Figures and figure supplements

Evolution of histone 2A for chromatin compaction in eukaryotes

Benjamin R Macadangdang, et al.
Figure 1. Histone H2A N-terminal sequence has co-evolved with genome size. Violin plots of the number of (A) arginines or (B) serines/threonines in the H2A NTD for species with small, medium, and large genomes. Plot widths correspond to species frequency within each group. (C) H2A NTD sequences for *S. cerevisiae* and *H. sapiens*. (D) Heat map of H2A NTD residue composition at the indicated positions ordered by genome size. Example species are shown.
Figure 1. Continued

shown with kingdom and genome size information. (E) Protein sequence motifs surrounding the four H2A NTD arginine residues. (F) Positioning of evolutionarily variable residues relative to the H2A N-terminus (left) or histone fold (right). See also Figure 1—figure supplements 1 and 2.

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Figure 1—figure supplement 1. Phylogenetic distribution of species analyzed in this paper.

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Figure 1—figure supplement 2. H2A arginines 3 and 11 are situated adjacent to DNA within the nucleosome.
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Figure 2  Ectopic expression of H2A NTD arginines causes compaction in yeast. (A) Schematic position of probes on chromosome XVI that were used for FISH. The letters correspond to the probe sets. (B) FISH images and (C) boxplot of the distributions of interprobe distances for probe set A in the indicated strains. (D) The mean interprobe distances for the indicated yeast strains for probe sets A, B, C, and D are plotted as a function of genomic distance. Solid lines are best fit equations. (E) Boxplot of the distributions of interprobe distances for probe set A in the indicated strains. Dashed lines mark the median value for the WT strain. The boxplot whiskers contain 90% of the data. All scale bars are 1 µm. Boxes are colored if the mean of the indicated strain is significantly different from WT. Red stars denote level of significance: *p<0.01; **p<0.001; ***p<0.0001 (For exact values, see Supplementary file 2). (F) Agarose gel electrophoresis of MNase-digested chromatin in the indicated strains including the densitometric profiles comparing the WT to each of the mutant H2A strains for a given amount of enzyme. See also Figure 2—figure supplement 1.

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Figure 2—figure supplement 1. Ectopic expression of H2A NTD arginines causes compaction in yeast. DOI: 10.7554/eLife.02792.009
Figure 3. Ectopic expression of H2A NTD arginines decreases nuclear volume in yeast. (A) Images of the nuclear envelope, as visualized by Nup49p-GFP, and boxplot of the distributions of nuclear volumes in the indicated strains in the TSY107 background (B) or the FY406 background (C). Dashed lines mark the median value for the WT strain. The boxplot whiskers contain 90% of the data. All scale bars are 1 µm. Boxes are colored if the mean of the indicated strain is significantly different from WT. Red stars denote level of significance: *p<0.01; **p<0.001; ***p<0.0001 (Supplementary file 3). Red dagger (†) indicates that mean nuclear volume of R11ΔS15 is significantly smaller than its isogenic WT strain (ΔS15; p<0.001). See also Figure 3—figure supplement 1.
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Figure 3—figure supplement 1. H2A arginines do not affect cell size.
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**Figure 4.** Loss of H2A NTD arginines decreases chromatin compaction in human cells. (A) FISH images of probes on chromosome 1 in normal primary IMR90 fibroblasts with HA-tagged WT or mutant H2A overexpressed as indicated. (B) Boxplot of the distributions of inter-probe distances. Note that R11K was only marginally significant at p=0.023. (C) Immunofluorescence images of IMR90 cells overexpressing HA-tagged WT or mutant H2A as Figure 4. Continued on next page
indicated. (D) Top: boxplot of the distributions of largest nuclear cross-sectional areas in the indicated H2A overexpressing cells. Bottom: boxplot of the distributions of α-HA fluorescence intensities. (E) Left: FISH images, as in (A), of IMR90 cells expressing a C-terminal FLAG-tagged WT or tailless (Δ1–12) H2A. Right: boxplot of the distributions of inter-probe distances. (F) Top: immunofluorescence images of IMR90 cells overexpressing FLAG-tagged WT or tailless H2A. Bottom: boxplot of nuclear areas and fluorescence intensities, as indicated. Dashed lines mark the median value for the WT strain. All scale bars are 10 µm. Boxes are colored if the mean of the indicated strain is significantly different from WT. Red stars denote level of significance: *p<0.01; **p<0.001; ***p<0.0001 (Supplementary files 4 and 5). See also Figure 4—figure supplement 1.

Figure 4. Continued

Figure 4—figure supplement 1. Loss of H2A NTD arginines decreases chromatin compaction in human cells.

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Figure 5. H2A NTD R11 directly modulates the compaction of chromatin fibers in vitro. (A) Polyacrylamide gel electrophoresis (PAGE) of ScaI-digested 601-177-12 DNA template assembled with octamers containing recombinant WT or ΔR11 H2A. As a control, 5% of the 601-177-12 DNA without octamers was also digested. (B) The distribution of sedimentation coefficients determined by van Holde-Weischet analysis plotted against the percent boundary fraction in the absence or presence of 0.8 mM MgCl₂ as indicated. S₂₀°C,W is the sedimentation coefficient corrected to water at 20°C. See also Figure 5—figure supplement 1.

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Figure 5—figure supplement 1. H2A NTD R11 directly modulates the compaction of chromatin fibers in vitro.

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Figure 6. Mutations to H2A NTD decrease the fitness of yeast. (A) Pearson correlations between the global gene expressions of the indicated strains grown in YPD. Correlations are calculated from an average of at least two experiments. (B) Growth curves of the indicated H2A yeast strains over 10 hr in YPD. (C) Spot tests with 10-fold serial dilutions for the indicated strains in the presence of different drugs. (D) The proportion of yeast cells in a co-culture of WT and the indicated mutant H2A carrying Pgk1 gene fusion to GFP (green) or RFP (red) as indicated by color.

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Figure 7. Mutations of H2A NTD found in cancers decreases chromatin compaction in human cells. (A) Schematic of the H2A NTD showing only the mutations within the arginine motifs found in various cancers as indicated by the colored shapes (Forbes et al., 2011). The letter within each shape represents the mutated amino acid. (B) FISH images of probes on chromosome 1 in normal primary IMR90 fibroblasts with HA-tagged WT or mutant H2A overexpressed as indicated. (C) Boxplot of the distributions of inter-probe distances. (D) Immunofluorescence images of IMR90 cells overexpressing HA-tagged WT or mutant H2A as indicated. Anti-HA primary and Alexa Fluor 647-conjugated secondary antibodies were used to determine expression. Figure 7. Continued on next page
in FISH images and for measurement of nuclear areas. (E) Top: boxplot of the distributions of largest nuclear cross-sectional areas in the indicated H2A overexpressing cells. Bottom: boxplot of the distributions of α-HA fluorescence intensities. Dashed lines mark the median value for the WT strain. All scale bars are 10 µm. Boxes are colored if the mean of the indicated strain is significantly different from WT. Red stars denote level of significance: *p<0.01; ***p<0.0001 (Supplementary files 4 and 5). DOI: 10.7554/eLife.02792.017