



## H2a histone function

PDF Split View Article Summary Figures and Tables Video Audio The additional data in Eukaryotes, Variations of Basic H2A histores are selectively incorporated in distinct functional domain, suggesting that each variant gives specific nucleosome properties. The chromatin of flowering plants contains four types of H2A variants, such as biochemical properties have not been characterized. We report that in contrast to animals, in Arabidopsis Thaliana H2A variants, such as biochemical properties have not been characterized. We report that in contrast to animals, in Arabidopsis Thaliana H2A variants define only four main types of omotic nucleosomes containing exclusively H2A, H2A.Z, H2A.X or H2A.W.W. The in vitro essays show that the L1 cycle and the docking domain give a distinct stability of the nucleoma. In vivo and the in vitro essays suggest that the L1 cycle and the docking domain cooperate with the C terminal queue to adjust the accessibility of the chromatin. On the basis of these results we conclude that the L1 cycle and the docking domain give a distinct stability of the chromatin. on its interaction with DNA and proposes that the H2A variants regulate the dynamics of chromatin accessibility. In plants, the predominance of omotic nucleelines with specific physical properties and their specific localization to distinct domains suggest that the H2A variants regulate the dynamic specific physical properties and their specific physical physic the histone are isoforms definite functionally based on basic histones and are mainly found in histone families H3 and H2A (1 "3). While there are a set of very limited histone variants in unicellular eukaryotes, progressive expansion in diversity and the number of genes that codify for the variants of the histone is observed in multicellular species of all taxa, with a particularly acute increase between vertebrates and terrestrial plants (2.4). Although most of the venes of the histone independently evolved, many acquired functions similar into plants and terrestrial animals. This degree of functional evolutionary convergence indicates that the versions of the histone give important functions (2.5 Å ° 7). This idea is supported by structural data showing that the variants of the idle histone nucleosome properties (3.8 Å ¢ â, ¬ "18). Some variants of the histone have evolved only between Taxa Specific, as H3 testicular in mammals (19,20), specific variants of the histone have evolved only between Taxa Specific variants of the histone have evolved only between Taxa Specific, as H3 testicular in mammals (19,20), specific variants of the histone have evolved only between Taxa Specific, as H3 testicular in mammals (19,20), specific variants of the histone have evolved only between Taxa Specific, as H3 testicular in mammals (19,20), specific variants of the histone have evolved only between Taxa Specific variants of the histone have evolved only between Taxa Specific, as H3 testicular in mammals (19,20), specific variants of the histone have evolved only between Taxa Specific, as H3 testicular in mammals (19,20), specific variants of the histone have evolved only between Taxa Specific variants of the histone have evolved only between Taxa Specific variants of the histone have evolved only between Taxa Specific variants of the histone have evolved only between Taxa Specific variants of the histone have evolved only between Taxa Specific variants of the histone have evolved only between Taxa Specific variants of the histone have evolved only between Taxa Specific variants of the histone have evolved only between Taxa Specific variants of the histone have evolved only between Taxa Specific variants of the histone have evolved only between Taxa Specific variants of the histone have evolved only between Taxa Specific variants of the histone have evolved only between Taxa Specific variants of the histone have evolved only between Taxa Specific variants of the histone have evolved only between Taxa Specific variants of the histone have evolved on H2A.The H2A.B in mammals (24) and in the specific variants of the sperm H2A.Q and H2A.R in mammals (25). The variants of the H2A family are mainly distinguished by reasons in their terminal tail C (2.4.7, 26). In terrestrial plants, between the H2A variants, H2A.W contains the queue of the terminal C-terminal with the KSPKK motif and is associated with constitutive heterochromatin (22). Contrasting H2A.Z and H2A are mainly to Euchromatin and decorate the gene bodies (22). H2A.X is