Purification of the H2A deubiquitinase Ubp-M

Heui-Yun Joo
The University of Alabama at Birmingham
Ling Zhai
The University of Alabama at Birmingham
Hengbin Wang
The University of Alabama at Birmingham

Method Article

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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. Here we report the identification and functional characterization of the major deubiquitinase for histone H2A, Ubp-M. Ubp-M prefers nucleosomal substrates in vitro, and specifically deubiquitinates histone H2A but not H2B in vitro and in vivo. Importantly, knockdowns of Ubp-M result in slow cell growth rates, which are due to defects in the mitotic phase of the cell cycle. Furthermore, we demonstrate that Ubp-M regulates Hox gene expression through H2A deubiquitination and that blocking the function of Ubp-M results in defective posterior development in _Xenopus laevis_. Therefore, this study identifies the major deubiquitinase for histone H2A and demonstrates that H2A deubiquitination plays a critical role in cell cycle progression and gene expression.

Reagents

HeLa S3 cells, Tris-HCl Hepes-KOH ammonium sulfate potassium phosphate KCl $CaCl_2$ EDTA DTT PMSF NP-40 glycerol

Equipment

FPLC \(Amersham Biosciences) P11 column \(Sigma) HPLC-DEAE-5PW column \(TOSOH Bioscience) Phenyl Sepharose column \(Amersham Biosciences) Sephacryl S-300 column \(Amersham Biosciences) hydroxyapatite column \(Bio-rad) Superose 6 column \(Amersham Biosciences) SDS-PAGE apparatus, semiphor transphor unit, power supply, and microcentrifuge

Procedure

1. Separate Hela nuclear proteins into nuclear extracts and nuclear pellets using a previously described procedure \(Dignam, 1983). 2. Load the Nuclear extract \(6 g) onto a 700 ml P11 column equilibrated with buffer C \(20 mM Tris-HCl, pH 7.9, 0.2 mM EDTA, 1 mM DTT, 0.1 mM PMSF, 0.025 % NP-40, 10 % glycerol) containing 100 mM KCl \(BC100). 3.Step elute proteins that bound to the column with BC300, BC500, and BC1000. 4. Dialyze the BC500 fraction against Buffer D \(40 mM Hepes-KOH, pH 7.9, 0.2 mM EDTA, 1 mM DTT, 0.1 mM PMSF, 0.025 % NP-40, 10 % glycerol) containing 20 mM ammonium sulfate \(BD20) and then load onto a 45 ml HPLC-DEAE-5PW column \(TOSOH Bioscience). 5. Elute bound proteins with an 8 column volume \(cv) linear gradient from BD20 to BD500. 6. Adjust fractions that contain H2A-deubiquitination activity to BD600 with saturated ammonium sulfate and loaded onto a 22

ml FPLC Phenyl Sepharose column \(Amersham Biosciences). Bound proteins were eluted with a 12 cv linear gradient from BD600 to BD0. 7. Apply the active fractions to a Sephacryl S-300 column \(Amersham Biosciences). The H2A-deubiquitination activity eluted between 443-670 kDa. 8. Dialyze these fractions against buffer P \(5 mM Hepes-KOH, pH 7.5, 0.04 M KCl, 0.01 mM CaCl2, 10 % glycerol, 1 mM DTT, 0.1 mM PMSF) containing 10 mM potassium phosphate and load onto a 1 ml hydroxyapatite column \(Bio-rad). 9. Elute the column with a 20 cv linear gradient from BP10 to BP600. 10. Load fractions that contain H2A-deubiquitination activity onto a Superose 6 column \(Amersham Biosciences) equilibrated with BC500. UBp-M eluted between 443-670 kDa.

Timing

3-4 months

References

Dignam, J., Martin, P., Shastry, B. & Roeder, R. Eukaryotic gene transcription with purified components. _Methods Enzymol_. **101**, 582-98 \(1983).