

Acknowledgments

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[19] Isolation and Assay of the RSC Chromatin-Remodeling Complex from *Saccharomyces cerevisiae*

By YAHLI LORCH and ROGER D. KORNBERG

RSC is the more abundant of two SWI/SNF-related, chromatin-remodeling complexes found in yeast, and conserved in humans and other eukaryotes.¹ Comprising 15 subunits, many encoded by genes essential for yeast cell growth,^{2,3} RSC has been implicated in both gene activation and repression.⁴ Purified RSC exhibits both a DNA-dependent ATPase activity and the capacity to perturb the structure of nucleosomes,¹ which is conveniently monitored by gel electrophoretic mobility shift analysis,⁵ by enhanced accessibility to nuclease digestion,^{1,5} or by histone octamer transfer from nucleosomes to naked DNA.⁶

Preparation of RSC

RSC can be purified from yeast extracts by conventional chromatography¹ or by tagging and affinity chromatography. In as much as RSC exists in two distinct forms,⁷ one containing only Rsc1 and the other only Rsc2, isolation on the basis of a tag on one of these subunits is expected to yield a homogeneous preparation. Isolation of the Rsc2-containing form is advantageous, since Rsc2 is more abundant than Rsc1 in yeast. A procedure

¹ B. R. Cairns, Y. Lorch, Y. Li, L. Lacomis, H. Erdjument-Bromage, P. Tempst, B. Laurent, and R. D. Kornberg, *Cell* **87**, 1249 (1996).

² B. C. Laurent, X. Yang, and M. Carlson, *Mol. Cell. Biol.* **12**, 1893 (1992).

³ Y. Cao, B. R. Cairns, R. D. Kornberg, and B. C. Laurent, *Mol. Cell. Biol.* **17**, 3323 (1997).

⁴ M. Damelin, I. Simon, T. I. Moy, B. Wilson, S. Komili, P. Tempst, F. P. Roth, R. A. Young, B. R. Cairns, and P. A. Silver, *Mol. Cell* **9**, 563 (2002).

⁵ Y. Lorch, B. R. Cairns, M. Zhang, and R. D. Kornberg, *Cell* **94**, 29 (1998).

⁶ Y. Lorch, M. Zhang, and R. D. Kornberg, *Cell* **96**, 389 (1999).

⁷ B. R. Cairns, A. Schlichter, H. Erdjument-Bromage, P. Tempst, R. D. Kornberg, and F. Winston, *Mol. Cell* **4**, 715 (1999).

for the isolation of RSC with the use of a TAP tag is presented here. Only aspects unique to RSC preparation are described, as general protocols for TAP-tagging and for the isolation of TAP-tagged proteins are available and in common use.⁸

RSC was TAP-tagged at the C-terminus of the second largest subunit of the complex, Rsc2.

TAP-tagging of Rsc2 was accomplished by integration of a PCR fragment encoding the TAP tag and a selectable marker in the yeast genome.⁹ PCR was performed with pBS1479, containing the TAP tag and TRP1 marker of *Kluyveromyces lactis*, and the synthetic oligonucleotides 5'ACGGCGCACAGACTCTCTATGCTGCGGCCTCCTTCGTCGTC-TTCATCCATGGAAAAGAGAAG3' with 45 residues at the 5'-end encoding the C-terminal 15 amino acids of Rsc2, and 5'AGGGTA-ATGCGCAATGGGAAGATATTATGCTGCCATTGCTTTTACTAC-GACTCACTATAGGG3' with 45 residues at the 5'-end representing the reverse complement of the sequence from 12 to 67 residues downstream of the RSC2 open reading frame (not including the termination codon).

The PCR product was transformed into the protease-deficient yeast strain CB010 (MAT a pep4::HIS3 prbl::LEU2 prc::HISG can1 ade2 trp1 ura3 his3 leu2-3, 112 cir^o GAL⁺ RAF⁺ SUC⁺) by the lithium acetate method. Successful integration and expression of the TAP-tagged protein were confirmed by PCR analysis of genomic DNA and immunoblot detection of a polypeptide of appropriate size (102 kDa).

