SUPPLEMENTARY INFORMATION

MATERIALS AND METHODS

DNA probes for assays on nucleosome arrays

A 381 bp region of the pG1E4T plasmid (Carey *et al*, 1990), containing part of the E4 promoter and a single Gal4 binding site, was amplified by PCR generating *Xho*I sites flanking this region. This DNA segment was used to replace the region between the *Xho*I sites of pG5E4-5s (Ikeda *et al*, 1999), generating the pG1-2n-5s plasmid. To prepare the end-labeled probe, pG1-2n-5s plasmid was digested with *Cla*I, end-labeled with [α-^{32}P]dCTP using Klenow and then sequentially digested with *Fsp*I and *Kpn*I. Then, the 2540 bp DNA segment, containing the 5s rDNA sequences and the Gal4 binding site, was purified from an agarose gel. To prepare internally labeled probe, pG1-2n-5s plasmid was digested with *Hind*III. This material was subjected to dephosphorylation using alkaline phosphatase, labeled using [γ-^{32}P]ATP and T4 polynucleotide kinase and then religated using T4 DNA ligase. Then, sequential digestion with *Bsp*I/*Cla*I and *Kpn*I was performed, followed by purification of the 2540 bp segment from agarose gel.

601 probes

The plasmid pGEM-3Z/601, containing the 601 nucleosome positioning sequence, was a kind gift from Dr. Jonathan Widom (Northwestern University, Evanston, Illinois, USA). The *Not*I-*Pst*I region of pGEM-3Z/601 was replaced by a sequence containing a single Gal4 binding site, locating this binding site 22 bp downstream the 601 nucleosome positioning sequence and generating the plasmid pGEM-3Z/601-Gal4. DNA probes were

generated by PCR from these two plasmids in the presence of [α-^{32}P]dCTP, amplifying a 216 bp region and locating the 601 positioning sequence to the 5' end in both cases.

Nucleosome Mapping

Histone octamer translational positions on dSHA probe were determined by combining micrococcal nuclease and restriction enzymes digestion as described previously (Juan *et al*, 1997). Additionally, the ratio of 5' lateral nucleosome population to 3' lateral population was determined by restriction enzyme digestions and direct loading of the digestion products on non-denaturing polyacrylamide gels (Studitsky *et al*, 1996). For this analysis, 5 µL probe were mixed with 9.5 µL Remodeling Buffer and 0.5 µL of 10 U/µL restriction enzyme (or restriction enzyme buffer, New England Biolabs, Beverly, MA), incubating for 20 min. at 30ºC. Digestion was stopped by addition of a mix containing 750 ng calf thymus DNA, 500 ng long oligonucleosomes and EDTA (20 mM final concentration), incubating for additional 20 minutes at 30ºC before gel electrophoresis. The dried gel was scanned using phosphor screen and Typhoon 9400 (GE healthcare). Quantitative analysis was performed using ImageQuant TL software (GE healthcare). Autoradiography was also performed.

FIGURE LEGENDS

Supplementary Figure 1 Gal4 binding site is accessible on most nucleosome populations generated upon dSHA probe reconstitution. (**A**) Mapping of the preferential translational positions adopted by the histone octamer upon dSHA probe reconstitution, performed by sequential digestion with micrococcal nuclease and restriction enzymes. The picture correspond to a sequencing gel electrophoresis of the restriction enzyme digestion products. Lanes 7-10 (named "Faster" on top) correspond to analysis of nucleosome populations linked to the faster migrating nucleosome band purified from non-denaturing gels (shown in Figure 1A, lane 4). Lanes 11-14 (named "Slower" on top) correspond to analysis of populations linked to the gel-purified slower migrating nucleosome band (shown in Figure 1A, lane 5). Analysis of dSHA probe just after its reconstitution (Figure 1A, lane 2) is shown on lanes 15-18 (named "Total" on top). Lanes 1-6 correspond to size markers derived from restriction enzyme digestions of the full-length DNA probe (named "F.L. dSHA" on top). Marker sizes are shown on the left of the gel. Sizes derived from the nucleosome mapping are displayed on the right. (**B**) Schematic representation of the nucleosome populations deducted from "A". The correlation between the bands obtained on "A" and the nucleosome populations illustrated here is coded by using the geometric symbols displayed inside the parentheses. (**C**) Determination of the relative abundance of 5' lateral and 3' lateral nucleosome populations by direct analysis of restriction enzyme digestion products. The assay was performed using gel-purified lateral nucleosome populations. The location of the restriction sites respect to both lateral nucleosome populations is shown schematically on top. Migrations of full-length (non-digested) DNA and non-digested nucleosome probe

