Reconstiuted nucleosomes containing ubiquitinated histones

Hengbin Wang (hbwang@uab.edu)
The University of Alabama at Birmingham

Method Article

Keywords: nucleosome reconstitution, ubiquitinated histones

Posted Date: October 8th, 2007

DOI: https://doi.org/10.1038/nprot.2007.436

Abstract

Introduction

Posttranslational histone modifications play important roles in regulating chromatin structure and function. One example of such modifications is histone ubiquitination, which occurs predominately on H2A and H2B. Although the recent identification of the ubiquitin ligase for histone H2A has revealed important roles for H2A ubiquitination in Hox gene silencing as well as in X inactivation, the enzyme\(s) involved in H2A deubiquitination and the function of H2A deubiquitination are not known. In order to identify the deubiquitinase for histone H2A, we developed an in vitro deubiquitination assay employing uH2A \(ubiquitinated H2A)-containing mononucleosomes as substrates.

Reagents

Hela cells stably expressing Flag-H2A, T85 yeast strain and YKH045 yeast strain, anti-Flag M2 resin, Flag-peptide Recombinant H3, H4, H2A, and H2B Tris-HCl Hepes-KOH Tris-acetate NaCl,KCl $CaCl_2$ Nonidet P-40\(NP40) deoxycholate SDS EDTA DTT PMSF glycerol

Equipment

Benchtop microcentrifuge and rotator.

Procedure

1. Purify Flag-H2A from HeLa cells stably expressing Flag-H2A and purify Flag-H2B from T85 yeast strain \(provided by Dr. Shelley L. Berger) and YKH045 yeast strain \(provided by Dr. Mary Ann Osley) with anti-Flag IP under denature conditions\(Wang _et al_., 2006; Robzyk, K, 2000). 2. Mix histone H2B purified from these two yeast strains at appropriate ratio to match the ratio of uH2A/H2A that were purified from HeLa cells, as judged by Coomassie blue staining. 3. Purify recombinant H3, H4, H2A, and H2B from _E. coli_ as described \(Luger _et al_., 1999). 4. PCR amplify rhe 5s DNA fragments from the XP-10 plasmid \(provided by Dr. Jeffrey J. Hayes) and the 5’ primer used for PCR amplification was biotinylated \(Wang _et al_., 2006). 5. Perform histone octamer refolding and nucleosome reconstitution as described \(Dyer _et al_., 2004). 6. To purify individual H2A, uH2A, H2B, uH2B, resolve the eluate from anti-Flag IP on a SDS-PAGE and excise gel slices containing H2A, uH2A, H2B and uH2B. 7. After extensive washing, electro-elute proteins out from these gel slices and further passed through a hydroxyapatite column to remove the excess SDS. 8. Combine fractions from the hydroxyapatite column and dialyze against water at 4 °C overnight and then lyophilize. 9. Use an aliquot to determine the amount of proteins as revealed by Coomassie blue staining. After dissolving in histone unfolding buffer \(6 M guanidinium chloride, 20 mM Tris-Hcl, pH 7.5, 5 mM DTT), perform histone octamer refolding and nucleosome reconstitution as described \(Dyer _et al_., 2004).

Timing

3-4 weeks

References

Dyer, P. _et al_. Reconstitution of nucleosome core particles from recombinant histones and DNA. _Methods in Enzymology_ **375**, 23-44 \(2004). Luger, K., TJ, R. & Richmond, T. J. Preparation of nucleosome core particle from recombinant histones. _Methods in Enzymology_ **304**, 3-19 \(1999). Robzyk, K., J. Recht, and M. A. Osley. 2000. Rad6-dependent ubiquitination of histone H2B in yeast. _Science_ **287**:501-504. Wang, H., L. Zhai, J. Xu, H.-Y. Joo, S. Jackson, H. Erdjument-Bromage, P. Tempst, Y. Xiong, and Y. Zhang. 2006. Histone H3 and H4 Ubiquitylation by the CUL4-DDB-ROC1 Ubiquitin Ligase Facilitates Cellular Response to DNA Damage. _Molecular Cell_ **22**:383-394.