A typical protein preparation begins with 200 g of cells, harvested from 7.5 L of culture, grown overnight at 30° in YPD medium. Cells are suspended in 100 ml of 0.45 M HEPES, pH 7.6, 0.15 M potassium acetate, 3 mM EDTA, 3 mM DTT, 30% glycerol, 3× protease inhibitors, and broken in a bead beater as described.¹⁰ Debris is removed by centrifugation at low speed (e.g., 7500 rpm in a Beckman JA-10 rotor for 30 min) and a clarified supernatant obtained by further centrifugation at 42,000 rpm in a Beckman Ti45 rotor for 30 min. The supernatant (9 g total protein, by Bradford determination) is applied to a 300 ml Bio-Rex 70 column equilibrated with 0.02 M HEPES, pH 7.6, 0.1 M potassium acetate, 1 mM EDTA, 1 mM DTT, 20% glycerol, 1× protease inhibitors, washed with the same buffer containing 200 mM potassium acetate, and eluted with the same buffer containing 600 mM potassium acetate. The

⁸ O. Puig, F. Caspary, G. Rigaut, B. Rutz, E. Bouveret, E. Bragado-Nilsson, M. Wilm, and B. Seraphin, *Methods* **24**, 218 (2001).

⁹ G. Rigaut, A. Shevchenko, B. Rutz, M. Wilm, M. Mann, and B. Seraphin, *Nat. Biotechnol.* **17**, 1030 (1999).

¹⁰ W. J. Feaver, O. Gileadi, and R. D. Kornberg, *J. Biol. Chem.* **266**, 19000 (1991).

eluate (protein-containing fractions, 220 ml) was diluted with the same buffer containing no potassium acetate to a conductivity of 100 μ S at a dilution of 1:200 and purified by IgG and calmodulin affinity chromatography exactly as described⁸ with the use of 300 μ l of affinity beads.

Assay of RSC Activity

Electrophoretic Mobility Shift. RSC binds naked DNA and nucleosomes with comparable affinities and the resulting complexes can be revealed by gel electrophoresis⁵ A system developed for the electrophoresis of nucleosomes, employing gels of high porosity and low ionic strength,¹¹ has proved effective. While gel shifted complexes of RSC with DNA and nucleosomes have comparable mobilities, only the RSC-nucleosome complex is further shifted by treatment with ATP (Protocol 1, Fig. 1).

DNase I Digestion. The first evidence for the perturbation of nucleosome structure by SWI/SNF complex came from an effect on the DNase I digestion pattern of the nucleosome. An alternating pattern of cleavage and protection, characteristic of the nucleosome, was replaced upon treatment with SWI/SNF complex and ATP by a more uniform pattern of digestion, similar to that of naked DNA. Treatment with RSC and ATP produces the same effect.¹ For assay by this approach, the nucleosomal DNA must be end-labeled and following digestion it must be analyzed by

PROTOCOL 1
Gel electrophoretic mobility shift assay

Sample number	1	2	3
³² P-labeled nucleosomes, 3.5 ng DNA/ μ l	1.8	1.8	1.8
1 mg/ml BSA	1.5	1.5	1.5
250 mM HEPES, pH 7.5, 50 mM MgCl ₂	1.5	1.5	1.5
200 mM potassium acetate	3	2.7	2.7
10 mM ATP	–	1	–
20 mM ATP γ S	–	–	0.5
RSC, 1 mg/ml in 375 mM potassium acetate	0.15	0.15	0.15
H ₂ O	12.1	11.4	11.9

Procedure: Nucleosomes are prepared as described (Lorch *et al.*⁵ #2368). All volumes are in microliters. Incubate 20 min at 30°. Add 1.6 μ l of 50% glycerol and subject to electrophoresis in a 3.2% polyacrylamide gel in 10 mM Tris, pH 7.5, 1 mM EDTA.

¹¹ A. J. Varshavsky, V. V. Bakayev, and G. P. Georgiev, *Nucleic Acids Res.* **3**, 477 (1976).