characterized by the SQEF/Y pattern in the tail of terminal C, which is phosphorylated in response to DNA damage in Arabidopsis (5,27,28) as in other eukaryotes (29). In addition to the distinctive badge of C-terminal tails, two other regions differentiate the four H2A variants. The L1 loop connects the two H2A variants in the nucleosome (4,30,31). The Arabidopsis genome contains 12 genes encoding four canonical H2A isoforms, two H2A.X isoforms, three H2A.Z isoforms, and three H2A.W isoforms (22). Among the three H2A's. Genera W, H2A.W.6 and H2A. W.7, which plays a role in response to DNA damage in heterochromate (5). Here, we establish that in Arabidopsis chromatin, nucleos contain only one type of H2A variants on the properties of nucleosomes. We show that the L1 and docking domains give clear nucleosm stability and impact on chromatin accessibility in vitro and in vivo. In addition, the C-terminal tail of H2A. W interacts with the DNA of the linker, suggesting the specific role in a higher order chromatin structure. MATERIALS AND METHODS Nucleus isolation, preparation of MNase nuclear extracts and immunoprecipitation of nucleosms For nucleus isolation followed by MNase digestion and immunoprecipitation four grams of 3-week old leaves were used. Nuclear isolation, MNase digestion and immunoprecipitation the beads were resuspended in 100 Î1/41 of 0.3Ã PBS, mixed with 30 Î1/41 of 5Ã load buffer, and 15 Î1/41 were loaded per lane (for variant-specific blots) or 10 11/41 for H3 blots. Nuclear protein extracts for Western Blot analysis (Figure S3) were prepared from 250 mg tissue (2-3 weeks of 10-12 days old seed leaves) as described in (5). Generation of transgenic plants expressing H2A. Mutant mutations W.6 into H2A. W.6 were introduced by gene synthesis (Invitrogen, GeneArt). It builds the expression of mutants under the control of H2A. W.6 promoters ( $a\Delta 1/41$  200 bp upstream of ATG) fused to tag HA have been inserted into the binary vector pCBK02. Heterozygous plants for h2a.w.7 and homozygous plants for h2a.w.6 were transformed by floral dip method (32) and T1 transgenic plants were selected on SM plates containing 10 11/4g/ml phosphonothricin. T1 transgenics were analyzed by Western blotting and only homozygous plants were selected for both h2a.w.6 and h2a.w.7. Transgenic plants T2 that segregated 3:1 for the selection marker were conserved and T3 generations that did not segregate for the selection marker (homozygous) were selected. for further analysis Digestion profiles MNase Nuclei from transgenic plants express H2A. The W.6 mutants were isolated from the NIB method (5), washed once in 10 ml buffer N (15 mM MgCl2, 1 mM CaCl2, 250 mM sucrose, 1 mM DTT, 10 mM mM mM protease inhibitors (Roche), once in a ml of buffer N and finally resuspended in 1 ml of buffer N (Total volume). Ten MNase microliters (0.01 u/µl) and 200 µl of aliquote were added to 0, 5, 10 and 15 min. DNA was phenol/chloropore extracted and precipitated with ethanol with addition of 2 µg of glycogen. The DNA was analyzed by the native 2% Agarose PAGE. Digested DNA gel images were acquired by ImageDoc (BioRad) and quantified using the ImageJ software. Immunostaining and Nuclei microscope isolated from 2 to 3 weeks old leaves were processed for immunosister as described above (22). H2A. The W.6 antibody was used in a dilution 1:200. Secondary antibody was Alexa 488 anti-corrected goat at 1:500 dilution. Nuclei was contracted with the DAPI to display DNA. The images were acquired with a confocal laser scanning microscope Zeiss and further elaborated in Photoshop for publication. Cloning, expression and purification of the recombinant istons Arabidopsis The fragment of DNA codifies Arabidopsis thaliana H2A.13, H2A.W.6, H2A.X.3, H2B, H3.1, H4 and H2A. The W.6 swap mutants were inserted into the pET15b vector (Novagen). H2A. The DNA fragment Z.9 was inserted in the pET28a vector (Novagen). The recombinant H4 was expressed with E. coli Rosetta-gami B pLysS or E. coli JM109 (DE3) which contain the expression vector of the minor tRNA (Codon (+) RIL, Stratagene). The purification of the istons Arabidopsis thaliana was performed by the methods. Preparation of nucleosis The iston ottamer was reconstituted as described above (33). The nucleosoma has been reconstituted with purified isthonic octamer and the fragment of DNA 193 (35) containing the sequence of Wisdom 601 (36) from the dialysis method of salt, as described above (33). Reconstituted nucleosis have been further purified by polyacrylamide gel electrophoresis, using