Research Square

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Assays of nucleosome assembly and the inhibition of histone acetyltransferase activity. (9) Plasmid super-coiling assay; and (10) Nucleosome assembly on a fragment of 5S DNA

Takahito Yamasaki
RIKEN BRC
Takehide Murata
RIKEN BRC
Chunyuan Jin
RIKEN BRC
Kohsuke Kato
Tsukuba University
Michiya Noguchi
RIKEN BRC
Koji Nakade
RIKEN BRC
Jianzhi Pan
RIKEN BRC
Kyousuke Nagata
Tsukuba University
Kazunari Yokoyama
RIKEN BRC

Method Article

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Abstract

Introduction

For a detailed introduction to assays of nucleosome assembly and the inhibition of histone acetyltransferase activity, please go here: "http://www.natureprotocols.com/2007/07/30/assays_of_nucleosome_assembly.php":http://www.natureprotocols.com/2007/07/30/assays_of_nucleosome_

Procedure

Plasmid Super-Coiling Assay[1,2] 1. In our experiments, we mixed 2 pmol of core histones from HeLa cells \(see “Preparation of Core Histones by FPLC”) with 4 pmol or 8 pmol of GST or GST-JDP2, respectively, in the assembly buffer \(tube A). The instructions that follow refer to a generalized assay. 2. Incubate the reaction mixture at 30 °C for 30 min. 3. In parallel, mix 100 ng of plasmid DNA \(see Note 1) and 5 units of topoisomerase I in topoisomerase I reaction buffer and incubate at 37 °C for 30 min \(tube B). 4. Combine the topoisomerase I-relaxed circular DNA \(in tube B) and core histones \(in tube A) that were pre-incubated with, in our experiments, GST or GST-JDP2 in 50 µl of assembly buffer. 5. Incubate the mixture at 37 °C for 1 h. 6. Add an equal volume of 2x stop buffer and continue incubation at 37 °C for 30 min. 7. Extract the plasmid DNA with phenol, chloroform and isoamyl alcohol. 8. Precipitate the DNA in cold ethanol. 9. Fractionate the purified DNA by electrophoresis on a 1.2% agarose gel \(see Note 2), and visualize by staining with ethidium bromide \(Figure 1). **Nucleosome Assembly on a Fragment of 5S DNA[3]** 1. Prepare 200 ng of a 197-bp-long fragment of 5S DNA by digestion of pB100-Uless/Strider DNA with EcoR I \(see Note 3). 2. Incubate the DNA in 10 µl of reaction buffer at 37 °C for 1 h with or without 200 ng of core histones that have been pre-incubated with increasing concentrations of, for example, either human His-TAF-1□□or GST-JDP2. 3. Fractionate samples on a 5% non-denaturing polyacrylamide gel in TBS buffer and visualize reaction products by staining with ethidium bromide or SYBR Gold \(see Note 4). 4. Excise gel portions that correspond to mononucleosomes. Allow them to equilibrate at room temperature for 15 min in the standard buffer for SDS-PAGE, prepared with 0.1 % SDS. 5. Transfer proteins to an ImmobilonTM membrane \(PVPF membrane; Millipore Corp., Bedford, MA, USA). 6. Analyze proteins by Western blotting with antibodies against histone H3 \(Figure 2).

Critical Steps

1. Plasmid DNA should be very pure and maintained at an appropriate molar ratio of open-circular to super-coiled DNA \(normally less than 1:9). Purification of closed circular DNA by equilibrium centrifugation in CsCl gradients is recommended. 2. To avoid formation of non-specific complexes of proteins with super-coiled DNA during electrophoresis, it is critical to avoid contamination by ethidium bromide of the agarose gel, the electrophoresis buffer, and the electrophoretic apparatus. 3. Purification of a 197-bp-long fragment of 5S DNA is not always required. A mixture of a fragment of 5S DNA and a 2-kbp-long fragment derived from the pB100-Uless plasmid can also be used for nucleosome assembly. 4. In general, two bands of DNA, corresponding to 197-bp-long 5S mononucleosomes, are detected because of the presence of two \(major and minor) differently positioned mononucleosomes on the fragment of DNA.

References

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Figures

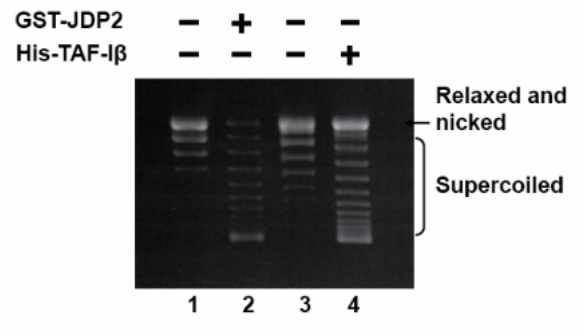

Figure 1

The histone-chaperone activity of JDP2, as detected by an assay of supercoiling. In the presence of core histones from HeLa cells, 8 pmole of GST-JDP2 (lane 2) and His-TAF-1β (lane 4) specifically introduced supercoils into circular DNA. Buffer (lane 1) and GST (8 pmole; lane 3) served as negative controls. The mobilities of relaxed and nicked DNA and of supercoiled DNA are indicated on the right.

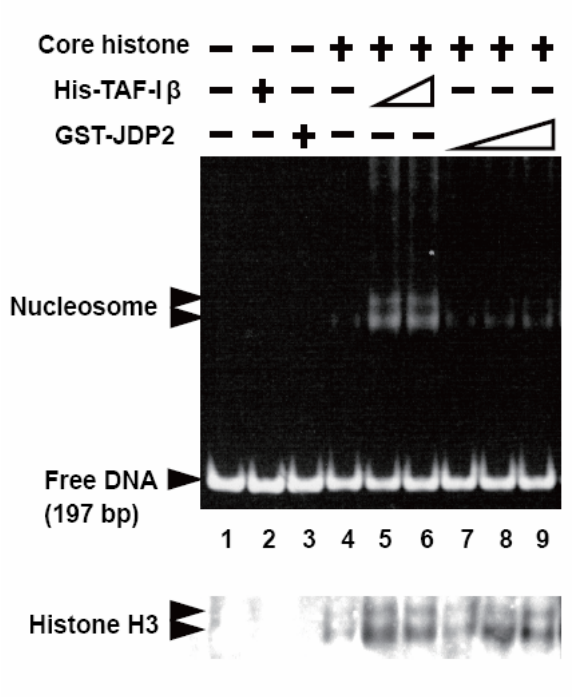

Figure 2

The dependence of histone-chaperone activity on histones. (Upper panel) Nucleosome assembly by JDP2 on a fragment of 5S DNA. 200 ng of a 197-bp-long fragment of 5S DNA were incubated without (lanes 1-3) or with 200 ng (lanes 4-9) of core histones, which had been preincubated without (lanes 1 and 4) or with 500 ng (lane 5) or 1.0 μg (lanes 2 and 6) of His-TAF-Iβ or with 300 ng (lane 7), 600 ng (lane 8) or 1.2 μg (lanes 3 and 9) of GST-JDP2, respectively. After incubation, samples were subjected to electrophoresis on a 5% non-denaturing polyacrylamide gel. The positions corresponding to the 197-bp-long fragment of 5S DNA and mononucleosomes are indicated. (Lower panel) The nucleosomes formed in the presence of JDP2 contained histone H3. Gel portions corresponding to mononucleosomes (upper panel) were excised and the proteins within them were subjected to Western blotting analysis with histone H3-specific antibodies.