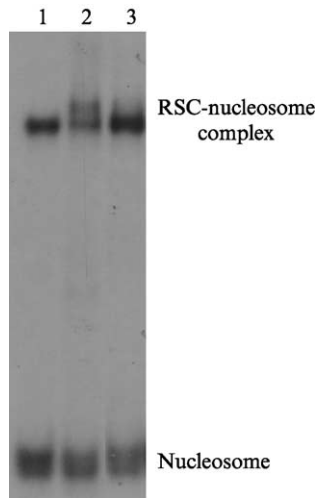


Fig. 1. Gel electrophoretic mobility shift assay (Protocol 1).

PROTOCOL 2
DNase I digestion assay

Sample number	1	2	3	4	5	6
³² P-end-labeled nucleosomes, 3.5 ng/ μ l	-	-	6	6	6	6
³² P-end-labeled DNA	5.3	5.3	-	-	-	-
17-residue oligonucleotide, 3.3 mg/ml	0.25	0.25	0.25	0.25	0.25	0.25
1 mg/ml BSA	2.5	2.5	2.5	2.5	2.5	2.5
250 mM HEPES, pH 7.5, 50 mM MgCl ₂	2	2	2	2	2	2
200 mM potassium acetate	5.6	1.85	5.6	1.85	4.65	3.7
10 mM ATP	-	1	-	-	1	1
RSC, 1.4 mg/ml in 375 mM potassium acetate	-	2	-	2	0.5	1
H ₂ O	4.35	5.1	3.65	5.4	3.1	3.55
Incubate 20 min at 30°C						
DNase buffer	4	4	2.2	2.2	2.2	2.2
DNase I, 13 μ g/ml in DNase buffer	1	1	2.8	2.8	2.8	2.8
Incubate rm. temp. for sec indicated	6	6	40	40	40	40

Procedure: Nucleosomes and DNA fragment were prepared as described. (Cairns *et al.*, 1996) The 17-residue oligonucleotide can be of any base sequence. DNase buffer is 25 mM HEPES, pH 7.5, 50 mM KCl, 5 mM MgCl₂, 3 mM CaCl₂, 0.2 mg/ml BSA, 20% glycerol. All volumes are in μ l. DNase I digestion is stopped by the addition of 2.5 μ l 100 mM EDTA. DNA is extracted and analyzed in a sequencing gel.

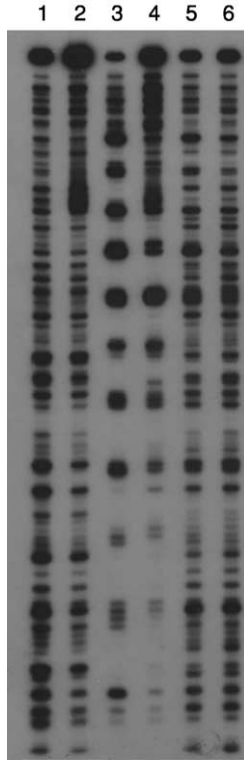


FIG. 2. DNase I digestion assay (Protocol 2).

PROTOCOL 3
Restriction enzyme digestion assay

Sample number	1	2	3	4
³² P-labeled nucleosomes, 3.6 ng/μl	1.7	1.7	1.7	1.7
1 mg/ml BSA	1.5	1.5	1.5	1.5
250 mM HEPES, pH 7.5, 50 mM MgCl ₂	1.2	1.2	1.2	1.2
200 mM potassium acetate	2	1.7	1.7	1.7
10 mM ATP	–	1	1	1
RSC, 1 mg/ml in 375 mM potassium acetate	–	0.15	0.15	0.15
H ₂ O	13.6	12.8	12.8	12.8
Incubate 15 min at 30°				
Dra I, 40 units/μl	1.5	1.5	1.5	1.5
Incubate 30° for minutes indicated	60	20	40	60

Procedure: Nucleosomes are prepared as described (Lorch *et al.*⁵ #2368). All volumes are in microliters. DNA is extracted and analyzed in a 7% polyacrylamide gel in TBE buffer.