the Prep Cell (Bio-Rad) apparatus. Analysis of thermal stability The analysis of thermal stability was performed as described above (37). The nucleosoma containing the 193 base-pair Widom 601 DNA was mixed with 5× SYPRO Orange, in 18 mM Tris-HCl (pH7.5) buffer containing 100 mM NaCl and 0.9 mM DTT. SYPRO Orange fluorescence signals have been detected with a StepOnePlus Real-Time PCR (Applied Biosystems) unit, with a temperature gradient from 26 to 95°C, at 1C/min degrees. The fluorescence intensity was normalized as follows: [F(T) - F(26°C)/[F(95°C) - F(26°C)]. F(T) indicates fluorescence intensity at a particular temperature. Or SYPRO orange fluorescence signal has been detected LightCycler 96 (Roche), with a temperature gradient from 37 to 95°C, in steps of 0.04°C/s. The fluorescence intensity was normalized as follows: [F(T) - F(50°C)/[F(95°C) - F(50°C)]. F(T) indicates fluorescence intensity A particular temperature. The MNASE nucleosomes (1.2 Î<sup>1</sup>/<sub>4</sub>g) treatment tests were incubated at 25Å ° C for 1, 3, 6, 9 and 15 min, in the presence of 0.006 U of MNASE (Neb). This analysis was performed in 60 1<sup>1</sup>/<sub>4</sub>l of reaction solution, containing 30 mm TRISâ € "HCL (PH7.5), 5 mm NACL, 2.5 mm CACL2 and 1.5 mm DTT. After incubation, 10 1<sup>1</sup>/<sub>4</sub>l of each rate was mixed with deproteinization solution, containing 20 mm TRIS<sup>â</sup> € "HCL (PH7.5), 80 mm EDTA, 80 The insulating DNA has been extracted from phenol-chloroforms and then analyzed by non-denaturing 10% polyacrylamide electrophoresis gel in 0.5-tbe (45 mm TRIS, 45 mm Boric acid and 1.6 mm EDTA). The gel was colored with Etidium bromide and DNA was displayed by Imagedoc (Biolad). The quantification was performed using Imagej software or software IMAGE Lab (Biorad). SDS-PAGE and WESTERN Blotting was performed according to the standard procedure with antibodies against the Istone variants of Arabidopsiss or are transferred to Nitrocellulose membrane (Protran, GE Healthcare). Western blotting was performed according to the standard procedure with antibodies against the Istone variants of Arabidopsiss or are transferred to Nitrocellulose membrane (Protran, GE Healthcare). (5.22) diluted at 1 Î<sup>1</sup>/4g / ml. The specific antibody H3 (ABCAM 1791) was used at 1: 5000 dilution. Anti-ha antibody rat (Roche 3F10) was used at 1: 5000 dilution. The secondary antibodies were goat's racing Igg (Biorad) and the Rat of goat IgG (Sigma) at 1:10 000 dilution. The blots have been developed with the enhanced chemilumin implant kit (Thermo Fisher Scientific) and the signals have been recorded using the Chemidoc (Biolad) tool. The quantification of the signals was made with the Chemidoc software using the volume tool. Arabidopsis nucleosomes are homotype compared to their composition in H2A heterotypic nucleosome variants have been recorded using the volume tool. reported in Drosophila, mouse, human and yeast in sprout (38â  $\in$  "40). In plants, the composition of nucleosomes are ported that H2A.X exists mainly in nuclosmas that are free of H2A.W and H2A.Z, suggesting that H2A.X exists mainly in nucleosomes are homotipic (5). Furthermore, the marked preferential occupation of distinct genomic domains for each type of Variant Arabidopsis H2A (22) has also suggested that most nucleosomes contain a single type of H2A variant and are therefore homotipic. To further test this hypothesis we obtained mononucleosomes from the MNASE digest chromatin and analyzed their composition from immunoprecipitation using specific antibodies against any type of H2A and H2A.X have been detected in H2A. Immunoprecipities W, which could be a small percentage of dinucleosomes in our chromatin preparations (Figure 1A). Co-precipitation of H2A.W.6 and H2A.W.7 (Figure 1B) (5) suggests that heterotypical nucleosome containing isoform isoforms The same family of variants could be formed, although there was a considerable predominance of homotypic nucleosomes containing only H2A.W.6 or H2A.W.7 (on average 19% of H2A.W. nucleosomes are heterotypical for both isoforms). Therefore, we conclude that the nucleosomes of Arabidopsis are mostly homotypical with respect to their content in the H2A variants, suggesting that the H2A variants confer specific properties to the nucleosomes. Open in a new tabDownload slideThe nucleosomes of Arabidopsis H2A are homotypical. (A) DNA isolated from digested MNase nuclei demonstrating almost complete digested into dinucleosomes (\*). A small portion of chromatin into mononucleosomes (\*). A small portion of chromatin was digested into dinucleosomes (\*). A small portion of chromatin was digested MNase nuclei demonstrating almost complete digested into dinucleosomes (\*). (B) Extracts of digested MNase nuclei were immunoprecipitated with antibodies against H2A variants W.6, H2A.W.7, H2A.1/13, H2A.XÅ<sup>°</sup> and H2A.Z.9 histone H2A and analysed by western blotting with indicated antibodies. The detection of H3 is used as a check for the integrity of the nucleosomes contained 15% of the heterotypical nucleosomes with H2A.W.6. (Note that the antibody against canonical H2A recognises two variants H2A.1 and H2A.13 (22).) H2A variants confer distinct stability to the nucleosome In Arabidopsis each class of H2A variant comprises two to four very similar proteins (2,4). We have already shown that the combination of three characteristics, the C-terminal (CT) tail amino acid sequence, the L1 loop, and the docking domain, unambiguously define each class of H2A variants in flowering plants (4) (Figure 2). So, to represent each class of H2A.X.3, H2A,X.3, H2A.X.3, H2A. and below we will only refer to H2A.Z, H2A.X, H2A.W and H2A, unless otherwise indicated. To compare the biochemical properties of homotypic nucleosomes in vitro using the salt dialysis method (Figures 3A and B and Supplementary Figure S1B). The stability of the nucleosomes was evaluated by the thermal stability test with the fluorescent dye SYPRO Orange. In this assay fluorescence is detected only when the orange SYPRO binds to the histories dissociated from the nucleosomes (Figure 3C) (37). H2A.Z-H2B dimers dissociated from the nucleosome at temperatures much lower than those of H2AâÅ<sub>i</sub>H2B and H2A.WÅ¢Å;H2B (Fig. Figure 3D) or H2A.X (Supplementary Figure S1B and C. In contrast, H2A-H2B dimers dissociated at slightly higher temperatures than H2A.X (Figure 3D) or H2A.X (Supplementary Figure S1B). Thus, each type of H2A variant confers distinct stability to the nucleosomes in vitro, with H2A.Z being the most unstable and H2A. the most stable. Open in a tabDownload slideThe alignment of the Istone Arabidopsis H2A.13, H2A.X.3, H2A.W.6 and H2A.Z.9. Amino acid residues, other than other H2A variants, are represented on a purple background. purple. The residues of amino acids, exchanged or deleted in this study, are coloured in red. The L1 loop, the hooking domain and the C terminal code are respectively in green, red and purple boxes. Open in a new tabDownload slideDosage of thermal stability of the nucleosomes assembled here contain about 24 bp DNA of the linker. (B) Native-PAGE analysis (left) and SDS-PAGE (right) of reconstituted nucleosomes containing H2A. W.6, H2A.W.6lCT (without the C-terminal tail), H2A.W.6L12 (loop W.6 L1 replaced by Z.9) sequences, H2A.Z.9 and H2A.13 variants. Note the slight differences in the migration of nucleosomes assembled on native PAGE. (C) Schematic presentation of the proof of thermal stability. The SYPRO Orange colorant is only linked to denatured proteins resulting in fluorescence. (D and E) Graphical presentation of the thermal stability essay of nucleosomes as indicated in the C panel in the presence of 100 mM NaCl. The normalized values against the increase in temperature were traced from 50°C to 95°C. In the lower panel, the derived values of the thermal stability curves shown in the upper panel are drawn for each temperature. The presented data is an average of 3 repeats with error bars representing SD. Note that the same results were used in D and E for nucleosomes containing H2A.13, H2A.Z.9 and H2A.W.6. The Tm values of the first peak corresponding to the dissociation of the H2Aâ dimer |H2B are 71â~72°C (nucleosoma H2A.I3), 63â~64°C (nucleosoma H2A.I3), 63a~64°C (nucleosoma H2A.I3), 63a~64°C (nucleosoma H2A of H2A. 2A.W, without the C terminal tail (H2A.W.6ÎCT), or with the L1 loop from H2A.Z (H2A.W.6L1Z), or with both the L1 loop and the dock domain. We assembled the nucleosomes containing these mutants and analyzed their thermal stability. The C-terminal tail deletion of the H2A.W (nucleosoma H2A.W.6ICT) did not affect the core stability (Figure 3E), suggesting that the extended C-terminal tail of the H2A. W.6 does not contribute to the stability of the H2A. W.6L1A) showed the same stability as the H2A.W wild type (S2). Conversely, nucleosomes containing H2A.W.6L1Z or H2A.W.6L1dZ1CT showed greater instability than nucleosomes containing H2A.W. wild type (Figure 3E and Additional Figure S2). These results suggest that it is L1 that the docking domain give greater