3.1 in both sperm cells and egg cells could simply result from an arrested cell-cycle progression before the S phase. However, male and female gametes initiate a new S phase before fertilization [16, 17]. These data thus suggest that, in the male and female gametes, H3.1 expression may be downregulated by a specific mechanism, which overrides the cell-cycle-dependent regulation.

Active Removal of Parental H3 Variants Inherited in the Zygote

H3.3, CENH3, and HTR10 are the sole H3 variants detected in the chromatin of the gametes that produce the embryo. We previously showed that paternally provided HTR10 was rapidly evicted in the zygote, probably through a DNA replication-independent mechanism [12]. To further test whether the remaining two H3 variants present in the sperm chromatin (H3.3 and CENH3) were inherited in the zygote, we first performed crosses between sperm cells expressing HTR5-GFP and ovules expressing mCherry fused to centromeric histone CENH3 (mCherry-CENH3) (Figure 2). We also performed crosses between sperm cells expressing HTR5-GFP and ovules from plants expressing the fusion protein HISTONE2B-RED FLUORESCENT PROTEIN (H2B-RFP) specifically

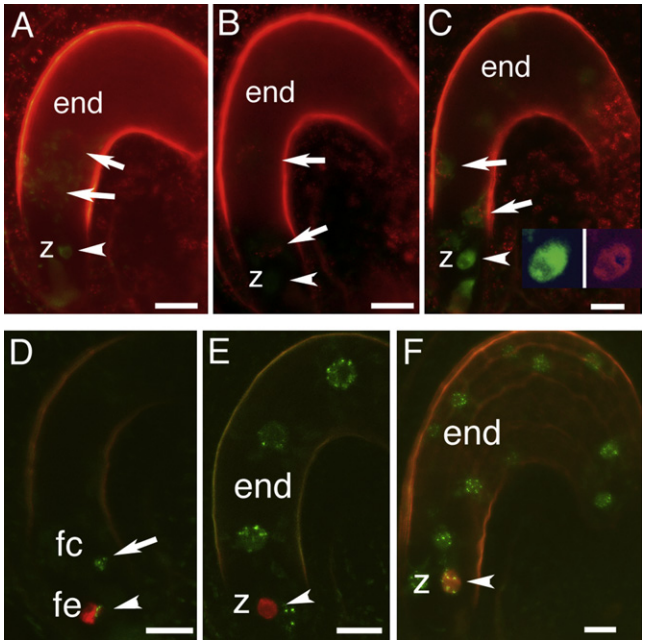

Figure 2. Dynamics of Paternally Provided H3 Variants

(A–C) Paternally provided HTR5-GFP (green) and maternally provided mCherry-CENH3 upon fertilization. The marker mCherry-CENH3 labels centromeres, which appear as faint red dots.

(A) Once karyogamy is complete, the chromatin of the zygote (z) (arrowhead) still shows HTR5-GFP contributed by the paternal chromatin. The two nuclei of the endosperm (end, arrows) already accumulate mCherry-CENH3.

(B) At 4 hours after fertilization (HAF), the HTR5-GFP signal becomes hardly visible in the zygote (arrowhead), and de novo-synthesized mCherry-CENH3 is observed in endosperm nuclei (arrows; two nuclei are out of focus).

(C) At 8 HAF, the zygote (inset shows the red and green channels separately) clearly shows a GFP signal corresponding to de novo synthesis of HTR5-GFP from the paternal allele as well as mCherry signal corresponding to de novo synthesis of mCherry-CENH3 from the maternal allele (arrowhead). Both tagged fluorescent H3 variants are also produced in the endosperm (arrows point at two nuclei).

(D–F) Dynamics of paternally provided CENH3-GFP (green) in embryo sacs expressing H2B-RFP (red) in the egg cell. The centromeric marker CENH3 labels the centromeres that appear as dots.