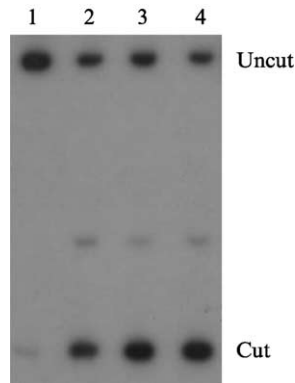


FIG. 3. Restriction enzyme digestion assay (Protocol 3).

PROTOCOL 4
Histone octamer transfer assay

Sample number	1	2	3	4	5
³² P-labeled DNA, 3.7 ng/ μ l	2	2	2	2	2
Nucleosome core particles, 0.19 mg DNA/ml	1	1	1	1	1
1 mg/ml BSA	1.1	1.1	1.1	1.1	1.1
250 mM HEPES, pH 7.5, 50 mM MgCl ₂	0.9	0.9	0.9	0.9	0.9
10 mM ATP	0.75	0.75	0.75	0.75	0.75
RSC, 0.29 mg/ml in 500 mM potassium acetate	0.1	0.1	0.1	0.1	0.1
H ₂ O	7.9	7.9	7.9	7.9	7.9
Incubate 5 min at 30°					
Incubate at 30° for minutes indicated	5	10	20	40	60
Bacterial plasmid DNA, 3 mg/ml	0.4	0.4	0.4	0.4	0.4

Procedure: ³²P-labeled DNA, a 154-bp fragment, was prepared as described.⁶ Nucleosome core particles were prepared as described.¹² All volumes are in microliters. Following reaction and incubation with unlabeled plasmid DNA, glycerol was added and electrophoresis was performed as in Protocol 1.

gel electrophoresis in single stranded form. A 17-residue oligonucleotide may be used as “carrier” in the DNase I digestion, since it is not expected to interfere with RSC-nucleosome interaction, as judged from the failure of such an oligonucleotide to stimulate RSC ATPase activity¹ (Protocol 2, Fig. 2).

Restriction Endonuclease Digestion. Perhaps the most convenient assay of RSC activity is based on restriction endonuclease digestion. Restriction sites normally protected in the nucleosome are exposed by treatment with

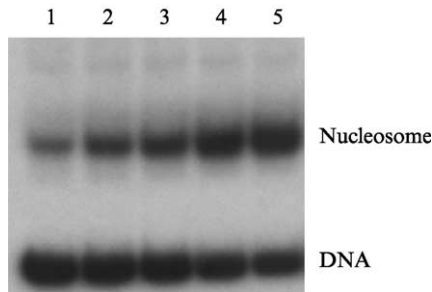


FIG. 4. Histone octamer transfer assay (Protocol 4).

RSC and ATP.⁵ The restriction enzyme must be present simultaneously with the RSC and ATP, as exposure of sites by RSC is only transient. Following digestion, nucleosomal DNA is extracted and analyzed by non-denaturing gel electrophoresis (Protocol 3, Fig. 3).

Histone Octamer Transfer. RSC catalyzes the transfer of a histone octamer from a nucleosome core particle to a naked DNA fragment, in an ATP-dependent manner.⁶ The DNA fragment is radiolabeled, and following the reaction, RSC is removed by the addition of excess unlabeled DNA, so the free, labeled nucleosome that is generated can be detected by gel electrophoresis in the same manner as for the electrophoretic mobility shift analysis described earlier (Protocol 4, Fig. 4).

¹² R. D. Kornberg, J. W. LaPointe, and Y. Lorch, *Methods Enzymol.* **170**, 3 (1989).

[20] DNA Translocation and Nucleosome Remodeling Assays by the RSC Chromatin Remodeling Complex

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The packaging of DNA into nucleosomes compacts and organizes the genome and also restricts access to enzymes involved in DNA and RNA metabolism. Chromatin has a highly dynamic structure; regions of nucleosomal DNA must be accessible at certain times and inaccessible at others. This selected and controlled access to chromatin is tightly regulated by ATP-dependent chromatin remodeling complexes. Chromatin remodelers