are displayed on the right of the gel, as well as the extent of digestion obtained by incubation with *Eco*RI and *Bam*HI. (**D**) Determination of accessibility of dSHA nucleosome populations to Gal4-DBD binding. Binding reactions were performed for gel-purified DNA (lanes 1-3), lateral (named “Laterals” on top, lanes 4-6) and central nucleosome populations (named “Central”, lanes 7-9). Migrations of the nucleosome populations and Gal4-DBD bound to these populations are shown schematically on the right of the gel, as well as migrations of naked DNA and Gal4-DBD bound to naked DNA.

Supplementary Figure 2 The nucleosome eviction effect of Gal4-VP16 requires the interaction of the transcription factor with the nucleosome probe. As VP16-SWI/SNF protein-protein interaction has been previously demonstrated (Neely *et al*, 1999), this interaction could eventually stimulate SWI/SNF activity, deriving in enhanced sliding and nucleosome eviction effect out of the context of transcription factor binding to the nucleosome probe. In that case, this stimulation would be observed when assaying a nucleosome probe lacking the Gal4 binding site. See legend in Figure 1 for a general description of remodeling assay figures. (**A**) Schematic representation of the probes used in these assays. The probes correspond to 216 bp segments, containing in one end the 601 nucleosome positioning sequence. Additionally, one of them (601_lat_G4) contains a single Gal4 binding site in the region predicted to be linker DNA. (**B**) Remodeling assay comparing 601_lat and 601_lat_G4 probes, performed under standard stringency. (**C**) Comparison of 601-lat and 601-lat-G4 probes under high stringency. This assay

additionally shows eviction and generation of central nucleosome populations in a probe (601_lat_G4) bearing a DNA sequence unrelated to the dSHA probe sequence.

Supplementary Figure 3 The nucleosome eviction effect is not derived from blocking octamer transfer from oligonucleosomes towards DNA probe. In these assays labeled probe, as free DNA, is incubated with a chromatin remodeling complex in the presence of cold nucleosomes. Generation of labeled nucleosomal DNA is indicative of octamer transfer activity. See legend in Figure 1 for a general description of remodeling assay figures. (**A**) ySWI/SNF octamer transfer activity observed by nucleosome formation on dSHA DNA probe, under different stringency levels. Remodeling incubation proceeded for two hours. Error bars correspond to one standard deviation from two independent assays. (**B**) Assay comparing the extent of nucleosome formation given by ySWI/SNF when the DNA probe is used as starting material (lanes 1-4 on both gel pictures) to the extent of nucleosome dissociation obtained in the presence of Gal4-VP16 and SWI/SNF when the nucleosome probe (non gel-purified reconstituted nucleosome) is used as starting material (lanes 5-8 on both gel pictures). The comparison was performed under standard (2 ng/μL SON) and high stringency (27 ng/μL SON). Remodeling incubation proceeded for 30 minutes. Error bars correspond to one standard deviation from two independent assays.