(D) Upon fertilization, the sperm chromatin labeled with CENH3-GFP (green, arrowhead) fuses with the chromatin of the egg cell (red) and the central cell (arrow).

(E) At 2 HAF, the paternally provided CENH3-GFP is no longer detected from the zygote nucleus (arrowhead). De novo synthesis of CENH3-GFP is observed in the endosperm.

(F) At 12 HAF, newly synthesized paternal CENH3-GFP is incorporated in the centromeres of the zygote. Autofluorescence (red) is used to visualize the contours of the embryo sac.

The follow abbreviations are used: end, endosperm; z, zygote; fc, fertilized central cell; fe, fertilized egg cell. Scale bars represent 15 μm. Further details on the eviction of paternal H3.3 are provided in Figure S2.

in the egg cell [18] and carrying a transgene encoding CENH3-GFP (Figure S2A). We observed that paternally inherited HTR5-GFP diffused into the egg cell chromatin at karyogamy (Figure 2A; Figure S2B) and became barely detectable in the zygote nucleus within 2–4 hr after fertilization (HAF) when the endosperm contained two to four nuclei (Figure 2B; Figure S2C). After 8 HAF, HTR5-GFP signal was detected again in the zygote nucleus, marking de novo synthesis of HTR5 (Figure 2C).

To study the fate of the paternal CENH3 upon fertilization, we analyzed the crosses between egg cells expressing the nuclear egg cell marker [18] and sperm cells labeled with CENH3-GFP (Figure 1B). Paternal CENH3-GFP, which was initially visible at the beginning of karyogamy in the zygote (Figure 2D), became undetectable in the zygote between 2 and 8 HAF when the endosperm contained two and eight nuclei, respectively (Figure 2E). Such observation could not be recorded in our previous study [12] because we lacked an egg cell marker allowing the precise monitoring of the onset of paternal CENH3-GFP expression after karyogamy. At the 16-nuclei stage of endosperm development, CENH3-GFP was again detectable in the zygote (Figure 2F), marking de novo synthesis of CENH3 in the zygote as reported previously [18].

We thus conclude that all H3 variants contributed by the paternal chromatin (HTR10, H3.3, and CENH3) are removed from the zygote nucleus within a few hours after fertilization. This confirms that active removal of paternal H3.3 variants is a common event at karyogamy in both plants and animals such as the nematode *C. elegans* [19]. However, it is still possible that residual parental H3 variants are transmitted to the progeny, which are impossible to detect as a result of technical limitations. This has recently been demonstrated for a limited fraction of paternal histones in animal embryos [20–22].

The zygotic inheritance of the maternal histone H3 contributed by the egg nucleus to the zygote has not been addressed in animals and plants. We performed fertilization experiments using sperm cells expressing HTR10-RFP and egg cells expressing HTR5-GFP, the only H3.3 detectable at maturity (Figure 1E). At karyogamy, we observed the male chromatin labeled by HTR10-RFP fusing with the egg cell chromatin marked by HTR5-GFP (Figure 3A). Maternally inherited HTR5-GFP was no longer detected from the zygotic chromatin within 4 HAF (Figures 3B and 3C), suggesting that H3.3, the sole H3 variant contributed by the egg cell, is not inherited by the zygote. Eviction of maternal HTR5-GFP from the zygote nucleus appeared coincident with removal of paternal H3 variants.

This brief transition phase where paternal and maternal H3 fluorescent fusion proteins are not detectable occurs between 2 and 4 HAF and must be accompanied by deposition of new H3 variants into the zygote chromatin. Several reports indicate that de novo transcription and translation are initiated a few hours after fertilization in the *Arabidopsis* zygote [18, 23, 24]. Accordingly, we observed that within 6–8 HAF, de novo synthesis of HTR5-GFP and CENH3-GFP occurred in the zygote when contributed by either the paternal genome (Figures 2C and 2F) or the maternal genome (Figure 3D). We estimate that this period corresponds to the time required for de novo synthesis of HTR5-GFP and CENH3-GFP fusion proteins, as well as folding and maturation of the fluorescent protein tag [25, 26], to reach protein levels sufficient for microscopic detection.