stability to nucleosomes containing H2A.W. on the docking domain give greater stability to nucleosomes containing H2A.W. from transgenic transgenic plants Mutants H2A.W.6. (A) Schematic presentation of the mutants H2A.W.6 in the LOOP L1 and in the docking of H2A.W.6 and H2A.Z. are also indicated. (B) Immunoprecipitation of H2A.W.6 after digestion of nuclei with mnase. Western blotting was performed with antibodies against H2A.W.6, H2A.Z.9, H2A.1 / 13, H3Å, and H2B spots represent their enrichment levels compared to H2A.W.6. Reports for the wild type were set to one and the others are expressed in relation to the wild type. (C) Immunoprecipitation of h2a.z.9 nucleosomes from transgenic plants expressing mutants H2A.W.6 indicated. The numbers below H3 and H2B Blots indicate their normalized levels at the enrichment of H2A.Z.9 in every IP, demonstrating that the instability of mutant nucleosomes H2A.W.6 is not due to the general effect of the Experimental circumstances. Importance of the L1 cycle, of the docking domain, and of the Cterminal tail of H2A.W in vivo to direct the function of the L1 cycle and the docking domain in the nucleosomes of H2A.W in vivo, we obtained transgenic plants expressing H2A.W in vivo, we obtained transgenic plants expressing H2A.W in vivo to direct the function of the L1 cycle and the docking domain in the nucleosomes of H2A.W in vivo, we obtained transgenic plants expressing H2A.W in vivo to direct the function of the L1 cycle and the docking domain in the nucleosomes of H2A.W in vivo, we obtained transgenic plants expressing introduced into plants H2A.W.6 +/- H2A.W.7 - / - and T1 plants that express each transgene in H2A.W.7 - / - have been Select to obtain dual mutant plants containing a copy of the transgene in H2A.W.7 - / - have been Select to obtain dual mutant plants containing a copy of the transgene in H2A.W.7 - / - have been Select to obtain dual mutant plants that express each transgene in H2A.W.7 - / - have been Select to obtain dual mutant plants containing a copy of the transgene in H2A.W.7 - / - have been Select to obtain dual mutant plants that express each transgene in H2A.W.7 - / - have been Select to obtain dual mutant plants that express each transgene in H2A.W.7 - / - have been Select to obtain dual mutant plants that express each transgene in H2A.W.7 - / - have been Select to obtain dual mutant plants that express each transgene in H2A.W.7 - / - have been Select to obtain dual mutant plants that express each transgene in H2A.W.7 - / - have been Select to obtain dual mutant plants that express each transgene in H2A.W.7 - / - have been Select to obtain dual mutant plants that express each transgene in H2A.W.7 - / - have been Select to obtain dual mutant plants that express each transgene in H2A.W.7 - / - have been Select to obtain dual mutant plants that express each transgene in H2A.W.7 - / - have been Select to obtain dual mutant plants that express each transgene in H2A.W.7 - / - have been Select to obtain dual mutant plants that express each transgene in H2A.W.7 - / - have been Select to obtain dual mutant plants that express each transgene in H2A.W.7 - / - have been Select to obtain dual mutant plants that express each transgene in H2A.W.7 - / - have been Select to obtain dual mutant plants that express each transgene in H2A.W.7 - / - have been Select to obtain dual mutant plants that express each transgene in H2A.W.7 - / - have been Select to obtain dual mutant plants that express each transgene in H2A.W.7 - / - have been Select to obtain dual mutant plants that express each transgene in H2A.W.7 - / - have b H2A.W nucleosomes after the MNASE digestion and found that no one of mutants H2A.W has co-precipitated significant quantities of H2A.Z or ROONIC H2A (Figure 4B). Small quantities of H2A.Z have been co-precipitated with most H2A.W mutants due to the low quantities of Dinucleosomes In the digested chromatin (see also discussion). Immunostaining of the isolated nuclei revealed that all the mutant forms of H2A.W specifically located with pericentric heterochromatin as Wild Type H2A.W (additional figure S4). The analysis of H3 and H2B co-precipitated with H2A.W has RIVEL ATo that the CT deletion has reduced the co-precipitation levels of both histores compared to the wild type (Figure 4B). Similarly, the mutation of each domain has reduced the quantity of H3 and H2B co-precipitated (Figure 4B). The combinations of the L1 cycle mutations and the coupling domain with the cancellation of the CT showed the increasing and cumulative