doi:10.1016/j.jmb.2007.05.063 J. Mol. Biol. (2007) 371, 11–18

JMB

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The Histone Domain of macroH2A1 Contains Several Dispersed Elements that Are Each Sufficient to Direct Enrichment on the Inactive X Chromosome

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Histone variants replace the core histones in a substantial fraction of nucleosomes, affecting chromatin structure and impacting chromatin-templated processes. In many instances incorporation of histone variants results in formation of specialized regions of chromatin. Proper localization of histone variants to distinct regions of the genome is critical for their function, yet how this specific localization is achieved remains unclear. macroH2A1 is enriched on the inactive X chromosome in female mammalian cells, where it functions to maintain gene silencing. macroH2A1 consists of a histone H2A-like histone domain and a large, globular C-terminal macro domain that is not present in other histone proteins. The histone domain of macroH2A1 is alone sufficient to direct enrichment on the inactive X chromosome when expressed in female cells, indicating that sequences important for correct localization lie in this domain. Here we investigate whether divergent sequences of the H2A variant macroH2A1 contribute to its correct localization. We mapped the regions of the macroH2A1 histone domain that are sufficient for localization to the inactive X chromosome using chimeras between H2A and the histone domain of macroH2A1. Multiple short sequences dispersed along the macroH2A1 histone domain individually supported enrichment on the inactive X chromosome when introduced into H2A. These sequences map to the surface of the macroH2A1/H2B dimer, but are buried in the crystal structure of the macroH2A1 containing nucleosome, suggesting that they may contribute to recognition by macroH2A1/H2B deposition factors.

Keywords: macroH2A; H2A; chromatin assembly; histone variants; X inactivation

In eukaryotes, chromatin is the basic platform for all DNA associated processes, such as replication, transcription, and genome segregation. The nucleosome is the fundamental particle of chromatin, consisting of DNA wrapped around two copies each of four core histones, H2A, H2B, H3 and H4. One mechanism by which chromatin structure is regulated is by changing nucleosome composition, through the incorporation of histone variants.[1] Assembly of nucleosomes containing histone variants into distinct regions of the genome is important for the formation of functional chromosomal domains, such as centromeres, as well as to delineate expressed or silent chromatin. How histone variants are correctly localized to their regions of action is poorly understood.

Nearly all of the core histones have variants, with histone H2A showing the greatest diversity in metazoans.[2] H2A exhibits uniform nuclear distribution.[3] The variant H2A.X is also present throughout the genome and its phosphorylation in response to

Abbreviations used: GFP, green fluorescent protein; FISH, fluorescence *in situ* hybridization; Xi, inactive X chromosome.

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DNA damage promotes the recruitment of DNA repair machinery.[4] H2A.Z is enriched on heterochromatic foci and is necessary for faithful chromosome segregation.[5,6] H2A.Bbd is depleted on the inactive X chromosome (Xi) in mammalian female somatic cells.[7] The macroH2A variants are enriched on the Xi in mammalian female cells, where they contribute to stable gene silencing.[8–12] Enrichment of macroH2A1 on the Xi requires *XIST* RNA, a non-coding RNA that coats the Xi and recruits a number of chromatin modifying activities to mediate stable transcriptional silencing of the Xi.[13,14] The molecular mechanisms underlying the *XIST* RNA-dependent enrichment of macroH2A1 on the Xi are unknown.

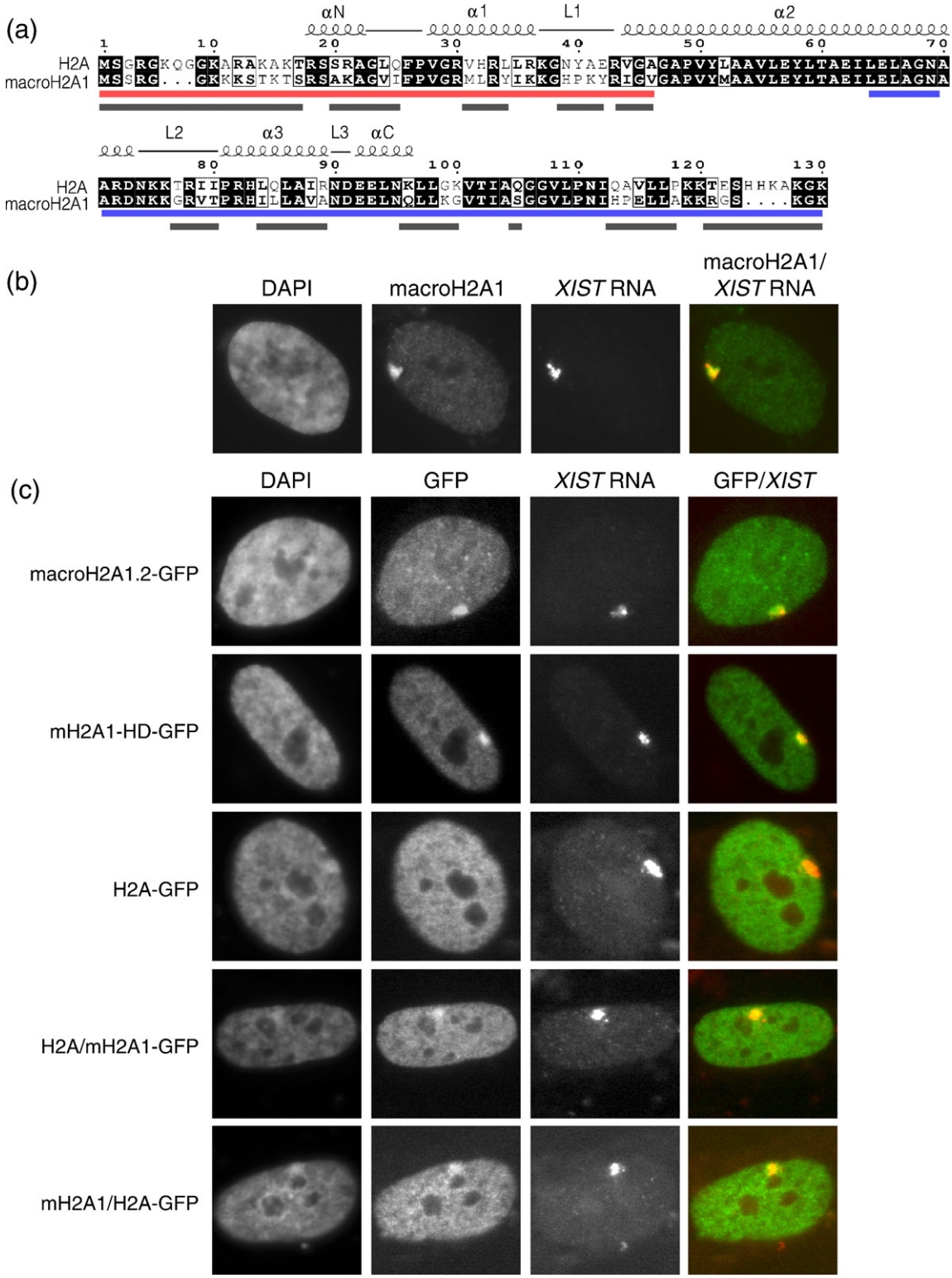

Figure 1 (*legend on next page*)