In the two-celled embryo, we observed de novo synthesis of canonical H3 variants initially absent in the sperm cells and the egg cells (Figure S3). Once the zygote has first replicated its DNA, chromatin composition in H3 variants in the embryo becomes similar to that observed in somatic cells in the seedling (Figure S1A). Our data support the idea that the majority of H3 variants contributed by the father but also by the mother are removed from the zygotic chromatin after karyogamy in *Arabidopsis*. The reprogramming of H3 variant composition during sexual reproduction takes place in three steps:(1) a few H3 variants are selectively expressed during male and female gametogenesis, (2) H3 variants inherited from both male and female gametes are removed from the zygote chromatin, (3) de novo synthesis of H3 variants restores the somatic H3 variant composition in the embryo. Although it is still unclear whether H3 variant removal also corresponds to nucleosome removal, such a phase likely corresponds to a reprogramming event, implying that at least a significant fraction of the epigenetic information carried by parental H3 variants is not transmitted to the progeny.

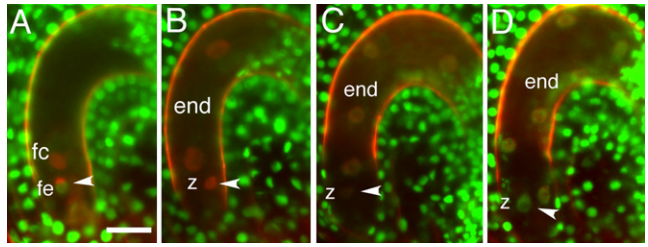

Figure 3. Dynamics of Maternally Provided H3 Variants upon Fertilization

(A) Upon fertilization, the egg cell chromatin labeled with HTR5-GFP (green) fuses with the sperm cell chromatin labeled with HTR10-RFP (red), producing the zygote nucleus (arrowhead). The spreading of the paternal chromatin is observed in the nucleus of the fertilized central cell (fc).

(B) At 2 HAF, HTR5-GFP is no longer visible in the zygote nucleus (arrowhead). HTR10-RFP signal remains visible in the endosperm (end) and the zygote nucleus (arrowhead).

(C) At 4 HAF, the paternal HTR10-RFP (red) and the maternal HTR5-GFP are barely detectable in the zygote nucleus (arrowhead).

(D) At 8 HAF, de novo-synthesized HTR5-GFP is detectable in the zygote (arrowhead) and the endosperm (end).

Autofluorescence (red) was used to visualize the contours of the embryo sac. The follow abbreviations are used: fc, fertilized central cell; fe, fertilized egg cell; end, endosperm; z, zygote. Scale bars represent 15 μm. Further details on de novo synthesis of H3 variants after the first zygotic division are provided in Figure S3.

Potential Mechanisms Reprogramming the Repertoire of H3 Variants in the Zygote

We investigated whether a dedicated mechanism removes H3 variants inherited from the chromatin of gametes. In mammals, H3.1 variants are incorporated in the chromatin by the chromatin assembly factor 1 (CAF1) complex during the S phase of the cell cycle [27]. In *Arabidopsis*, the CAF1 complex is represented by the core subunits FASCIATA1 (FAS1) and FASCIATA2 (FAS2) [28]. In silico expression analyses of *FAS1* and *FAS2* in the female gametes were performed on the available transcriptome of microdissected egg cells and central cells [29]. However, it was not possible to obtain conclusive evidence for the expression of CAF1 subunits in the female gametes (Table S2). To determine the expression of the CAF1 complex in the female gametes, we performed RT-PCR using cDNA from isolated egg cells and central cells and imaged the fluorescence from transgenic plants expressing a genomic fusion of *FAS2* with the *GFP* reporter gene. Transcripts of *FAS1* and *FAS2* were detected in the maturing