Supplementary Figure 4 Model for transcription factor enhancement of SWI/SNF nucleosome eviction activity. **(A)** In the absence of transcription factor, reassociation of another section of the same DNA chain (small arrow) is favored over the entrance of

acceptor DNA in *trans*. **(B)** The presence of the transcription factor and its interaction with SWI/SNF enhance DNA unpeeling from the nucleosome and/or reduce the reassociation rate of the unpeeled DNA, favoring the entrance in *trans* of acceptor DNA. AD = activation domain. The black star stands for the activation domain interaction with SWI/SNF, which would be required during at least part of this nucleosome remodeling process.

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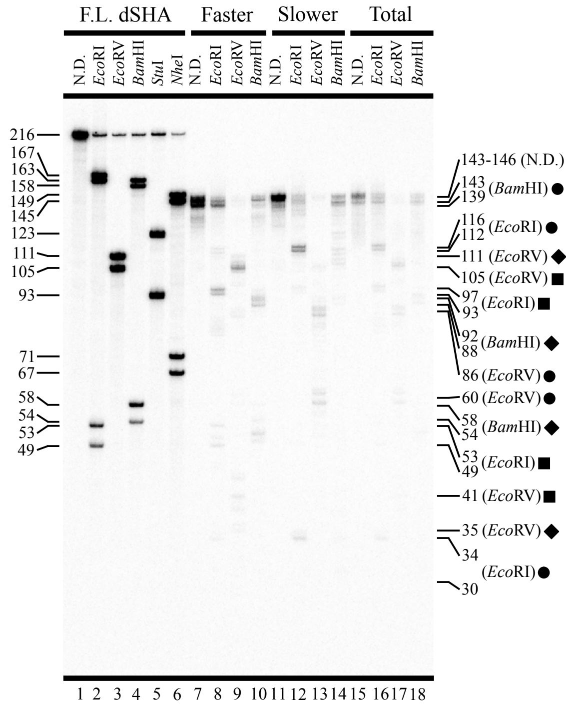
Studitsky VM, Clark DJ, Felsenfeld G (1996) Preparation of nucleosomal templates for transcription in vitro. *Methods Enzymol*, **274:** 246-256

Supplementary Figure 1

A



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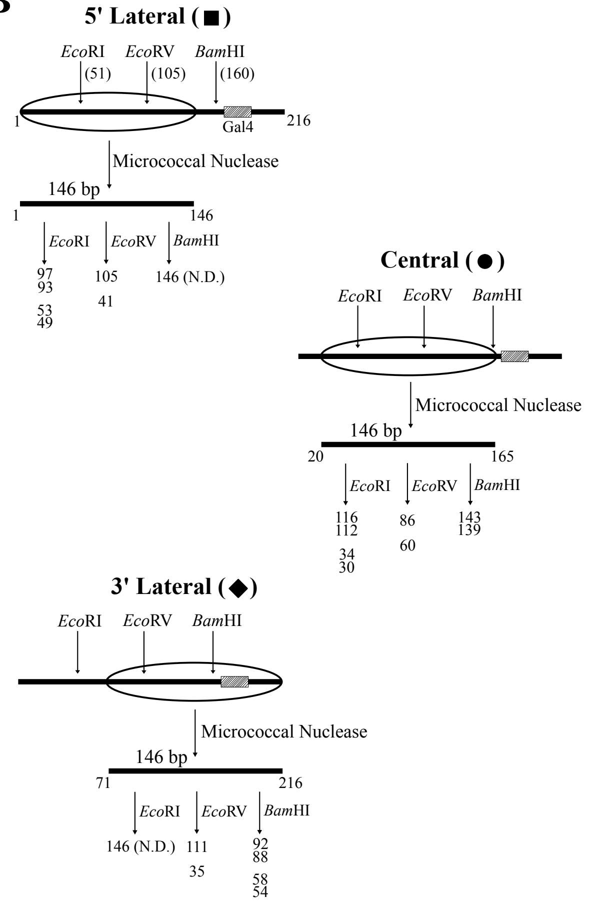

