Immunofluorescence staining for studying histone deubiquitination

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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. 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

nocodazole hypotonic buffer \(10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 5 mM MgCl2) KCM-T solution \(10 mM Tris, pH 8.0, 120 mM KCl, 20 mM NaCl, 0.5 mM EDTA, 0.1% Tween 20) with or without 0.1% Triton X-100 2.5% BSA primary and secondary antibodies DAPI mounting solution

Equipment

Shandon Cytospin3 Olympus immunofluorescence microscope

Procedure

Perform immunofluorescence staining performed essentially as described \(Perez-Burgos et al., 2003). 1. Treat HeLa cells \(70–80% confluence) with nocodazole \(400 ng/ml, Sigma) vernight. 2. Collect by trypsinization. 3. Resuspend cells in hypotonic buffer \(10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 5 mM MgCl2) and incubate at 37°C for 15 min. 4. Add 1/10 volume of 3% Tween 20 and centrifuge the cells onto Superfrost slides using a Shandon Cytospin3 \(Shandon, UK) for 5 min at 1000 rpm. 5. After centrifugation, incubate the slides with KCM-T solution \(10 mM Tris, pH 8.0, 120 mM KCl, 20 mM NaCl, 0.5 mM EDTA, 0.1% Tween 20) containing 0.1% Triton X-100 at RT for 10 min. 6. After washing with KCM-T buffer \(without Triton X-100) for three times, fix the cells in freshly prepared 4% para-formaldehyde \(in PBS, pH 7.4) at RT for 10 min. 7. After washing with KCM-T buffer for three times, block the slides with KCM-T buffer containing 2.5% BSA and 10% goat serum at RT for 30 min. 8. Incubate the slides with primary antibody \(1:200 dilution, Anti-phospho-Histone H3-Ser10, Mitosis Marker, Upstate, Catalog # 06-570; 1: 500, Anti-ubiquityl-Histone H2A, clone E6C5, Upstate, Catalog # 05-678) at RT for 1 h. 9. After washing the slides with KCM-T buffer for three times, incubate them with secondary antibodies at 1:500 dilutions \(FITC conjugated goat anti-rabbit IgG, Texas Red conjugated goat anti-mouse IgG, Santa Cruz) for an additional 1 hr. 10. After washing the slides with KCM-T for three times and followed by washing with PBS for one more time, add DAPI at a concentration of 0.5 μg/ml and incubate for 1 min. 11. After

washing with PBS twice and water once, mount the slides with vectorshield mounting media \(Vector Laboratories). 12. Capture images with an Olympus microscope with 20×objective.

Timing

2-3 days

References

Perez-Burgos, L. _et al_. Generation and Characterization of Methyl-Lysine Histone Antibodies. _Methods in Enzymology_ **376**, 234-254 \(2003).