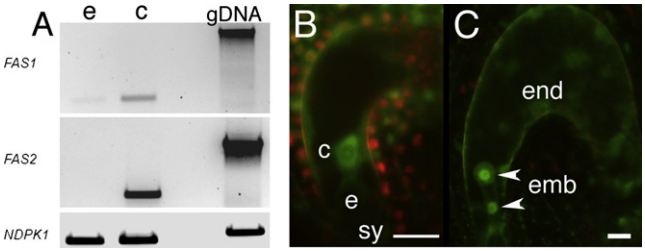

Figure 4. Expression of Two Subunits of the CHROMATIN ASSEMBLY FACTOR 1 Complex

(A) Transcripts encoding the two subunits *FAS1* and *FAS2* are mainly detected in isolated central cells (c), but not in isolated egg cells (e). A control experiment with genomic DNA (gDNA) is shown. The standard used *NUCLEOSIDE DIPHOSPHATE KINASE TYPE 1* (*NDPK1*; At4g09320) shows about 7.5% more transcripts in central cells than in the egg cell.

(B and C) Confocal sections of seeds obtained by self-fertilization of a transgenic line carrying FAS2-GFP (green fluorescent signal) and p35S-H2B-RFP, which marks somatic ovule integument cells (red fluorescent signal). The follow abbreviations are used: c, central cell; e, egg cell; sy, synergids; end, endosperm; emb, embryo. Scale bars represent 15 μm.

(B) In the mature embryo sac, fluorescence of FAS2-GFP is not detected in the egg cell (e) and is limited to the central cell (c).

(C) FAS2-GFP is expressed after the first zygotic division (arrowheads showing the two nuclei of the embryo) and the developing endosperm (end). Further details on the characterization of the *athira* mutant and expression of other putative H3 chaperones are provided in Figure S4 and Table S2.

central cell, but not in the maturing egg cell (Figure 4A). Likewise, the FAS2-GFP fusion protein was not detected in the egg cell but was clearly expressed in the central cell (Figure 4B). After fertilization, FAS2-GFP became detectable in the two-celled embryo (Figure 4C) when de novo synthesis of H3.1 fluorescent fusion proteins was observed (Figure S3). Our observations are in agreement with the model that the CAF1 complex loads de novo-synthesized H3.1 variants in the embryo chromatin.

We observed histone turnover before the first division in the zygote, indicating that distinct histone deposition machineries other than the DNA replication-dependent CAF1 may be recruited. In all animal model systems tested thus far, H3 provided by the female gametes is loaded onto the male chromatin upon fertilization [19, 30–32]. In *Drosophila*, the histone chaperones HIRA and the SWI2/SNF2 chromatin remodeling factor CHD1 are critical for maternal H3.3 deposition and chromatin remodeling during egg fertilization and embryo development [30, 33, 34]. In *Arabidopsis*, data from the transcriptome of laser-captured egg cells and central cells [29] indicate expression of the putative *Arabidopsis* homolog of *HIRA*, *AtHIRA* (At3g44530) [35], but not of the *CHD1* homolog *PICKLE RELATED 1* (At5G44800) [36] in female gametes (Table S2). We obtained a null mutant *hira* allele caused by insertion of a T-DNA in the fifth intron (*athira-1*, WiscDslox362H05) (Figure S4A). Although no *AtHIRA* transcripts were detected in the homozygous *athira-1* mutant plants (Figure S4B), no defects were noticed during sexual reproduction (data not shown). The absence of effect of the *HIRA* knockout on sexual reproduction was confirmed by the Mendelian genetic transmission of the T-DNA from self-fertilized *hira-1*/+ mutants (25.50% homozygous; standard deviation 5.57%; n = 702). These results thus indicate that *Arabidopsis HIRA* does not play a crucial role during fertilization, in contrast to the role played by *HIRA* homologs in animals [37]. The mechanism responsible for H3 variant replacement in the zygotic nucleus thus remains to be discovered in plants.