effect on the nucleosoma stability (Figure 4b). The relatively strongest impact observed on H2B compared to H3 is probably explained by the fact that the antibody recognizes only three out of a total of eleven H2B variants in the arabidopsis. These data suggest that the CT, L1 loopÃ, and the H2A.W coupling have distinct non-overlapping effect on in vivo nucleosome stability. I live. Under the same experimental conditions, we did not observe a comparable instability of the H2A.Z nucleosome (Figure 4C), suggesting that the mutations directly affect the H2A.Z nucleosomes and do not affect the H2A.Z nucleosomes and the CT of H2A.W and H2A.Z confer distinct properties to the nucleosomes but do not affect localization and incorporation into chromatin. H2A C-terminale tails protect DNA linker To assess the impact of MNase digestion on chromatin from transgenic lines expressing the wild type H2A.W.6, H2A.W.6L1Z and H2A.W.6L1ddZ1CT. We performed MNase digestion limiting the amount of MNase followed by DNA extraction and profile analysis. We found that CT cancellation and L1 and binding domain mutations lead to increased access to MNase cumulatively (Figure 5), suggesting that these three regions of H2A may contribute to its role in establishing a higher order chromatin structure. Open in a new tabDownload slideMAChromatin profiles from transgenic plants access in plants expressing H2A.W.6 mutants indicated. The isolated nuclei were digested for 15 minutes with 0.1 units of MNase at RT and the DNA was extracted and analysed by 2% agarose gel electrophoresis. (B) Quantified profiles of digested MNase nuclei for 15 min. The crystalline structure of the nucleosome suggests that the H2A CT locates at the DNA entry/exit sites in the nucleosome (41). In addition, the impact of CT suppression on nucleosome stability suggested that the extended CT of H2A.W might interact with the linker's DNA. To study the association of H2A.W CT with linker DNA, we performed the MNase treatment test on in vitro reconstituted nucleosomes containing H2A.W, H2A.WéÂ|CT, H2AÃ" and H2A.Z (Figure 6A). In the case of nucleosomes containing H2A and H2A.WI¢CT, MNase digestion produced mainly fragments between 140 and 150 bp (Figure 6B, asterisk) corresponding to the nucleosome nucleosome of 155 base pairs or 165 base pairs was detected in the H2A.W nucleosome (Figure 6B, C, red arrows, D, E and F), suggesting that the extended C-terminal tail of H 2A.W Interacts with Linker DNA and Protects Collects DNA from MNase cleavage. At the same time, the 130 bp fragment, which originates from over-digestion due to respiration of the DNA ends, was more prominent in H2A.W. and C, blue arrows). Consistent with the presence of a very short C-terminal tail on H2A.Z, the strong 155 bp fragment, resulting from DNA protection by the C-terminal tail in the H2A.W nucleosome, was almost undetectable in H2A.Z H2A.Z assays. (Figure 6B, CÃ ¢ and f). Together, these results strongly suggest that the C-terminal queues of the H2A variants have distinct capabilities to interact with the Linker DNA. Open in the new test of the Slidemnase treatment treatment. (A) The nucleosomes assembled in vitro containing H2A variants indicated and mutants H2A.W.6 were treated with MNASE for the times indicated at 25 ° C and the digested DNA was analyzed by the native page. (B) Analysis of the native page of the FRAGMENTS OF DNA First (left panel) or after 15 minutes MNASI digestion (panel to right) of nucleosomes containing indicated or mutant h2a.6 variants. The red and blue arrow indicates the upper and lower bands, used respectively for the quantification shown in the panel C. (c) graphic presentation of the distribution of DNA fragments after 15 minutes digestion with mnase shown in the panel B. (d) schematic representation of the 165, 155 or 145 BP fragments obtained by means of MNASI treatment. (E and f) graphic representation of the addition of mnasis at times indicated. The intensity of each band was normalized to the 193 fragment of the data of the base couple corresponding to the time point 0 in the panel A. Discussion We refer here in vivo and in vitro analysis of nucleosomes containing the four different H2A variants present in Arabidopsis Chromatin , H2A, H2A. X, H2A.ZÃ ¢ and H2A.W. Our data reveal the overwhelming predominance of omotic nucleosomes most likely reflect the presence of mnasi heterochromatin dinnerosomes, which is mainly occupied by H2A.W but also contains H2A.X and H2A (5.22). However, we

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