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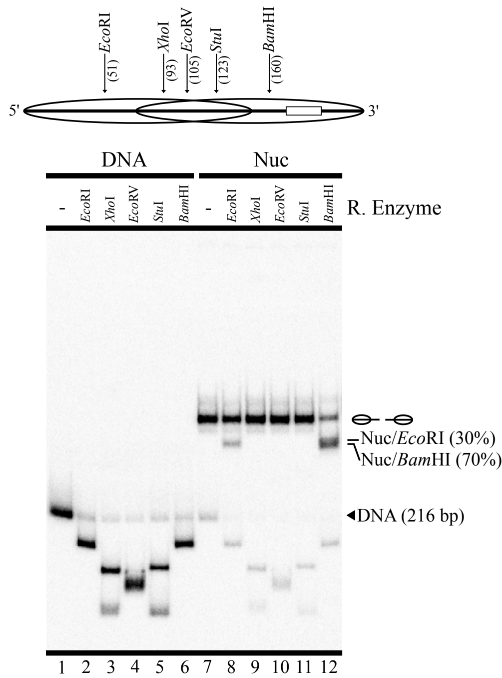

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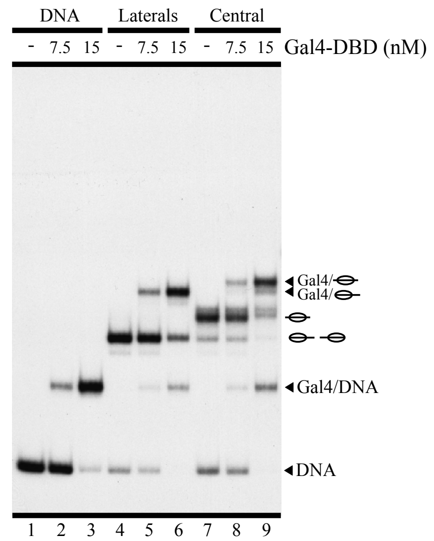

Supplementary Figure 2

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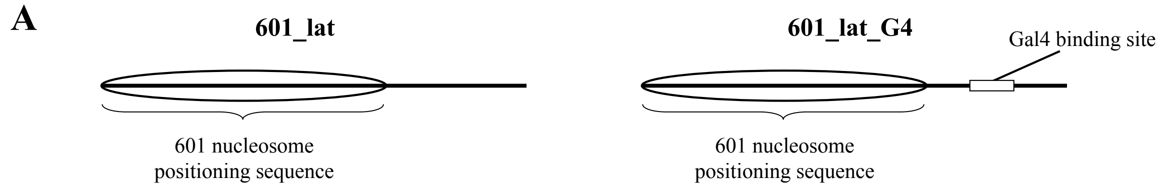

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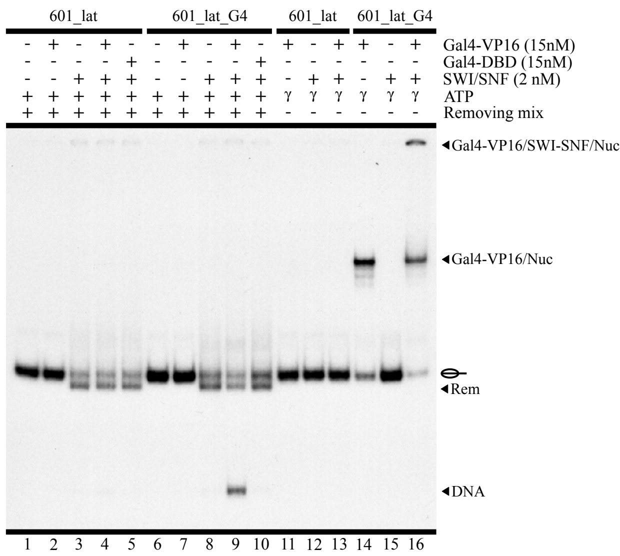