Conclusions

In summary, our data show that during gametogenesis, H3 variant expression becomes restricted to only a few genes encoding H3.3 in both sperm cells and egg cells. In addition, sperm chromatin still contains the sperm-specific HTR10 and CENH3, whereas remarkably, CENH3 appears to be absent from the egg cell chromatin. After fertilization, all H3 variants contributed by each parent are removed from the zygotic nucleus in a DNA replication-independent manner. The absence of CAF1 expression in the egg cell and the zygote further supports the idea that parental H3 variants are not diluted passively by incorporation of zygotic H3.1 during DNA replication but are removed by an active mechanism. Yet it is not clear which potential plant homologs of the H3.3 chaperones HIRA [27], ATRX, Daxx [1], or DEK [38], which were identified in animals, is involved in the remodeling of the composition of H3 variants that takes place at fertilization in *Arabidopsis*. H3 variants carry epigenetic information in the form of covalent modifications [39, 40]. It has been proposed that, in addition, the mosaic pattern of distribution of H3.3 and H3.1 variants and of CENH3 represents a form of epigenetic information [6, 41]. Our results suggest that the removal of parental H3 variants followed by de novo synthesis of H3 variants in the zygote limits the transmission of H3-dependent epigenetic information across generations. A recent study on the dynamics of the H2A gene family during mouse fertilization and early embryogenesis similarly reported a major resetting of maternal H2A isoforms in the zygote [42]. The *Arabidopsis* genome comprises 12 genes encoding H2A variants (http://www.chromdb.org/), and resetting of H2A zygotic composition may also occur in plants. We propose that these reprogramming events could participate in the acquisition of zygotic totipotency and the initiation of embryogenesis.

Experimental Procedures

Plant Material and Growth Conditions

The marker lines HTR10-RFP [12], CENH3-GFP [43], and pEC1-H2B-RFP [18] were used in this study. The wild-type accession Columbia (Col-0) and the *athira-1* insertional mutant line WiscDsLox362H05 were provided by The Arabidopsis Information Resource (http://www.arabidopsis.org). After 3 days at 4°C in the dark, seeds were germinated and grown on soil or plates. Plants were grown at 18°C in a growth room with an 8 hr day/16 hr night cycle until they formed rosettes. Flowering was then induced at 22°C in Conviron growth chambers with a 16 hr day/8 hr night cycle. All images of fluorescent fusion proteins were obtained from open flowers for pollen (anthesis) and from pistils emasculated the evening prior to anthesis and pollinated 1 to 1.5 days after emasculation. In the case of HTR5 and HTR8, we provide precise measurement of the impact of emasculation and maturation (Table S1). Pollination was performed at the physiological time 12 hr after emasculation.

Isolation of *Arabidopsis* Female Gametes for RT-PCR

Egg cells and central cells were isolated from excised ovules of *Arabidopsis thaliana* (Col-0) 2 days after emasculating stage 11 flowers, as described previously [13].

Molecular Analyses

See details in the Supplemental Information available online.

Microscopy

Emasculated pistils expressing the reporter line mCherry-CENH3 were crossed with pollen from transgenic lines carrying pHTR5-HTR5-GFP. Expression of reporter lines was analyzed in the resulting seeds with a Zeiss LSM 510 laser scanning confocal microscope with selective settings for DAPI (excitation 405 nm, emission band-pass 420–450 nm), CFP (excitation 458 nm, emission band-pass 475–510 nm), GFP (excitation 488 nm,

emission band-pass 505–530 nm), and mRFP1 (excitation 543 nm, emission band-pass 560–615 nm).

Feulgen-stained material was obtained and observed as reported previously [44].

Supplemental Information

Supplemental Information includes four figures, four tables, and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cub.2010.11.012.

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