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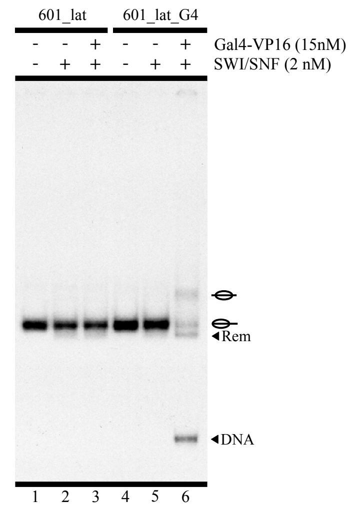
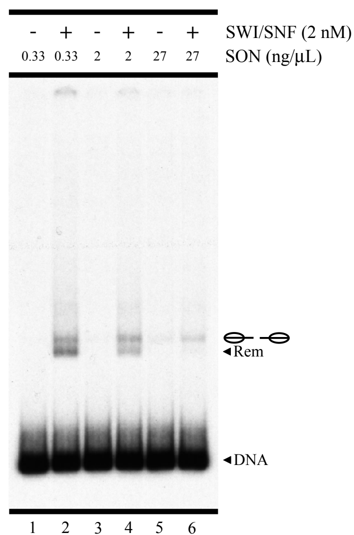
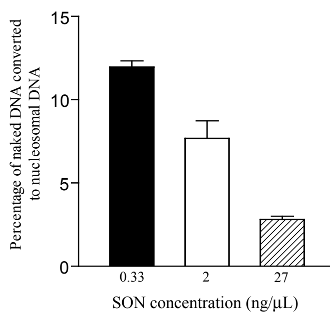


Supplementary Figure 3

A





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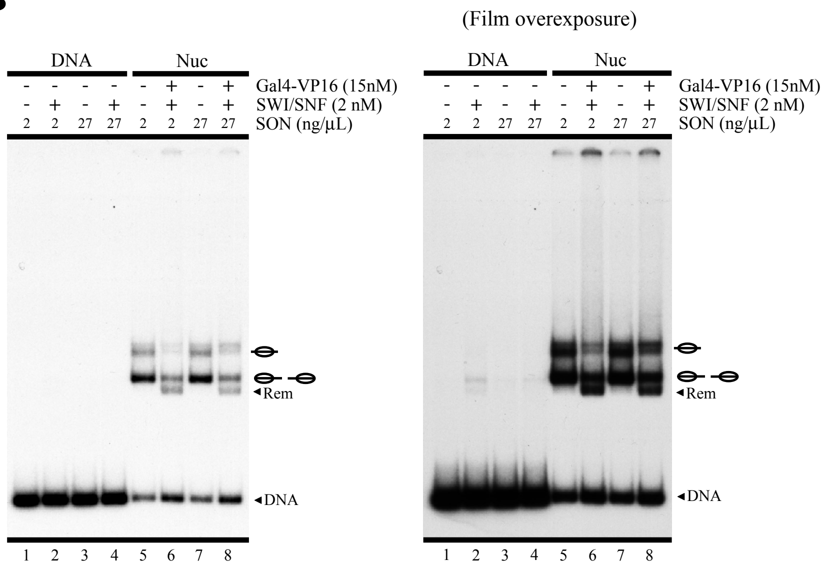

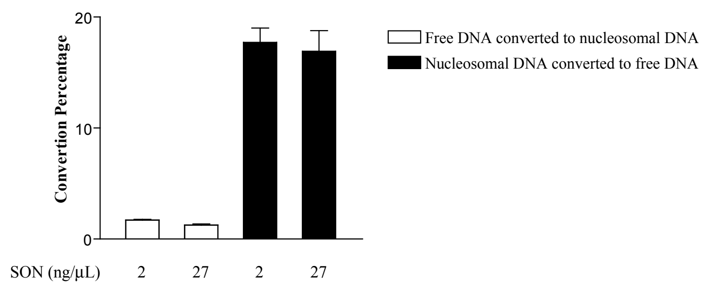

Supplementary Figure 4